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CHRONOTHERAPY WITH 5-FLUOROURACIL (5-FU) AND FOLINIC ACID (FA) IN ADVANCED COLORECTAL CARCINOMA: RESULTS OF A CHRONOPHARMACOLOGIC PHASE I/II TRIAL.

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Most attempts to improve the survival of patients with advanced colorectal cancer have been frustrating. 5-FU ist still the single most effective drug which induces tumor remissions in <20% of the cases. Its antineoplastic activity is enhanced by the concomitant application of FA. This combination, however, has considerable side effects. The circadian timing of antineoplastic drug application (= chronotherapy) is a rather new strategy of reducing cytotoxic side effects. Stimulated by observations of a higher efficacy and lower toxicity of 5-FU by an appropriate circadian timing, we conducted a chronopharmacologic phase I/II trial with 5-FU and FA in 8 patients with advanced colorectal cancer. Patients (pts) received 5-FU and FA at starting doses of 500 mg/m^2/d and 20 mg/m^2/d over 5 consecutive days per treatment course. Treatment courses were repeated after 28 days. Dose escalations of 250 mg/m^2/d 5-FU and 10 mg/m^2/d FA per course were performed in the absence of any toxicity ≥ WHO grade III. Using a portable, ambulatory drug delivery system allowing rectangular changes of the infusion rate (Chronomat, Fresenius, Germany), 75% of the daily doses of 5-FU and FA were given as constant i.v. infusion from midnight to 7h00, and the remaining 25% during the rest of the day.

Dose-limiting toxicity WHO grade III was observed at 5-FU and FA doses of 750 and 30 mg/m^2/d in 5 pts, and 1000 and 40 mg/m^2/d in 3 pts, respectively. Mucositis was the dose-limiting toxicity in 6 pts. A partial clinical remission was achieved in 1 pt, and a stabilization in 2 pts. In the remaining 5 pts, a disease progression occured despite treatment with maximally tolerated doses. The maximally tolerated doses were slightly higher than the average doses reported by conventional phase I/II trials with 5-FU and FA, but clearly lower than those recently reported in a chronotherapeutic trial in which a different, sinusoidal mode of drug application was used (Lévi, Cancer 70:893, 1992). Therefore, we feel justified to caution that the circadian modulation of a given treatment protocol such as 5-FU plus FA may not always allow the safe application of very high doses. Specific delivery systems may be needed in order to make chronotherapy with 5-FU and FA relevant for patients with colorectal carcinoma.

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Identification of the mast cell progenitor as a c-kit$^+$, CD34$^+$, CD14$^-$, CD17$^-$, Ly$^-$, colony forming cell.

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Mast cells (MC) belong to the hemopoietic system and arise from hemopoietic precursor cells. In contrast to other hemopoietic cells, MC are found in extravascular areas but not in peripheral blood stream. So far, the nature and phenotype of the mast cell progenitor cells remain largely unknown. In this study, the identity of the circulating MC-progenitor, previously felt to be a monocyte (Mo) or basophil granulocyte (Ba) was investigated. For this purpose, CD14$^+$ peripheral blood (pb) monocytes, CD17$^+$ pb basophils and CD34$^+$ cord blood CFU were purified from mononuclear cells (normal adult donors, n=17, cord bloods, n=2) by counter-flow centrifugation followed by cell-sorting with mAb. In the presence of novel mast cell growth factor, rhKL (kit ligand also termed stem cell factor SCF, or steel factor, SL), MC developed in long term suspension culture (LTC) from 95% pure CD34$^+$ cells (up to 80% MC on day 80) but not from 99% pure CD14$^+$ monocytes, enriched CD17$^+$ basophils, or lymphocytes (mast cell tryptase levels on day 42: CD14$^+$ Mo: 3.7±0.8 vs CD17$^+$ Ba: 3.2±0.5 vs Ly: 2.0±1.5 vs control: 196.5±92.5 ng/ml, p<0.001). Depletion of CD 34$^+$ cells from MNC (by beads or sorting) resulted in a decrease of MC in LTC (22.8±7% of control), whereas depletion of either Mo (CD14 plus complement, C), Ba (CD17 + C), or Ly, did not. Moreover in methyl-cellulose culture, in the presence of rhKL, MC and tryptase could be detected in pure (CFU-mast) and mixed (CFU-myeloid/mast) mast cell colonies. Together, mast cells do not originate from blood monocytes, basophil granulocytes or from circulating lymphocytes. The circulating MC-progenitor is a CD34$^+$, c-kit$^+$, Ly$^-$, CD14$^-$, CD17$^-$, multipotent colony-forming cell. Mast cells are replenished directly from early (circulating) hemopoetic progenitors and form a unique cell lineage within the hemopoetic system.

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IL-3 AND GM-CSF INDUCE RAPID SERINE-PHOSPHORYLATION OF THE SMALL STRESS PROTEIN (hsp27) INVOLVING ACTIVATION OF THE MAPKAP-KINASE 2.

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Both Interleukin-3 (IL-3) and granulocyte-macrophage colony-stimulating factor (GM-CSF) have been previously identified to induce rapid phosphorylation of the MAP-kinase (Blood 79:2880,1992). However, little is known about signalling events initiated by IL-3/GM-CSF which occur downstream of the MAP-kinase. MAP-kinase has been shown to phosphorylate the AP-1 transcription factor and to activate two kinases designated insulin-stimulated protein kinase-1 (ISPK-1) and MAPKAP- kinase 2 . We here report that IL-3 and GM-CSF induce MAPKAP-kinase 2 activity in the human megakaryoblastic leukemia cell line MO7E and phosphorylate the human small heat shock protein hsp27 on serine residues. In contrast, neutrophils failed to phosphorylate hsp27 upon IL-3, while GM-CSF induced hsp27 phosphorylation in a similar range as observed in MO7E cells suggesting that MAPKAP-kinase 2 mediated hsp27 activation occurs independently of proliferation. Hsp27 phosphorylation is dose-dependent and occurs as early as 5 minutes following exposure to IL-3 or GM-CSF. Moreover, hsp27 phosphorylation is inhibited by tyrosine kinase inhibitors such as genistein or herbimycin A. In addition, we show that protein tyrosine phosphatase and protein phosphatase 2A (PPA2) interfere with the ability of IL-3 or GM-CSF to induce serine phosphorylation of hsp27. Taken together, our findings indicate that tyrosine phosphorylation of MAP-kinase is a prerequisite for serine phosphorylation of hsp27 mediated by MAPKAP-kinase 2. Hsp27 is predominantly localized in the nucleus and has been linked to the cellular stress response. Its precise function, however, is largely unknown. Our data identify hsp27 as a nuclear target of IL-3/GM-CSF stimulation via MAP-kinase and MAPKAP-kinase 2. Furthermore, our results indicate that hsp27 may also exert phosphorylation-activation functions involved in growth signalling pathways in unstressed cells.

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NOVEL GROWTH FACTOR RECEPTOR TYROSINE KINASES IN MEGAKARYOBLASTIC DIFFERENTIATION AND ANGIOGENESIS

Kari Alitalo

In order to get insight into the growth regulation of hematopoietic and leukemia cells and to characterize growth factor receptors of megakaryoblasts, we have cloned novel tyrosine kinase cDNAs from human leukemia cells having a bipotential erythroid/-megakaryoblastoid differentiation potential. This resulted in the identification of several novel tyrosine kinases including a potential anti-oncogene and members of the fibroblast growth factor (FGF) and vascular endothelial growth factor (VEGF) receptor families. One of the novel genes encodes a receptor expressed in fetal endothelial cells and upregulated during neovascularization associated with the ovulatory cycle and wound healing. These receptors may be involved in megakaryopoiesis and in tumor angiogenesis. They provide a potential to prevent the growth of several types of solid tumors by strategies that will be discussed.

Proc. Natl. Acad. Sci. 87: 8913, 1990; ***EMBO J.*** 10: 1347, 1991; 11: 2919, 1992; in press; ***Oncogene*** 6: 2013, 1991, in press; ***Nucl. Acids Res.*** 19: 5096,1991; ***Mol. Cell Biol.*** 12: 1698, 1992; ***Cancer Res.*** 52: 746, 1992; ***Progr. Growth Factor Res.*** 4: 69, 1992.

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IFN-α DIFFERENTIALLY REGULATES CYTOKINE AND GROWTH FACTOR PRODUCTION IN HUMAN BONE MARROW STROMAL CELLS. A POTENTIAL MECHANISM OF INTERFERON ACTION IN CML.

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Bone marrow stromal cells support the differentiation and self-renewal of hematopoietic progenitor cells in long-term cultures. This effect is thought to be at least partially mediated by production of colony-stimulating factors and other cytokines which are produced either constitutively or in response to a variety of exogenous stimuli. Therefore the pattern of cytokine production by stromal cells can be of great importance in the regulation of normal and malignant hematopoiesis. In the present study we investigated cytokine gene expression in human bone marrow stromal cultures in response to interferon-α alone or in combination with inducing agents such as TNF-α, IL-1α and LPS. In all experiments we observed a differential effect of interferon on cytokine expression in these cells. The expression of GM-CSF, IL-1β and IL-8 genes was dramatically inhibited by interferon, while TNF-α and IL-1 receptor antagonist were induced and the expression of IL-6 and TGF-β mRNA remained unaffected. These effects were accompanied by marked induction of the antiviral gene MxA, providing evidence for a specific effect of interferon. Recently published reports suggest the involvement of IL-1 and GM-CSF overproduction in progression of chronic myelogenous leukemia. Regarding these reports downregulation of these cytokines by IFN-α and simultaneous induction of a negative regulatory factor of hematopoiesis like TNF-α could be involved in mechanism of action of IFN-α in IFN-responsive CML patients.

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IMMUNOREGULATORY EFFECTS OF GRANULOCYTE-MACROPHAGE COLONY-STIMULATING FACTOR AND GRANULOCYTE COLONY-STIMULATING FACTOR

M.J. Aman, A. Thews, K. Stockdreher, K.H. Kienast, J. Lorenz, H. Lange, L. Färber, B. Koci, W.E. Aulitzky, C. Huber, C. Peschel

Administration of G-CSF or GM-CSF provides beneficial effects as supportive therapy in patients undergoing cytotoxic cancer treatment. The activity of both hemopoietic factors is largely overlapping with some differences in their hemopoietic profile. A specific clinical application of each CSF in defined clinical settings might depent on additional biologic properties which distinguish the factors such as immunomodulatory properties. Therefore we studied the effects of G-CSF and GM-CSF on regulation of production of cytokines (TNF, IL-1, IL-8, IL-6) and soluble receptors (sCD25, sTNF-R) at the RNA and protein level and investigated the effect of both factors on expression of activation markers and adhesion molecules in PBMNC. Patients with small cell lung cancer (GM-CSF) or bladder cancer (G-CSF) received a single dose of CSF before chemotherapy (CT) and were treated with factor subsequent to CT. Ex vivo evaluations were performed before and after growth factor application. In PBMNC expression of proinflammatory cytokines predominantely produced by monocytes was induced in approximately one third of both patient groups after one single administration of G-CSF or GM-CSF demonstrating no significant difference between the two CSFs. However, serum protein levels of IL-6, IL-8 and IL-1RA were moderately induced in more patients by GM-CSF. sCD25 was markedly induced in all GM-CSF treated patients whereas G-CSF caused no induction. In T cells of GM-CSF treated patients expression of surface CD25 was not induced suggesting that the monocytes are the main source of sCD25. Surface analyses of PBMNC showed that GM-CSF but not G-CSF induced significant expression of MHC class II on monocytes. Adhesion molecules (CD54, CD44, CD11b, CD49d) were differently regulated by G-CSF and GM-CSF. This study indicates a divergent immunomodulatory profile of G-CSF and GM-CSF in vivo. Future studies should provide evidence whether these subtle differences can translate into a specific use of the different CSFs in certain clinical settings.

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EFFICACY OF AN IDARUBICIN-BASED PROTOCOL IN ACUTE MYELOGENOUS LEUKEMIA. Annaloro C., Pozzoli E., Oriani A., Soligo D., Cortelezzi A., Lambertenghi Deliliers G.

Between 1987 and 1992, 60 previously untreated adult AML patients (35 male, 25 female, median age 44 years, range 16-64) received Idarubicin (IDR) (12 mg/m²/day, days 1-3) in sequential combination with Ara-C (120 mg/m²/12 h, days 4-10) as induction therapy; post-remission therapy included two courses of IDR (8 mg/m²/day, days 1-2) and Ara-C (150 mg/m²/days 1-5) in alternation with two courses of VP-16 (150 mg/m²/day, days 1-3) and Ara-C (as above). Patients still in CR received either high-dose Ara-C or bone marrow transplantation (BMT) (autologous or allogeneic) as late intensification, depending on their age and the availability of an HLA-matched donor. CR was achieved in 50 patients (83.3%), 43 after one induction course, 7 after two courses. None of the common clinical and hematological parameters showed any prognostic relevance for CR at univariate analysis; a discriminant function (predictive level 78.33%) for CR was calculated taking into account age, hemoglobin and platelet levels, and the presence of DIC, lymphoadeno- and splenomegaly. As of April 15, 1993, median follow-up was 17 months (range 3-72): 23 patients had relapsed 1-68 months after CR, 7 had died in CR and 20 were alive in first CR. Twelve patients had received autologous BMT as late intensification after a median interval of 11 months from the achievement of CR; two of them had relapsed, one had died in CR, and 9 were alive in first CR. Median disease-free survival (DFS) was 28 months. Univariate analysis did not identify any of the investigated variables as having prognostic significance in predicting DFS. Patients achieving CR after one course had a significantly better DFS than those requiring two courses (median DFS 67 vs 13 months). A discriminant function (predictive level 76.74%) for DFS was calculated taking into account sex, lymphoadenomegalies and the duration of post-chemotherapeutic myelodepression. The DFS of patients undergoing autologous BMT was compared with that of the other patients whose CR duration exceeded 11 months, the median time from CR to autografting; the two groups were comparable for all clinical and hematological parameters except for age. The analysis of DFS favours autologous BMT (6-year DFS chance 76.19 vs 48.53%).The antileukemic activity of the present IDR-based protocol is testified by the high CR rate (mainly after one induction course), and the possibility of minimizing the role of prognostic factors in achieving CR . The better outcome of patients attaining CR after one course supports the opinion that the intensity of the induction treatment offered by an agent as potent as IDR significantly influences DFS; the importance of VP-16 in postremission therapy must also not be overlooked. Furthermore, the present results suggest that autologous BMT may play a role in prolonging EFS in AML patients.

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EXTRACTION OF PROGNOSTIC FACTORS IN IFN-α TREATED PATIENTS WITH CHRONIC MYELOGENOUS LEUKEMIA

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One of the important features in the analysis of prognostic factors is the identification of risk groups to compare results of different clinical trials. A further question is whether the selected factors have the same importance in other patients with the same disease (validity).

In the last two decades several prognostic models have been published to identify different risk groups in patients with chronic myelogenous leukemia (Tura 1981, Sokal 1984, Kantarijan 1990).

The German CML-trial is a multicentre three-arm randomized clinical trial. 622 patients from 60 centres in Germany and Switzerland were randomly allocated to either IFN-α (164) , Busulfan (226) or Hydroxyurea (232).

An initial pool of 17 potential prognostic factors for survival time in the Ph-positive population was selected by combining clinical relevance (clinicians´opinion) and statistical significance (univariate Kaplan-Meier analyses). Six initial variables (age, Karnofsky-Index, blasts, erythroblasts in peripheral blood, extramedular manifestation and organomegaly-related symptoms) were selected as prognostically significant using stepwise Cox-regression. An individual risk function , termed "score1", was constructed using these six variables, which is published elsewhere (Hehlmann 1992).

Applying score1 and two other staging systems, namely Sokal and Kantarijan, to our IFN-α treated population we could not reproduce the same results as in chemotherapy patients. Thus a new extraction of prognostic factors in the IFN subgroup became necessary. 123 IFN-α treated Ph positive CML patients were included in this analysis of prognostic factors. In a stepwise Cox-model only three of the 17 initial variables were statistically significant (eosinophils and blasts in peripheral blood and extramedullar manifesation). The aim of this paper is to present the results of our analyses and to compare them with other staging systems.

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ALLOGENEIC BONE MARROW TRANSPLANTATION IN CML PATIENTS: RISK FACTORS FOR RELAPSE AFTER BMT?

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Between 1982 and May 1993 82 adult patients received an allogeneic bmt for treatment of chronic myeloid leukemia. Disease status at bmt was first chronic phase in 60 patients, accelerated phase (n=13), blast crisis (n=3) and second chronic phase (n=6). The majority of patients were transplanted from an HLA identical, MLC negative sibling (n=67). Other donors were haploidentical family donors (n=6), matched unrelated donors (n=8) and an identical twin. The further analysis deals with 48 CML patients in first chronic phase transplanted from an HLA identical sibling donor. The conditioning regimen consisted of TBI (10 Gy resp. 12 Gy) and Cyclophosphamide 2x60 mg/kg bw. GvHD prophylaxis was Methotrexate alone (n=4), consecutively followed by ex vivo T-depletion (Campath group n=14) and Cyclosporin A/MTX (n=30). The probability of survival, of relapse and of disease-free survival were determined by Kaplan-Meier analysis. Probability of survival at 3950 days is 46 %, probability of relapse is 52 % and probability of DFS is 35 %. To clarify this high relapse rate we analysed single prognostic factors for relapse. Time from diagnosis to bmt and leukocyte or platelet count or organomegaly at bmt were of no significance.

Analysis of GvHD prophylaxis revealed the following: ex vivo T-cell depletion (Campath group, n=14) had a high probability of relapse (59%) and a probability of DFS of 29 % at 3179 days post bmt. With a shorter follow up (2095 days post bmt) GvHD prophylaxis with CSA/MTX had a identical high probability of relapse (66 %) and a probability of DFS of 26 %. When all modalities of GvHD prophylaxis were pooled together, patients without acute GvHD (n=23) had a high probability of relapse (65%) and a probability of DFS of 27 % in contrast to patients with a GvHD grade I and II (n=24), who had a probability of relapse of 34 % and a probability of DFS of 48 %. In patients with chronic GvHD (n=20) the probability of relapse was 63 % with a DFS of 30 %, where in patients without cGvHD probability of relapse was 49 % and DFS 46 %.

In contrast to bmt in acute leukemia these data stress the importance of acute GvHD for the maintenance of remission after bmt in CML patients. To improve the bmt results in CML a combination of intensification of chemotherapy before conditioning and immunomodulation after bmt seem to be promising approaches.

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ELIMINATION OF DETECTABLE RESIDUAL DISEASE IN PATIENTS WITH BCR / ABL - POSITIVE ACUTE LYMPHOBLASTIC LEUKEMIA AFTER AUTOLOGOUS BONE MARROW TRANSPLANTATION.

J. Atta, H. Martin, J. Bruecher, R. Claudé, S. Elsner and D. Hoelzer.

Conventional chemotherapy does not eradicate disease in the vast majority of patients with Ph-pos-bcr/abl-pos. ALL. Most patients who initially enter complete remission will inevitably relapse and succumb to their disease. However, dose intensification including BMT may eliminate leukemia in a proportion of patients. We autografted patients with bcr/abl pos. ALL in first CR using immunomagnetic beads (CD10-, CD19- and HLA-DR(AB-4)-Dynabeads™) -purged marrow following induction chemotherapy and consolidation with HD-AraC/Mitoxantrone according to the German multicenter ALL/AUL trial (04/89) . To evaluate the quality of remission after chemotherapy and after autologous BMT, we analyzed minimal residual disease (MRD) using a semiquantitative PCR assay (SQ-PCR). First, bone marrow samples from patients were ficolled and diluted in 1-log steps in normal ficolled buffy coat cells (limiting dilution), followed by RNA-extraction using a modified guanidine thiocyanate method and random primed reverse transcriptase c-DNA synthesis. PCR was then performed with 35 cycles at 92°C/62°C/72°C denaturation/annealing/extension temperature using *M*ajor- and *m*inor-bcr/abl specific nested primers. The following preliminary results are expressed as $\log_{10}$ of the highest sample dilution with a positive PCR-signal.

Time of sampling		median	(range)
Prior HD-AraC/Mitox	(n=4) :	-4	(-3 to -5)
Post HD-AraC/Mitox	(n=4) :	-4	(-3 to -5)
Purged autol. BM-graft	(n=4) :	-1	(-1 to -2)

The PCR-signal was negative posttransplant in 3 pts on d+29, d+109 and d+165, respectively, and detectable in undiluted BM-samples only in 1 patient on 3 occasions between d+13 and d+75. We conclude that SQ-PCR is highly sensitive to detect MRD in bcr/abl-pos. ALL-patients, that residual leukemia in cytological CR after chemotherapy is median 4-log above the limit of detection, and that consolidation chemotherapy does not significantly decrease MRD. We further conclude that ABMT with purged marrow eliminates detectable leukemia in a substantial proportion of patients despite the high pretransplant residual tumor load and the presence of a low residual SQ-PCR-signal in the reinfused marrow. The follow-up, however, is yet to short to conclude on clinical cure.

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ALPHA-INTERFERON, INTERLEUKIN-2 AND 5-FLUOROURACIL AS A PROMISING BIOCHEMOTHERAPY REGIMEN FOR THE MANAGEMENT OF ADVANCED RENAL CELL CARCINOMA

J. Atzpodien, H. Kirchner, E. Lopez Hänninen, M. Fenner, M. Deckert, and H. Poliwoda.

Recent clinical trials for the biological therapy of solid tumors have used recombinant human cytokines in combination with conventional chemotherapy.

In patients with metastatic renal cell carcinoma, we established a 3-drug combination of subcutaneous recombinant human interferon-α (IFN-α), interleukin-2 (IL-2) and 5-fluorouracil (5-FU) as outpatient regimen. Treatment consisted of eight weeks each of IFN-α (10 million U/m² x3 per week SQ) combined sequentially with IL-2 (5-20 million IU/m² x3 per week SQ for four weeks) and 5-FU (750 mg/m² IV weekly for four weeks).
Among 39 consecutive patients treated, there were 6 complete (15.4%) and 12 (30.8%) partial responders, with an overall objective response rate of 46.2% (95% confidence interval, 30-63%). Median response duration was calculated at 10+ months, and no relapse has occurred among complete responders.
Systemic toxicity was mild to moderate, with no severe 5-FU related mucositis or diarrhea. There were no dose limiting adverse effects of SQ IL-2 and no toxic deaths.

In summary, this outpatient biochemotherapy was as effective as the most aggressive inpatient IV IL-2 regimen; it appeared to significantly improve the therapeutic index in patients with metastatic renal cell carcinoma.

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BIOCHEMOTHERAPY OF ADVANCED MALIGNANT MELANOMA

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The combination of systemic chemotherapy and immunotherapy comprising interleukin-2 and alpha-interferon leads to significant tumor regressions in patients with advanced malignant melanoma. In contrast to chemotherapy by itself, the combination produces a significantly extended remission duration in the majority of treatment responders.
We conducted 2 phase II studies to assess the potentially additive or synergistic effects of chemotherapy and immunotherapy in metastatic malignant melanoma patients:
The first study comprised two cycles of carboplatin (400mg/m²) and dacarbazine (750mg/m²); the sesond study included up to four cycles of cisplatin (25mg/m² x3 days), dacarbazine (220mg/m² x3 days), BCNU (150mg/m², cycle 1+3) and tamoxifen (20mg daily). Chemotherapy was followed by up to 2 cycles of a 6-week immunotherapy comprising interleukin-2 (5-20 million IU/m² sc 3x weekly) and alpha-interferon (3-6 million U/m² sc 3x weekly).
Among 25 evaluable patients in study I, there were 9 (12%CR, 24%PR) objective responders; median remission duration was 18+ months for complete, and 10+ months for partial responders. Chemotherapy intensification in study II lead to an increased response rate of 50% (9 out of 18 patients). In both studies, the progression free interval was significantly extended when compared to patients who received chemotherapy, only (historic controls).
The role of immunotherapy as consolidation in patients with advanced metastatic malignant melanoma is currently being evaluated in a prospective randomized trial.

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VIRUS MODIFIED CANCER VACCINES FOR THE ADJUVANT TREATMENT OF LOCALLY ADVANCED RENAL CELL CANCER

J. Atzpodien, H. Kirchner, U. Zorn, E. Lopez Hänninen, M. Deckert, P. Anton, U. Jonas, and H. Poliwoda.

Patients with locally advanced renal carcinoma are at high risk of relapse after initial radical surgery. We initiated a clinical phase II trial using autologous tumor vaccines for the surgical adjuvant therapy of renal cancer patients.

Seventy-two patients (pts) (25 female, 47 male; median age, 56 yrs; range, 28-77 yrs) with locally advanced renal carcinoma (pT3b-4pN0 or pTxN1-2M0) received autologous Newcastle Disease Virus modified and lethally irradiated tumor vaccines in combination with 1.8 million IU of IL-2 and 1.0 million U of IFN-α2, once weekly over 10 consecutive weeks. Toxicity was very mild with transient flu-like symptoms. Among 55 evaluable patients, there were 5 relapses (2pts, pT3aN1-2; 3pts, pT3bN0); the median relapse-free survival was 22+ months with a range from 6 to 41+ months; survival probability in this vaccine treated cohort was significantly better than in all historic controls.

Using Western blot analyses, we could demonstrate a vaccine specific in-vivo B-cell response in all patients receiving NDV tumor vaccine.

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LOW DOSE INTERFERON GAMMA FOR TREATMENT OF METASTASIZING RENAL CELL CARCINOMA

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We retrospectively analysed four different phase II trials using low dose IFN gamma. 115 patients suffering from advanced renal cell carcinoma were treated with 50-400 µg IFN gamma once per week and response rates ranging from 3-22% were observed. A total of 16 patients achieved either a partial or complete remission (14%, 8-20% 95CI) and no severe acute or chronic side effects of this regimen were observed. Exclusively patients with localized metastatic disease responded to therapy. No response was observed in 27 patients with more than 2 organs affected by the disease. 14 of the remissions were observed in 68 patients with disease confined to one organ with or without involvement of the respective lymph node area (20%, 12-32 95CI). The varying response rates observed in the four low dose IFN gamma studies closely paralleled the proportion of patients recruited with localized disease. We conclude that IFN gamma is active in patients with a low tumor burden of metastasizing RCC. The overall response rate is identical to that reported recently for 327 patients treated with high dose IL-2 (Jones et al, 1993). In addition, it has to be concluded that selection of patients is critical for the response rates observed after treatment of RCC with IFN gamma. These IFN gamma sensitive RCC patients represent approximately 20% of the patients with localized metastatic disease. Similar features have been reported to be predictive for beneficial response to IL-2. Therefore it seems likely that BRM sensitive RCC might represent a distinct entity of kidney cancer. To further characterize this BRM sensitive RCC phenotype seems of critical importance for a rational development of treatment strategies for this malignant disease.

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Computer Assisted Therapy planning In Pediatric Oncology

A. Bachert[1], F. Schilling[2]

CATIPO is a computer program, which has been developed to be used in pediatric oncology (p.o.). It allows the computer assisted transposition of the applied chemotherapy protocols, whereby a high contribution to therapy quality is achieved. It also has a high work and time saving capacity.
This projekt is now over 2 years old, in the course of which the work procedures have proved to be very efficient. On the one hand, there exists a workgroup, called "applied informatics in pediatric oncology", headed by Prof. Michaelis from the institute of med. statistics and documentation in Mainz. A subgroup under the direction of Dr. Schilling, Stuttgart, consisting mainly of medical experts and statisticians, is working out and defining standards for computer applications in the special field of therapy planing in p.o. On the other hand, the university children's clinic Heidelberg started the parallel development of CATIPO. The system was distributed from the beginning to the workgroup members, to test it in the practice. In this way, problems and errors were detected in early stages and could be solved successively. Now, a program has been systematically built up, that, respecting individual wishes and supplements, is universally applicable in any clinical environment. The use of CATIPO isn't even confined to p.o., as any given therapy can be determined with it.
To do this, the user decides which cytostatica or other medicaments are needed and how the necessary infusions are combined. Then these elements are put in a chronological plan. To achieve an individual patient's plan, one only has to choose the corresponding therapy and the starting time.
The printout contains a complete work guide for the nursing staff, including infusion labels for every single bottle. One can also get a cytostatica synopsis in text and graphic form. Aditionally, all the calculations are stored in an ASCII file and therefore can be transferred to a clinic data base, if available.
CATIPO runs on all DOS machines, without requiring any supplementary hard- or software.

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MODULATION OF CYTOKINE RELEASE BY CYSTEAMINE IN IRRADIATED SAMPLES OF WHOLE BLOOD IN VITRO

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Cytokines such as Interleukin-1 and Tumor necrosis factor-alpha (TNF-alpha) have been implicated in protection of normal hematopoiesis from irradiation damage possibly by inhibiting the cell cycle in hematopoietic progenitor cells. Sulfhydryl-compounds have also been used successfully as radioprotective agents in biological systems. In this study we examined the effect of cysteamine on the release of TNF-alpha, Interleukin-1-alpha (IL-1-alpha), IL-1-beta, IL-2 and Interferon-gamma (IFN-g) in an in vitro assay.
Whole blood samples from 8 healthy persons were stimulated with 7.5 g/ml PHA in 5% CO_2 at 37°C. Cysteamine (CM) was added at concentrations of 2,4, 8 and 16 mmol/l. The samples were irradiated with 18 Gray isodose. **IL-1-alpha, IL-1-beta and Il-2:** Compared to controls (PHA stimulation and irradiation) addition of 2 mmol CM first increased cytokine concentrations significantly. But with increasing CM concentrations cytokine values declined dose dependent reaching even lower values than controls at 8 and 16 mmol/l. **TNF-alpha:** The decreasing values according to CM dosage paralleled the course seen in IL-1. The initial increase compared to control was not significant. **IFN-g:** Compared to control significant dose dependent decrease of cytokine release was already demonstrated at an addition of 2 mmol CM. We conclude that low doses of CM are able to induce an increase of radioprotective cytokines in vitro whereas high doses suppress cytokine production in general.

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IN VITRO EFFECTS OF STEM CELL FACTOR AND INTERLEUKIN -11 IN NORMAL AND "PRELEUKEMIC" HAEMATOPOIESIS

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Haematopoietic growth factors (HGFs) represent a new therapeutic tool for hematologists and oncologists. G-CSF and GM-CSF have proven to shorten neutropenia after intensive chemotherapy and to enhance neutrophil count in myelodysplastic syndromes. Several factors are under investigation to stimulate erythropoiesis and/or megakaryopoiesis.

The aim of our study was to investigate a panel of recombinant cytokines for their potential to stimulate haematopoiesis in bone marrow gained from healthy individuals and from patients suffering from myelodysplastic syndrome. The following HGFs / cytokines were applied alone or in combination in a soft agar culture system: stem cell factor (SCF; supplied by Amgen), IL-3, IL-6, IL-11 (supplied by Schering Plough), G-CSF, GM-CSF and EPO. A significant stimulation of granulopoiesis was detected with SCF alone or in combination with other cytokines. SCF given alone demonstrated no effect regarding erythropoiesis, but was highly effective in the presence of EPO. IL-11 applied alone demonstrated no effect regarding granulopoiesis and erythropoiesis, but a significant stimulation of megakaryopoiesis was apparent in normal and "preleukemic" bone marrow. A further increase of megakaryopoiesis was detected in combination with IL-3, but not with IL-6 or SCF.

Our results suggest, that IL-11 might be an interesting factor to induce megakaryopoiesis in vivo, while erythropoiesis might be stimulated by the combination of SCF and EPO even in patients not responding to EPO alone.

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Characterization of a novel Hodgkin cell line HD-MyZ with myelomonocytic features and xenotransplantation into SCID mice

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A novel Hodgkin cell line, designated HD-MyZ, was established from the pleural effusion of a 29-year-old patient with Hodgkin's disease (HD) of nodular sclerosing type. The majority of cells grow adherently and display typical morphological characteristics of Reed-Sternberg (RS) and Hodgkin (H) cells, i.e. large multi- and mononucleated cells with prominent nucleoli. Immunofluorecence analysis revealed a myelo-monocytoid immunophenotype (expression of CD13, CD68 and lack of lymphoid markers). HD-MyZ cells strongly expressed restin a recently described intermediate filament associated protein, the expression of which is associated with H, RS cells and in vitro cultivated peripheral blood monocytes. In addtion mRNA expression of c-fms (CSF-1 receptor) could be induced in HD-MyZ cells by PMA stimualtion. Southern blot analysis did not detect rearrangement of TCR-β and IgH loci.

HD-MyZ cells constitutively express mRNA's for IL-1α, IL-1β, IL-5, IL-6, IL-7, IL-8, IL-10, IL-1 receptor (type I) and IL-6 receptor. Stimulation of cells with PMA increased mRNA expression as well as the secretion of IL-1β, IL-6, IL-8 and induced the de-novo expression of IL-8 receptors.

Xenotransplantation into SCID mice by intravenous or subcutaneuos inoculation led to development of disseminated tumors with infiltrative and destructive growth. In addition lymphadenopathy, pleural effusion and infiltration of spleen were observed.

Proliferation of HD-MyZ cells could be inhibited in vitro by 3'modified phosphothioate antisense-oligonucleotides (AS) against IL-6, whereas control sense oligonucleotides or IL-1β AS did not affect spontaneous proliferation of HD-MyZ cells.

Our SCID mice model might prove helpful in developing new therapeutic strategies in vivo.

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APOPTOTIC CELL DEATH DURING NEOPLASTIC PROGRESSION

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To study the susceptibility of cells at difference stages of neoplastic progression to apoptotic cell death, we used a cell culture model of neoplastic transformation. Following carcinogen exposure, the first preneoplastic stage in this model is cells that have lost one or more senescence genes and become immortal in contrast to the normal Syrian hamster embryo (SHE) cells that senesce after <40 population doublings. The immortal cells retain the ability to suppress tumorgenicity in cell hybrids with tumor cells and are termed sup^+. At a later stage, the cells loose tumor suppressor activity (termed sup^-) but are still nontumorigenic. The sup^+ and sup^- preneoplastic cells both have wild-type P53 and RB genes, suggesting that the sup^+ gene is another, yet unidentified gene. Normal cells in low serum were growth arrested with a low labeling index (0.2%) and little cell death. The immortal sup^+ cells also had a low labeling index in low serum (1.6%) but in contrast to the normal cells, these preneoplastic cells died by apoptosis at a high rate. The cell number decreased by 55% in 48 hr., and apoptosis was clearly evident as detected by electron microscopic examination, formation of DNA ladders, and in situ detection using terminal transferase with biotinylated dUTP to end label fragmented DNA. In contrast, the sup^- cells in low serum maintained a high labeling index (40%) even though the cell number remained constant due to a balance between cell growth and cell death. The cells died, however, predominantly by necrosis. The tumor cells grew in low serum with a high labeling index (40%) and a low degree of cell death. The data support the concept that cancer arises due to alterations in control of cell proliferation and cell death. The surprising finding is that preneoplastic cells have an increased susceptibility to apoptotic cell death due to serum deprivation, which is consistent with the hypothesis that apoptosis is initiated by an imbalance in growth signals. Neoplastic progression can result from the progressive loss of cell death signals.

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CORRELATION OF CLINICAL, HISTOPATHOLOGICAL AND CYTOGENETIC DATA IN 196 PATIENTS WITH MALIGNANT LYMPHOMA

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In a prospective study, the clinical, histopathological and cytogenetic findings in 196 consecutively analyzed patients with malignant lymphomas were collected. 119 patients suffered from B cell lymphoma (77 low grade, 42 high grade), 28 patients from T cell lymphoma (low grade 13, 14 high grade, 1 unclassified) and 32 patients from Hodgkin's disease. Sufficient chromosome analysis was possible in 91% of the cases. 65% of the cases revealed chromosomally aberrant clones, which in most cases showed complex karyotypes. Normal metaphases or single cells with different chromosome abnormalities were mostly found in B-CLL/immunocytoma and in Hodgkin's disease. Recurrent chromosome aberrations were t(14;18)(q32;q21) in centroblastic-centrocytic and centroblastic lymphoma, t(11;14)(q13;q32) in centrocytic and centrocytoid-centroblastic lymphoma, t(8;14)(q24;q32) in Burkitt's lymphoma, trisomy 12 in B-CLL and immunocytoma, inv(14)(q11q32.1) and i(8q) in T-CLL/T-PLL as well as t(2;5)(p23;q35) in large cell anaplastic lymphoma. Translocations t(14;18) and t(11;14) were always accompanied by nonrandom secondary chromosome aberrations. These were +X, +5, +7, +12, 6q- and +der(18)t(14;18) in case of t(14;18), 1p-, +3/der(3), 6q- and der(12) in case of t(11;14).

The impact of the cytogenetic findings on remission rates and survival is currently evaluated in detail.

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SIGNIFICANCE OF CYTOGENETIC DIVERSITY IN TWO RENAL CELL CARCINOMA CELL LINES

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We present cytogenetic and biological data of two newly established renal carcinoma (RCC) cell lines: MZ 1795 and 1257. Both cell lines have been generated from clear cell carcinomas of different morphology. The tumors differed significantly in their clinical and biological behavior. The tumor of MZ 1795 had a much more aggressive clinical course compared with the tumor of MZ 1257. This difference was in accordance with the cytogenetic aberrations and biological features observed.
Both cell lines were studied at the same passage number. They showed very different numerical and structural chromosomal aberrations. MZ 1795 was hyperdiploid/hypotetraploid and had a high degree of genetic instability with some marker chromosomes, e.g. two isochromosomes. No 3p deletion was seen. In contrast to MZ 1795, the cell line MZ 1257 showed a 3p deletion which is the chromosomal hallmark for clear cell type of RCC. The modal chromosome number was hypodiploid. Interestingly, in MZ 1257 an isochromosome was seen too. Morphology and mitotic activity of the cell lines differed significantly.
There is evidence that genetic and clinical distinct subentities of the clear cell type of RCC exist. We conclude that cytogenetic aberrations including the degree of genetic instability seen in cell lines reflect the biological/clinical potency of the tumors they have been generated from. Therefore, chromosomal characterisation of RCC, cell lines as well as primary tumors, may become a prognostic factor.

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CONTINUOUS VERSUS INTERMITTENT CHEMOTHERAPY WITH EPIRUBICIN AND IFOSFAMIDE IN ADVANCED BREAST CANCER

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A total of 239 patients with histologically proven advanced and progressive breast cancer received chemotherapy with Epirubicin (30 mg/m^2) and Ifosfamide (2g/m^2) together with dexamethasone days 1+8, q22. After 6-8 courses, when the maximum response was achieved, patients were randomized to either continuation or interruption of treatment. In all patients with CR, treatment was stopped. The overall response rate was 48%. Patients without prior cytostatic treatment (n=101) achieved 60% objective response (CR + PR). Results were clearly poorer in those patients (n=71) who relapsed after adjuvant chemotherapy or had had chemotherapy (n=56) without anthracyclines and/or Ifosfamide in the metastatic stage before, with a response rate of 42 % and 38 % respectively. At present, 92 patients are randomised (46 in each arm). There were no significant differences neither in time to progression nor survival. Patients relapsing after treatment interruption (n=23) received reinduction with the prior chemotherapy schedule, resulting in 5 objective responders (22 %), 12 cases with NC (52 %) and 6 unresponsive patients (26 %). Toxicity of this regimen was mild with comedication of ondansetron. Alopecia developed in almost all patients. The treatment limiting factor was leucopenia. The Epi/Ifo protocol is effective in advanced breast cancer. There is no indication that prolongation of treatment beyond 6 courses is necessary.

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TOPOISOMERASES AND ANTICANCER DRUG RESISTANCE

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We are studying in human leukemic cells the biochemical and molecular lesions associated with a form of "natural product" multidrug resistance (MDR) that is characterized by alterations in DNA topoisomerase II (topo II) activity or amount (at-MDR). These cells display cross-resistance to many anticancer drugs that interact with topo II. We are also studying mechanisms of resistance to inhibitors of topo I. In several at-MDR cell lines the topo IIα gene harbors point mutations in an ATP binding sequence as well as in the DNA binding region. We have used single-strand conformational polymorphism (SSCP) analyses to screen many cell lines and leukemic blasts from patients for mutations. We have identified mutations by this method (confirmed by sequencing) in ~25% of the "at-MDR-like" cell lines analyzed, suggesting that mutations in these regions of the structural gene are not uncommon. However, we have not found SSCPs in the ATP- and DNA-binding domains of the topo IIα gene of 15 ALL and 13 AML relapse patients who had received etoposide as part of their treatments. By contrast, we have identified a mutation in the blasts from the AML admission of one of eight ALL->AML lineage switch patients. In other studies, we have found that traditional anti-topo drugs such as VM-26 and camptothecin stimulate *c-jun* transcription in drug-sensitive cells. In at-MDR cells, however, the VM-26-stimulated expression of *c-jun* is attenuated in proportion to their resistance, apparently due to a decrease in both *c-jun* mRNA transcription and stability. These data suggest that transcription factors and early response genes may be important in mediating the cytotoxicity of topoisomerase inhibitors and may also play a role in at-MDR. (We are currently evaluating *c-jun* induction by topo I inhibitors in topotecan-resistant leukemic cells.) We have found that the at-MDR cells, while cross-resistant to topo II inhibitors that stabilize covalent DNA-topo II complexes, are sensitive to a wide variety of non-complex-forming topo II inhibitors, suggesting that such agents may afford a novel approach to the circumvention of at-MDR. Finally, the studies described above constitute part of our attempts to develop microdetection assays for drug resistant tumor cells. Other efforts involve development of single-cell functional assays for topo II or topo I activity and resistance that are based on topoisomerase immunostaining and "comet" assays. Results of these and other studies will be reported. (Supported in part by research grants CA30103, CA40570, and CA47941, program project grant CA23099, and CORE grant CA21765, all from NCI, Bethesda, MD, and in part by ALSAC)

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IMPROVED RESULTS OF ALLOGENEIC MARROW TRANSPLANTATION FOR CHRONIC PHASE CHRONIC MYELOID LEUKEMIA

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Study objective: To evaluate the influence of transplant treatment strategies on the major endpoints of allogeneic bone marrow transplantation (BMT) for patients (pts.) with chronic myeloid leukemia in first chronic phase (1st CP-CML). Study design: Single center retrospective multivariate analysis (proportional hazards general linear model [PHGLM] using categorized explanatory variables. Study population: 106 pts. (F/M 40/66, median age 33 [17-57] yrs.) with 1st CP-CML, who underwent BMT with HLA-identical sibling donors (93 pts.) or HLA-compatible (i.e. not more than 1 class-I antigen difference) family donors (13 pts.) between 5/82 and 5/93. Results: With a median follow-up of 3.3 yrs., 58/106 (54%) pts. are alive and in hematologic remission with a projected disease-free survival (DFS) estimate of 50% (95%-confidence interval [95%-CI] 40-60%) at 10 yrs. post BMT. The most important independent predictor for DFS in the PHGLM was the time period of BMT with a 2.0-fold higher probability (95%-CI 1.4-3.0) of DFS for pts. transplanted since 1990 compared to those who underwent BMT between 1982 and 1989 (p=.0006). Transplant-related mortality (TRM) was increased in pts. who contracted acute graft-versus-host disease (aGvHD) of grades II-IV (relative risk 3.5 [95%-CI 1.8-6.6]) (p=.0002) and in pts. treated before 1990 (relative risk 2.2 [95%-CI 1.4-3.4]) (p=.0007). The type of immunoprophylaxis was identified as the most important predictor for grades II-IV aGvHD with a 1.6-fold higher risk (95%-CI 1.1-2.2) (p=.01) for pts. who did not receive a combined short course methotrexate and cyclosporine (sMTX/CSA) prophylaxis. A stratified analysis using these treatment-related prognostic factors revealed a DFS estimate of 90% (95%-CI 76-100%) at 3 yrs. for the 23 pts. who underwent BMT since 1990 and received sMTX/CSA as prophylaxis for aGvHD compared to a DFS estimate of 53% (95%-CI 41-65%) for all other pts. (p<.005). Conclusions: This single-center analysis demonstrates that the outcome of allogeneic BMT in pts. with 1st CP-CML has been considerably improved by modifications of transplant-associated treatment strategies. Most importantly, this was achieved by a significant reduction of TRM, which was not offset by an increased risk of disease recurrence.

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ENDOTOXIN CONCENTRATION IN NEUTROPENIC PATIENTS WITH SUSPECTED GRAM-NEGATIVE SEPSIS: CORRELATION WITH CLINICAL OUTCOME AND THERAPY WITH POLYCLONAL IGM-ENRICHED IMMUNOGLOBULINS

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We carried out a study in patients with severe neutropenia from hematologic malignancy and suspected gram-negative sepsis to evaluate the clinical significance of endotoxin plasma levels before and during therapy with polyclonal IgM-enriched immunoglobulins (Pentaglobin). The immunoglobulin was administered every 6 hours for 3 days (7.8 g IgM, 7.8 g IgA, 49.4 g IgG). Concentrations of endotoxin and anti-endotoxin core antibodies were determined by a chromogenic Limulus amebocyte lysate test and ELISA, respectively, before each immunoglobulin infusion and during the following 25 days. Of the 21 patients enrolled into the study, 17 were endotoxin-positive, and in 5 gram-negative microorganisms could be detected in blood cultures. Overall mortality from endotoxin-positive sepsis was 41% (7/17), and 64% in patients with septic shock (7/11). Nonsurvivors revealed significantly higher maximum concentrations of endotoxin compared with those of survivors at the first study day (medians 126 vs 34 pg/ml, $P < 0.05$) and during the whole septic episode. In survivors, endotoxin levels decreased significantly within the initial 18-h treatment period from a median of 28 pg/ml to 8 pg/ml, whereas in nonsurvivors no significant change of endotoxin levels was observed. IgM and IgG antibodies against lipid A and Re LPS increased significantly under immunoglobulin treatment. These data strongly suggest a prognostic significance of endotoxin plasma levels and stimulate a prospective placebo-controlled trial to assess the impact of a therapeutic intervention with polyclonal IgM-enriched immunoglobulins on the clinical course.

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PROGNOSTIC RELEVANCE OF THROMBOSIS OR EMBOLISM IN PATIENTS WITH HIGH-GRADE MALIGNANT NON-HODGKIN LYMPHOMA (h-NHL)

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As reported previously, 593 patients (pts) with h-NHL enrolled in the prospective response-adapted COP-BLAM / IMVP-16 trial had been evaluated for the occurrence and outcome of thromboembolic complications (TEC). The total frequency of TEC was 6.6 % (39/ 593 pts) and TEC-related fatality rate figured with 0.67 % (4/593 pts). To evaluate the prognostic significance of TEC in h-NHL, TEC-patients were now stratified for the phase of polychemotherapy at the time of occurrence of TEC into a pre-treatment, a during- and post-treatment group. These three groups were analyzed separately for the outcome of lymphoma and causes of death and compared to non-TEC pts. Correction for known risk factors (age, stage and dose-intensity of chemotherapy) was performed. TEC occurred in 12/39 pts before and in 21/39 pts during treatment whereas in the remaining 6/39 pts chemotherapy had already been discontinued for a median of 6.5 months. All four cases, in whom TEC was the main or a contributory cause of death belonged to the during-treatment group. Two of these patients had progressive disease while the other two were in partial remission. Overall survival (OS), remission rates (RR) and relapse-free survival (RFS) did not differ between the pre-treatment TEC and the non-TEC group. For the during/post treatment TEC group univariate statistical analysis revealed a significantly lower OS and RFS as well as an inferior RR. These results persisted after correction for age, stage and dose-intensity.

Thus, 1) TEC in hNHL-patients is rare and seldom fatal. 2) The occurrence of TEC during/post treatment in hNHL-patients may constitute rather an additional prognostic indicator of an unfavorable course of lymphoma than an additive dangerous complication.

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EFFECT OF POLYCHEMOTHERAPY ON COAGULATION INHIBITORS IN PATIENTS SUFFERING FROM PULMONARY CANCER

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Multiple mechanisms are involved in the pathophysiology of hypercoagulability in cancer. Protein S (PS) and protein C (PC) are vit.k-dependent coagulation inhibitors. Bound to C4b-binding protein (C4bBP), PS looses its cofactor activity for the anticoagulatory active PC. In patients suffering from pulmonary cancer we have examined the change of PS, PC and C4bBP during four cycles of polychemotherapy (c1-c4).

Methods: Patients: 52 normals, 43 patients with pulmonary cancer were examined. Only patients with intact liver function were included in the study. Protein C: ELISA protein C, Boehringer, Mannheim. Protein S: EID protein S, Boehringer, Mannheim. Free protein S were determined after precipitation of C4bBP-bound protein S with C4bBP-ASSERA-PLATE test, Diagnostika Stago, FRA.

Results: Normals (n=52): PS total 109.7 ± 17.7, PS bound 68.1 ± 15.6, PS free 41.8 ± 9.6, PC 93.8 ± 14.3, C4bBP 119.0 ± 28.1.

	c1	c2	c3	c4	p
PC	103.95	100.75	103.25	106.70	0.736
PStotal	159.50	149.50	150.65	150.95	0.373
PSbound	128.55	118.55	117.50	119.00	0.330
PSfree	30.35	33.75	35.40	33.60	0.015
C4bBP	158.45	150.95	148.00	142.35	0.081

Conclusion: Patients suffering from pulmonary cancer have increased levels of PS total,PS bound and C4bBP and decreased levels of PSfree compared to healthy group. Treatment with polychemotherapy results in significant increase of PS free leading to decreasing thromboembolic risk.

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DETECTION OF THE BCR-ABL FUSION ON BLOOD SMEARS USING FLUORESCENCE IN SITU HYBRIDIZATION

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The Philadelphia (Ph[1])-chromosome contains the ABL protooncogene of chromosome 9 fused with the BCR gene on chromosome 22. It is present in 95% of patients with CML. In adult ALL, the t(9;22) is the most frequent chromosomal abnormality and associated with poor prognosis. So far, Ph[1] has been detected either by G-banding analysis, polymerase chain reaction or Southern blot analysis. These methods are time consuming and patient material has to be specially prepared. We demonstrate the rapid detection of the BCR-ABL fusion on blood smears by fluorescence in situ hybridization (FISH) using the yeast artificial chromosome (YAC) clone D107F9 (kindly provided by Dr. H. Riethman, Philadelphia and Dr. T. Cremer, Heidelberg). In case of the BCR-ABL fusion, part of its sequences are translocated to the 9q+ chromosome resulting in an additional hybridization signal. The usefulness of this clone has been evaluated on 6 controls and 17 pts (12 with CML and 5 with ALL, 2 with the breakpoint in M-bcr and 3 in m-bcr). In the controls, the majority of cells (mean 95.4%, range 93.4% to 98.1%) had two signals whereas in pts with CML and ALL the majority of cells exhibited 3 fluorescence signals (CML: mean 84.5%, range 77% to 97%, ALL: mean 72.3%, range 60% to 79.5%). Subsequently the probe was applied to blood smears of pts with CML. Again, a high percentage (mean.73.8, range 62% to 86%) of cells exhibited 3 signals. To further increase the sensitivity and specificity of this approach, dual color hybridization was performed using the YAC derived probe in combination with cos-abl-8, a cosmid flanking the breakpoint region on chromosome 9. Conclusion: In contrast to previously published in situ hybridization experiments for the detection of the BCR-ABL fusion, our data demonstrate that Ph[1]-positive leukemias can be rapidly detected directly on blood smears. As the YAC clone spans both the major and the minor breakpoint cluster region, this is possible in CML and ALL.

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CHEMOTHERAPY FOR CANCER PATIENTS: CAN HEMATOPOIETIC GROWTH FACTORS HELP?

Wolfgang E. Berdel

Interleukin-3 (IL-3), granulocyte-macrophage colony-stimulating factor (GM-CSF), granulocyte-CSF (G-CSF) and macrophage-CSF (M-CSF) belong to a family of glycoproteins that control growth and differentiation of hematopoietic progenitor cells, modulate functioning of their mature progeny and can modulate apoptosis. Among various other possible indications for their clinical use, recombinant human (rh) IL-3, GM-CSF and G-CSF can accelerate bone marrow recovery after myelotoxic therapy in cancer patients. Randomized studies have shown that G-CSF and GM-CSF have a clear impact on the supportive care for patients under chemotherapy when hematopoietic recovery and clinical parameters such as neutropenic fever and use of antibiotics are evaluated. Furthermore, an area of interest for the clinical application of these and other CSF is their potential role in dose escalated cytotoxic chemotherapy with or without radiotherapy. Four avenues of research are discussed for the CSF in this respect:

1. The possible role of CSF such as rhGM-CSF and rhG-CSF in dose escalation of chemotherapy without stem cell support.
2. The role of rhGM-CSF and rhG-CSF with or without cytotoxic treatment in harvesting autologous peripheral blood stem cells (PBSC) to allow PBSC support after high dose chemotherapy.
3. The role of multiple CSF active in the hematopoietic system to expand hematopoietic precursor cells ex vivo before their use for stem cell support.
4. The role of rhGM-CSF and rhG-CSF in accelerating recovery of reinfused autologous PBSC or bone marrow cells after high dose chemotherapy.

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Chemotherapie bei Tumorpatienten: Können hämatopoetische Wachstumsfaktoren helfen?

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INFLUENCE OF RECOMBINANT HUMAN (RH) INTERLEUKIN 10 (IL-10) ON CLONAL GROWTH OF HUMAN MALIGNANT LYMPHOID CELL LINES UNDER DEFINED GROWTH CONDITIONS IN VITRO.

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IL-10 is a member of the cytokine network which among other activities can act as a cytokine synthesis inhibitory factor (CSIF) and as growth and differentiation cofactor for various cells. The IL-10 gene shows homology to the Epstein-Barr virus gene. Here, we describe results testing rhIL-10 (PeproTech, Rocky Hill, NJ, USA) on the clonal growth of different human lymphoma and leukemia cell lines in vitro. Cell lines (plating efficiency of controls in %, range) tested were Raji (8.4-29.9), Daudi (14.7-23.3), CCL 87 (4.8-6.6), U 698 (4.1-9.3), DHL 4 (0.5-39.8), U 937 (0.2-21.0), LiA (16.9-39.1), CEM (9.3-22.0), Molt-4 (10.1-24.5), and Jurkat (8.5-18.3). IL-10 (0, 0.2, 2, 20, 200 ng/ml) was tested in a human tumor cloning assay (HTCA) in methylcellulose. HTCA has previously been shown to reliably detect positive and negative growth control by cytokines. Cells were continuously exposed to the cytokine for the complete assay period. Clonal growth of one of the cell lines (U 698, Burkitt)) was significantly stimulated by rhIL-10 to >1.5-fold of the controls, however, without clear dose-relationship. Modulation of clonal growth in the other cell lines by rhIL-10 was only minor (>0.5 - <1.5-fold), although significant growth stimulation was seen also in CCL 87 (Burkitt). Further experiments testing these lymphoid cell lines for potential autocrine loops involving IL-10 are underway.

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THE PROXIMAL FOREARM, THE DISTAL UPPER ARM, AND THE CHEST WALL AS IMPLANTATION SITES FOR A NEW CENTRAL VENOUS ACCESS MINI-PORT.

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A new mini-port system (Pharmacia Deltec, Erlangen, FRG) was used for central venous access in 61 patients. Fifteen patients (with solid tumors x8, lymphomas x4, acute leukemias x3) had the port positioned on their proximal forearm, 24 (with solid tumors) on the distal upper arm, and 22 (with solid tumors x13, lymphomas x7, acute leukemia x1, CML x1) on the chest wall. Observed life-span of mini-ports was 201 days (range 28-549) for the proximal forearm port, 130 days (range 29-261) for the distal upper arm port, and 87 days (range 0-480) for the chest wall port with cumulative observation periods of 3778, 3120 and 3358 patient-days respectively. Mini-ports were used for chemotherapy, supportive treatment including parenteral nutrition and transfusion of blood products and for taking blood samples. No complications were observed in 8 of 15 patients with the proximal forearm port, 23 of 24 patients with the distal upper arm port, and 18 of 22 patients with the chest wall-positioned port. There were 6 patients with peripheral vein thrombosis, 3 with reversible port occlusion, and 3 with port infections for the proximal forearm port. One port infection occurred in the group with the distal upper arm port. For the chest wall position we have found 1 patient with port infection, 1 with dislocation of the catheter tip, 1 with unsuccessful implantation, 2 patients with paravasations, and 1 patient with port occlusion. However, loss of function and/or explantation were the consequences for only 6 mini-ports (proximal forearm port x2, distal upper arm port x1, chest wall port x3). A new electromagnetic catheter tracking system (Cath-Finder, Pharmacia) was used in 32 patients for implantation of peripheral access port. When compared with x-ray detection the system predicted the catheter tip accurately in 29 patients and changed surgical procedure by predicting wrong positioning in 4 patients. The new mini-port can be used like the older and larger systems with less cosmetic damage. Positioning of the port on the distal upper arm or on the chest wall was accompanied with less frequent complications. Acceptance for the 2 more central positions was better than for the position on the proximal forearm by the patients, physicians, and nurses interviewed.

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NERVE GROWTH FACTOR (NGF) STIMULATES CLONAL GROWTH OF HUMAN LUNG CANCER CELL LINES AND A HUMAN GLIOBLASTOMA CELL LINE EXPRESSING THE HIGH AFFINITY BUT NOT THE LOW AFFINITY NGF RECEPTOR.

WE Berdel, L Sreter, B Wiedenmann, D Oberberg, B Reufi, and E Thiel

The growth of a panel of 22 different human tumor, leukemia and lymphoma cell lines was examined in a human tumor cloning assay (HTCA) in agar or methylcellulose and a tritiated thymidine uptake assay. The cultures were performed in the absence or presence of increasing concentrations (0.5 - 500 ng/ml) of NGF. The growth of 17 out of the 22 cell lines was not significantly and reproducibly affected by NGF. There was minor (1.2-fold) but reproducible stimulation of clonal growth in 1 glioblastoma cell line (86-HG-39) by NGF, but in this cell line NGF induced no growth modulation in a tritiated thymidine uptake assay. However, clonal growth of another glioblastoma cell line (87-HG-31) and all 3 lung cancer cell lines tested (HTB 119, HTB 120, CCL 185) could be stimulated up to 3-fold by NGF with a dose-response relationship for the growth factor. Growth stimulation by NGF could be completely reversed by neutralizing anti-NGF antibody. Evaluation of secondary plating efficiency (PE_2) revealed the stimulation of colony formation as representing self renewal and not differentiation. Out of the 5 responding cell lines only 86-HG-39, the cell line with the lowest responsiveness, revealed low-affinity NGF receptors (LNGF-R), the other 4 cell lines with high responsiveness, including the 3 lung cancer cell lines, expressed no LNGF-R as shown by FACS analysis and immunoprecipitation using the ME 20.4 antibody. However, binding studies with iodinated NGF showed only LNGF-R on the 86-HG-39 cell line and only high-affinity NGF receptors (HNGF-R) without LNGF-R on the the high-responder cell lines CCL 185 and 87-HG-31. Our data suggest that NGF can be operative in stimulation of clonal growth of malignant tumor cells, and that HNGF-R are sufficient to mediate signal transduction for clonal growth.

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PRODUCTION OF IL-1ß AND CD23 BY HUMAN B LYMPHOCYTES: COOPERATIVE EFFECTS OF HIV-1 AND EBV

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B cell lymphomas are frequently occurring in patients infected with the human immunodeficiency virus type 1 (HIV-1). To assess a possible role of HIV-1 in the activation of B cells and to identify critical elements in B cell proliferation, we infected primary human B cells from HIV-1-seronegative, Epstein-Barr virus (EBV)-seropositive donors by cell-free culture supernatants of HIV-1 strain 3B. 48 hours after infection HIV-1-specific mRNA was detectable by Northern blotting and virus capsid protein (p24) was secreted 4 days after onset of cultures. A profound proliferative response as revealed by ^{3}H-thymidine incorporation was also seen in these cultures. In a second set of experiments, recombinant HIV-1 proteins were used instead of infectous virus. While the addition of gp120, p24, p55, rev or nef had no effects, the transactivating protein tat led to a significant uptake of ^{3}H-tymidine. Moreover, tat-stimulated B cells continued to proliferate and finally resulted in EBV-positive B cell lines. To analyze the production of cytokines relevant for B cell growth, ELISA's specific for IL-1ß and sCD23 were used. Kinetic studies revealed that immediately after infection none of these cytokines were produced. After 2 weeks, however, high amounts of IL-1ß and sCD23 could be detected in culture supernatants and paralleled the occurrence of an EBV-encoded antigen (EBNA-2), known to upregulate CD23-expression in B cells. Both cytokines are induced by EBV rather than by HIV-1, since transfection of B cells with plasmids encoding the EBNA-2 gene efficently induced CD23 as well as IL-1ß secretion in B cells, while tat-encoding plasmids failed to do so.
Based on our results it is tempting to speculate, that HIV-1 and HIV-1/tat activates endogenous EBV. This is further strengthened by recent results of cotransfection experiments in which transfection of HIV-1/tat- and EBV-BMRF1-promotor/CAT-containing plasmids into B cells led to an activation of the BMRF1-promoter. We therefore propose, that B cell proliferation seen in AIDS is mediated via cooperative effects between HIV-1 and EBV leading to the secretion of cytokines important for B cell growth.

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INTERLEUKIN-2 (IL-2) IS A FEASIBLE CONSOLIDATION TREATMENT AND MAY PROLONG SECOND COMPLETE REMISSION (CR) OF ACUTE MYELOCYTIC LEUKEMIA (AML)

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Elimination of minimal residual disease (MRD) in patients (pts.) with AML remains a major problem for long-term disease-free survival (DFS) and the induction of graft versus leukemia (GvL) like reactions with IL-2 may be an interesting new therapeutic approach. We conducted a multicenter phase II trial with IL-2 late consolidation in pts. with 1st relapse of AML. Patients with secondary AML were not included in the study.
The patients receive two cycles of intermediate high-dose cytosine arabinoside (iHIDAC, 600 mg/m² q12h x 8) and VP-16 (100 mg/m² d1-7) as induction therapy and a third cycle as early consolidation. After a rest of 4 weeks, 1h infusion of 9x10^6 IU/m² rIL-2 (EuroCetus, Frankfurt, FRG) is administered on day 1-5 and 8-12. After a rest of 4 weeks, this cycle will be repeated up to 4 cycles totally. Pts. undergoing autologous bone marrow transplantation (ABMT) receive IL-2 starting latest 6 weeks after ABMT, too.
Up to now, 47 patients were included into the study. 25/42 (58%) evaluable patients achieved CR after chemotherapy. 14 pts. received IL-2 late consolidation, 4 after ABMT., 6 pts relapsed before IL-2 therapy, 3 pts refused further therapy and 2 pts are too early for evaluation. The median remission duration of pts, who received IL-2, is 19 months. 4/14 pts (29%) achieved a longer 2nd than 1st remission duration. The toxicity was moderate and the schedule feasible. Only two patients after ABMT developed severe infections (sepsis, pneumonia) after IL-2. The monitoring of immunological parameters revealed a induction of endogenous TNF-alpha, IFN-gamma, IL-6 and adhesion molecules. In conclusion, the preliminary data suggest a benefit of IL-2 for prolongation of relapse free survival in AML with 2nd remission.

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ANTIPROLIFERATIVE ACTION AND MODULATION OF CYTOSOLIC Ca^{++} CONCENTRATION BY ETHER PHOSPHOLIPID ANALOGUES

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Antitumour ether lipids are structurally related to the naturally occurring substances lysolecithin and platelet-activating factor.
Numerous ether lipids have been synthesized and some of them were found to be selectively cytotoxic to tumour cells. However, the molecular mechanisms of action are not yet fully understood.
We have synthesized structurally related alkylphosphocholines and alkylphosphoserines and investigated the structure-activity relationships with respect to their antiproliferative action and their effect on the cytosolic Ca^{++} concentration (fura-2 fluorescence measurement in cell suspension) in different normal and transformed cell types.
The ether phospholipid analogues investigated induce a transient and/or a sustained increase in cytosolic Ca^{++} concentration. Interestingly, the compounds increase not only the cytosolic Ca^{++} concentration but also inhibit the transient $(Ca^{++})_i$ response induced by a standard ether phospholipid (1-O-hexadecyl-2-chloro-2-deoxyglycero-3-phosphocholine). The antiproliferative action seems to be related to the last-mentioned effect.

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EFFECTIVE CHEMOTHERAPY WITH A COMBINED THERAPY OF VINCRISTINE, ADRIAMYCIN, CYCLOPHOSPHAMIDE, PREDNISONE AND ETOPOSIDE (VACPE) IN HIGH-GRADE NON-HODGKIN LYMPHOMAS - RESULTS OF A MULTICENTER PHASE-II STUDY.

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In a pilot phase-II trial in patients (pts.)with high-grade NHLs we tested the feasibility and response rate of an intensified chemotherapy. Pts. with high-grade NHLs stage I_E-IV and age 18-75 years were included. One therapy cycle consisted of vincristine d1 i.v., adriamycin 25 mg/m² d1-3 i.v., cyclophosphamide 800 mg/m² d1 i.v., prednisone 60 mg/m² d1-7 p.o. and etoposide 120 mg/m² d1-3 i.v. (VACPE). For pts. >60 years, adriamycin was administered only two days and etoposide was reduced to 100 mg/m² d1-3. Most pts. received GM-CSF beginning on day 4 of each cycle or have been included into a randomized trial (± GM-CSF). The cycles were repeated every three weeks. Pts. with stage IV received six cycles of VACPE, all other pts. received five cycles VACPE followed by a consolidating radiotherapy.
Up to now, 61 pts. have been included. 7 pts. are too early for evaluation, 3 pts. had an early death (1x stroke, 2x tumor-related) and 5 pts. had major protocol violations. 45 pts. are presently evaluable for response (22 cb, 4 ibl, 4 Ki1, 9 pleomorphic T-cell, 2 anaplastic, 4 others). The median age was 55 years (15-77), 3 pts. had stage I_E, 15 stage II, 9 stage III and 18 stage IV. 68% of pts. were symptomatic or had elevated serum LDH. 39/45 pts. (85%) achieved CR, 5/45 achieved PR. 8 patients with CR meanwhile relapsed. The CCR after 42 months is 63%, the probability of overall survival 50% after 48 months. So far, VACPE seems to be a tolerable and highly effective schedule for treatment of high-grade NHLs.

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REVERSAL OF MULTIPLE DRUG RESISTANCE (MDR) BY ANTISENSE PHOSPHOROTHIOATE OLIGONUCLEOTIDES AND RIBOZYMES

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The outgrowth of multiple drug-resistant tumor cells is a major cause for the failure of cytotoxic chemotherapy in cancer. Selective reduction of mdr-expression with antisense oligonucleotides or ribozymes in combination with chemotherapy could present an outlet to this probleme. We studied the effectiveness of 14-, 15- and 18-mer antisense phosphorothioate oligonucleotides (S-ODN) directed to different regions of the published mdr-1 gene sequence (S-ODN1, 600 nucleotides upstream of the AUG translation start codon; S-ODN2, translation start region; S-ODN3, nucleotides 2420-2434 and S-ODN4, nucleotides 2990-3007) in reducing mdr-1 levels and MDR function in the mdr-overexpressing cell lines S180Dox^R, KBCH^R and LoVoDox^R. The effect of antisense oligonucleotides in comparison with sense or random sequences was monitored by a cell proliferation assay (^{3}H-thymidine incorporation), by Western-blotting and using a functional assay. S-ODNs were applied up to 2 μM final concentration and incubated with mdr-overexpressing cells (5x10^4/ml) for 18h in Clicks/RPMI-medium without FCS. Then FCS was added to 10% final concentration. After 72h cells were subjected to ^{3}H-thymidine assays or Western-blotting. A maximal reduction (50%) in ^{3}H-thymidine incorporation was obtained with S-ODN3. The functional assay was also used to compare antisense oligonucleotide mediated mdr down-regulation with modulation of MDR by verapamil or tamoxifen.
A ribozyme directed to the same mdr-region as S-ODN3 was tested in vitro for its ability to degrade mdr-mRNA in total RNA or mRNA isolated from overexpressing cells. Ribozymes are interesting antisense agents which may be effective at reduced doses as compared to S-ODNs.

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VP16, IFOSFAMIDE, AND CISPLATIN CHEMOTHERAPY (VIP) FOR THE MOBILIZATION OF PERIPHERAL BLOOD PROGENITOR CELLS IS AN EFFECTIVE ANTI-TUMOR REGIMEN IN A VARIETY OF MALIGNANCIES.

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At our institution, peripheral blood progenitor cells (PBPCs) are mobilized following chemotherapy with VP16 (500 mg/m^2), ifosfamide (4 g/m^2), and cisplatin (50 mg/m^2) (VIP) plus colony-stimulating factors (CSFs). Advantages of this method - as compared to the mobilization with CSFs alone - include the application of a therapeutic chemotherapy preceeding PBPC harvest, the possibly reduced risk of tumor cell contamination, as well as a more efficient way for the recruitment of PBPCs. Since July 1991, we have analyzed 106 patients eligible for high-dose chemotherapy as to the antitumor effect of the VIP regimen. Tumor evaluation was performed after two cycles of VIP chemotherapy. 40 patients with small cell lung cancer (SCLC; extensive-disease n=28; limited-disease n=12), 22 patients with non-operable non-small cell lung cancer (NSCLC), 12 patients with relapsed high-grade Non-Hodgkin's or Hodgkin's lymphoma (NHL), 11 patients with relapsed Stage IV breast cancer, 11 patients with non-operable head and neck cancer and 10 patients with other tumors were evaluated. Patients with extensive-disease SCLC revealed an objective response rate of 75% (complete remission [CR] in 57% and partial remission [PR] in 18%). Patients with limited-disease SCLC had a CR + PR rate of 83% with CR in 75%. The median survival in ED-SCLC patients was 12 months, the median survival in LD-SCLC patients is not reached yet. Patients with NSCLC had an overall response rate of 32%. Advanced NHL patients had a 66.6% objective response rate and 6/12 patients achieved CR (50%) and subsequently received high-dose chemotherapy. 3/11 stage IV breast cancer patients were chemosensitive (PR in 27%). Patients with head/neck or other solid tumors responded in 38% with 2/21 in CR and 6/21 in PR. Hematological toxicity showed WHO grade III neutropenia and WHO grade I thrombocytopenia. Other toxicities were $\leq$ WHO grade II. In conlcusion, VIP chemotherapy, which we have shown to be highly effective in PBPC recruitment[1], has broad antitumor activity. Moreover, it does not induce severe thrombocytopenia which might impede apheresis at the optimal time point for PBPC collection. Addition of anthracyclines to VIP chemotherapy might be synergistic in selected patients.

[1] W. Brugger et al, Blood 79, 1193 (1992).

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2-CHLORODEOXYADENOSIN (2-CDA) IS PREFERENTIALLY ACTIVE IN LOW GRADE LYMPHOMAS AND HAS A HIGH RATE OF INFECTIOUS COMPLICATIONS. RESULTS FROM A SAKK PILOT STUDY.

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We treated 31 patients (pt) with Non Hodgkin's lymphoma (NHL)with the purine analogue 2-CDA. The pt had previously received a median of 3 regimens. The histology according to the International Working Formulation (IWF) was low grade in 17 (including 1 pt with Waldenströms disease), intermediate grade in 8 and high grade in 6 pt. A comparison to the Kiel classification will be presented. The pt either had refractory or relapsing disease. 2-CDA was given at 0.1mg/kg/m2 body surface/day as a continuous infusion for seven days. A total of 65 cycles of 2-CDA is evaluable.

Results:	CR	PR	NC	PD
Low Grade (IWF A-C)	3	9	3	2
Intermed.Gr (IWF D-G)	-	4	2	2
High Grade (IWF H-J)	-	1	-	5

(CR=Complete remission, PR=partial remission, NC=no change, PD=progressive disease).
Details on response duration are currently not available due to the short follow-up. Immediate treatment tolerance was good with no alopecia, no GI-, neuro-, nephro- or hepatotoxicity. Phlebitis and nausea occurred in <10% of the cycles. Hematological toxicity (WHO grade III and IV): granulocytopenia in 2 of 25, lymphopenia in 12 of 23 and thrombopenia in 5 of 27 evaluable cycles. Cytopenias seem to correlate with the degree of bone marrow infiltration by the respective tumor. Major infectious episodes in the 30 days post 2-CDA were observed in 16 patients. Bacterial (E.coli and Staph. septicemia) and viral (Herpes simplex, Varicellla zoster, Cytomegalovirus) infections were seen. Older patients seemed to be more prone to such infections.
Conclusion: 2-CDA is an active agent mainly in low grade NHL even if patients are heavily pretreated. The main side effect consists of cytopenia and especially lymphopenia with subsequent infections. The rate of infections was high in our pt group. The question of antiinfectious prophylaxis needs to be assessed (acyclovir, cotrimoxazole, fluconazol?) if heavily pretreated pt. are given 2-CDA.

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PERIPHERAL BLOOD STEM CELLS AS RESCUE AFTER HIGH-DOSE CHEMOTHERAPY.

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Peripheral blood stem cells (PBSC) are increasingly used as rescue after high-dose chemotherapy resulting in a more rapid immediate hematological recovery when compared to bone marrow stem cells (BMSC). However, the effects of the way of mobilisation, the timing of the collections and the administration of cytokines after stem cell reinfusion have been equivocal in retrospective studies. Therefore, we prospectively evaluated the use of BMSC versus G-CSF mobilized PBSC.
Methods: Between 1/92 and 12/92, 35 patients (pts) with relapsed or refractory germ cell tumors were stratified according to prior cisplatin treatment and randomly assigned for bone marrow harvest or PBSC collection. Mean age was 31 years (range 20-49). In 17 pts bone marrow harvest was followed by one or two cycles of conventional chemotherapy. In 18 other pts PBSC were collected after an identical chemotherapy regimen plus subcutaneous administration of G-CSF 5 μg/kg bodyweight. Between two and four (median three) stem cell collections were performed via a double lumen Hickman catheter when > 1500 /ml circulating CD34 positive progenitor cells could be demonstrated in the peripheral blood by FACS analysis. In these patients stem cell collections were again followed by one or two conventional treatment cycles. The high-dose regimen consisted of carboplatin 1500 mg/m^2, etoposide 1200-2400 mg/m^2 and ifosfamide 0-10 g/m^2 given over four days followed by ASCR two days later on day 0. After ASCR intravenous G-CSF 5 μg/kg bodyweight/24h i.v. was administered in all pts from day +1 until leukocyte counts > 1000 /μl for two consecutive days. **Results:** All patients survived and had a complete hematological recovery to their pre HDT values. Pts with PBSC received more mononuclear cells per kg/bodyweight and more CFU per kg/bodyweight compared to pts with BMSC resulting in a significantly faster recovery of leucopoesis, granulopoesis and thrombopoesis. There was also a trend for better clinical parameters such as less days on antibiotics, i.v. alimentation or inpatient days. **Conclusion:** ASCR after HDT can safely be performed using PBSC or BMSC. The use of PBSC seems to be associated with a faster hematological recovery and a better clinical outcome.

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PRIMARY NON-HODGKIN'S LYMPHOMA OF THE LIVER

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1976 Kim et al. first described primary lymphomas of the liver. In the international literature 48 cases have been published up to now. An additional case is reported on an 71-year-old patient who was operated for a space-occupying tumor of the left liver lobe diagnosed by ultrasound. Preoperative investigation by digital subtraction angiography (DSA) and angio-CT confirmed a 5x5 cm solid tumor as well as kidney cysts and a liver cyst in the right lobe. An explorative laparotomy with lobectomy of the left hepatic lobe and segmentectomy (VII) followed. Postoperative course with CHOP-cycles followed as well as regular staging. Although the number of this patients are small, the data suggest surgical resection were possible in conjunction with chemotherapy. Two years after the additional chemotherapy the patients is alive and without any symptoms.

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WEEKLY THERAPY WITH FOLINIC ACID AND 5-FLUOROURACIL IN PATIENTS WITH BILIARY TRACT, PANCREATIC AND HEPATOCELLUALAR CARCINOMA

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No effective chemotherapy is known for patients with metastatic and inoperable carcinoma of pancreas, liver and biliary tract. We have conducted a phase II trial to study the efficacy of weekly 24 hours infusion of high-dose 5-FU (2,6 g/m²) with folinic acid (500 mg/m²) stimulated by the positive results in metastatic colorectal carcinoma (Ardalan et al. ICO 9, 1991, 625-630).

Between 4/92 and 12/92 we administered folinic acid (500 mg/m²) as short infusion (1 hour) followed by high dose 5-fluorouracil (2,6 g/m²) as 24 hours infusion using an implantable drug delivery system (Port-A-Cath). If the therapy was well tolerated this course was repeated once a week. After 6 applications response to therapy was evaluated. A second course was planned in cases of remission or stable disease with clinical improvement.

27 consecutive patients with inoperable biliary tract (10), pancreatic cancer (11) and hepatocellular carcinoma (7) untreatable by chemoembolisation were included.

Results: All patients - 1 male, 9 female with biliary tract, 7 male, 1 female with HCC, 6 male and 5 female with pancreatic cancer - are evaluable for response and toxicity. Mean age was 54 years (range 28 - 67) for pancreatic, 61 (51 - 70) for biliary tract and 51 (47 -61) for liver cancer. Over all remission rate was 7 %. The only partial remissions were seen in the group of biliary tract cancer (20 %). In 7 cases stable disease was observed (5 pancreatic, 1 liver, 1 biliary tract cancer. Yet the survival rate is not evaluable.

Toxicity: The therapy was usually well tolerated. One patient developed vomiting grade III. Grade II toxicity was observed in 17 cases (3 stomatitis, 4 nausea, 10 diarrhea). Neurotoxicity was observed in three cases and a pneumothorax occurred two times during implantation of the drug delivery system.

After the second course one patient died of ventricular fibrillation probably induced by therapy.

Conclusion: Based on our experience in biliary tract carcinoma a phase II trial including more patients with this regime seems feasible. The side effects are comparable to other 5-FU/FA regimens.

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MODULATION OF TUMOR GROWTH BY CYTOKINES EXEMPLIFIED BY GENE TRANSFER EXPERIMENTS

Thomas Blankenstein and Tibor Diamantstein

Cytokines (about 30 are currently known) regulate cell proliferation, differentiation and activation. Their relationship to tumor growth is extremely complex. At the extreme ends, aberrant cytokine production contributes to malignant transformation (e.g. as autocrine growth factors or due to their immunesuppressive properties) or, alternatively, constitutive expression of transfected cytokine genes can powerfully suppress tumor growth. Evidence for the first possibility results from either the identification of oncogene-like activation of cytokines in malignant cells (e.g. IL6) or cytokine gene transfection into non-malignant, cytokine-dependently growing cells. By the latter approach it is possible to demonstrate that 'in vitro' growth autonomy conferred by the transfected cytokine results in a malignant phenotype (e.g. IL5). In sharp contrast, expression of certain cytokines in tumor cells leads to a high local cytokine concentration at the tumor site 'in vivo' which causes a strong inflammatory response and eventually rejection of the tumor (e.g. by IL2, IL4, IL7, TNF, IFN). These experiments may have therapeutical implications and provide a powerful tool to analyse the function of cytokines 'in vivo'. Together, these transfection studies illustrate the 'Janus-faced' appearance of cytokines.

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PREDICTING BENCE JONES PROTEIN NEPHROTOXICITY: RELEVANCE OF PH-CHARGE RELATIONS AND PROTEIN DIMERIZATION.

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Bence Jones proteins cause nephropathy due to physico-chemical properties not yet clearly identified. A reliable prediction of nephropathogeneity, which is prerequisite for a possible therapeutic prevention of nephropathy, has not yet been established. In search of criteria predictive for Bence-Jones nephropathy we analyzed pI, molecular weight, and pH-dependent charge distribution (by titration curve (TC)-electrophoresis) of Bence-Jones proteins from 40 patients suffering from multiple myeloma, lymphoplasmocytoid lymphoma, or chronic lymphocytic leukemia. Renal condition of the patients was defined by creatinin retention and renal proteinuria. It could be differentiated into (1) absence of nephropathy; (2) severe creatinin retention without renal proteinuria; (3) tubular or glomerular proteinuria with mild creatinin retention. To each of these clinical types of nephropathy specific structural features of the individual Bence-Jones protein could be assigned. These were best characterized by TC-analysis but could not be detected by isoelectric focussing. We discriminated three TC-types: (i) proteins with low pI and a strong negative charge at pH>pI. These were associated either with creatinin retention (8/14) or with absence of nephropathy (6/14); (ii) Bence-Jones proteins with neutral pI, weak positive charge at pH<pI, and weak negative charge at pH>pI were predominantly associated with glomerulopathy (9/12); (iii) alkaline pI and a weak positive charge at pH<pI were typical for proteins causing tubulopathy (12/14). Additionally, a low degree of protein-dimerization (< 20%) was significately ($p < 0.05$) associated with creatinine retention.

Conclusion: Our data support a protein charge-related theory of Bence-Jones nephropathy. The charge distribution appears to be superior to pI in predicting nephropathogeneity. Additionally, sulfhydryl-interchange reactions might be involved in Bence-Jones nephropathy.Therapeutical consequences could include manipulation of urinary pH and treatment with reducing agents such as thiosulfate.

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TARGET-RESISTANCE OF TOPOISOMERASE II: ROLE OF DNA-CATALYSIS, NUCLEAR DISTRIBUTION, AND DNA-AFFINITY.

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Topo(isomerase) II a key enzyme in cell division, gene transcription, cell differentiation, and genetic recombination is the target for anthracyclines, podophyllotoxines and many other cytostatics. Topo II inhibition is a highly effective strategy for tumor cell killing. However, leukemic cells can escape by altering the sensitivity of the target enzyme. Little is known about the mechanisms regulating drug-sensitivity of topo II. Here, we screened human HL-60 leukemic cells for structural heterogeneity of topo II. We chromatographically resolved the known α- and β- isoenzymes and in addition a late-eluting α-form. In a HL-60 clone resistant to topo II inhibitors the late α-form is predominant (80%). In sensitive cells topo IIα is prevalently (80%) early-eluting. Functional differences of early and late topo IIα: (i)pH-optimum of decatenation activity; (ii) ortho-vanadate sensitivity; (iii) drug-sensitivity: the late eluting form ist 100-fold more resistant to mAMSA and etoposide. We monitored SDS-induced covalent attachment of the purified enzyme fractions to calf thymus DNA by disappearance of the immuno-reactive protein band from Western blots. With the early topo IIα low levels (10%) of residual complex formation (by SDS alone) and a pronounced drug-induced increase (90%) were observed. In contrast, the late form exhibited high levels of residual complex formation (80%) and no additional drug-effect. In sensitive cells topo IIα was diffusely distributed in the nucleoplasm and topo IIβ was enriched in the nucleoli. In resistant cells both subforms were nucleolar enriched. Sensitive and resistant cells contained similar levels of topo II. Apparently, HL-60 cells can increase the level of topo II- DNA-attachment. In the DNA-bound state the enzyme is not accessible to inhibitors. Predominance of this form renders cells resistant. Modulation of topo II-DNA attachment is expressed in sensitive and resistant cells to a different extent.*Supported by Wilhelm-Sander Stiftung, grant 90.038.1. and Deutsche Forschungsgemeinschaft, SFB 172, C9.*

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CEFOTAXIME/PIPERACILLIN VERSUS IMIPENEM/CILASTATIN FOR INITIAL ANTIMICROBIAL THERAPY AND EARLY TREATMENT WITH ITRACONAZOLE IN FEBRILE NEUTROPENIC PATIENTS

Böhme,A.[1], Just,G.[2], Bergmann,L.[1], Shah,P.[2], Hoelzer,D.[1], Stille,W.[2]

182 febrile episodes of neutropenic patients with hemtalogic malignancies were initially treated with cefotaxime/piperacillin (cef/pip) vs imipenem/cilastatin (imi/cil) in a prospective, randomized trial.Totally the response was 53/88 (60%) and 55/94 (58,5%) respectively, for FUO (fever of unknown origin) 37/48 (77%) vs 25/35 (71%), for bacteremias without any localization of infectious focus 9/18 (50%) vs 23/29 (79%). For secondarly developed pneumonias the total response was 2/15 (13%) under cef/pip vs 3/24 (12,5%) under imi/cil, only radiologically documented pneumonias responded in 2/5 vs 3/9 cases, whereas the response of microbiologically documented pneumonias was 0/10 vs 0/15. Infections with other localization (mostly abdominal foci) had a recovery in 5/7 vs 4/6 cases.
If no microorganisms were found, non-responders under initial therapy additionally received gentamicin and were randomly assigned to treatment with or without itraconazole. Totally the response was 11/17 (65%) with vs 12/23 (52%) without itraconazole; the favourable groups FUO and bacteremias responded in 8/8 (100%) with, in 10/14 (71%) cases without, the pneumonias in 3/8 with, in 2/9 cases without itraconazole .
By adjusting antibiotic treatment to antibiogram or addition of amphotericin B/5-flucytosine, the overall response of FUO was 83/83 (100%), of bacteremias 45/47 (96%), of pneumonias 27/39 (69%) totally, with only radiological documentation 12/14 (86%), with microbiological documentation 15/25 (60%), and of other infections 12/13 (92%).
35 patients with initially diagnosed pneumonia were randomized to treatment with imipenem/cilastatin or imipenem/cilastatin/itraconazol. The total response under imipenem was 10/19 (53%), with itraconazole 11/16 (69%); only radiologically documented pneumonias responded in 8/10 (80%) vs 5/6 (83%), microbiologically documented pneumonias in 2/9 (22%) vs. 6/10 (60%) cases.
In conclusion there was no different efficacy between cefotaxime/piperacillin and imipenem/cilastatin except a small benefit of bacteremias under imipenem/cilastatin.The results of initially diagnosed pneumonias suggest a benefit of combination imipenem/cilastatin/itraconazole .

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ITRACONAZOLE AS ANTIFUNGAL PROPHYLAXIS FOR NEUTROPENIC PATIENTS WITH HEMATOLOGIC MALIGNANCIES

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73 neutropenic patients with hematologic malignancies (48 AML,12 NHL,9 ALL,2 M. Hodgkin,1 aplastic anemia,1 plasma cell leukemia) were treated with itraconazole 2x 400 mg per day as antifungal prophylaxis from 10/92 to 3/93.
Most of the administered cytostatic regimens were highly aggressive; the mean duration of granulocytopenia < 100/µl was 13 days, < 500/µl 14 days. G-CSF was given in most cases.
The total incidence of mycoses was 6/73 (8%), with oropharyngeal candidiasis in 3, aspergillosis in 3 cases (4%). The probable diagnosis of aspergillosis based on thoracal CTscan, the fungus was documented in 2 cases by transbronchial biopsy and autopsy.
Under therapy with amphotericin B/5-flucytosine 2 patients responded completely.
The incidence of adverse events was 8/73 (11%), elevated ALT in 6 patients, nausea and sweat in 1 patient, respectively.
The incidence of aspergillosis was compared to a historical group of patients with the same diagnoses, cytostatic regimens and intensity and duration of granulocytopenia, who received oral polyenes as antifungal prophylaxis. The evaluated time period for the historical control (10/91 - 3/92) corresponded to the study period, because of the seasonal occurance of aspergillus. In 3/67 (4,5%) episodes Aspergillus was documented.
Until now there was no difference in the incidence of invasive aspergillosis under antifungal prophylaxis with itraconazole or oral polyenes in the evaluated groups. A conclusive assessment of the value of antimycotic prophylaxis with itraconazole will require prospective studies with a larger number of patients .

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STIMULATION OF T-CELLS FROM PATIENTS WITH B7 POSITIVE B CELL LYMPHOMA WITH CD3XCD19 BISPECIFIC ANTIBODIES IN COMBINATION WITH CD28 ANTIBODIES

H.Bohlen, O.Manzke, V.Diehl and H.Tesch

Bispecific antibodies can be used to target T cells to autologous tumor cells. However, the activation of resting human T cells requires two independent signals namely the crosslinking of the TCR-CD3 complex together with the CD28 homodimer. The activation can be achieved by costimulation with CD3xCD19 bispecific and CD28 bivalent antibodies.
It has been described that tumor cells expressing the B7 protein, the natural ligand for CD28, could also coactivate T cells. In contrast, B7 positive B lymphoma cells may provide inhibitory signals to the redirected T cell.
Single-cell suspensions from freshly isolated lymphoma invaded lymph nodes were monocyte depleted and stimulated with CD3xCD19 bispecific antibodies alone or in combination with CD28 antibodies. It was shown, that the stimulation by CD3xCD19 antibodies alone resulted in a transient T cell activation, while stimulation with bispecific antibodies plus CD28 antibodies induced a long-lasting T cell activation. It will be pointed out whether the unresponsiveness correlates to T cell anergy.

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A NEW NON-RADIOACTIVE METHOD TO DETECT CYTOLYTIC ACTIVITY AGAINST B-CLL CELLS USING ELECTROPORATION OF EUROPIUM-DTPA COMPLEXES

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The labelling of B-cells from patients with chronic lymphocytic leukaemia (CLL) with ^{51}Cr or other non-radioactive dyes is difficult to achieve by standard protocols. The use of Europium -DTPA as a marker for cell mediated cytotoxicity has been described in other system (NK-function). A method was developed to label B cells from patients with CLL with the non-radioactive label Europium-DTPA by means of electroporation.

By standard protocols (dextransulphate permeabilisation) no labelling of CLL B-cells with the Europium-DTPA complexes could be achieved. However, by the use of a standardised electroporation protocol using a twin pulse we were able to label CLL B-cells in approximately 70% of those cases (7/10) tested. The results obtained with this protocol suggest an appropriate method to test specific anti-tumor response against primary B-cell neoplastic target cells which are usually difficult to label with ^{51}Cr.

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APPLICATION OF FIRST LINE DOSE INTENSIFIED PEI-CHEMOTHERAPY WITH SEQUENTIAL HARVESTING AND REINFUSION OF PBSC IN PATIENTS WITH ADVANCED TESTICULAR CANCER

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Dose intensification of the platinum / etoposide / ifosfamide (PEI)-regimen may improve the outcome in patients with advanced (according to Indiana University classification) testicular germ cell tumors. During the initial phase of the study 44 pts received P 30mg/m2, E 200mg/m2, and I 1,6g/m2 (dose level 3), day 1-5, q 22 days for 4 cycles followed by GM-CSF 5 or 10 μg/kg s.c. per day. The dose limiting toxicities were mucositis WHO ° 3/4 in 33% of pts and prolonged thrombocytopenia (> 10 days). In the current phase of the study 7 pts were treated by the same dose of the PEI-regimen followed by G-CSF (5μg/kg per day) and retransfusion of peripheral blood stem cells (PBSC) at the second day after chemotherapy. PBSC were separated during a prephase after stimulation with G-CSF alone (4 pts) and after the 1st (5 pts) and/or the 2nd cycle (6 pts) of PEI-therapy. 20 chemotherapy cycles with PBSC retransfusion are evaluable. Pts received a mean of 2.0 x 10^8 MNC/kg (0.3-3.8), 21.6 x10^4 CFU-GM/kg (2.1-49) and 3.3 x 10^6 $CD34^+$/kg (0.3-16.1).

Hematological toxicity:

	cycles			
GM-CSF alone (N=44 pts)	1	2	3	4
med.days with ANC <500/μl	5	6.5	9.5	12
med.days with plts.<25000/μl	2	4	6	11
G-CSF + PBSC (N=7 pts)				
med.days with ANC <500/μl	2.3	6.7	6.5	6.3
med.days with plts.<25000/μl	1.2	4.4	3.0	3.3

Infections WHO°3/4 occurred in 3/20 cycles with PBSC as compared to 59/161 cycles with growth factor application alone (p=0.06). 1/7 pts (14%) receiving PBSC developed mucositis WHO°3/4 in comparison to 15/44 (33%) without PBSC retransfusion. **Conclusions**: The harvesting and retransfusion of PBSC after dose intensified PEI-chemotherapy is feasible without major complications. This approach reduces the duration of granulocytopenia and particularly thrombocytopenia and may thereby also reduce the incidence of infections and mucositis compared to the application of hematopoietic growth factors alone. Consecutively, the achievement of an upfront higher dose intensity of active chemotherapeutic agents may improve the cure rates of pts with advanced testicular germ cell tumors.

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EFFECT OF POLYCHEMOTHERAPY ON COAGULATION INHIBITORS IN HODGKIN LYMPHOMA PATIENTS

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Introduction: In malignant lymphoma, the disease itself as well as its treatment may induce coagulation activation. To find out, whether alterations of coagulation inhibitors protein C and S or increases of coagulation activation marker TAT (thrombin-antithrombin III-complex) could be observed, we studied patients suffering from Hodgkins Lymphoma during polychemotherapy with COPP-regimen (Endoxan^R, Vincristin, Procarbazin, Prednison).
Patients: 15 COPP-cycles in 5 patients were examined. Blood samples were taken at the 1st and 8th day of each cyclus immediately before and after infusion. **Methods:** Protein C antigen and activity (ELISA and PTT-dependent method); Protein S (EID Protein S, Boehringer Mannheim, Germany); C4b-binding protein (ASSERA-PLATE-test, Diagnostika Stago, France); TAT-complexes (Enzygnost TAT Mikro, Behringwerke Marburg, Germany).
Results: 1. TAT-complexes were elevated in all patients, there was no significant difference before and after chemotherapy. 2. Protein C was within the normal range, no alterations during chemotherapy were observed. 3. Free protein S levels were diminished (1st day:26.5±3.9) during the whole therapy (8th day:28.7±5.6 % of normal total protein S). 4. C4b-binding protein decreased significantly during COPP-therapy (1st day: 137,4±27,5%; 8th day 107,5±18,6 % of normal).
Conclusion: Coagulation activation is observed in all Hodgkin patients examined. During polychemotherapy, TAT-complexes did not increase significantly indicating that coagulation activation is mainly dependent on Hodgkin's lymphoma itself.

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TRANSCRIPTIONAL ACTIVATION OF THE M-CSF GENE BY IL-2 IS ASSOCIATED WITH SECRETION OF BIOACTIVE M-CSF PROTEIN BY MONOCYTES AND INVOLVES ACTIVATION OF THE TRANSCRIPTION FACTOR NF-κB

M.A. Brach, Ch. Arnold, M. Kiehntopf, H.-J. Gruß, and F. Herrmann

Human peripheral blood monocytes (Mo) constitutively display the β-chain of the receptor (R) for interleukin-2 (IL-2), while expression of the IL-2R α-chain is not constitutive but inducible with IL-2. Here we report that binding of recombinant human (rh) IL-2 to its binding site leads to transcriptional activation of the macrophage colony-stimulating factor (M-CSF) gene in Mo resulting in accumulation of M-CSF mRNA and subsequent release of bioactive M-CSF protein as demonstrated by enzyme linked immunosorbent assay (ELISA) and inhibition of IL-2-induced release of an activity stimulating growth of monocyte-type colonies by a neutralizing anti-M-CSF antibody. Transcriptional activation of the M-CSF gene by IL-2 is preceded by enhanced binding activity of the transcription factor NF-κ B to its recogniton sequence in the 5' regulatory enhancer region of the M-CSF gene. Moreover, using a heterologous promoter (herpes thymidine kinase, HTK) construct containing the NF-κ B consensus sequence, it is shown that NF-κ B binding by an IL-2-induced monocyte-derived nuclear protein confers reporter gene (human growth hormone, HGH) activity. Taken together, our findings indicate that IL-2 induces gene expression of M-CSF in human blood-derived Mo and provide evidence for involvement of NF-κ B in transcriptional regulation of this gene.

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IONIZING IRRADIATION INDUCES EXPRESSION OF THE IL-6 GENE BY HUMAN FIBROBLASTS INVOLVING ACTIVATION OF THE NUCLEAR FACTOR-κB.

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We here report that human lung fibroblasts respond to X-ray treatment (XRT) with release of Interleukin (IL)-6. Synthesis of IL-6 upon ionizing radiation is preceded by an increase of IL-6 transcript levels resulting from transcriptional activation of the IL-6 gene. Analysis of deleted fragments of the IL-6 promoter indicated that transcriptional activation of the IL-6 promoter was due to enhanced binding activity of the transcription factor nuclear factor (NF)-κB. Although activation protein (AP)-1 did not participate in the rapid induction of the IL-6 promoter, its binding activity was also enhanced by XRT. In contrast to binding kinetics observed with NF-κB, AP-1 binding following XRT was biphasic with the second peak being dependent on de novo protein synthesis. In contrast, however, NF-IL-6 activity was not enhanced by XRT in fibroblasts. The introduction of both the NF-κB- and the AP-1 recognition sequence, conferred inducibility by XRT to a heterologous promoter, with reporter gene activity being maximal 24 hours or 48 hours following XRT, respectively. Sequential activation of two distinct transcription factors might thus contribute to synchronize transcriptional activation of different genes participating in the X-ray (XR) response.

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MOLECULAR ANALYSIS OF THE SYNERGY OF IL-3 AND TNF-α IN STIMULATING PROLIFERATION OF HEMATOPOIETIC PROGENITOR CELLS.

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An optimal proliferative response is achievable in both normal CD34+ hematopoietic progenitor cells and blast cells of patients with acute myelogenous leukemia (AML) by combinational treatment with interleukin (IL)-3 and tumor necrosis factor (TNF)-α. Analysis of the molecular mechanisms mediating this synergism revealed that TNF-α, but not IL-3, enhanced binding activity of the c-jun/AP-1 transcription factor. In order to further elucidate the importance of c-jun/AP-1 for the capacity of TNF-α to synergize with IL-3, the antisense-technique was employed. Treatment of AML blasts with an antisense oligomer directed against the translation initiation site of c-jun, but not with the corresponding sense or an unrelated nonsense oligonucleotide resulted in intracellular RNA/oligomer duplex formation followed by efficient inhibition of c-jun/AP-1 protein synthesis. Elimination of c-jun/AP-1 by antisense oligomers relieved TNF-α/IL-3-mediated synergism, while IL-3-induced growth stimulation remained unaffected. Molecular analysis of the mechanisms governing TNF-α-induced c-jun/AP-1 binding revealed that TNF-α induced a signalling cascade leading to posttranslational modification of pre-existing c-jun/AP-1 protein and thereby allowed binding of c-jun/AP-1 to its recognition site, while IL-3 did not. Moreover, TNF-α transcriptionally activated the c-jun gene by enhancing binding activity of the NF-jun transcription factor which recognizes a palindromic sequence within the c-jun promoter located immediately 5' of the SP-1binding site. Activation of NF-jun and thus expression of c-jun/AP-1 is a prerequisite for TNF-mediated growth-stimulation of IL-3 treated hematopoietic progenitor cells in that deletion of the NF-jun recognition sequence abolished TNF-mediated activation of a reporter gene linked to the c-jun promoter. Moreover, accumulation of c-jun mRNA was achievable in TNF-stimulated progenitor cells but not in cultures that had received IL-3 only. In contrast, more mature myelopoietic cells - though responding to TNF with functional activation - failed to synthesize DNA on exposure to TNF and also did not exhibit NF-jun binding activity.

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LEUKOTRIENE B_4 TRANSCRIPTIONALLY ACTIVATES INTERLEUKIN-6 EXPRESSION INVOLVING NUCLEAR FACTORS (NF)-κB AND NF-IL6 IN A H_2O_2-DEPENDENT PATHWAY.

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Leukotriene B_4 $(LTB)_4$ is a notable participant in inflammation and chemotaxis. It is, however, still unclear whether LTB_4 acts in this regard directly or indirectly by stimulating the release of chemotactic and inflammatory cytokines. Here we report that LTB_4 induces synthesis of interleukin (IL)-6 by human blood monocytes through transcriptional activation of the IL-6 gene. We furthermore demonstrate that this process involves activation of the transcription factor NF-κB and, to a lesser extent, of NF-IL6, while the activity of the transcription factor AP-1, shown to otherwise confer IL-6 inducibility, appeared to be unaffected by LTB_4. Involvement of NF-κB and NF-IL6 in induction of IL-6 transcripts by monocytes was demonstrated using deleted forms of the IL-6 promoter. Activation of the IL-6 promoter by LTB_4 was not only associated with accumulation of the respective transcripts but resulted in synthesis of functional IL-6 protein as well. In addition, LTB_4 mediated transactivation of a heterologous promoter construct containing the NF-κB or the NF-IL6 enhancer, but not the AP-1 enhancer. The signalling events mediating this effect appeared to involve the release of H_2O_2, since LTB_4 failed to induce NF-κB or NF-IL6 in the presence of the scavenger of H_2O_2, N-acetyl-L-cysteine.

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PROTEIN-A-IMMUNOADSORPTION TREATMENT OF REFRACTORY ADULT IMMUNE THROMBOCYTOPENIC PURPURA (ITP) PATIENTS

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ITP in adults is considered to be an autoimmune disorder in which platelets are sensitized by association with antiplatelet antibodies and/or circulating immune complexes (CIC) resulting in shortened platelet survival due to splenic hypersequestration. Recently, staphylococcal protein A-silica bead columns have become commercially available for immunoadsorption treatment of ITP as well as other diseases of the immune system for use in therapeutic removal of IgG and IgG-containing CIC. Extracorporeal immunoadsorption of plasma (EIP) has been initiated in 1o adults with treatment-resistant ITP, who failed at least long-term (9-58, median 14 months) corticosteroid-treatment, and completed in 5 patients at present. They received 6 EIP-treatments of 0.6-1.0 L plasma per procedure over a 2- to 3-week period. Initial platelet counts of 5,000 - 66,000/ul (median 27,000) rose to 20,000 - 153,000/ul (median 43,000) at the end of EIP-treatment. There was 1 complete and 2 partial responses of 1 month duration in these 5 patients. Thrombocytopenia remained unchanged in the other 2 patients. Arthralgia, adynamia, angina pectoris and petechiae have been observed during EIP-treatment. The effectiveness of EIP-treatment of adult refractory ITP may be enhanced by increasing the adsorbed plasma volume per procedure or by a second EIP-treatment period.

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RAG-1 TRANSCRIPTION IN ACUTE MYELOID LEUKEMIA

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The recombination activating genes (RAG) are involving in the physiological regulation of immunoglobulin (Ig) and T cell receptor (TCR) gene rearrengements. High transcription rates have been reported in patients with acute lymphoblastic leukemia consistent with their lineage committment. Cell biological markers of lymphoid cells have also been observed in subsets of patients with acute myeloid leukemia, including expression of TdT, of lymphoid lineage-associated cell surface antigens, as well as Ig or TCR gene rearrangements. In order to investigate different stages of gene regulation in early hematopoiesis, we studied the transcription of RAG-1 in myeloid leukemic blasts. Peripheral blood samples or bone marrow aspirates from 17 patients with acute myeloid leukemia were analyzed. All samples constained more than 80 % leukemic cells. RAG-1 transcripts were amplified using primer-specific reverse transcription PCR. The leukemic pre-pre-B cell line Reh served as positive, mouse fibroblasts as negative controls. A specific amplification product of 365 base pairs was obtained in 7 of 17 samples. All products were of equal length, identical to the expected transcript in the positive control. Four of the 7 positive AMLs had been classified as FAB M5, two as M4Eo, one as M2. None revealed an IgH or TCR delta gene rearrangement by Southern blot analysis.
We conclude that transcription of recominase activating gene 1 is a rather frequent phenomenon in AML without correlation to Ig or TCR gene rearrangement.

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TRANSFORMING GROWTH FACTOR (TGF-β_1) TRANSCRIPTION AND RELEASE BY LEUKEMIC CELLS MAY PROVIDE AN EXPLANATION FOR IMMUNOSUPPRESSION IN LEUKEMIA

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In leukemic patients cytotoxic activity of peripheral mononuclear cells is generally reduced. This observation might be correlated with synthesis and release of immunosuppressive cytokines by leukemic cells. Since TGF-β_1 is known to be an inhibitor of lymphokine activated killer (LAK) cell activation, the present study was designed to investigate the expression of TGF β_1 in various types of human leukemias. For this purpose leukemic blast cells were isolated from bone marrow or peripheral blood in acute phase of AML (>80 % blast cells) or high leukemic CLL, respectively. Total RNA was extracted and assessed for TGF-β_1 transcription using RT-PCR and Northern blotting. For measurement of TGF β_1 release, four representative AMLs were cocultered with Interleukin 2 for 24 hours and evaluated using a bioassay.
In 13/14 AMLs, 2/5 ALLs and 1/5 CLLs studied, specific transcripts for TGF-β_1 were detectable by PCR and/or Northern blotting. In 1/4 culteres - investigated so far - TGF-β_1 protein release was significantly increased. Our observations suggest that production and release of immunosuppressive cytokines like TGF β_1 by leukemic cells may be responsible for the inhibition of cytotoxic mechanisms observed in these patients.

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EXPRESSION OF THE WILMS TUMOR GENE (WT 1) IN HUMAN LEUKEMIAS MAY PROVIDE EARLY DETECTION OF MINIMAL RESIDUAL DISEASE

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The detection of minimal residual desease (MRD) in acute leukemias is an important and still unresolved subject of clinical research. Persistence of MRD my be important for relapse rate and in autololgus bone marrow transplantation (ABMT). Another approach is the control of MRDs in patients with acute leukemias receiving immunotherapy. Up to now , for the detection of MRDs in acute myolocytic leukemias only a limited number of genetic markers are available. Since the WT-1 gene has been described to be expressed in human leukemias, it may be useful as a marker for the detection of MRDs. To obtain preliminary evidence for this hypothesis leukemic blast cells were isolated from bone marrow or peripheral blood from patients with various leukemias. Preparations of mononuclear cells and bone marrow from healthy persons were used as negative controls. Total- RNA was extracted and WT-1 transcription was assessed via RT-PCR using WT-1 specific oligonucleotides.
In 9/21 AMLs (43 %) and 4/5 ALLs a signal was obtained. Neither in five CLLs studied, nor in the 8 controlpreparations transcripts of WT-1 were detectable. Follow ups from patients in complete remission are in progress. Our observations suggest that analysis of WT-1 Gene-expression via PCR may be a powerful tool for early detection of MRDs in acute leukemias compareable to bcr/abl in ALL.

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VASCULAR INVASION: AN OLD OR A NEW SIGNIFICANT AND INDEPENDENT PROGNOSTIC FACTOR IN EARLY-STAGE BREAST CANCER?

B. Brockmann

Axillary lymph node state has been the most important prognostic factor in operable breast cancer, but it does not fully account for the varied disease outcome, especially in lymph node negative patients.

In 194 patients with operable breast cancer and negative lymph node state the importance of vessel invasion with respect to survival was retrospectively and double-blindly investigated. Our recent findings indicated that vascular invasion is significantly associated with relapse-free survival and overall survival and thus may be a prognostic indicator. The vascular invasion was in life table analysis, Cox regression analysis, and multivariate discrimination analysis the only statistically significant prediction of 5-years survival and tumor recurrence. According to life table analysis we found highly significant differences between patients with vascular invasion grade 0 versus grade II (p=0,0009) versus grade III (p=0,0075). A significant difference in overall survival and relapse-free survival with histological grading (mitotic rate and degree of tubular formation) could not be recognized.

Our recent findings indicated a prognostic factor, the vascular invasion, would help selection of patients at high risk for disease recurrence and death in lymph node negative patients who are candidates for systemic adjuvant therapy.

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IMMEDIATE CYTOTOXICITY OF FLUDARABINE AND POTENTIAL INDUCTION OF MDR EXPRESSION IN CHRONIC LYMPHOCYTIC LEUKEMIA

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Fludarabine (F-ara) is an effective drug in the treatment of CLL. As a basis for the design of combination chemotherapy including Fludarabine we investigated the kinetics of drug induced cytoreduction and the potential induction of MDR protein. Peripheral blood from CLL patients was analysed by multiparameter flow cytometry. CLL cells were identified by gating on lymphoid cells and by the coexpression of CD 20 and CD 5. MDR was detected using the monoclonal antibody HYB 241. 17 therapy courses were monitored in 7 patients. All patients had been pretreated with different regimens including alkylating agents and anthracyclines. They received a 5 day course of 20-25 mg/m² F-ara monotherapy. The mean WBC decreased by 23,5 % (4,2-60,5 %) from day 1 - day 5. The mean percentage of CD 5/CD 20 positive cells was 88 % (range 40,5-99,2 %) at start of therapy. The relative number of CD 5/CD 20 positive cells decreased in 4 of 15 courses, while the absolute number decreased in 10 of 15 courses. MDR expression was found in all samples. The median percentage of positive cells was 79 % (7-83 %) prior to F-ara-A therapy. In 5 of 17 courses a significant increase of MDR positive cells was observed, while it decreased in 4 cycles. 6 patients were reanalysed 4 weeks later, MDR expression had increased in 2 and decreased in 4 patients.
Our results show that the absolute number of CLL cells decreases by about ¼ during a 5 day course of Fludarabine (F-ara-A). Fludarabine may increase MDR expression. This is significant for the design of combination therapies including F-Ara-A and anthracyclines.

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IMMUNOHISTOCHEMICAL EXAMINATIONS OF TENASCIN IN PATIENTS WITH STOMACH CANCER

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The production of TENASCIN, an extracellular matrix glycoprotein, is induced by epithelial growth and differentiation during embryonic development, but also by skin injuries and epithelial tumor growth. Obviously it is important for the epithelial/mesenchymal interaction between tumorcells and surrounding extracellular matrix.
We examined the distribution of TENASCIN in paraffin sections of 25 patients with stomach cancer. To identify the polyclonal anti-TENASCIN antibody we used the APAAP-method.
The muscularis mucosae was TENASCIN-positive stained in all sections, especially in areas close to the tumor. In this region we found the muscularis mucosae broader and loosened. The stroma around the tumorcells was also positive stained. and often so intensiv, that this could indicate the direction of tumorgrowth. It was impressive to see a positive reaction especially in regions, where the tumorcells had just invaded through the muscularis mucosae into the submucosa. The TENASCIN-positive staining of focal tumorcells in lymphnodes is remarkable; it is possible to detect a small number of tumorcells too.
Obviously TENASCIN might play an impotant role concerning the tumorcell invasion into the submucosa and the development of lymphnode metastases. Larger series, especially in cases with early cancer, are necessary in order to proof, whether TENASCIN is a prognostic tumor-marker and to enlighten its role in tumor invasion.

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CIRCULATING TUMOR CELLS IN PERIPHERAL BLOOD OF PATIENTS WITH MALIGNANT MELANOMA.

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Recently a highly sensitive assay combining reverse transcription and polymerase chain reaction (RT/PCR) to assess for melanoma cells in peripheral blood has been developed. The detection of tyrosinase mRNA, a tissue spezific enzyme in melanocytes and melanoma cells in peripheral blood indicates the presence of melanoma cells.
We used RT/PCR assay to determine malignant melanoma cells in peripheral blood of 43 patients with malignant melanoma in different stages of disease. In none of 8 patients with stage I (localised tumor) but in 5 of 14 patients in stage II (regional lymph node metastases) tyrosinase transcripts were detected. Tyrosinase mRNA was found in all 21 patients with distant metastases (stage III). This method may be helpful to define a group of patients at high risk for development of hematogenous metastases, that would be a possible target group to explore adjuvant treatment strategies.
We then examined blood samples and bone marrow aspirates of 28 patients with metastatic malignant melanoma for presence of melanoma cells prior to and after therapy with IFN-a and IL-2. 10 patients showed antitumor response to immunotherapy: 3 complete remissions (CR) and 7 partial remissions (PR). 4 patients (3 PR, 1 stable disease) underwent subsequent resection of residual tumor lessions and had no clinical evidence of disease after the surgery. Tyrosinase mRNA was detected in blood and bone marrow samples from all patients with malignant melanoma prior to and after immunotherapy, including the patients without any clinical evidence of disease (median disease free survival 21 month, range 19-28 month). Tyrosinase transcripts were also detected in all patients with amelanotic melanoma. In contrast, no tyrosinase mRNA was determined in any of 30 healthy persons or 6 patients with other malignancies. The presence of residual melanoma cells in patients without clinical evidence of disease after successful immunotherapyover a prolonged time periods may confirm the assumption of sustained immunosurvaillence.

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THE ADVANTAGE OF PERIPHERAL BLOOD STEM CELL TRANSPLANTATION (PBSCT) COMPARED TO AUTOLOGOUS BONE MARROW TRANSPLANTATION (ABMT) REMAINS HIGHLY SIGNIFICANT EVEN WHEN USING POSTTRANSPLANT G-CSF.

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In order to compare the difference in speed of hematopoietic recovery after PBSCT and ABMT and to quantify the impact on the need for supportive care and the time of hospitalization, we retrospectively analyzed the posttransplant clinical course of a total of 20 patients autografted with advanced lymphoid malignancies (≥CR2) at our institution. Eleven patients (median age 34±2.5 years) with Hodgkin's disease (HD) were treated with PBSCT and 9 patients (median age 26±2.8 years, 3/9 HD, 2/9 NHL and 4/9 ALL) were treated with ABMT. All 20 patients received posttransplant G-CSF 10µg/kg s.c. from day +1 until the neutrophil count exceeded 1000/µl (ANC_{1000}). We evaluated the time to recover to 500/µl neutrophils (ANC_{500}), the time to recover to unsupported platelet counts of >20000/µl ($pltls._{20000}$), the days of G-CSF application until ANC_{1000}, the number of transfused single-donor platelet units (SDPT), the number of transfused red blood cell units (RBCT), the days of total parenteral nutrition (TPN), the days of i.v. antibiotics and the time of hospitalization in the BMT-unit (MEAN±SEM).

	PBSCT	ABMT	p-value
ANC 500 (d)	9.4±0.5	14.8±1.2	0.0005
Pltls. 20000 (d)	12.7±1.7	>30.3±3.4	<0.0007
G-CSF application (d)	10.5±0.6	21.9±2.1	0.0002
SDPT (units)	5.7±1.2	15.7±3.3	0.006
RBCT (units)	3.8±0.9	8.3±1.6	0.03
i.v.-antibiotics (d)	5.8±1.2	14.1±2.7	0.02
TPN (d)	5.1±1.2	14.0±3.0	0.005
Hospitalization (d)	19.3±1.5	30.3±3.5	0.01

Hematopoiesis recovers more rapidly after PBSCT compared to ABMT and the difference is highly significant, even with posttransplant stimulation using G-CSF. Consequently the reduction in transplant-related risks and in procedure-related costs favor PBSCT significantly.

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RECRUITMENT OF TUMOR CELLS INTO PERIPHERAL BLOOD OF PATIENTS WITH SOLID TUMORS CONCOMITANT TO THE MOBILIZATION OF PERIPHERAL BLOOD PROGENITOR CELLS.

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The reduced risk of tumor cell contamination in peripheral blood progenitor cell (PBPC) preparations is one advantage over the use of autologous bone marrow. The actual level of tumor cell contamination, however, is still a matter of debate. We evaluated peripheral blood and apheresis samples from 40 patients with Stage IV (n=6) or high-risk Stage II/III (n=2) breast cancer, newly diagnosed small cell (SCLC, n=16) or non-small cell (NSCLC, n=11) lung cancer as well as advanced head and neck cancer (n=5) for the detection of circulating tumor cells. Monoclonal antibodies against cytokeratin components (AE1-3/KL-1) and epithelial antigens (HEA) were used in an alkaline phosphatase-anti-alkaline phosphatase (APAAP) assay with a sensitivity of 1 tumor cell within $4x10^5$ total cells. At diagnosis, circulating tumor cells were detected in 33% of patients with Stage IV breast cancer and in 13% of SCLC patients. Patients with high-risk breast, NSCLC or head/neck cancer were negative at diagnosis. Following VP16, ifosfamide and cisplatin (VIP chemotherapy) + G-CSF induced mobilization of PBPCs, between 1 and 1,400 tumor cells per $4x10^5$ blood mononuclear cells were detected in 100% of Stage IV breast cancer patients, 31% of SCLC and 9% of NSCLC patients, respectively. Kinetic analyses revealed a maximum early after chemotherapy (between day 1-7) and in addition, in patients with bone marrow infiltration, between day 10-16, i.e. within the optimal time period for the collection of PBPCs. In summary, we conclude that a substantial risk of tumor cell contamination of PBPC harvests exists, particularly in patients with bone marrow infiltration. These data argue for the positive selection of peripheral blood $CD34^+$ cells. Moreover, our data suggest that induction chemotherapy should be administered before PBPCs are harvested.

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HEMATOPOIETIC RECONSTITUTION AFTER HIGH-DOSE CHEMOTHERAPY IS IDENTICAL IN PATIENTS GIVEN POSITIVELY SELECTED PERIPHERAL BLOOD $CD34^+$ CELLS AND UNSEPARATED PERIPHERAL BLOOD PROGENITORS.

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In order to reduce the number of potentially contaminating tumor cells in peripheral blood progenitor cell (PBPC) preparations, we have positively selected $CD34^+$ progenitor cells in an ongoing study in patients with advanced solid tumors. Up to now, 12 patients were included. PBPCs were mobilized following chemotherapy with VP16, ifosfamide and cisplatin (VIP) and G-CSF administration. $CD34^+$ cells were positively selected by a large-scale avidin immunoadsorption column with a capacity > 10^{10} cells. One leukapheresis product with a median number of $1.3x10^8$ mononuclear cells/kg or $3.2x10^6$ $CD34^+$ cells/kg was labeled with a biotinylated anti-CD34 monoclonal antibody (12.8) and subsequently processed. The overall duration of the procedure was less than 2 hours. The yield of $CD34^+$ cells was 77% (range 37-99), the purity of the $CD34^+$ cell fraction was 71.5% (range 30.1-80.9). The total number of $CD34^+$ cells recovered was $2.6x10^8$/kg (range 0.79-9.51). The characterization of these cells has shown predominantly committed progenitor cells as analyzed in clonogenic assays for CFU-GM, BFU-E and CFU-GEMM as well as by the coexpression of lineage-associated molecules such as CD33, CD38, or HLA-DR (multi-color flow cytometry). However, $CD34^+$ cells also comprised primitive progenitors as demonstrated by the presence of $CD34^+$, lineage-negative cells, 4-HC resistant cells as well as long-term culture initiating cells (LTC-IC), indicating that very early progenitors are not lost upon this selection procedure. Up to now, cells were retransfused in 5 patients after high-dose VP16 (1,500 mg/m²), ifosfamide (12 g/m²), cisplatin (150 mg/m²), and epirubicin (150 mg/m²). Patients were reconstituted with $1.8x10^6$ $CD34^+$ cells/kg (range 0.79-3.5). As compared to historical control patients at our institution given comparable numbers of unseparated PBPCs, time to neutrophil recovery or platelet recovery was identical in both groups (ANC > 500-/uL: day +11; PLT > 50,000/uL: day +15). These data demonstrate the feasibility of large-scale $CD34^+$ cell selection and their efficacy to rapidly reconstitute hematopoiesis after high-dose chemotherapy.

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LARGE-SCALE EX VIVO EXPANSION OF PERIPHERAL BLOOD $CD34^+$ PROGENITOR CELLS FOR CLINICAL USE AFTER HIGH-DOSE CHEMOTHERAPY.

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In order to provide sufficient numbers of peripheral blood progenitor cells (PBPCs) for repetitive use after high-dose chemotherapy, to provide sufficient numbers of progenitor cells in patients with low progenitor cell yield, or to possibly avoid leukapheresis, we investigated the ability of hematopoietic growth factors to expand PBPCs ex vivo. Chemotherapy + G-CSF mobilized PBPCs from 10 patients with advanced chemosensitive solid tumors were collected by one single leukapheresis containing a median of $3.2x10^8$ $CD34^+$ cells/kg (range 0.6 - 12.6). Subsequently, $CD34^+$ cells were positively selected without further processing (i.e. no Ficoll separation, no red blood cell lysis) by an avidin-biotin immunoaffinity column device (provided by Dr. R.J. Berenson, CellPro, Bothell, USA). The purity of the target-cell population was 69.6% (range 46.5 - 81.9). $3x10^7$ selected $CD34^+$ cells, which represent 15% of the original leukapheresis preparation, were cultured in Fenwal bags or tissue culture flasks in the presence of 1% autologous plasma. A combination of 5 growth factors including stem cell factor (SCF), erythropoietin (EPO), interleukin-1ß (IL-1), IL-3, and IL-6 was identified as the optimal combination for the expansion of clonogenic progenitors. Proliferation peaked at day 12-14 with a mean 190-fold increase (range 46-930) of clonogenic cells (CFU-GM, BFU-E, CFU-GEMM). Interferon-gamma synergized with this combination, whereas the addition of GM-CSF or G-CSF decreased the number of clonogenic progenitors. Morphological as well as phenotypical analyses after ex vivo expansion revealed that promyelocytes and monocytes were present in less than 3% of the total cells. The majority of cells were still immature blast-like cells. The number of early progenitor cells were also expanded, though the number of $CD34^+/Lin^-$ or 4-hydroperoxycyclophosphamide [4-HC] resistant cells was variable upon ex vivo expansion. Our data indicate the feasibility of large-scale expansion of PBPCs, starting from small numbers of $CD34^+$ cells. The number of cells generated ex vivo should be sufficient for clinical use.

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THE ROLE OF CYTOKINES IN THE TREATMENT OF AML

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How can more patients be cured from AML? This secret seems to be hidden in residual rather than in overt disease. Some cures may be gained by minimizing residual disease using early intensification strategies. Most patients, however, relapse even after myeloablative treatment requiring autotransplant. There is clear evidence that leukemia survives in a - may be temporarily - therapy resistant state. Ways to overcome this situation are either controlling residual disease by the graft versus leukemia effect or related immune modulation by IL-2 or by aggressing leukemic cells when they leave the resistant state by giving repeated postremission chemotherapy. A novel approach aims at recruiting residual leukemic cells into a sensitive state by the use of growth factors before and together with (priming) chemotherapy. In vitro, the recruitment of leukemic blasts to colony forming cells in presence of GM-CSF (Blood 67:1448, 1986), G-CSF or IL-3 (Blood 70:657, 1987) is documented by numerous reports. Given to patients with newly diagnosed AML GM-CSF priming decreased the proportion of leukemic cells in G_0 and increased cells in sensitive cycle phases within 24-48 h (Blood 77:700, 1991). A similar priming with preinfusion of GM-CSF for a variable period of 0-8 days before chemotherapy started resulted in significantly inferior outcome and more persistent leukemias than in historical controls (Blood 79:2246, 1992). In a randomized study in patients with newly diagnosed AML we give GM-CSF from 24 h before chemotherapy and then on to neutrophil recovery which is repeated in each of the initial 5 treatment courses and is compared to chemotherapy alone. 2 1/2 years from study start and after entering 72 patients present update in the GM-CSF group and controls shows 78% vs 81% CR, 2 vs 5 persistent leukemias, a clearence of b.m. blasts on day 16 in 59% vs 40% of pts, and a longer remission duration significant at least in pts under age 60 (p = .03). In contrast, leukemic blasts grown in GM-CSF showed a lower response to a 20 min. pulse of Ara-C than those grown in G-CSF (Leukemia 5:789, 1991). GM-CSF can also inhibit apoptotic cell death induced by Ara-C in murine myeloid leukemic cell lines (Blood 80:1750, 1992), but a GM-CSF/IL3 fusion protein can enhance apoptosis by Ara-C in two human AML cell lines (Blood 80:2883, 1992). So, whether GM-CSF priming and longterm administration ultimately improves the cure rate in AML should be shown some later from the multiple course strategy used in our study.

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Tumor registry Donaustauf (TR) - a cancer registry for clinical and scientific use

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Since 1980 a clinical tumor registry has been established at the chest hospital Donaustauf (FRG) recording all tumor patients (pt) according to ADT-rules. The system is suitable for hospitals with stand-alone data processing. Costs and staff requirements are low. TR is pt related, enables quality control of therapies, monitoring of follow-up and appointment dates, and statistical analysis of anonymized data.
Ca. 3000 pt have been registered. The generation of medical reports can be programmed. It is a single user system. Access is password-protected. Pt consent is achieved by signature in the follow-up booklet. Pt identification is gained by basic pt data and follow-up number.
Hardware consists of a CPU 486 33 MHz, 200 MB hard disc, 16 MB RAM, VGA color monitor, mouse and a fast matrix printer. The software was developed using MS-DOS version 3.31 and the object orientated programming language smalltalk. TR has a grafical user interface. Data are collected without questionaires or codes either menue-based, or with mnemotechnical abbreviations or in open text. An automatic coding is possible. Important data, e. g. histology, grading, beginning of therapy, death date, can be checked on plausibility.
The TR Donaustauf adds well to a regional cancer and epidemiological registry. It suits for intern clinical evaluation of therapies and disease courses. Data transmission is feasible.

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SEA-BLUE HISTIOCYTES IN SECTIONS OF BONE MARROW BIOPSIES FROM PH1-POSITIVE AND PH1-NEGATIVE PATIENTS OF THE GERMAN CHRONIC MYELOID LEUKEMIA TRIAL

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Sea-blue histiocytes (SBH) are known in bone marrow biopsies (BMB) from patients with various hematological diseases, especially in chronic myeloid leukemia. A better prognostic course of CML has repeatedly been reported if histiocytes, such as SBH, can be detected in BMB. However, these reports based on small numbers of cases and differ by the reported percentage of sea-blue histiocytes (Takahashi et al., 1977; Kelsey and Geary, 1988; Thiele et al., 1992). Therefore, a total of 189 BMB from patients recruited into the trial before any medication was given were researched for sea-blue histiocytes.
Thus, the incidence of SBH in CML can be determinated as 37 % (69 of 189 biopsies), regardless of evidence of Ph1-Chromosome. However, in 43 % of positive cases (30 of 69 biopsies), only few of these cells could be detected (< 5 cells per 10 low power fields).
A connection between the incidence of SBH and the subtypes of CML (Hannover Classification), the evidence of myelofibrosis or of blast excess could not be detected ($p < 0.05$). Furthermore, the percentage of biopsies with SBH was not higher if other histiocytes which are often seen in BMB of CML, the Pseudo-Gaucher cells, are detected ($p < 0.05$), although some histiocytes could be seen which showed features of sea-blue histiocytes and morphological resemblance to Pseudo-Gaucher cells as well.
Afterwards, 22 diagnostic biopsies of Ph^1-positive CML taken by random selection and 3 diagnostic biopsies of bcr-abl-positive CML were mixed with 22 biopsies of Ph^1-negative CML, taken before any treatment had been given, and then examined without knowing cytogenetical results. A remarkable difference between the incidence could be detected: while 24 % (6/25) of biopsies of Ph^1- or bcr-abl-positive CML showed SBH, 55 % (12/22) of biopsies of Ph^1-negative CML contained these cells ($p < 0.05$).
The results show that sea-blue histiocytes are usual findings in bone marrow biopsies among untreated CML patients.

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HISTOPATHOLOGICAL DIFFERENCES BETWEEN PH1-POSITIVE AND PH1-NEGATIVE CML IN BIOPSIES OF BONE MARROW FROM PATIENTS OF THE GERMAN CHRONIC MYELOID LEUKEMIA TRIAL

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22 biopsies of Ph^1-negative CML, 24 biopsies of Ph^1-positive CML taken by random selection and 3 diagnostic biopsies of bcr-abl-positive CML taken before any medication was given were mixed and then examined by 3 investigators independently without knowing cytogenetical results. In spite of a striking resemblance of the histological pictures, certain differences between Ph^1-negative CML and Ph^1-positive CML could be detected in bone marrow biopsies: Ph^1-positive CML shows a slightly stronger increase of granulopoiesis, while Ph^1-negative CML more often shows monocytoid cells. Erythropoiesis is macrocytic or megaloblastoid, i. e. disturbed in maturation, while the megakaryocytes are slightly larger and show a stronger lobulation of nucleus. Furthermore, the megakaryocytes seem to be slightly smaller in number in biopsies of Ph^1-negative CML.
A remarkable difference between Ph^1-negative and Ph^1-positive CML could be detected regarding the incidence of Pseudo-Gaucher cells and sea-blue histiocytes: while 67 % (18/27) of biopsies of Ph^1-positive or bcr-abl-positive CML showed Pseudo-Gaucher cells, only 18 % (4/22) of biopsies of Ph^1-negative CML contained these cells ($p < 0.005$). On the other hand, sea-blue histiocytes could be detected in 55 % (12/22) of biopsies of Ph^1-negative CML, whereas only 24 % (6/25) of biopsies of Ph^1-positive or bcr-abl-positive CML showed these cells ($p < 0.05$).
Thus, differences between the histopathology of Ph^1- (or bcr-abl-) positive CML and Ph^1-negative CML can be considered to support the assumption that these are two different diseases. However, the histopathological picture varies remarkably between single cases, so that it is not possible to draw a conclusion whether a particular case is a Ph^1-negative CML or not by histopathology.

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Antileukaemic effector mechanisms after buffycoat therapy for relapsed CML after BMT: evaluation by limiting dilution analysis and coculture studies.

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We have analysed the immunological effector mechanisms in 4 pts who achieved a molecular remission (PCR negative) after treatment with donor buffycoat. All four pts developed acute and/or chronic GvHD. Donor peripheral blood mononuclear cells (PBMNC) were used as responder cells. They were obtained prior to the buffycoat transfusion and during the period preceding the bone marrow hypoplasia/aplasia which always occurred prior to remission. The T cell response was evaluated by stimulating with irradiated host CML-PBMNC in the presence of low dose IL-2 (10 U/ml) for 14 days in a limiting dilution culture (LDC). After that period the T cells were evaluated for cytotoxicity and IL-2 production. LAK-activity was evaluated by stimulating donor PBMNC with high-dose IL-2 (100-500 U/ml) in bulk culture or in a LDC and measuring cytotoxicity against CML-PBMNC after 5-7 days. No cytotoxic T-Lymphocyte-precursor (CTL-p) or LAK-p were detected at any time point. Host-reactive helper T-lymphocyte -precauser (HTL-p) were detectable in 3 of the 4 donors prior to therapy. After buffycoat therapy very high frequencies (>1/10000) of HTL-p could be detected in the peripheral blood of all 4 pts. In fact the frequencies were almost an order of magnitude larger than those after allogeneic bone marrow transplantation. These results indicate that CML-PBMNC express host minor H antigens and stimulate in vivo expansion of host-reactive HTL-p. Furthermore our studies provide strong indirect evidence that T cells are likely to be the main effector cells of the GvL effect in this model. More direct evidence for the role of T cells is provided by the preliminary results of coculture studies we performed with two T cell lines established from one of the pts mentioned above. These T cell lines appear to inhibit leukaemic CFU-GM and long-term culture initiating cell (LTCIC).

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ASSOCIATION OF HEREDITARY PROTEIN C AND PROTEIN S DEFICIENCY AND ISCHEMIC STROKE

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Hereditary deficiency of protein C and protein S are well known disorders associated with venous thromboembolism. Their role in arterial ischemic diseases such as stroke is much less clear and still controversially debated. We report on a family with hereditary deficiency of both protein C and protein S including family members presenting with cerebral ischemia in young age.
Proposita was a 40 year old woman admitted because of a severe left sided hemiparesis. A year ago she had suffered from a transient ischemic attack with left sided symptoms. At the age of 18 she had experienced a venous thrombosis of her right leg. She had smoked 10 cigarettes/day for 20 years but had not taken any hormonal contraceptives. Further evaluation revealed radiomorphologic evidence of a recent cerebral infarction in the territory of the right anterior cerebral artery and of several old ischemic lesions of both parietal lobes. Arteriosklerotic and intracardiac causes of thromboembolism were excluded. Blood and cerebrospinal fluid examinations were normal including ESR, plasma coagulation tests and test for anticardiolipin antibody. Protein C and protein S were determined on three occasions using functional tests. Protein C activity was between 37 and 45%, protein S between 38 and 56%.
Family history revealed that the father had died of pulmonary embolism at the age of 65. The sister had experienced a transient ischemic attack at the age of 25 and a cerebral infarction one year later. She smoked as well. Her protein C and protein S activity were both reduced to a half of normal. In several clinically healthy family members decreased levels of either protein S or protein C were found confirming the hereditary nature of the deficiencies.
Our observation suggests that a combined deficiency of protein S and protein C may be an independent risk factor for cerebral infarction in young adults. The contribution of these and of additional known risk factors should be evaluated in prospective studies to especially define individual risks in asymptomatic patients.

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LONG - TERM CELL GROWTH OF B - CELL LYMPHOMA AND ACUTE LYMPHOBLASTIC LEUKEMIA IN LONG - TERM BONE MARROW CULTURES

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The lack of standardized in vitro culture systems for long - term growth of malignant pre - B cells is a major obstacle for research on the regulation of proliferation and differentiation of these malignant cells. Stroma cells have been shown to be a major factor in the induction of short - term proliferation and were therefore used for coincubation of malignant pre - B cells from 13 patients with acute lymphoblastic leukemia or malignant lymphoma. Long - term cultures were successfully established in four patients comprising one patient with lymphoblastic lymphoma, one patient with B - ALL, one patient with immunoblastic lymphoma and one patient with lymphoplasmocytic immunocytoma. The cell cultures characteristically show a decline in the number of viable cells within the first month of coculture, then after a latent phase of up to two months proliferation of small lymphoid cells is observed. Immunophenotyping with monoclonal antibodies against B - cell specific or - associated antigens confirmed the identity of all the malignant B - cells. In the patients with B - ALL the translocaton 8;14 could be consistently detected in the original material as well as in the cell culture specimens. The proliferation of these cells was further increased using IL-7 and/or IL-3. None of the four long - term cell cultures was EBV positive.
Detailed analysis of leukemia and lymphoma cells is often hampered by lack of sufficient material and lack of a long - term in vitro culture system. The coincubation with stroma cells can successfully induce long - term proliferation in a significant number of patients with different B - cell malignancies.

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PROLIFERATION OF HUMAN LEUKEMIC PRE-B CELLS IN A STROMA - DEPENDENT IN VITRO SYSTEM: THE ROLE OF EXTRACELLULAR MATRIX COMPONENTS, STROMA CELL MEMBRANES AND INTERLEUKIN-7.

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Stroma cells have been recognized to be essential for B - lymphocytopoiesis in vivo. However, the exact role of the different elements of the stroma microenvironment for the induction of B-cell differentiation and proliferation has not been completely elucidated. We had previously shown that the proliferation of leukemic pre-B cells can be stimulated by human stroma cells or mouse fibroblasts. We have now investigated the different components of this system. Collagen I, III, IV and V as well as the complex matrix gel Matrigel® did not induce proliferation of the leukemic pre-B cells. Also coincubation with soluble heparane sulfate did not influence proliferation. However, membrane fragments of the murine fibroblast cell line L929 prolonged survival of the leukemic pre-B cells. A strong synergism between membrane fragments and interleukin-7 was observed in the induction of proliferation. This effect was similar to the synergism between intact stroma cells and IL-7.
These data underline the essential role of stroma cells for the induction of pre-B cell proliferation. The effect of intact stroma cells can be substituted by membrane fragments, however isolated extracellular matrix proteins were not sufficient as substitutes. Binding of growth factors to cell membranes or membrane associated glycosaminoglycans may be one of the relevant mechanisms in the activation of pre-B cells.

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Detection of Rearrangement of the Heavy Chain Gene and the Bcl-2 Gene in Mononuclear Cells from Blood and Bone Marrow in Patients with cc/cb and cb Lymphomas

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Five patients with cc/cb-lymphomas and four patients with cb-lymphomas, classified according to the Kiel-classification, were investigated for rearrangement of the heavy chain gene in peripheral blood and bone marrow mononuclear cells.
One patient was tested in addition for bcl-2 rearrangement by Southern blot analysis and polymerase chain reaction.
Four cc/cb-patients were positive for JH-rearrangement in bone marrow, one of which was negative in peripheral blood, whereas the two others were positive in peripheral blood, too, and one was not evaluable.
One patient was negative for JH-rearrangement in bone marrow, however, positive in peripheral blood.
Two of four cb-patients were positive for JH-rearrangement in bone marrow, one of which was positive in peripheral blood, too.
The other two patients were negative in bone marrow as well as in peripheral blood.
Southern blot analysis of the bcl-2 gene in one of the cc/cb-lymphomas which had been positive for JH-rearrangement, showing three rearranged bands, revealed comigration of bcl-2 with one these bands.
At reanalysis of the same patient after three cycles of NOSTE, the rearranged clone had disappeared in bone marrow as far as evaluable by Southern blot analysis. In the peripheral blood, in contrast, two new rearranged bands were detectable with the JH-probe, and the same DNA showed several rearranged bands with the bcl-2 probe none of which comigrated with the JH-bands.
Four months later bone marrow still showed germline for both JH and bcl-2.
PCR analysis of the bone marrow samples showed two amplification products identical at all three times which hybridized with JH-probe as well as the bcl-2 probe, indicating that both alleles were rearranged differently, and the same clone persisted even during complete remission. The relevance of Southern blot as compared to PCR analysis with respect to clonal evolution is discussed.

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LUNG FUNCTION IMPAIRMENT FOLLOWING AUTOLOGOUS BONE MARROW TRANSPLANTATION AND PERIPHERAL BLOOD STEM CELL TRANSPLANTATION

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Autologous bone marrow transplantation (BMT) or peripheral blood stem cell transplantation (PBSCT) are associated with multiple risk factors that may affect pulmonary function including prior high dose chemotherapy (HDCT), radiotherapy, and/or infections. To assess an association between BMT/PBSCT and abnormal pulmonary function, 12 patients (pts; 4 ♀, 8 ♂, mean age (SEM) 33±4 yrs) with Hodgkin's (n=11) or Non-Hodgkin's lymphoma (n=1) undergoing BMT (n=3) or PBSCT (n=9) in ≥ 2nd complete or partial remission were followed for changes in pulmonary function. Pts were tested within 4±1 weeks (wks) prior to HDCT followed by BMT/PBSCT and again for 35±9 wks after treatment. In 8/12 pts vital capacity declined from 93±6% to 81±7% ($p<0.01$), and FEV1 from 97±7% to 79±6% ($p<0.001$) within 9±1 wks after BMT/PBSCT. 14±1 wks after BMT/PBSCT both parameters had nearly returned to baseline (VC: 90± 6%, $p>0.1$; FEV1: 93± 5%, $p>0.05$; all comparisons to baseline). 5/8 pts recovered spontaneously without therapy. 3 pts required systemic corticosteroids. In conclusion, a majority of pts undergoing BMT or PBSCT developed a significant, temporary reduction of pulmonary function. Since none of the pts had signs of pulmonary infection or tumor progression, the causes of these changes in pulmonary function may involve cumulative toxicity from chemo- and/or radiotherapy, and/or other effects associated with BMT/PBSCT. Pulmonary function should be monitored closely following BMT/PBSCT to early identify pts at risk for pulmonary complications requiring further diagnostic evaluation and therapeutic intervention.

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THE HEPATOCYTE GROWTH FACTOR RECEPTOR: STRUCTURE AND FUNCTIONS

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Hepatocyte Growth Factor (HGF) and Scatter Factor are identical glycoproteins secreted by cells of mesodermal origin. The factor has several activities on hepatocytes and other epithelial cells, including mitogenesis, dissociation of monolayers, stimulation of cell motility, and promotion of matrix invasion. HGF is the ligand for $p190^{MET}$, the receptor tyrosine kinase encoded by the *MET* proto-oncogene, a heterodimer of two ($\alpha\beta$) disulfide-linked protein subunits.

HGF binding triggers tyrosine autophosphorylation of the receptor β subunit at Tyr^{1235}. Autophosphorylation upregulates the kinase activity of the receptor, increasing the V_{max} of the phosphotransfer reaction. Negative regulation of the receptor kinase activity occurs through distinguishable pathways involving protein kinase C activation or increase in the intracellular Ca^{2+} concentration. Both lead to serine-phosphorylation of a unique phosphopeptide of the receptor and to a decrease in its kinase activity.

Receptor autophosphorylation also triggers the signal transduction pathways inside the target cells. The phosphorylated receptor associates Phospholipase C-γ, *src*-related tyrosine kinases, Phosphatidylinositol 3-kinase, and activates *ras* by stimulation of a guanine nucleotide exchanger, indicating that different downstream signaling pathways mediates the motility and/or the growth response to HGF.

The $p190^{MET}$ HGF receptor is expressed in several epithelial tissues and it is often overexpressed in neoplastic cells. In some tumors of the gastro-intestinal tract the *Met* tyrosine kinase is constitutively activated, either by overexpression of the amplified c-*MET* gene or by lack of cleavage of the receptor precursor, due to defective post-translational processing. Transfection of genetically engineered HGF receptors, mutated at critical residues or truncated at the N-terminus, induces transformation and tumorigenicity of recipient cells, indicating that *MET* is a potent oncogene.

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REARRANGEMENT OF THE T CELL RECEPTOR GAMMA CHAIN IS A RARE EVENT IN HODGKIN AND REED-STERNBERG CELLS AS SHOWN BY SINGLE CELL PCR

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The cellular origin of the Hodgkin and Reed Sternberg (H&RS) cells remains a mystery. Since the tumour cells rarely represent more that 1% of the cells present in a Hodgkin's lymphnode, tests in situ or at the single cell level are the methods of choice to examine H&RS cells. Since a lymphoid origin for H&RS cells has been proposed, and T cell receptor gamma chain (TCRγ) rearrangements can serve as markers for origin and clonality in T lymphocytes, we developed a PCR based assay at the single cell level to detect TCRγ rearrangements in single H&RS cells. Southern Blot studies had previously shown TCRγ rearrangements in approx. 10% of Hodgkin's cases. Four different primers corresponding to the variable region of the TCRγ gene were used as forward primers, and two primers corresponding to the joining region were used as reverse primers. PCR products of 350 bp length were obtained from single cells of different lymphoid cell lines and Non Hodgkin's lymphomas, and specifity of PCR was demonstrated by hybridization of the PCR products to an internal oligonucleotide probe. Single H&RS cells from 6 patients with Hodgkin's disease were isolated by micromanipulation from glass slides. PCR products were obtained from none of the H&RS cells examined so far, demonstrating that TCRγ rearrangements are infrequent events in Hodgkin's lymphomas. These results show that rearrangements previously reported from Hodgkin's cases were probably due to reactive T lymphocytes and not to neoplastic H&RS cells in these nodes.

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INDUCTION OF APOPTOSIS IN LYMPHOID LEUKEMIAS: BASIC PRINCIPLES AND THERAPEUTIC PERSPECTIVES

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The selective induction of apoptosis or programmed cell death in tumor cells may become a new perspective in tumor therapy. In lymphoid cells, apoptosis may follow withdrawal of crucial growth factors ("death by default"). Alternatively, apoptosis may be induced via cell surface molecules such as APO-1, a new 48kd member of the TNF/NGF-receptor superfamily ("triggered death"). In normal T cell ontogeny, expression of APO-1 is moderate on immature (triple negative, double positive) thymocytes, weak on resting mature T cells but strong on activated T cells. Triggering of APO-1 by the monoclonal antibody anti-APO-1 induces apoptosis in most APO-1 positive cell lines. However, in activated T cells apoptosis sensitivity is not fixed and depends on the state of activation. In T cell leukemias, a distinct pattern of sensitivity is found. Mature T cell leukemias such as adult T cell leukemia cells (ATL) are sensitive to anti-APO-1 induced apoptosis. In contrast, the majority of APO-1 positive T-ALL cells representing T cell precursor phenotypes is apoptosis-resistant. This resistance is independent of the expression of the antiapoptotic protooncogene bcl-2 which is differentially expressed during the discrete stages of T cell maturation within the thymus. However, APO-1 resistance in T-ALL is turned into sensitivity by inhibition of protein synthesis. Thus, resistance towards induction of apoptosis is actively maintained by cellular programs and can be modulated. Heterogeneous apoptosis sensitivity of T-All cells is also demonstrated in a model of an established human T-ALL in SCID mice. Injection of leukemia bearing SCID mice with anti-APO-1 leads to rapid induction of apoptosis only in a fraction of leukemic cells. The identification of molecular determinants of sensitivity and resistance towards apoptosis will give new insights into the biology of leukemias and should provide key molecules as targets for rational therapeutic approaches in the future.

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CYTOKINE SECRETION BY PURIFIED CD4 POSITIVE T CELLS FROM PATIENTS WITH B-CLL IN VITRO

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In B cell chronic lymphocytic leukemia the absolute number of the non-malignant T cells is significantly elevated. Several studies in the past described certain functional abnormalities in the T cell population of B-CLL. We investigated the proliferative capacity and cytokine secretion patterns in non-malignant T cells of B-CLL patients in various clinical stages and normal age-matched individuals as controls. CD4 positive T cells were highly enriched by several separation procedures. Such purified cells consisted of >95% CD4 positive T cells. T cells were stimulated with combinations of soluble or immobilized antibodies, including CD3, a combination of stimulating CD2 antibodies, and CD28 in costimulation with PMA or autologous antigen presenting cells (APC). In proliferation assays maximal stimulation was achieved with immobilized CD3 in combination with CD28 or CD2 or APC. We observed no difference between the proliferative capacity of normal and CLL-derived T cell populations. In our studies on cytokine secretion patterns, identical stimulatory substances were used as described for the proliferation assays. We measured secretion of IL-2, IL-4 IFN-g and TNF in supernatants of such stimulated T cells. Our results demonstrate that T cell cytokines are differently produced by cells from B-CLL patients as compared to normal controls. The cytokine secretion pattern was dependent on the kind of stimulatory antibodies and the source of APCs used. This study suggests functional interactions between malignant B cells and T lymphocytes. Reciprocal regulation of lymphotropic cytokine secretion might contribute to the expansion of both cell populations.

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MOLECULAR CYTOGENETICS (FICTION) IN HODGKIN'S DISEASE REVEAL CHROMOSOMALLY ABERRANT TUMOR CELLS IN 100 % OF STUDIED CASES

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Many investigators know to their cost that cytogenetic analysis in Hodgkin's disease means frustrating labor. The low yield of analyzable metaphases hampers in many cases the determination of aberrant tumor clones. In view of the low number of cases with detected clones it is unclear wether all rather than only a proportion of cases do have cytogenetically detectable chromosome aberrations. Problems in cytogenetic analysis could be mastered by the developing technique of interphase cytogenetics which allows detection of numerical chromosome aberrations also in interphase cells. The newly reported FICTION-technique, which simultaneously combines fluorescence immunophenotyping and interphase cytogenetics, additionally allows immunophenotypical characterization of aberrant cells. This strategy enables rapid identification of CD30 positive Hodgkin's and Reed-Sternberg cells on a slide and subsequent interphase cytogenetic evaluation. This holds also true if the total number of malignant cells is very low (e.g. <1%). We have investigated 12 cases of Hodgkin's disease with different histological subtypes, after unsuccesful tumor cytogenetic analysis. In every single case we were able to find aberrant chromosome numbers which were consistent in and restricted to the CD30 positive HRS-cells.

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MECHANISM OF THE CHROMOSOMAL TRANSLOCATION t(14;18): NUCLEASE-SENSITIVITY OF THE MAJOR BREAKPOINT REGION AND BINDING OF A 45kDa PROTEIN

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The translocation t(14;18) occurs in early B cell development at the time of the first immunoglobulin rearrangement and juxtaposes the BCL-2 oncogene with the immunoglobulin heavy chain locus. While V(D)J-recombinase is likely to be involved on the chromosome 14 (IgH)-part, little is known about the mechanism of breakage on chromosome 18. Using B cell nuclei and supercoiled plasmids we found that the BCL-2 major breakpoint region (mbr) contains an S1-nuclease sensitive site, suggesting the structural vulnerability of this genetic locus. Moreover, an endogenous nuclease from B cell extracts is able to cleave the BCL-2 mbr.

Using gel retardation assays as well as Southwestern blotting we have also identified a 45kDa protein (bp45) which binds to minisatellite (CHI) elements in the major and minor breakpoint regions of BCL-2. Similar sequences present around the DH and JH regions on chromosome 14 cross-compete with a 20bp fragment from the mbr, indicating that they all bind the same protein. bp45 is predominantly expressed in early B cells, but can also be found in various other cell lines. Our data suggest (1) that the BCL-2 mbr can assume alternative DNA structures and is highly susceptible to endonucleolytic cleavage; and (2) that the binding of bp45 to the t(14;18) breakpoints maybe part of the translocation process.

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FLOW CYTOMETRIC DETECTION OF MYELOPEROXIDASE AND OTHER ENZYMES OF PRIMARY AZUROPHILIC GRANULES FOR IMMUNOPHENOTYPING OF ACUTE MYELOID LEUKEMIAS

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Immunophenotyping of acute leukemias is usually performed by flow cytometry using clustered antibodies targeted against cell surface antigens (CD). This approach is useful in subtyping of acute lymphoblastic leukemias but unsatisfactory in cases with AML for the fact that 1) no single AML-specific CD antibody is available and 2) most membrane markers are not lineage-specific or confined to only certain AML-subtypes. Recently, antibodies against myeloid-specific enzymes of azurophilic granules like myeloperoxidase (MPO) and neutrophil elastase (EL) have become available. Due to their intracellular localization, these proteins were mainly detected by immunocytochemistry so far. However, this method is time-consuming and does not allow exact quantification of obtained immunoreactivities. Recently, various protocols for cell membrane permeabilisation and antigen fixation for flow cytometry have been reported. We have thus utilized this method as an amenable alternative for the routine laboratory where large amounts of samples have to be analyzed. We used indirect immunofluorescence and flow cytometric quantitation of MPO and EL. In addition, another enzyme of the primary granules, cathepsin G (CG) was investigated.
Peripheral blood as well as bone marrow aspirates (whole blood and Ficoll-separated mononuclear cells) of leukemia patients were examined for MPO, EL and CG. In parallel, the same samples were investigated by immunocytochemistry (APAAP) using the same antibodies. The results obtained so far show that all of the three enzymes can reliably be detected by flow cytometry. A good correlation was obtained between the two methods. In most of the AML's investigated by flow cytometry, expression of at least one of the enzymes was observed. Thus, myeloid lineage could be confirmed immunologically. Significantly more samples could be analyzed in the same time compared to the immunocytochemical technique.

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INHIBITION OF TUMOR COLONY FORMING UNITS FROM FRESHLY EXPLANTED TUMOR SPECIMENS BY TRANSFORMING GROWTH FACTOR-ß (TGF-ß)

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Transforming Growth Factor-ß (TGF-ß) is a tumor growth-modulating peptide with pleiotropic activity. Depending on the experimental system used it can either inhibit or stimulate tumor cell proliferation. It may play an important role in autocrine tumor growth control. The purpose of our study was to determine the effects of TGFs-$ß_1$ and -$ß_2$ on soft agar colony formation of freshly explanted human tumor specimens in vitro. Concentrations ranged from 0.1 ng/ml to 10 ng/ml. Fifty of 81 tumor specimens tested were evaluable (62%). At 10 ng/ml, concentration-dependent inhibition (colony survival $\leq$ 50% of control) by TGF-$ß_1$ was observed in 12 specimens (5 renal cancers, 1 breast cancer, 4 Non-Hodgkin's lymphomas, 1 ovarian cancer, 1 melanoma) for a total response rate of 24%. TGF-$ß_2$ appeared to be more active against colorectal cancer (3 of 6 specimens inhibited) with otherwise similar spectrum of activity at 10 ng/ml. A total of 17/50 specimens (34%) were inhibited by this peptide. Combination of TGF-$ß_1$ and Epidermal Growth Factor or Platelet-Derived Growth Factor reversed the inhibitory activity in 4 of 5 specimens (80%) and 2/5 specimens (40%), respectively. We conclude that TGFs-$ß_1$ and -$ß_2$ negatively modulate growth of a subgroup of freshly explanted tumor colony forming units. However, their activity appears to be influenced by other growth factors.

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FAILURE OF TYPE I INTERFERONS TO INHIBIT IL-3-INDUCED PROLIFERATION OF MYELOID PROGENITOR CELLS

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The clinical efficacy of Interferon (IFN)-a in chronic phase of Ph1 positive CML is well established, however, the mechanism(s) of action of IFN in CML is still poorly understood. The antiproliferative capacity of Type I IFNs in hematopoietic progenitor cells (HPC) might contribute to the therapeutic effects without explaining a differential effect in normal and CML hematopoiesis. We investigated the effect of different type I IFNs on in vitro proliferation of subpopulations of HPC separated from bone marrow or peripheral blood of CML patients and normal individuals. G-CSF stimulated proliferation of CFU-GM day 7 and BFU-e stimulated with IL-3 and EPO were inhibited by IFN-a and IFN-b in a dose dependent fashion. In CML cells the antiproliferative effect of IFN-b was more pronounced as compared with IFN-a. By contrast, IL-3 induced proliferation of CFU-GM day 14 was not inhibited by both IFNs up to a concentration of IFN 3000 U/ml in 12 patients with CML. In cultures stimulated with IL-3 and EPO an increase of myeloid colony formation was observed with increasing doses of IFN which correlated with the suppression of erythroid colony formation by IFN. In bone marrow samples of normal individuals IL-3 induced colony formation was not suppressed up to a concentration of IFN of 1000 U/ml and only slightly inhibited at higher doses. In contrast, CFU-GM from normal peripheral blood were completely inhibited by IFNs suggesting functional differences of subpopulations of CFU-GM day 14 circulating in PBL or resinding in bone marrow. In highly enriched CD34 positive cells from CML PBL or normal BM a similar effect was observed. CD34+ CML cells were not inhibited by IFNs upon stimulation with IL-3. In normal CD34+ cells IL-3 provided only a limited growth signal for CFU-GM. The combination of stem cell factor and IL-3 as optimal stimulus was not inhibited by IFNs. Dose titration experiments of IL-3 with constant doses of IFN-b (1000 U/ml) revealed that at suboptimal concentrations of IL-3 (< 1 ng/ml) CFU-GM formation was reduced by 50 % in presence of IFN. However, at optimal doses IL-3 appears to protect CFU-GMs from the antiproliferative effect of IFNs.

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NEW CONCEPTS IN THE TREATMENT OF HODGKIN'S DISEASE.

V. Diehl for the German Hodgkin Study Group (GHSG)

Radiotherapy (RT) as single treatment modality is the standard therapy for pts. with **limited stages** CS/PS I, II without risk factors. Long term 10-year survival rate of more than 85% can be achieved. Beside the question of introducing mild chemotherapy (CT) to prevent relapse, optimization of RT is under debate. The aim of the ongoing HD4 trial is to define the appropriate dose of RT in the extended field (EF) (40 Gy EF vs. 30 Gy EF plus 10 Gy involved field (IF)). Treatment of choice for pts. in **intermediate stages** is combined modality therapy which results a in 5-year survival rate of about 85%. Current strategies aim at reducing toxicity without compromising efficacy. The ongoing HD8 trial compares EF vs. IF radiotherapy after 2 double-cycles COPP-ABVD. Half of the pts. in **advanced stages** relapse within 10 years. One approach to improve treatment results is intensification of CT. In the HD9 trial the GHSG compares standard CT with a new timely intensified protocol (BEACOPP) on two different dose levels. The higher dose level is given with G-CSF support. Only about 35% of **pts. who relapse** after CT can still be cured. The ongoing HDR-1 trial compares 4 cycles of dose escalated DexaBEAM with 2 cycles of DexaBEAM followed by high dose myeloablative BEAM plus PSCT.

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WEEKLY THERAPY WITH FOLINIC ACID AND HIGH-DOSE 5-FLUORO-URACIL 24-HOUR INFUSION IN PREVIOUSLY UNTREATED PATIENTS WITH METASTATIC COLORECTAL CARCINOMA: A CONFIRMATORY PHASE-II STUDY

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Recently Ardalan et al (JCO 9, 1991, 625-630) reported the high remission rate of 58 % in 12 previously untreated patients with metastatic colorectal carcinoma, using a weekly 24-hour infusion of high-dose 5-FU (2600 mg/m²) with folinic acid (500 mg/m²).

Between 1/92 and 12/92 we have conducted a confirmatory phase-II study using the same regimen with the slight modification that folinic acid was given as 1-hour infusion prior to 5-FU. The main inclusion criteria were: proven progressive and/or symtomatic metastatic colorectal carcinoma and no previous chemotherapy. After 6 infusions (one course) response to therapy was evaluated. If partial remission (PR) or stable disease (SD) with improvement of the patients' condition was achieved, therapy was continued with a second course and stopped thereafter. In cases of SD without clinical improvement or progressive disease (PD) treatment was stopped.

Results: 27 consecutive patients have entered the study of whom 21 are evaluable for response and toxicity until now. Mean age of the 13 male and 8 female patients was 59 years (range: 38-78 years). Therapy resulted in 38 % (8/21) PR, 38 % SD and 19 % (4/21) PD; one patient died due to therapy-related toxicity. So far, probability of median duration of PR/SD is 6 months, duration of survival is not evaluable, yet.

Toxicity: one patient developed grade III diarrhea, grade IV mucositis and grade II leukopenia after 6 infusions and died subsequently due to therapy-induced toxicity. In the other patients 17 toxic side effects , grade II-III were seen: diarrhea (8), nausea (7) and stomatitis (2). Four patients suffered from hand-foot syndrome, cardiotoxicity (angina pectoris) was observed in one case and neurotoxicity in three other.

Conclusion: this regimen is undoubtedly an effective treatment in metastatic colorectal carcinoma, although we could not confirm the previously reported high remission rate. Toxicity is moderate and comparable to conventional 5-FU/FA regimens. However, hand-foot syndrome and neurotoxicity, seen in 7 patients, seem to be correlated to high-doses of 5-FU.

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CELLULAR CHANGES DUE TO CYTOSTATIC DRUG RESISTANCE

M. Dietel

Chemotherapy of hematological malignancies has considerably improved over the last decade. However, complete therapeutic success continues to be frustrated by the appearance of tumor cell clones resistant to antitumor drugs and drug combinations. These clones develope specific mechanisms to prevent drug activity, mechanisms such as overexpression of intramembrane efflux pumps (P-glycoprotein, P-Gp), enhancement of detoxifying enzymes, acceleration of nuclear DNA repair, and intensified intracellular compartmentalization. In addition, structural changes of the nuclear targets, e.g. altered or mutated topoisomerase II, contribute to resistance. It is likely that two or more mechanisms work together.

The transfer of this knowledge into improvements of chemotherapy appears to be more difficult than expected. One major problems is the lack of standardized assays to determine or, even better, to predict chemoresistance. It has, for example, become clear that using one assay to determine P-Gp is insufficient and that immunocytochemistry *and* RT-PCR or Northern Blot are needed and, if possible, substantiated by a functional assay. Since the agreed-upon standardization is lacking here, controversial results are reported when researchers attempt to correlate resistance parameters raised in the laboratory with clinical follow-up.

The example is not random, since a large amount of data on P-Gp has been gathered, and most authors agree that the clear detection of P-Gp in a given tumor means that the tumor is at least to some extent resistant to MDR cytostatic drugs. Our own experiments have confirmed these results. Twenty-two tumors found positive for P-Gp have been shown to be refractory to anthracycline therapy. On the other hand, P-Gp negativity does not guarantee chemosensitivity.

Although approaches to reverse drug resistance are numerous, so far, in clinical settings, only the deactivation of P-Gp has gained some relevance. When a chemosensitiver is combined with a cytostatic, the success of growth inhibition may be improved. A rationale for the combined therapy is the pretherapeutical diagnosis of P-Gp as outlined above.

Despite the described difficulties, there is no doubt that a better understanding of resistance, as well as improved methods for detection of resistant cells, would aid therapists in predicting the effectiveness of cytostatics, and help them to design more effective chemotherapy.

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HIGH LEVELS OF CIRCULATING SOLUBLE RECEPTORS FOR TUMOR NECROSIS FACTOR IN HAIRY CELL LEUKEMIA AND TYP B CHRONIC LYMPHOCYTIC LEUKEMIA

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Tumor necrosis factor (TNF) is a pleiotropic cytokine that plays a major role in inflammation,and host defence to infection; it has also been shown that TNF stimulates leukemia growth in patients with hairy cell leukemia (HCL), type B chronic lymphocytic leukemia(B-CLL), and acute myelgenous leukemia.We have investigated the presence of soluble tumor necrosis binding proteins (TNFBP) in the sera of healthy volunteer blood donors and cancer patients.Two distinct types of TNFBP, Type A and B, which are immunologically related to the cellular 75-kD TNFR and the cellular 55-kD TNFR, respektively, were assessed by immunoassays using nonblocking anti-receptor antibodies and 125I-rhTNF-alpha. As compared to the titers observed in 25 healthy controls, TNFBP types A and B titers were found to be elevated in almost all sera obtained from patients with underlying malignant disease.The highest amount of TNFBP were seen in the sera of patients with B cell malignancies including HCL and B-CLL. The most notable result was the excepionally high TNFBP with predominance of type A over type B TNFBP in the sera of most HCL and B-CLL patients.Effective treatment of HCL patients with rhIFN-alpha was associated with decrease of circulating TNFBP. Analysis of the cellular source of TNFBP indicated, that the neoplastic B-cells are the producer cells of type A TNFBP.TNF serves as an autocrine growth factor in both disease states and thus TNFBP may play an important role in the regulation of neoplastic cell growth.

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PROSPECTIVE RANDOMIZED TRIAL OF CONVENTIONAL HIGH DOSE CISPLATIN PLUS CYCLOPHOSPHAMIDE VERSUS CISPLATIN PLUS CARBOPLATIN IN EPITHELIAL OVARIAN CANCER PATIENTS: PRELIMINARY RESULTS ON TOXICITY AND DOSE INTENSITY

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Between 6/90 and 12/92, 189 patients with epithelial ovarian cancer FIGO stages IC-IV were entered into a multicenter nation-wide prospective randomized trial. In this preliminary analysis, the toxicity profile and the degree of platinum dose intensity reached of the combination of cisplatin/carboplatin in comparison to the standard therapy with cisplatin /cyclophosphamide were evaluated. 100 mg/m2 cisplatin plus 600 mg/m2 cyclophosphamide or 100 mg/m2 cisplatin plus 300 mg/m2 carboplatin were applied every 4 weeks for 6 cycles. Up to now, 116 patients (58 in each treatment arm) completed combination therapy. Under the therapy with cisplatin/carboplatin and cisplatin/cyclophosphamide, thrombocytopenia WHO grade 4 occurred in 12.6% and 1.3%, respectively, and granulocytopenia WHO grade 4 in 22.2% and 9.2%, respectively. Neurotoxicity, nephrotoxicity and nausea/vomiting were comparable between the two regimens. No treatment related deaths occurred. In the cisplatin/carboplatin therapy group, 80.4% of the planned cisplatin dose intensity (mg/m2/week) and 71.3% of the planned carboplatin dose intensity were reached. The dose intensity reached in the cisplatin/cyclophosphamide therapy group were 80.4% for cisplatin and 84.6% for cyclophosphamide. Overall, the ratio of total platinum to cisplatin between the cisplatin/carboplatin therapy and the cisplatin /cyclophosphamide therapy was about 2:1.

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MOLECULAR CYTOGENETIC STRATEGIES FOR DETECTION OF CHROMOSOME ABNORMALITIES IN CHRONIC LYMPHOID LEUKEMIAS

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In B-cell chronic lymphoid leukemias cytogenetic analysis has been hampered by the low in vitro mitotic activity of the leukemic cells. In order to achieve a higher accuracy regarding the incidence of specific numerical and structural chromosome abnormalities, we performed a combined metaphase and interphase cytogenetic analysis. We specifically examined the incidence of some of the most frequent aberrations: (i) trisomy 12; (ii) abnormalities of 13q, especially of chromosomal band 13q14 to which the retinoblastoma tumor suppressor gene (RB-1) has been mapped; and (iii) abnormalities of 17p, site of the p53 tumor suppressor gene. Seventy-two pts with chronic lymphoid leukemias of B-cell origin (chronic lymphocytic leukemia=68; prolymphocytic leukemia=4) have been collected prospectively. For in-situ hybridization (ISH) the following probes were used: D12Z3, biotin-labeled (Oncor Sciences, Gaithersburg, MD), identifying the centromere of chromosome 12; 16 lambda-phage clones spanning the whole 200kb of RB-1 (kindly provided by Dr. T.P. Dryja, Boston, MA); and three overlapping cosmids recognizing the entire p53 gene. So far, 52 pts have been analyzed by G-banding: no metaphases=14%; normal karyotype =42%; clonal chromosome abnormalities=44%. The following table summarizes the presently available G-banding and ISH data with respect to the three regions of interest:

Chromosome Aberration	*G-Banding*		*ISH*	
Trisomy 12	5/52 pts	(10%)	9/67 pts	(13%)
13q	5/52 pts	(10%)	13/59 pts	(22%)
17p	5/52 pts	(10%)	4/16 pts	(25%)

Our data demonstrate (i) a higher frequency of specific chromosome aberrations in chronic lymphoid leukemias than previously assumed based on G-banding analysis and (ii) the applicability of ISH on a routine basis which now allows the more accurate correlation of specific aberrations with patient characteristics, response to therapy and survival.

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QUANTITATIVE ANALYSIS OF CIRCULATING t(14;18)-POSITIVE LYMPHOMA CELLS IN PATIENTS WITH FOLLICULAR LYMPHOMA AFTER RADIOTHERAPY AND AUTOLOGOUS BONE MARROW TRANSPLANTATION

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The t(14;18) translocation has been detected cytogenetically in about 85% of patients with follicular lymphomas. Applying a two-step PCR with nested primers we were able to detect this translocation in about 50% of patients with advanced stage malignant lymphoma. Minimal numbers of circulating t(14;18)-positive lymphoma cells were found in 38% (8/21) of patients with cbcc NHL being in CR for 2-12 years after radiotherapy for localized lymphoma. A quantitative analysis of t(14;18)-positive cells during a follow-up period of 2-3 years showed, that 7 of 8 patients had t(14;18)+ cells circulating in the blood at almost constant (n=6) or even decreasing (n=1) levels in the range of one positive cell per 1 million cells (n=2) or one per 10.000 to 100.000 (n=5).
Five patients were treated with autologous bone marrow transplantation. Only one patient relapsed at day +280, a quantitative analysis of the lymphoma cells showed a logarithmic increase in the peripheral blood starting already on day +192. The other four patients are still in CR (n=3) or stable PR (n=1): in all cases t(14;18)-positive cells reappeared in the circulating blood several months after BMT, but they did not increase in number during the past 7-24 months. Therefore, a quantitative PCR analysis of circulating minimal residual lymphoma cells seems to be of prognostic significance.

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SUPPRESSION OF THE TUMORIGENIC GROWTH OF BURKITT'S LYMPHOMA CELLS IN NUDE MICE BY COINOCULATION OF AUTOLOGOUS LYMPHOBLASTOID CELLS

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Somatic cell hybrids between the malignant Burkitt's lymphoma (BL) cell line BL 60 and the autologous EBV-immortalized lymphoblastoid cell line (LCL) IARC 277 demonstrate the non malignant phenotype of the parenteral LCL cells despite deregulated c-myc expression: After s.c. inoculation into nude mice the BL 60 cells consistently show progressive tumor growth, while the LCL and the hybrid cell tumors regress after an initial growth phase.
Now we demonstrate, that it is possible to suppress the malignant growth of the Burkitt's lymphoma cells just by coinoculation of the autologous LCL in a mixture-suspension. Even in a mixture ratio of 1:10 the LCL cells could suppress the malignant growth of the Burkitt's lymphoma cells in 50% of the cases. The same result can be obtained with other Burkitt's lymphoma/LCL constellations. When BL cells and LCL were inoculated s.c. into contralateral flanks of the nude mice tumor suppression was observed only in a minority of cases. Therefore the tumor suppression effect might be locally restricted rather than acting in a systemic manner.
Our results suggest that cytokines secreted by the LCL's mediate tumor suppression of BL cells probably by induction of the host's immune response. Interestingly BL cells are susceptible to these anti-tumor cytokines despite deregulated c-myc expression and latent infection with EBV. Since IL 6, IL 4 and TNF-alpha are secreted by the LCL used in our studies but not by the BL cells, these cytokines might be involved in tumor suppression.

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DOES ADDITIONAL CYTOTOXIC TREATMENT IMPROVE THE YIELD OF G-CSF-INDUCED MOBILISATION OF PERIPHERAL BLOOD PROGENITOR CELLS?

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Peripheral blood progenitor cells (PBPC) have successfully been employed for restoring haematopoiesis after myeloablative chemotherapy. PBPC for autologous transplantation can be mobilised effectively by cytotoxic therapy. While it is well documented that the addition of G-CSF strongly increases the efficacy of cytotoxic PBPC mobilisation, investigations indicating the augmentation of G-CSF-mediated mobilisation by cytotoxic therapy are sparse. Thus, we compared the PBPC yields of 13 patients who were treated with myelosuppressive chemotherapy (Dexa-BEAM) plus G-CSF for PBPC mobilisation (group A) with those of 8 patients who were mobilised with G-CSF alone (group B). All patients suffered from Hodgkin's disease or non-Hodgkin's lymphoma and were intensively pretreated. Progenitor cells were assayed by measurement of CD34+ cells and CFU-GM.
Results: Although both periphal blood CD34+ cell maxima (3.9-258 vs. 6.7-88.6 x10^3/mL) and average CD34+ cell yield per 10L leukapheresis (3-122.9 vs. 2.2-34.6 x10^5/kg) were higher in group A than in group B, these differences were not statistically significant. The numbers of CFU-GM paralleled those of CD34+ cells very closely. With three 10L leukapheresis procedures, a sufficient amount of PBPC (>1x10^4 CFU-GM/kg) could be collected in 11/13 patients of group A and 6/8 patients of group B. In both groups, B cells were barely detectable in the collection products (<1% of nucleated cells in the vast majority of patients).
Conclusions: Addition of Dexa-BEAM to G-CSF does not substantially improve the yields of PBPC mobilisation as compared to G-CSF-induced mobilisation alone. Provided that larger patient numbers will confirm these results, PBPC harvesting may be performed independent from chemotherapy, allowing a much more precise scheduling of leukapheresis procedures.

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CYTOGENETIC RESULTS IN PHILADELPHIA CHROMOSOME POSITIVE CHRONIC MYELOGENOUS LEUCEMIA PATIENTS TREATED WITH RECOMBINANT INTERFERON ALPHA-2C.

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In a multicenter phase II study 90 patients with Philadelphia-chromosome (Ph) positive chronic myelogenous leukemia (CML) were treated with recombinant interferon alpha-2C (rIFN). (Thaler et al.: Dtsch.Med.Wschr.: 116(1991), 721-728)
We report the cytogenetic data for 85 of these patients. Detailed analysis at start of therapy revealed chromosomal aberrations additional to the Ph-translocation in sixteen (19%) patients, including complex translocations involving the Philadelphia-chromosome and single chromosome aberrations, a result that was significantly correlated with poor clinical outcome. Twenty patients (24%) showed a cytogenetic response, including 3 complete cytogenetic remissions. In eight (9%) patients additional chromosomal aberrations, including only single chromosome aberrations, occured during rIFN therapy. Five of these patients remained in a well controlled stable clinical condition for one year or longer. The remaining three patients developed myeloid blast crisis.

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SELF-RENEWAL AND DIFFERENTIATION OF PGM-2 CELLS, A TRANSPLANTABLE MURINE PROGENITOR CELL LEUKEMIA

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The PGM-2 leukemia is a variant of the PGM-1 leukemia (Leukemia 3:796,1989). Like the original cells, PGM-2 cells could initially not be maintained in vitro, but had to be propagated by continuous subcutaneous transplantation in C3H/He mice. In contrast to PGM-1 cells, they had lost responsiveness to GM-CSF and M-CSF, but showed colony formation in agar cultures in response to a multitude of other myeloid and lymphoid growth factors, including interleukins (IL) 2, 3, 4, 5, 6 and 7 as well as Steel factor and G-CSF. While IL-3 induced the formation of macrophage colonies lacking the capacity for self-renewal, IL-7 stimulated 2 types of mononuclear cell colonies. The first one was characterized by rapidly degenerating dispersed cells. The second type was compact, continuously increased in size with extended culture and induced the development of typical PGM-2 leukemias after transplantation to syngeneic mice. When these putative stem cell colonies were recultured in secondary IL-7-stimulated agar cultures, their progeny reproduced the same 2 types of IL-7-colonies as seen in primary PGM-2 cultures. In IL-3-stimulated secondary cultures large numbers of macrophage colonies were formed, suggesting that the IL-3-responsive progenitor cells were the progeny of the IL-7-responsive stem cells. When PGM-2 cells were cultured in agar at extremely high density, spontaneous formation of compact colonies similar to those stimulated by IL-7 was observed. This could be specifically inhibited by an IL-7-antibody, suggesting that IL-7 also played a role as an auto- or paracrine stimulator in the natural expansion of the leukemia. When primary cells from subcutaneous PGM-2 tumors were cultured at very high density in unstimulated suspension culture, they underwent a crisis followed by the formation of an adherent layer from which IL-3- and IL-7-responsive colony forming cells could be recovered over prolonged periods of time. The generation of colony forming cells in these long-term leukemia cultures was also inhibited by added IL-7-antibody.

PGM-2 is a leukemia with the capacity for macrophage differentiation in which IL-7 plays a vital role as a stem cell self-renewal factor. This leukemia model and 4 recently derived cell lines with similar properties could be useful for the study of the mechanisms underlying self-renewal and differentiation of leukemic stem cells.

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TETRAPLOIDY IN ASSOCIATION WITH NUMERICAL AND STRUCTURAL CHROMOSOME ABERRATIONS - A CYTOGENETIC MARKER FOR AML-M7?

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Acute megakaryoblastic leukemia (M7 according to FAB classification) is a rare subtype of AML for which there is hardly any information on cytogenetic findings reported. We had the opportunity to karyotype a female patient with de novo AML/M7. She presented with mild leucocyto- and thrombocytopenia of 1.9 and 69 G/l, respectively. Morphological examination of marrow smear and histology exhibited the picture of an immature leukemia with about 50% atypical and polymorphical blast cells; these cells showed a low nucleocytoplasmic ratio, intensive basophilic reaction with less perinuclear stain, cytoplasmic vacuolisation and a high proportion of binuclear cells without Auer rods. Morphological and cytochemical (94% of blasts were POX negative) evaluation allowed to define myeloid, megakaryocytic and monocytic characteristics. Diagnosis was confirmed immunocytologically as leukemic cells reacted with anti-megakaryocytic antibodies (CD41a) and with early myeloid antibodies (CD33, CD34). Cytogenetic analysis concerning ploidy revealed beside normal female mitoses in 14/25 metaphases hypotetraploidy (chromosome number 80 -87). For simplification basing on a tetraploid chromosome set (92,XXXX) we found the following composite karyotype representing the malignant clone within the hyperdiploid cells: **80-87,XXXX,-3,-4,del(5)(q22q33),del(5)(q22q33),-del(6)(24),del(6)(24),-9,-12,-12,-17,-17,-18,-18,+19,-22,+mar1,+mar2 [cp7]**. In a recent report on cytogenetic evaluation in cultured M7 leukemic cells also near tetraploidy in combination with numerical and structural aberrations was found. Therefore we suggest, that tetraploidy in association with structural and numerical aberrations could be a chromosomal pattern associated with the rare M7 subtype in AML.

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CELLULAR EXPRESSION OF P-GLYCOPROTEIN CORRELATES TO TUMOR PROGRESSION IN RENAL CELL CARCINOMA PATIENTS

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The phenomenon of multi drug resistance (MDR) in human tumors has been addressed to the expression of a 170 kD membrane glycoprotein (P-glycoprotein). Immunophenotyping of untreated renal cell carcinomas detected P-glycoprotein expression in 48-100%. It has been suggested that P-glycoprotein contributes at least partially to the high degree of intrinsic chemoresistance of renal cancer. Furthermore, in vitro experiments demonstrated a possible relationship between high levels of P-glycoprotein expression and increased resistance to NK mediated cytotoxicity.
We evaluated 25 patients with advanced renal cell carcinoma for the pretreatment P-glycoprotein expression of tumor cells by immunocytochemistry. Positive staining results were found in twenty tumor specimens (80%) using the monoclonal antibody C219. P-glycoprotein expression of 0% or <1% of the tumor cells was found to be associated with an improved progression-free survival interval of tumor patients when compared to those with 1% or more positive tumor cells (Kaplan-Meier survival analysis; $p<0.005$).
These results suggest a possible role of P-glycoprotein as a correlate to tumor dissemination potential in vitro.

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PRETREATMENT CD56 ANTIGEN DENSITY ON CIRCULATING NK CELLS CORRELATES TO CLINICAL RESPONSE IN TUMOR PATIENTS RECEIVING LONG-TERM SUBCUTANEOUS rIL-2 and rIFN-α

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A small subset of peripheral blood natural killer (NK) cells has been hypothesized to exhibit high density surface expression of the NK associated CD56 antigen. It has been suggested that these NK cells respond to lower concentrations of IL-2 than those needed to stimulate the majority of NK cells. We evaluated density of the CD56 antigen on circulating NK cells of 47 patients with advanced renal cell carcinoma by flow cytometry. Patients received a combination of low-dose subcutaneous recombinant IL-2 (rIL-2) at 18 millions IU/m²/day on days 1 and 2, followed by 3.6 millions IU/m²/day, 5 days per week, over 6 consecutive weeks, in combination with recombinant IFN-α (rIFN-α) at 5 millions U/m², three times weekly. Antigen density of CD56 before therapy was 2.2-fold higher ($p<0.005$) in patients who subsequently achieved a complete or partial remission (n=10) when compared with patients who presented with progressive disease on therapy (n=11). After a 6-week treatment cycle, NK cells of treatment responders expressed significantly (2.1-fold; $p<0.05$) more CD56 antigens than NK cells in nonresponding patients.
These results suggested a potential role of both pre- and posttreatment NK antigen density levels as a biologic correlate to treatment response.

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ANTI-TUMOUR ACTIVITY OF ALL-TRANS RETINOIC ACID ALONE AND IN COMBINATION WITH IFN α2b AND CISPLATIN IN HUMAN TESTICULAR CANCER CELL LINES

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Among novel approaches being explored for the chemotherapy of cancer, systemic treatment with retinoic acid (RA) derivatives is one of the most exciting since 13-cis retinoic acid has been used successfully in the treatment of acute promyelocytic leukemia, and dramatic clinical results have been observed in combination with IFNα in advanced squamous cell carcinoma of the skin and cervix. All-trans RA (atRA) has been shown to induce neuroectodermal differentiation of a clone of the human embryonal carcinoma cell line, N-TERA II. We have been testing the anti-tumour activity of various combinations of DDP (24 hour exposure), atRA and clinically relevant doses of IFN α2b (100IU/ml) using a 5 day sulforhodamine B cytotoxicity assay, in 2 human testicular cell lines with intrinsic sensitivity (H12.1) or resistance (H32) to DDP. The IC50 concentrations of atRA were 50-100 μM for both cell lines. The dose response curve to 24 hour DDP exposure (0.01-100μM) was not modified by co-incubation with atRA at < 1μM. Combination of atRA (0.03-150μM) with 0.1μM DDP revealed antagonism at > 3μM atRA for H32, but additivity for H12.1. Pre-treatment of cultures for 2 passages with 10μM atRA, a dose able to induce differentiation in human embryonal carcinoma cell lines, prior to 24hour DDP exposure increased the IC50 value of DDP 2-3 fold in both cell lines. Similar pre-treatment, and then co-exposure to DDP plus atRA/IFN α2b produced a 5-10 fold increase in the IC50 of DDP compared to control (DDP alone).These results indicate that there is no positive interaction between DDP and atRA or atRA+IFN α2b in the testicular cell lines studied, and that careful preclinical investigations are necessary before attempting clinical trials in testicular cancer patients.

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CYTOTOXIC ACTIVITY OF IFN α2b AND IFN GAMMA (γ) ALONE AND IN COMBINATION WITH MITOMYCIN C OR CISPLATIN IN GASTRIC AND COLON CARCINOMA HUMAN CELL LINES

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The combination of interferons (IFN) with conventional cytotoxic agents offers a promising therapeutic approach for the treatment of some cancer types. We have studied the potential modulation of cisplatin (DDP) and mitomycin c (Mito C) cytotoxicity by IFN α2b and IFN γ in 2 gastric carcinoma cell lines (M2, M51) and 2 colon adenocarcinoma cell lines, ATCC-HTB38 (HT-29) and ATCC-CL187 (LS 180). The following IC50 values for DDP and Mito C alone or in combination with a clinically relevant dose of IFN α2b (100IU/ml) were evaluated using a 96 hour incubation schedule in a 5 day sulforhodamine B cytotoxicity assay:

IC50 (μM)

	M2	M51	HTB 38	CL187
DDP	1-3	0.3-1	1-3	1-3
DDP+IFN α2b	1-3	0.3-1	1-3	1-3
DDP+IFN γ	nd	0.3-1	0.03-1	nd
MitoC	0.1-0.3	0.1-0.3	0.03-0.1	0.1-0.3
MitoC+IFNα2b	0.1-0.3	0.1-0.3	0.03-0.1	0.1-0.3
MitoC+IFN γ	nd	0.1-0.3	0.01	nd

The cell lines differed in their sensitivity to the cytotoxic effects of IFN α2b alone (dose range 10 - 30000IU/ml), M2 being the most sensitive (cell survival approximately 85% at 100IU/ml) and M51 being resistant (cell survival approximately 94% even at the highest dose tested of 30000IU/ml). Preliminary data on the cytotoxicity of IFN γ alone show that M51 is rather resistant to it (dose range 1- 10000IU/ml) whilst HTB 38 showed greater sensitivity (cell survival approximately 60% at 100IU/ml). A significant synergistic activity with both cytotoxic agents was observed in combination with a clinically relevant IFN γ dose (100IU/ml) in HTB 38. Further experiments are underway to investigate the cell line and IFN γ dose dependency of this interaction which is of potential clinical importance.

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PRELIMINARY RESULTS OF A PROGNOSTICALLY/STAGE ORIENTATED MULTIMODALITY TREATMENT INCLUDING SURGERY FOR SELECTED SUBGROUPS OF LIMITED DISEASE SMALL CELL LUNG CANCER (SCLC).

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Since 6/91, 20 patients (pts) with limited disease SCLC (mediastinoscopy obligatory) have been entered into an ongoing multimodality trial. Pts with stage I and II were treated with 4 cycles cisplatin (P) (50mg/m^2 d 1+7) and etoposide (E) (170 mg/m^2 d3,4,5) q d 22 followed by restaging and surgery. IIIa pts were treated with 3 cycles P (50 mg/m^2 d 1+7) and E (170 mg/m^2 d 3, 4,5) q d22 followed by one cycle simultaneous RTx/CTx (45Gy, 1,5 Gy twice daily within 3 weeks; P 50 mg/m^2 d 2+9 of RTx, E 100 mg/m^2 d 4,5,6 of RTx) followed by remediastinoscopy and operation. Stage IIIb pts were treated with 3 cycles P (50 mg/m^2 d 1+7) and E (170 mg/m^2 d 3,4,5) q d22 followed by one cycle simultaneous RTx/CTx (60 Gy, conventional fractionation, 2 Gy daily for 6 weeks); (P 50 mg/m^2 d 2+9 of RTx, E 100 mg/m^2 d 4,5,6 of RTx) without OP.

17 pts are currently „off treatment". THEIR CHARACTERISTICS: m/f 12/8; age 55(33-69); PS 1(0-1); Stage I 5, II 2, IIIa 9, IIIb 4. RESULTS after CTx±RTx:cCR 7; PR 10, CR/PR 17(85%), MR 2. TOXICITY(WHO): leucopenia 3° 25%, 4° 10%, infection 3° 10%, 4° 5%; thrombocytopenia 3°/4° 20%; diarrhea 3° 5%; one pt died in septic shock during the first CTx cycle; one pt experienced a cerebral infarction after the first CTx cycle. RESULTS of all 17 „off-treatment" pts: pCR 6(35%); R0/NED 5(29%), pCR/NED 11 (65%); overall resection rate 65%; treatment not completed (medical reasons) 2, irresectable after CTx/RTx 1; CTx/RTx only 3 pts. Median observation time 8 months(1-24)(mo). The calculated 75% survival rate is 12 mo(1+-24) for all pts and 14 mo(7+24+) for pts with resection. 4/11 resected pts have relapsed so far (4 CNS).

CONCLUSIONS: This intensive stage orientated multimodality program is tolerable and highly effective for LD SCLC. However, the observed high cerebral relapse rate still seems to be a major problem. Pt accrual continues and elective cranial irradiation is now integrated early in the treatment program. Whether the inclusion of surgery in such a multimodal treatment program contributes to these good results cannot be determined yet.

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HIGH EFFICACY OF AN INTENSIVE PREOPERATIVE CHEMO - RADIOTHERAPY FOR LOCALLY FAR ADVANCED (LAD) NSCLC.

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Since 3/91, 50 patients (pts) with LAD-NSCLC (mediastinoscopy obligatory) have been entered into an ongoing trial with 3 cycles cisplatin (P) (60 mg/m² d 1+7) and etoposide (E) (150 mg/m d² 3, 4, 5), q d 22 followed by one cycle simultaneous RTx/CTx (45 Gy,1,5 Gy twice daily within 3 weeks; P 50 mg/m² d 2+9 of RTx, E 100 mg/m² d 4,5,6 of RTx) followed by remediastinoscopy and operation.

42 pts are currently off treatment. THEIR CHARACTERISTICS: m/f 31/ 11 ;age 53(31-67);PS 1(0-1);IIIa(>2 mediastinal lymph node stations involved) 21, IIIb 21; squamous cell ca 21, adeno ca 14, large cell anaplastic ca 7.

RESULTS after CTx: cCR 3; PR 26, CR/PR 69% (95% conf.lim 55-83%), MR 9; NC 2; PD 2. TOXICITY (WHO): leucopenia 3° 24%, 4° 6%; infection 3° 6%, 4° 3%; thrombocytopenia 3° /4° 16%; diarrhea 3° 3%; no other severe toxicity. RESULTS after CTx/RTx: further significant response improvement in 5 pts; Toxicity (WHO): leucopenia 3° 53%; thrombocytopenia 3° 18%; esophagitis 3°/4° 32%. RESULTS after OP: (n=28) pCR: 12(43%); R0-resection/NED 12 (43%), pCR/NED 86%, R1-resection 2, R2-resection 2; 2 delayed postoperative deaths; 8/28 pts have relapsed so far (4 CNS, 1 liver, 1 supraclav.LN, 1 lymphangiosis carcinomatosa lung, 1 local). RESULTS of all 42 "off-treatment" pts: pCR 12(29%); R0/NED 12(29%), pCR/NED 24 (58%); overall resection rate 64%; treatment not completed (medical rea- sons) 3, PD during CTx +/- RTx 3; CTx/RTx refused 2; OP refused 2; irresectable after CTx/RTx 4; median observation time 9 months (4-27)(mo). median survival time of all pts 16 mo (4-27+), IIIa 21 mo (6-27+), IIIb 16 mo (6-24+), pts with resection: 16 mo (6-27+).

CONCLUSIONS: This intensive preoperative program is tolerable and highly effective for locally far advanced (LAD) NSCLC.Because of these results, further pts will be accrued in an extended phase II trial (IIIb, pancoast tu). A randomized trial with this program versus surgery + postop. RTx versus preop. CTx + surgery + postop. RTx in pts with stage IIIa (except pancoast tu) is in preparation.

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INFLUENCE OF CYCLOSPORIN A ON THE PHARMACOKINETICS AND PHARMACODYNAMICS OF DOXORUBICIN AND EPIRUBICIN

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Cyclosporin A (CYC) has been shown to modulate multidrug resistance (MDR) in tumor cells *in vitro*. However, in a phase I-trial with etoposide (VP16) the area under the concentration-time curve (AUC) was significantly increased by CYC with subsequent augmentation of myelosuppression and response. We have initiated a phase I-study with 80 mg/m^2 doxorubicin (DOX) or 120 mg/m^2 epirubicin (EPI) as 30min iv infusion in pts with soft tissue sarcomas, mesotheliomas, CUP and solid tumors refractory to anthracyclines either alone or after dose escalation of CYC (loading dose of 2, 4, 6, and 8 mg/kg/2h followed by 6, 12, 18, and 24 mg/kg/24h for 48h by continuous iv infusion, respectively). Up to now, the addition of CYC caused a significant increase in myelosuppression in nearly all pts and tumor regressions in 2/3 pts with DOX and 1/7 pts with EPI. Blood levels of CYC were in the range of 0.8, 1.5, 2.2, and 3.0 μM, respectively. During the first 48h after administration plasma AUCs as well as urinary excretion were not altered for DOX/EPI by CYC. However, both were maximally increased by ≥ 400 % for DOXol/EPIol and by about 300 % for the glucuronides of EPI and EPIol (-Glu), respectively. As with VP16, CYC may significantly contribute to the myelotoxicity of DOX and EPI by an inhibition of their biliary excretion. However, in contrast to VP16, DOX and EPI are more intensively metabolized to DOXol/EPIol and EPI-Glu/EPIol-Glu leading to a subsequent increase of these metabolites in plasma despite higher renal excretion. Thus, the amelioration of myelosuppression and tumor response caused by the addition of CYC cannot be solely attributed to a modulation of MDR but may be due to an effect on the pharmacokinetics and metabolism of both anthracyclines.

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IN SITU-QUANTITATION OF NUCLEAR AND CYTOPLASMIC ONCOPROTEINS: COMPARISON OF SEMIQUANTITATIVE - WITH COMPUTER-ASSISTED IMAGE - AND FACS ANALYSIS

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Activation of protooncogenes includes structural alterations of encoded proteins, but also mechanisms causing overexpression of structurally normal proteins. Therefore, quantitative analysis of oncoproteins is essential and may require *in situ* investigation, particularly when small numbers of tumor cells are present in a given tissue. We have recently suggested a semiquantitation system for nuclear oncogenes which is based on 4 semiquantitative categories defined by Computer-assisted Image Analysis (CAS) and supported by results of immunoblotting. We now intended (a) to determine the influence of detection methods of oncoproteins i.e. the various steps of immunocytochemistry as compared to indirect immunofluorescence, which is only driven by antigen/antibody interactions, (b) to assess the comparability and the influence of three quantitation systems (semiquantitation, CAS, FACS) and (c) to extend the analyses of the nuclear *c-Myc*- and *c-Fos*- to the cytoplasmic oncoproteins *c-Ras* and *c-Raf*. All tests were performed and analysed on resting- and PHA-activated T-cells. Our results demonstrate: (1) Indirect immunofluorescence and immunocytochemistry,though dependent on highly different variables, gave the same relative ranking in staining intensity of *c-Fos* abs (i.e. DCPI> 416>413*). This was also true for three different anti-*c-Myc* abs (152=157<155*). (2) Similar findings were obtained concerning cytoplasmic proteins, with the staining intensity of *c-Raf* being constantly below that of *c-Ras*(*). This held true independent from whether staining intensity was analysed by FACS or by CAS, where maximal cytoplasmic grey values were used as the sole initial parameter. This is equivalent to microscopical evaluation of "staining intensity". (3) Surprisingly, semiquantitative categories based on estimations of the percentage of positive nuclear areas (in case of nuclear oncogenes) and on maximal cytoplasmic staining in case of *c-Ras* and *c-Raf* were most strongly correlated with peak grey values measured by CAS (p<.0001 and <.0002, respectively). In contrast, estimations of the amounts of cytoplasmic proteins by the formula {(mean grey value x (total cell - nuclear area)} did not correlate with semiquantitation categories at the same discriminative level. (*). We conclude, that immunocytochemistry and indirect immunofluorescence yield comparable and reliable results in defining oncoprotein expression. In addition, a simplified CAS and microscopical semiquantitation can be used for reliable comparison of oncoprotein rankings. Staining and evaluation techniques can be applied in cells with highly different nucleus/plasma ratios. * Significant at the 95% confidence level.

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NEOADJUVANT CHEMO-/RADIOTHERAPY IN LOCALLY ADVANCED NON-SMALL CELL LUNG CANCER

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Purpose: This phase II trial was designed to evaluate the feasibility, toxicity and response rates for neoadjuvant chemotherapy and radiotherapy followed by surgical resection in newly diagnosed patients with surgically staged III NSCLC (N2/3).
Patients and Methods: Neoadjuvant treatment consisted of two cycles of polychemotherapy (ICE) followed by concurrent chemotherapy and radiotherapy. Chemotherapy included Ifosfamid 1.500 mg/m² i.v. d. 1, 3, 5; Mesna 400 mg/m², time 0, 4, 8 h i.v., d. 1, 3, 5; Etoposid i.v. 100 mg/m² d. 1, 3, 5 and G-CSF 5 µg/ kg/KG. Concurrent chemo-/radiotherapy included Carboplatin 100 mg/m² d. 1, 8, 15 i.v.; Vindesin 3 mg d. 1, 8, 15 i.v. plus simultaneous RTX 45 Gy. within three weeks (1,5 Gy 2 x daily, 5 times a week). Since 04/92 21 patients were included. Patients' characteristics: Male/female 19/2, age 60 years (45 - 68); squamous cell Ca. 12, adeno-Ca. 6, large cell Ca. 3.
Results after CTx/RTx: too early 6; CR 3, PR 7, NC 2, PD 3. 1 patient with CR after CTx/RTx refused further treatment.
Results after surgery: (n=9): R0-resection 8 (pCR 3, microscopic disease 4), R1-resection 1. 1 treatment related death (pop pneumonia) was observed.
Conclusion: This multimodal treatment is feasible and effective for locally advanced NSCLC.

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SUCCESSFUL CONTROL OF STEROID-RESISTENT ACUTE GVHD AFTER ALLOGENEIC BMT FROM AN UNRELATED DONOR BY AN ANTI-CD3 MONOCLONAL ANTIBODY.

H.Einsele, G.Ehninger, H.Schmidt, B.Berner, M.Reuss-Borst, H.D.Waller, C.A.Müller

Acute graft-versus host reaction (GvHD) is one of the major complications of allogeneic bone marrow transplantation (BMT), especially after grafting from an unrelated or HLA-mismatched family donor. It occurs in 30-50% of all the patients transplanted from HLA-identical sibling donors and in 50-80% of all the patients transplanted from an HLA-matched unrelated or HLA-mismatched family donor despite the GvHD-prophylaxis with methotrexate (MTX) and ciclosporin (CSA). Therapeutic strategies in the treatment of acute GvHD include the application of corticosteroids and polyclonal anti-T cell globulin (ATG).
Here we report our experience with OKT3 treatment in patients with severe acute GvHd following allogeneic BMT from an unrelated donor. 8 patients who underwent allogeneic bone marrow transplantation from an HLA-matched unrelated donor developed severe acute GvHD grade II-IV on day 10-28 after BMT in spite of prophylaxis with methotrexate and CSA. Clinical manifestations in these patients did not respond to prednisolon in a dosage of 5 mg/kg body weight administered in 3 doses per day. When clinically no response could be demonstrated after 3 days of corticosteroid therapy in a dosage of 5 mg/kg body weight the patients received OKT3 monoclonal antibody as a 14-day course of 5 mg/day as a bolus injection while maintaining first line immunosuppression with CSA and corticosteroids. In all these patients acute GvHD markedly improved. Only in one of these patients chills, fever and tachycardia following the first injection of OKT3 was observed. Only mild forms of a microangiopathy were observed in these patients. A marked decrease of the CD3+-T-cells and a significant increase in the number of CD56+-T-cells could be demonstrated by immunophenotyping of the peripheral blood mononuclear cells in these patients. The T cells recovered 21 - 28 days after cessation of OKT3-treatment. Further data on the immune reconstitution in these patients will be presented at the meeting.

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PCR-BASED PRE-EMPTIVE THERAPY TO REDUCE THE MORBIDITY AND MORTALITY OF CMV DISEASE AFTER ALLOGENEIC BMT

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Even new strategies in the treatment of CMV disease, especially of CMV-induced interstitial pneumonia have failed to significantly improve the outcome CMV disease after allogeneic BMT. In a recent European survey, only 31% of the patients with CMV-induced interstitial pneumonia survived the first 3 months after allogeneic BMT. Thus, in 2 studies performed in the USA, antiviral treatment was introduced at the earliest positive finding of CMV infection in a marrow transplant recipient. The positive demonstration of the virus was based on culture technique. These studies showed a significant reduction in the patients who received this so-called pre-emptive therapy in comparison to the patients who received antiviral therapy only when symptoms of CMV disease were present. But a minority of patients developed CMV disease in spite of the fact that the recived culture-based pre-emptive therapy or in spite of being culture-negative prior to the onset of CMV disease. In these patients culture technique obviously detected the virus too late for early therapeutic intervention. Thus, here a PCR-based pre-emptive therapy was compared to a pre-emptive therapy based on culture technique to analyse whether earlier detection of CMV infection and thus earlier pre-emptive therapy might help to reduce the incidence and mortality of CMV disease. 54 patients (27 in each group) were treated with this pre-emptive therapy. A significvant reduction of CMV disease could be shown for the patients who received antiviral therapy based on PCR technique.

No increase in mortality due to bacterial and fungal infections following earlier and more frequent courses of antiviral therapy were found among the patients receiving PCR-based pre-emptive therapy compared to the ones treated after a positive culture from any site.

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VIRUS AND HOST FACTORS INFLUENCE THE OUTCOME OF CMV INFECTION FOLLOWING ALLOGENEIC BONE MARROW TRANSPLANTATION

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In spite of new developments in the diagnosis and therapy of CMV infection CMV disease, especially CMV pneumonia is still affected by a high mortality in patients who have undergone allogeneic bone marrow transplantation. But only a minority of patients with CMV infection after BMT develop CMV disease. Different detection methods were assessed for their positive and negative predictive values for the development of CMV disease in patients with CMV infection after BMT.

PCR technique was found to provide an optimal sensitivity and negative predictive value, but the positive predictive value for the onset of CMV disease was only 61%. Unfortunately positive culture from urine samples or throat washings (64%) as well as from blood samples (69%) did not provide significantly higher positive predictive values.

Thus, other parameters were looked for which might influence the onset of CMV disease in a patient with culture-proven CMV infection. The immune reconstitution was found to have a major impact on the development of CMV disease in patients after allogeneic BMT. A significant drop in the lymphocyte count, especially in the CD4+ T-cell count, was found to be significantly associated with the onset of CMV disease. Additionally the cytokine network in these patients seems to be dramatically altered, including serum levels of IL-6.

Apart from host-derived also virus-derived factors were found to influence the virus-host interaction. By amplifying specific functionally relevant regions of the CMV genome, the association of various virus strains and the clinical course of the virus infection were analysed. An association of mutations in the major transactivationg domain of the immediate early gene and the develpment of CMV disease could be demonstrated.

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MAGNETIC RESONANCE IMAGING REVEALS DELAYED HAEMATOLOGICAL RECONSTITUTION AFTER AUTOLOGOUS BMT AND TRANSPLANTATION FROM AN UNRELATED DONOR

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Magnetic resonance has become a powerful tool for diagnostic imaging of soft tissue. Red bone marrow of healthy persons has considerable contents of water and lipids. The cellularity and the corresponding fat-water ratio within the marrow show clear changes in hematological diseases and following cytotoxic therapy and thus may be used to evaluate haematological reconstitution in patients after bone marrow transplantation. Magnetic resonance (MR) methods use the signals of the protons of water and lipids. Here different standard magnetic resonance techniques and recently developed water- and fat-selective imaging methods were used to analyse the bone marrow changes that occur following autologous bone marrow transplantation. Additionally volume-specific magnetic resonance spectroscopy was applied to evaluate the fat-water ratio and additional qualities of water and lipid protons.

Patients were followed up after BMT by NMR chemical shift imaging and 1H localized spectroscopy and were seen after discharge from the marrow transplant unit and for further controls in the later post-transplant period. The peripheral heamatological parameters, in some patients marrow cellularity and the NMR data were compared in these patients.

In spite of the rapid increase in the white blood cell count, in contrast to the rapid normalization fo the lipid/water ratio in recipients of an allogeneic transplant, patients after autologous marrow transplantation showed a marked increase in the lipid/water ratio indicating a low marrow cellularity in the early phase after autologous BMT.

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PROGNOSTIC RELEVANCE OF IMMUNOLOGICAL MARKERS IN B-CLL AND IMMUNOCYTOMA

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Using cryostat sections and an immunoperoxidase technique bone marrow biopsies from 160 patients with B-cell chronic lymphocytic leukemia (B-CLL) and 89 patients with generalized immunocytoma (LP-IC) were analysed to study the prognostic value of immunophenotype (CD5, CD6, CD23, DRC-1, Ki-67) in comparison with well established clinical parameters. In B-CLL patients Rai stage, Binet stage, presence of thrombocytopenia and anaemia, pattern of bone marrow infiltration and age were significantly asssociated with survival, whereas immunological markers did not discriminate subgroups with different clinical outcome. Similarly, in LP-IC patients Rai stage, Binet stage, anaemia, extent of bone marrow involvement and age correlated with survival. Additionally immunohistological markers displayed significant prognostic influence on survival. So, the presence of follicular dendritic cells detected by the monoclonal antibody DRC-1 correlated with longer survival (p=0.0367, univariate analysis). Positivity for DRC-1 proved its prognostic relevance also in multivariate analysis (Cox regression model, p=0.0370). Furthermore CD6 antigen expression was associated with benign clinical course (p=0.048 in multivariate analysis).

Including all patients studied (n=249) the variables age, Rai stage, extent of bone marrow involvement, Ki-67, but not histological subtype revealed significance in Cox regression analysis.

In summary, our results verify the diagnostic and prognostic value of established morphological and clinical parameters for B-CLL and LP-IC. Nevertheless, immunological markers (DRC-1, CD6, Ki-67) show significant association with survival and may help to identify patients with indolent or aggressive clinical course.

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Altered Adhesion To Fibronectin In A K562 Cell Line And A Variant U937 Cell Line By Low Dose Ionizing Radiation

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Low dose ionizing radiation induces protein kinase C (PKC), which is known to increase the receptors for extracellular matrix (ECM) proteins like fibronectin (FN). The effect of low dose ionizing radiation on the adhesion to FN as one of the major ECM proteins in the human haematopoetic cell line K562 and a variant promonocytic cell line U937V was studied. Gamma irradiation with 0.5 Gy and 1 Gy was applied and adherence of cells to FN coated and uncoated surfaces was examined 2, 24, and 96 hours postirradiation. Here we provided strong evidence that the binding of K562 and U937V cells to FN coated surfaces is transiently altered by ionizing radiation. 2 hours postirradiation the binding capacity to FN increased up to 2.5 times in K562 cells and up to 4 times in U937V cells. This upregulation in the binding to FN was followed by a significant decrease of adherence in both cell lines. 96 hours postirradiation the extent of cell adhesion reverted to control levels. Furthermore, the influence of Interferon (IFN) alpha, IFN-gamma, PMA as a PKC-activator and Staurosporin as a PKC-inhibitor on adhesion of cells to FN which were exposed to irradiation was examined.

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20-EPI-VITAMIN D3 ANALOG: AN EXTRAORDINARILY POTENT INHIBITOR OF LEUKEMIC CELL GROWTH IN VITRO

Elena Elstner, Y. Y. Lee, H. K. Koeffler

Acute myelogenous leukemia (AML) arises from neoplastic transformation of a myeloid stem cell, leading to a block in cellular maturation at an early stage of development. High-dose chemotherapy has improved survival of AML patients but normal hematopoiesis is dangerously depressed by this treatment. An ideal alternative therapy for these patients is to induce differentiation and/or inhibit clonal proliferation of their leukemic cells without toxic effects on their normal hematopoietic stem cells. 1,25 dihydroxyvitamin D3 (vitm D3) has some of these qualities, but causes hypercalcemia. We have analyzed a variety of vitm D3 analogs. The 20-epi-1,25(OH)$_2$D3 (code name IE) showed extraordinary activity with 87% inhibition of clonal growth of HL-60 and 50% inhibition of fresh myeloid leukemic clonogenic cells (CFU-L) from peripheral blood of patients with AML at 10^{-11} M. Effect of compound IE on induction of differentiation of HL-60 and AML blast cells as measured by generation of superoxide and nonspecific esterase production paralleled its antiproliferative activities. In contrast, this analog stimulated CFU-GM growth from normal bone marrow as well as from long-term bone marrow cultures.
In order to gain insights into the remarkable antileukemic activities of compound IE, we examined its ability to enter HL-60 cells, bind to vitm D3 receptors, and interact with a transfected vitm D3 response element (VDRE) attached upstream of a TK promoter-driven receptor gene [chloramphenicol acetyl transferase (CAT)]. The IE compound potently increased CAT activity (>30 fold as compared to receptor with no VDRE), but paradoxically it was of equal potency to vitm D3, even though compound IE had 1000-fold greater antileukemic effect as compared to vitm D3. In summary, we have identified an extremely potent vitm D3 analog IE, which may be clinically useful. Further studies are required to elucidate the mechanism by which this analog produces its prominent activity.

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RISK EVALUATION AND THERAPY STRATEGIES IN HIGH-GRADE MALIGNANT NON-HODGKIN LYMPHOMAS

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In the treatment of advanced high-grade malignant non-Hodgkin lymphomas (NHL), the development of efficient polychemotherapy regimens has led to complete response rates of 50 to 80 % but at least one third of these patients have to be expected to relapse and finally to succumb to progressive disease. The modification of dosages and schedules and moderate increases in dose-intensity as examplified in "third generation" regimens have not substantially improved these overall results. In the evaluation of treatment data it has become evident that the prognosis of the individual patient is critically influenced by a series of initial parameters assessing the patient's status at diagnosis, all measures of tumor extension and indicators of sys-temic activity. In an international meta-analysis a prognostic index was derived from relevant para-meters and applied to a recently completed compara-tive treatment trial. Thus, prognostic risk seems to be more important for outcome than the choice of conventional treatment regimen. Risk stratification implies avoidance of unacceptable toxicity for low-risk and novel approaches for high-risk patients. Treatment options for the latter include variants of dose-intensified regimens aided by hematopoietic growth factors and/or progenitor cell support. Ultimately, however, the search for entirely new therapeutic principles seems to be mandatory.

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NEW IMMUNOTHERAPEUTIC STRATEGIES IN HODGKIN´ S DISEASE

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Monoclonal antibody (MoAb) technology has supplied oncologists with a limitless supply of tumor-selective reagents. A large number of MoAbs have been prepared particularely for hematopoietic malignancies, many of which were linked to toxins like the ribosome damaging A-chain of ricin or others like abrin, saporin, pseudomonas-exotoxin, or diphteriatoxin to form immunotoxins which combine the selectivity of the antibody moiety with the potency of the toxin. Hodgkin´s disease is an ideal candidate for immunotherapy since the putative malignant Hodgkin/Reed-Sternberg (H-RS) cells express high amounts of target antigens like CD25 and CD30 which are present only on a small minority of normal cells. Clinical trials using an anti-CD25-ricin A-chain immunotoxin or an anti-CD30 Saporin-6 immunotoxin are ongoing in patients with resistant Hodgkin´s disease. Other immunotherapeutic strategies involve the use of Interleukin-4 (IL-4) which is currently being tested in a phase I/II trial in Hodgkin´s patients or the possible use of anti-idiotypic antibodies for active immunotherapy.

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PHASE-II STUDY OF IDARUBICIN, IFOSFAMIDE, AND VP-16 (IIVP-16) IN PATIENTS WITH RELAPSED AGGRESSIVE NON-HODGKIN'S LYMPHOMA

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The prognosis of patients with relapsed agressive Non-Hodgkin's lymphoma (NHL) is usually poor. Comple remission rates of second-line therapy range between 24 and 40%. In an ongoing non-randomized phase-II study, we evaluate the feasibility of a combination of Ifosfamide, VP-16 and Idarubicin, a new anthracycline which has been demonstrated to be effective against NHL. The IIVP-16 protocol consists of 1000mg/m^2 Ifosfamide i.v. (day 1-5), 10mg/m^2 Idarubicin i.v. (day 1+8), and 150 mg/m^2 VP-16 i.v. (day 1-3). So far (4/93), 18 patients with refractory or relapsed aggressive NHL at a medium age of 55,5 years (range 31-73) have been enrolled. Fourteen of the 18 patients had two or more different prior treatments. Serum levels of Idarubicin and the main metabolite with anti-tumor activity, Idarubicinol, were measured. Toxicity analysis of a total of 64 cycles revealed that the major toxicities were WHO grade-4 leukocytopenia in 45% and thrombocytopenia in 16%. WHO grade-3 toxicities included alopecia (49%), anemia (18%), infection (3%), and fever (2%). We conclude that IIVP-16 can be safely administered in heavily pretreated patients with NHL.

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PATHOBIOLOGY OF MALIGNANT LYMPHOMAS

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The biology of interaction between different cellular compartments is in part regulated by cytokines as potent modulators of the physiological immune response. A number of reactive processes are associated with a deregulation of this cytokine expression. Animal models with transgenic mice or transfection experiments have shown that an autocrine or paracrine pathway might be involved in tumor progression and/or tumorigenesis.

The study of cytokine expression in malignant lymphomas revealed a heterogenous expression pattern with a number of cases which quantitatively show indications for a marked deregulation of the cytokine expression. The expression of IL-7 and IL-9 as well as of IL-9R seems to be a special feature of Hodgkin's disease and large cell anaplastic lymphomas. Transfection experiments with IL-9 into mouse T-cells result in an autocrine loop and tumorigenicity of the transfected cells. These tumors share a high number of similarities to Hodgkin's disease and large cell anaplastic lymphomas in humans including the CD30 expression in the mouse tumors.

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CLINICAL RESULTS AND IMMUNOSUPPRESSIVE EFFECTS OF FLUDARABINE PHOSPHATE IN PRETREATED ADVANCED CHRONIC LYMPHOCYTIC LEUKEMIA - A PHASE II TRIAL.

K. Fenchel [1], L. Bergmann [1], A. Engert [2], PS.Mitrou [1], V. Diehl [2], D.Hoelzer [1].

Fludarabine phosphate (FAMP) has been shown to be effective in pretreated chronic lymphocytic leukemia (CLL) and to induce even complete remissions (CR). Here we report treatment results in 34 patients (pts.) with advanced and resistant CLL, 32 with B-CLL, 2 with T-CLL. FAMP was administered at a dosage of 25 mg/m^2 as a bolus infusion daily for 5 days and repeated every four weeks. Dosage and time course were adapted according to toxicity. After 3 and 6 cycles reevaluation was performed. 157 cycles of FAMP were administered. 1 of 33 (3%) evaluable pts. achieved complete remission, 19 of 33 pts. (58%) achieved partial remission, 7 of 33 (21%) had stable disease, and 6 of 33 (18%) showed progressive disease (PD). In most cases, PR was achieved within 2 cycles of FAMP. The duration of partial remission was in median 6 months, with a range of 2-12+ months. 2 of the patients in PR relapsed after 2 and 7 months, 8 patients in PR died due to infection. Major toxic effects included infections in 14 patients of WHO-grade 3 and 4 and nausea in 6 patients of WHO-grade 1. Among the severe infections, germs like pneumocystis carinii and aspergillus fumigatus could be observed. In one case a tumor lysis syndrome was observed. The development of pulmonary, even opportunistic infections can possibly be explained by FAMP-induced reduction of CD 4+ positive cells down to minimal counts of 17 cells/yl, namely in patients achieving PR or CR. In conclusion, fludarabine is highly effective in patients with advanced CLL, but severe opportunistic infections due to CD4 reduction requires antibiotic prophylaxis.

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PROGNOSTIC VALUE OF SIMULTANEOUS EXPRESSION OF CD7 AND CD33 ON LEUKEMIC BLASTS IN AML

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Therapeutic regimens in the treatment of acute myelocytic leukemia (AML) have been intensified in the last decade resulting in improved induction of complete remissions. However, we still look for clinically useful prognostic criteria, which would predict response to therapy . This may be helpful to choose the most appropriate therapy for an individual patient. A panel of 32 monoclonal antibodies reactive with normal lymphoid and myeloid cells at various stages of differentiation were used to characterize leukemic blasts of patients (pts.) with acute myelocytic leukemia (AML) to evaluate the prognostic relevance of phenotypical parameters. Mononuclear cells were recovered from heparinized bone marrow aspirated at diagnosis. After density gradient sedimentation using Ficoll-Hypaque, the cells were stained by double color direct immunofluorescence (PE-or FITC-labeled) with a series of monoclonal antibodies and studied immediately. Up to now, 63 adult pts. with AML, 35 of them with de novo AML, 14 had a history of an antecedent haematolgic disorder (AHD), and 14 pts. were in first relapse of AML (FR). Diagnosis war based on Wright-Giemsa-stained bone marrow smears and cytochemistry, according to the French-American-British (FAB) Group criteria. The achievement of complete remission (CR) was significantly correlated with a high (>50% of cells) coexpression of CD7/CD33. All pts. with de novo AML and high expression of CD7/CD33 achieved CR, so far ($p<0,05$, according to the Fisher's exact test). High expression of CD7 alone was correlated with the achievement of CR with a sifnificance of $p<0,02$. A marginal significance for predicting response to therapy was observed for the high coexpression of CD15/CD33. In contrast to recent reports, we found no correlation between CD34 surface expression and the prognosis of the disease.

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METHYLPENTYLAMINOPROPYLIDENEBISPHOSPHONATE (BM 21.0955), A NEW BISPHOSPHONATE IN THE MANAGEMENT OF TUMOR OSTEOLYSIS

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We investigated safety and efficacy of 1-hydroxy-3-(methylpentylamino)propylidenebisphosphonate (BM 21.0955), which seems to possess 50-/500-fold activity in animals compared to pamidronate and clodronate, respectively. As part of a multicenter randomized double-blind phase II trial 10 normocalcemic patients with breast cancer metastatic to bone were treated in our department with BM 21.0955 orally (capsulae) with three different dosages (10 mg, n=4; 20 mg, n=3; 50 mg, n=3 daily) over a period of 4 weeks. The bone resorption index (BRI) decreased significantly from 0,31 ± 0,15 to 0,19 ± 0,13 (mean ± SD) after two weeks and did not raise the following weeks. Urinary excretion of pyridinium cross links, which are more specific for bone resorption than hydroxyproline, showed a marked decrease. 3 of 10 patients experienced an episode of gastrointestinal toxicity, 2 of these being diagnosed as eosophagitis (WHO grade II), requiring discontinuation of study medication and symptomatic therapy. Overall BM 21.0955 was effective orally in reducing the bone resorption, as measured by BRI and pyridinium cross links. A trend to better response with 50 mg was observed. Additionally, in multicenter phase I/II trials, 10 patients (myeloma, n=8; squamous cell carcinoma of head and neck, n=2) with 13 episodes of malignancy-associated hypercalcemia were treated in our department with a single infusion of BM 21.0955 (doses between 1,1 and 2,0 mg). Serum calcium corrected for albumine was lowered by rehydration with saline over at least 24 hours to a mean of 3,34 ± 0,49 mmol/L. After administration of BM 21.0955 (mean dose 1,6 mg) 8 of 9 evaluable patients became normocalcemic (serum calcium: 2,41 ± 0,47 ; 2,37 ± 0,29; 4 and 6 days after BM 21.0955, respectively). One patient with excessive osteolysis as judged by the BRI required a second treatment with 2,0 mg to reach normocalcemia. There was no difference of response between myeloma patients and patients with epithelial neoplasia. The highly significant fall of serum calcium was associated by a marked decrease in serum phosphate, BRI, and pyridinium cross links. The response was better in the patients treated with higher doses (2 mg). We conclude that BM 21.0955 is an effective agent for reducing bone resorption and treatment of hypercalcemia. Better tolerated oral BM 21.0955 formulations are needed. Meanwhile, film coated tablets have been developed and are subject of ongoing clinical trials.

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DTIC PLUS 5-FU/FOLINIC ACID (5-FU/FA) AS SECOND LINE TREATMENT AFTER 5-FU/FA FOR METASTATIC OR LOCALLY ADVANCED COLORECTAL CANCER

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21 patients with locally advanced or metastatic colorectal carcinoma, who were progressive to treatment with 5-FU/FA, received additional DTIC. The monthly application consisted of 5-FU (750 mg day 1-5 continous infusion),folinic acid (4x50 mg d 1-5) and DTIC (100-200 mg/sqm d 1-3).

Patients characteristics: m/f=13/8, median age 55y (41-74), adenocarcinoma of the colon (13) and of the rectum (8), locally advanced (2) and metastatic tumors (19), median Karnofsky Index 70%(100-40).

Toxicity: Under antiemetic protection of ondansetron the treatment was well tolerated in most of the patients. 1 pt received only 1 course because of nausea WHO III, 1 pt because of nausea WHO III and diarrhea. Hematologic toxicity > grade II did not occur. In 1 pt treatment was discontinued during the first course because of ileus due to diffuse peritoneal metastases.

Results: 20 pts were eligible for response. 40 % (8/20) pts could achieve MR and SD. CR and PR 0 pt,MR 3 pts (15%),SD 5 pts (25%),PD 12 pts (60%).
The median progression free intervall was 5 months (range 1-8 months). Median survival time for DTIC -non-responders was 8 months, for the responders it is not yet reached (16+ months). 3 of 8 patients, who failed primary treatment with 5-FU/FA responded to the combination with DTIC. There was a trend to better outcome for the patients treated with a higher dosage of DTIC, but statistically the difference was not significant.

Conclusions: Addition of DTIC to 5-FU/FA seems to be effective in patients with advanced colorectal cancer progressive to 5-FU/FA. Toxicity is acceptable at the dosage used. The further role of DTIC in the treatment of colorectal cancer is still to be determined.

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GM-CSF SECRETION IS INDUCED BY TNF-α IN LEUKEMIC CELL LINES U937 AND KG-1a

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Myeloblasts of some patients with AML constitutively secrete GM-CSF. Recently it has been shown that IL-1 and TNF-α can induce GM-CSF production in fresh leukemic cells of patients who do not release GM-CSF spontaneously. To further characterize this phenomenon we investigated GM-CSF induction by TNF-α in two leukemic cell lines, U 937 and KG-1a. KG-1a constitutively expresses GM-CSF m-RNA as demonstrated by Northern blots and PCR analysis. Unstimulated U 937 cells contained no detectable GM-CSF transcripts. After incubation with TNF-α, GM-CSF specific m-RNA was found in U 937 cells. Only slight increases of GM-CSF transcripts were noted in KG-1a cells after TNF-α treatment. In unstimulated cultures, GM-CSF concentrations were below 1 pg/ml. After 3 days of culture detectable levels of GM-CSF were found after stimulation with 1 ng/ml TNF-α and reached a mean of 11.9 pg/ml for U937 and 59.3 pg/ml for KG-1a after incubation in 50 ng/ml TNF-α. Therefore mechanisms of GM-CSF expression are regulated differently in both cell lines.

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STUDIES ON BCL-2 PROTEIN IN CULTURED LYMPHOID CELLS

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BCL-2 protein was analysed in cell cultures derived from non-Hodgkin Lymphomas and Chronic Lymphocytic Leukaemia patients. Cytospin preparations of separated fresh and cultured cells were prepared in all cases incl.control blood donors. The presence of bcl-2 gene encoding oncoprotein has been demonstrated using monoclonal antibody to the BCL-2 molecule /DAKO/ and immunocytochemical staining with biotinylated rabbit anti mouse immonoglobulin followed by streptavidin-HRP conjugate. We investigated the correlation between the expression of the BCL-2 protein and the appearance of proliferation- or differentiation related features in defined culture conditions. The reported BCL-2 up regulation in malignant lymphoid cells was observed in haemo- and lymphopoetic progenitor cells as well as in PHA/TPA activated peripheral blood lymphocytes. The outcome of these studies enclosed in the final discussion was to understand possible mechanisms whereby the inappriopriate expression of the BCL-2 protein plays a central role in the pathogenesis of lymphoid malignancy.

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MONOALLELIC EXPRESSION OF *BCR* PROVIDES FURTHER EVIDENCE THAT THE GENE IS IMPRINTED

Monika Fink and Oskar A. Haas

Based on chromosome banding polymorphisms our group recently showed that in Philadelphia-chromosome (Ph)-positive leukemias the translocated chromosome 9 is generally of paternal and the translocated chromosome 22 of maternal origin (Haas et al., Nature 359:414-416,1992). This parental of origin bias of the involved chromosomes suggests that the genes affected by the translocation,*BCR* and *ABL*, might be imprinted. However, although these two genes are expressed in all normal tissues and leukemic cell populations, it is not yet known whether the paternal, maternal or both allels are transcribed. With the recent detection of a polymorphic trinucleotid sequence in the transcribed part of the *BCR*-gene the distinction between the two alleles became possible (Riggins et al., Nature Genetics 2:186-191, 1992). We therefore have analysed DNA and RNA obtained from peripheral blood, isolated granulocytes and EBV-transformed B-cell lines of ten normal individuals and have found that in all instances only one allel of the *BCR* gene is transcribed. Thus, this privileged monoallelic expression strongly indicates that the *BCR* gene is imprinted. To elucidate the parental origin of the transcribed allel we are currently performing pedigree studies in normal families as well in patients with Ph-chromosome positive leukemias and their parents.

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NEOADJUVANT CHEMOTHERAPY IN LOCALLY ADVANCED GASTRIC CANCER WITH ETOPOSIDE, ADRIAMYCIN AND CISPLATINUM FOLLOWED BY TOTAL GASTRECTOMY.

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Despite the decreasing incidence gastric carcinoma is still a devastating disease with the worldwide second highest deathrate of cancer patients. At the time of diagnosis nearly 50% of patients with gastric cancer present with an advanced stage (UICC IIIB and IV). The generally poor prognosis in this group of patients can only be significantly improved, if a complete resection (R0-Resection, UICC 1987) is performed.
To increase the number of R0-resections we performed a phase II trial using *neoadjuvant chemotherapy* with etoposide, adriamycin and cisplatinum (EAP). Pretherapeutic staging included endoscopy with biopsy, *endoscopic ultrasound*, cat-scan, chest x-ray and percutaneous ultrasound to evaluate TNM-Stage and surgical laparoscopy including preparation ot the lesser sac to rule out peritoneal carcinosis *Gastrectomy with extended lymphadenectomy* concluded the therapy.
30 patients (22m, 8F; med. age 51.8 years; clinical stage (AJCC 1987); IIIA:8, IIIB:12, IV:10) were evaluable for response, toxicity and survival after an average of 3 cycles Ctx (1-4 cycles). Toxicity (WHO grade) included: leucopenia 3° (40%), 4° (16,6%), thrombocytopenia 3° (20%), 4° (26,6%); nausea and emesis 3° (33%); alopecia 3° (100%).
RESULTS AFTER EAP AND SURGERY: Complete resection (total gastrectomy and radical lymphadenectomy) 24/27 (88,9%), incomplete resection 3/27 (11,1%). Morbidity was not increased and no mortality was observed after surgery. Median follow up was 24 months (4-42 months). 13 patients are alive with NED, 16 patients died. Survival for all patients was 16 months and 23 months after complete resection.
CONCLUSION: These data indicate that neoadjuvant chemotherapy is feasible and effective in patients with locally advanced gastric cancer, if complete resection is achieved. However, in patients with locally advanced stages (i.e. T2N2, T3N0, T3N1, T3N2, T4N0), a randomized trial that compares primary surgery with neoadjuvant chemotherapy followed by surgery is warranted.

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ROLE OF CHEMOTHERAPY IN PRIMARY GASTRIC CARCINOMA.

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The prognosis of patients with gastric carcinoma is dismal. This is because 50-60% of patients with gastric carcinoma present with locally advanced tumors and a complete tumor resection, i.e. a R0-resection (UICC/AJCC 1987), is possible in only 40-50% of these patients. The current treatment options to improve survival in patients with locally advanced gastric carcinoma are:

- multivisceral resections and extended lymphadenectomy,
- palliative resections (i.e. R1/R2-resections) followed by additive chemotherapy and/or radiation,
- adjuvant postoperative chemotherapy in high risk patients after complete tumor resection, and
- preoperative neoadjuvant chemotherapy in patients with locally advanced tumors (i.e. T3/T4 N+ M0 tumors).

At the present time aggressive surgery can increase the rate of R0-resections and improve long term survival in only a small group of patients. Cytoreductive surgery does not increase the efficacy of postoperative additive chemotherapy. Adjuvant chemotherapy has failed to show a clear survival benefit and cannot be recommended outside of clinical trials. The role of postoperative chemotherapy in patients with locally advanced gastric carcinoma is unsettled.
In patients with irresectable disease (proven by explorative laparotomy or by clinical staging including endoscopic ultrasonography and surgical laparoscopy) preoperative chemotherapy allows a R0-resection in about 50% of patients with a median survival of 24 months. In patients with disseminated peritoneal carcinosis or linitis plastic preoperative chemotherapy is ineffective. In patients with locally advanced but resectable tumors (i.e. stage IIIa) a beneficial effect of preoperative chemotherapy over primary surgery has to be proven by prospective randomized trials.

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Cytology Trainer
An Interactive Computer Based Tutorial

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Senior haematologists train junior staff using a multi-view microscope. Frequently, senior staff capacities and technical facilities are less than demand. Furthermore, systematical teaching sessions are the exception rather than the rule. Therefor we have developed a computer based learning tutorial guiding the user from simple features (i.e. setting a microscope) to more complex ones (making a ctology report). The program is targeted at lab technicians, medical students, junior staff and physicians preparing for specialty licenses. The program consists of 5 sections: 1. A tutorial demonstrating how to prepare a cytology slide, how to identify and categorize physiological and pathological blood cells (cell "tree") and how to systematically develop a complete cytrology report. 2. A guided tour navigates the user through structured analysis of 40 specimens including quantiative assessment of WBCC. 3. Non-guided routine-like development of a cytology report from scratch including description of findings and establishment of final diagnosis. 4. An image archive with some 1000 indexed hematology cytology specimens including short description of the findings. Any two specimens can be displayed simultaneously on the screen. 5. Referenced glossary with indexed standard literature.
The program was written for Apple Macintosh and IBM-compatibles with 256 colours (8 bit). An authoring system was used (SuperCard®) in combination with an image database. Images were taken from Kodak Photo-CD® based cytology sliede archives. The final application runs from a CD-ROM.
This application is the first of series of modules. The algorithms developed in this version are used to develop a generic shell application for teaching and reference in other cytology and histolgy compartments (bone marrow, spinal fluid etc.).

Keywords: haematology, cytology report, Kodak-Photo CD, Apple Macintosh, IBM-compatible, CAI (computer aided intstruction, software, blood slides, image database)

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COMPARISON OF THIRD GENERATION PROTOCOLS WITH STANDARD CHOP: RESULTS OF THE NATIONAL HIGH PRIORITY LYMPHOMA STUDY

R.I. Fisher, E.R. Gaynor, and S. Dahlberg.

In order to make a valid comparison between first and third generation regimens, the Southwest Oncology Group and the Eastern Cooperative Oncology Group initiated a randomized Phase III comparison of CHOP vs. m-BACOD vs. ProMACE-CytaBOM vs. MACOP-B. Each treatment arm contained between 218 and 233 eligible patients. Known prognostic factors were equally distributed. The initial results of this study were recently published (N.Engl.J.Med. 1993;328:1002-6). There were no significant differences in either the partial or complete response rates between treatment arms. After a median follow-up of 49 months and a maximum follow-up of 84 months, there is still no difference in time to treatment failure (p = .40) or overall survival (p = .68). No subset of patients was found to have significantly improved survival with the third generation regimens. The received dose intensity data was comparable to the data previously published data for these regimens. Fatal toxicity was 1% for CHOP, 3% for ProMACE-CytaBOM, 5% for m-BACOD, and 6% for MACOP-B. Based on similar failure-free and overall survival with lower cost and lower severe toxicity, CHOP remains the standard chemotherapy for patients with advanced stage, intermediate or high grade non-Hodgkin's lymphoma. New treatment strategies need to be developed to improve the prognosis of these patients.

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Mutations of the p53 tumor suppressor gene and the mdm-2 gene are rare in human testicular tumors

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The p53 tumor suppressor gene is a nuclear phosphoprotein that appears to be a transcriptional activator. The protein appears to suppress the activity of a variety of promoters. Mutations of the gene result in a protein with altered or lost suppressor activity. The mdm-2 gene is a gene which was recently described to be functional in the regulation of p53 activity. Mutations of the p53 gene are perhaps the most common genetic alterations in a variety of cancers. We analyzed a large cohort of testicular tumors for mutations of the p53 gene by polymerase chain reaction (PCR) and single strain conformation polymorphism analysis (SSCP). Fresh testicular tumor tissue from 40 patients was obtained at the time of primary surgery. DNA and RNA were extracted from these specimens, purified and quantitated by fluorometry prior to gel electrophoresis as described (Strohmeyer et al., PNAS 88: 6662, 1991). PCR-SSCP analysis was performed using oligonucleotide primers and amplification of exons 4-8 of the p53 gene. Samples with a slight migration shift detected by PCR-SSCP analysis were amplified via PCR and sequenced using the deaza sequencing kit (USB, Cleveland, OH). In any case the result of the PCR-SSCP was confirmed and only wild type sequences were oberserved. Northern-blot analysis showed an expression of p53 in most of the samples analyzed, as well as in four human tumor cell lines. Dot blot analysis of all samples gave no evidence of amplification of the mdm-2 gene.
It is concluded from our studies that the p53 gene as well as the mdm-2 gene are rarely mutated in human testicular germ cell tumors. Therefore other factors than exogenous carcinogens seem to be responsible for the induction of malignancy in human testis cells and this development does not necessarily involve a mutation of the p53 gene.

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INTERFERON-ALPHA-2C FOR THE TREATMENT OF PATIENTS WITH PH-NEGATIVE CML: REPORT OF SIX CASES

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Between 1985 and 1993 a total number of 148 CML patients were treated with interferon-α-2C according to consecutive protocols of the Austrian CML study group. Six (4,1%) out of these were Philadelphia (Ph) chromosome negative. Median age of the six patients was 51 (16-63), two were female, four were male. All patients were in chronic phase of the disease and two had received a pretreatment, busulfan in one case and hydroxyurea following splenectomy in the other. The median WBC at the beginning of the interferon treatment was 49 G/L (7-195). Initial median values for platelets were 425 G/L (119-510), hemoglobin of 11,3 g/dL (10,3-15,5), monocytes 7% (0-19,5), ALP-score 8 (1-217) and LDH 396 U/L (191-1.079).

Three patients received interferon-α-2C (Berofor®) subcutaneously (s.c.) at an average dose of 10,5 MU per week as monotherapy, the other three patients were treated with a combination of interferon-α-2C 24,5 MU/week s.c. and low dose AraC (Alexan®) at a dose of 10mg/m2 /day s.c. for ten days a month.

Complete hematological response (CHR) was reached in three cases and partial hematological response (PHR) in two patients. Median duration of interferon therapy was 10 months (3-52). Three patients went off study at 3, 6 and 45 months, respectively, because of primary nonresponse (n=1) or disease progression (n=2), while the disease is under control in the other patients at 4, 14 and 52 months.

Although based on a small cohort of patients, these data demonstrate that it is worthwhile to further investigate the role of interferon-α for patients with Ph-negative CML.

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CYTOGENETICS OF MALIGNANT LYMPHOMAS: DIAGNOSTIC AND PROGNOSTIC SIGNIFICANCE

Ch. Fonatsch

In malignant lymphomas (ML) non random chromosome abnormalities correlate with histopathologic and morphologic features, with immunophenotype, and with progression of the disease. In general, rather simple karyotype deviations are found in low-grade ML, whereas in high-grade ML multiple (complex) chromosome abnormalities are observed. One of the most significant aberrations, a translocation between the long arms of chromosomes 14 and 18 - t(14;18)(q32;q21) - is found specifically in follicular centroblastic-centrocytic lymphomas, but does also occur in about 20 % of high-grade lymphomas. In most of these cases which are characterized by additional specific karyotype changes a histological transformation from a low-grade follicular lymphoma can be proved. In ML, chromosome bands in which cell type specific differentiation genes are located, as for example immunoglobulin and T-cell receptor chain genes, are known to be preferentially involved in translocations and inversions. The diagnostic and prognostic significance of those chromosome aberrations will be discussed. Moreover, a recently described translocation t(2;5)(p23;q35), specific to Ki-1-positive anaplastic large cell lymphomas, will be presented as well as cytogenetic findings in other types of ML whose clinical importance remains to be established. Since the detection of characteristic primary as well as secondary karyotype aberrations plays an essential role in diagnosis and prognosis of ML and may elucidate the clinical course, the delineation of new typical chromosome abnormalities represents one of the most important tasks of tumor cytogenetics.

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INTERFERONS IN LOW GRADE MALIGNANT NON-HODGKIN'S LYMPHOMAS. A CRITICAL REVIEW.

M. Freund

Interferon alpha (IFN α) is a therapeutic principle with well established activity in malignant Non-Hodgkin's lymphomas. Although IFN α has an antiproliferative effect in vitro, the in vivo basis of its effect is still poorly understood. The most favourable results have been achieved in low grade malignant non-Hodgkin's lymphomas. Objective responses of about 40% have been reported in patients with nodular histology (mostly centrocytic-centroblastic according to the Kiel classification). There is some evidence for a dose-response relationship. In advanced chronic lymphocytic leukemia, responses have been noted in 15% of patients only. There are data demostrating an *in vitro* growth-stimulation of CLL-cells by IFN α. In early stages (mostly Binet stage A) the disease seems to be sensitive to IFN α with a response rate of 70 percent. Complete responses have not been reported with IFN α in CLL. As the prognosis of CLL in the early stages is favourable, the impact of IFN α in early CLL is still open. There are controversial data on the treatment of cutaneous T-cell lymphoma and mycosis fungoides with IFN α. Based on pooled published data, the overall response-rate is about 40%. IFN α has an interesting activity in angioimmunoblastic lymphadenopathy (AILD, lymphogranulomatosis X). It may be due to a suppression of the cytokine expression in the tumor cells.

The effect of IFN α as a monotherapy is comparable to that of some cytotoxic agents. Despite this fact its final place in the therapeutic strategy is controversial. The combination of IFN α with alkylating agents has been effective in some reports, but additional hematotoxicity may evolve. In other studies IFN α is given for maintenance after conventional chemotherapy-induction. There is evidence that the remission duration may be improved. However, an improvement of survival still has to be demonstrated.

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HIGH-DOSE CHEMOTHERAPY WITH G-CSF AND PERIPHERAL STEM CELL SUPPORT IN RELAPSING OR REFRACTORY HIGH-GRADE MALIGNANT NON-HODKGIN'S LYMPHOMAS.

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13 patients (med. age 39 years, range 22-58 yrs.) have been treated with a high-dose chemotherapy protocol. 7 had a centroblastic NHL, one had a large cell mediastinal B-NHL, two high-grade pleomorphic or anaplastic T-NHL, one an immunoblastic and two more a high-grade not classifiable NHL. All patients had advanced disease and all have been extensively pretreated with chemotherapy and irradiation. 8 have been refractory to pretreatment, the others had relapsing disease. After a pre-phase of VCR 1.4 mg/m² (max. 2 mg) IV days 1, 8 and prednisolone 60 mg/m² PO days 1 - 10 the high-dose chemotherapy consisted of prednisolone 60 mg/m² PO days 1 - 4, ifosfamide IV days 1 - 4, methotrexate 5,000 mg/m² day 1 as a 24 h-infusion, cytosine-arabinoside 1,000 mg/m² IV days 3 + 4, and etoposide IV days 3 + 4. Etoposide has been escalated from 170 mg/m² to 500 mg/m² at the present time. The dose of ifosfamide is currently escalated from 1,500 mg/m² to 2,500 mg/m² and finally 3,500 mg/m² as a continuous 24 h-infusion. The high-dose chemotherapy is repeated for a maximum of 4 times. During the pre-phase 12 μg/kg G-CSF are given twice daily. Apheresis of APBSC is done on days 5 - 7. APBSC are reinfused after the high-dose chemotherapy and G-CSF is given at a dose of 5 μg/kg. With WBC rising to >1,000/μl aphereses are performed repeatedly. The pre-phase for induction of PBSC was excellently tolerated and a median of 10.4×10^4/kg (1.3-91.2) CFU-GM collected. After the first and second hd-chemotherapy course the stem cell yield was 20.6×10^4/kg (0.2 - 113.5) and 52.9×10^4/kg (0.2-95.8) respectively. The high-dose chemotherapy was associated with significant toxicities. In 36 courses WHO grade 3 and 4 side effects have occurred in the following number of courses: mucositis 14, diarrhea 3, vomiting 5, sepsis 5, GOT/GPT 8. Granulopenia grade 4 and thrombopenia grade 4 lasted for a median of 7 and 4 days after course 1 and for 3 and 1 days respectively after course 2. Results: 3 CR, 7 PR, 2 PD, 1 patient is too early to evaluate. The high-dose regimen is tolerable and efficient when given with APBSC support. Sufficient numbers of peripheral stem cells could be collected after a prephase after preparation with G-CSF only and after chemotherapy. The protocol should be incorporated into the primary treatment of high-risk patients.

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TREATMENT OF CHRONIC MYELOGENOUS LEUKEMIA (CML) WITH INTERFERON α-2b AND CYTOSINE ARABINOSIDE

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IFN α induces complete and partial cytogenetic remissions in 15-30% of the patients. To improve these results, we are currently treating patients with Ph'+ CML with a combination of cytosine-arabinoside at a maximum dose of 20 mg/m² SC on 5 days per week and IFN α-2b. IFN α-2b is started at a dose of 3 MU/m² SC daily and escalated to the maximum tolerated dose. 48 patients (25 male, 23 female, median age 44 years) have been entered into the trial. 15 patients have been pretreated with other regimen for a median time of 32 months. 33 patients are without pretreatment. The treatment has been well tolerated. Besides the IFN α related side-effects some patients experienced gastrointestinal toxicity with nausea and vomiting after prolonged Ara-C application. The median observation time in the study is now 8 months and patients are still entered. Up to now complete hematologic remissions have been achieved in 24 patients, and partial ones in 15 patients. The rate of complete hematologic remissions was higher in patients without pretreatment (55%) compared to patients who have been pretreated (40%). Five partial cytogenetic remissions have been observed and 3 minor reductions in the Ph'+ cell clone. All cytogenetic responses have been found in patients without pretreatment. We conclude that a combination of cytosine-arabinoside and IFN α-2b is well tolerated in patients with CML. Early results are encouraging. Longer follow up times are necessary to evaluate whether combination therpy will give superior results compared to a treatment with IFN α alone.

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UPDATE OF THE GERMAN MULTICENTER TRIAL OF ALG AND CYCLOSPORINE VERSUS ALG FOR TREATMENT OF APLASTIC ANEMIA.

N. Frickhofen and J.P. Kaltwasser* for the German AA Study Group.

The German Aplastic Anemia Study Group demonstrated that cyclosporine A (CsA) significantly improves the response rate of patients with aplastic anemia to antilymphocyte globulin (ALG) (N.Engl.J.Med. 324:1297;1991). The trial, which included 84 patients, randomly treated with either ALG, CsA and methylprednisolone (study group) or ALG and methylprednisolone (control group), has recently been updated. Complete follow-up of 2-6 years (median 40 months) is now available from all surviving patients. As reported previously, significantly more patients treated with CsA reached complete or partial remission at 3 months (65% vs. 39%, $p<0.03$) and 6 months (72% vs. 49%, $p<0.05$). Response rates were no longer different at 12 months (74% vs. 59%, $p<0.2$) due to late responses of control patients after secondary treatment. Monthly follow-up showed, that blood counts improved much faster in patients treated with CsA, thereby substantially reducing the time at risk from cytopenia. Longterm survival did not differ between the two treatment groups, again due to successful secondary treatment of nonresponsive control patients. With longer observation time, the actuarial risk of relapse increased to 32% at 4.5 years in the study group and to 52% in the control group ($p<0.5$). CsA delayed relapses by the time the patients were treated with the drug. Remission could be reinduced in most patients who relapsed. One patient each developed laboratory signs of PNH or oligoblastic leukemia. A 30 yo patient developed oral squamous cell carcinoma, probably associated with treatment. Gastric cancer and breast cancer in two 67 and 68 yo patients 9 and 3 months after treatment almost certainly occurred independently from treatment. This data demonstrates, that "triple drug treatment" produced superior short term results; however many nonresponders of the control group could be rescued by secondary treatment. Relapse is a significant problem of successful treatment protocols and the rate of secondary diseases is likely to increase over time. Immunosuppressive treatment is thus very effective but not curative in the majority of the patients.

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CIRCULATING CLONOTYPIC DNA IN PATIENTS WITH MALIGNANT B CELL DISORDERS FOR MONITORING RESPONSE TO TREATMENT.

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Rapid and complete response to treatment is one of the most powerful favorable prognostic factors for long term survivalof patients with cancer. This has been demonstrated for many malignancies including aggressive lymphoma and lym-phoblastic leukemia. Whereas response to treatment can conveniently be measured in patients with tumor masses assessable by CT scanning or ultrasound, quantification of response is more difficult in patients with sy-stemic disease such as leukemia or lymphoma with bone marrow involvement. We have found that clonotypic DNA, as evaluated by nested primer polymerase chain reaction (PCR) of rearranged immunoglobulin genes, can be demonstrated in the serum of more than 80% of patients with B cell lymphoma or leukemia, whose DNA is informative with the PCR method employed (again about 80% of the patients). Clonotypic DNA does not seem to be liberated into serum by tumor cells in vitro, since patients have been found with negative peripheral blood cell DNA but positive serum. Specific DNA has been demonstrated in serum of patients with localized lymphoma and in patients with normal LDH and normal $\beta 2$ microglobulin, suggesting that it is a more sensitive marker for active disease than these parameters. To date, only a limited number of patients has been followed during treatment. There have been patients who rapidly responded clinically and also rapidly cleared circulating rearranged DNA. Two patients had a very good clinical response but remained positive for circulating clonotypic DNA; both patients relapsed within 6 months of treatment. Patients resistant to treatment remained positive in serum. We were unable to extend these observations to clonotypic RNA, which is degraded within seconds in fresh serum. This data suggests, that circulating clonotypic DNA can be used as a parameter for active disease. Use of this marker may be particularly useful for monitoring systemic disease. Preliminary results in patients with leukemia suggest, that this method is less sensitive than analysis of DNA derived from bone marrow cells. However it is less prone to sampling error and it may be sufficiently sensitive for measuring clinically meaningful response.

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A NEW HYBRID REGIMEN (CEOP-IMVP-DEXA) IN THE TREATMENT OF HIGH-GRADE NON-HODGKIN'S LYMPHOMAS (NHL). AN AUSTRIAN MULTICENTER TRIAL.

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In this prospective multicenter trial patients with untreated high malignant NHL according to the Kiel Classification and measurable disease were included. Ten Austrian centers entered 81 patients; 68 were evaluable (male/female rate 40/41, median age 55.5 years). Two non-cross-resistant regimens, CEOP (Cyclophosphamide 750mg/m^2 iv. d1, Epidoxorubicin 70mg/m^2 iv. d1, Vincristin 1,4mg/m^2 iv. d1+8 and Prednisolone 100mg po. d1-5) and IMVP-Dexa (Ifosfamide 2g/m^2 with Uromitexan uroprotection iv. d15-17, VP-16 100mg/m^2 iv. d15-17, Dexamethasone 40mg po. d15-19 and Methotrexate 800mg/m^2 iv. with Ca-folinat rescue po. d22) were combined and repeated in 4 week intervals.

Complete remission rate with 81.5% was excellent, the projected overall survival and time to relapse after 3 years was 68% and 64%, respectively (median observation time 25.9 months). Age>60 and stage III or IV were the only independent risk factors for a high relapse rate. Toxicity was primarily hematological with a median granulocyte nadir of 0.5x10^9/L. Seventy-one percent of patients had infections, but only 26% of them required hospitalization. Toxic death rate was 4.4%.

A comparision with the International NHL Prognostic Factors Project (Int. Index), is listed as the follows:

Risk Category Int.Index	% patients Int.Index	% patients own data	2-a survival Int.Index	2a-survival own data
Low	35 %	19 %	84 %	93 %
Intermed.-low	27 %	35 %	66 %	75 %
Intermed.-high	22 %	22 %	54 %	56 %
High	16 %	24 %	34 %	56 %

CEOP/IMVP-Dexa is a safe and highly effective regimen for high grade NHL.

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CD34-ENRICHMENT FROM LEUKAPHERESIS PRODUCTS IS EFFECTIVE FOR LYMPHOMA CELL PURGING.

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Peripheral blood stem cells are increasingly used for autografting after high dose consolidation therapy in lymphohematopoietic malignancies and solid tumors. This is partly due to a more rapid time to engraftment than following autologous bone marrow transplantation. However, leukapheresis products as well as bone marrow harvests may be contaminated by malignant cells. In low-grade non-Hodgkin's lymphomas and solid tumors normal hematopoietic progenitors may be separated from tumor cells by CD34-selection.
Follicular lymphoma cells (line K422) marked with the fluorescent dye PKH26 were added in varying amounts to leukapheresis products. Immunomagnetic CD34-selection (IMB) was compared to a biotin-avidin column system (BAC). The starting preparation contained 2.4% $\pm$ 1.5% (n=6, SE) CD34+ cells. In the enriched fraction comparable CD34-purities were observed after IMB and BAC selection (60.7% + 28.8%, n=6 vs. 66.1% + 11.1%, n=5). In both groups, only 16% + 3% (IMB) and 18% + 9% (BAC) of the initial CD34+ cell number could be recovered. The purging efficiencies for lymphoma cells in the CD34-enriched fraction were log 3.25 + 0.28 (IMB) and log 3.02 + 0.67 (BAC), respectively. Pilot experiments with monocyte depletion before CD34-enrichment showed that a CD34-purity of 90% and a purging efficiency of log 4.8 can be achieved, but only at the expense of the CD34-recovery (6.5%). In conclusion, CD34-enrichment is an effective approach for lymphoma cell depletion from leukapheresis products. However, due to the significant loss of progenitor cells only patients with a good hematopoietic reserve will be eligible for future clinical trials.

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HBsAg AND HBVDNA DETECTION IN HAEMATOPOIETIC PROGENITOR CELL CULTURES OF PATIENTS WITH CHRONIC ACTIVE HEPATITIS B

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Several studies suggested that patients with chronic active hepatitis B are carrying HBVDNA in their peripheral blood MNC (PB-MNC). Disagreement exists whether replication of HBVDNA takes place in haematopoietic precursor cells or is restricted to differentiated MNC. Using a microagar culture system for circulating haematopoietic progenitor cells 10 HBs, HBe and HBVDNA positive patients with cAHB were investigated. The effect of monocytes (mo) and T-lymphocytes (T-ly) on the growth of BFU-E, CFU-GM and CFU-Meg was tested by sequent depletion of mo and T-ly from MNC. Readdition of autologous T-ly resulted in a decreased progenitor cell growth suggesting suppressor T-ly which inhibit haematopoiesis. A titrated addition of autologous HBVDNA positive serum inhibited proliferation and differentiation of CFU-GM, BFU-E and CFU-Meg. These results suggest that haematopoiesis in HBs carriers can be affected in a dual mode first by the virus itself and second by suppressor T-ly. Culture supernatants were screened for HBsAg, HBeAg and HBVDNA. HBsAg could be detected in the liquid overlayer of cultured MNC of all patients (10/10). The detection of HBsAg was not dependent on the cytokine used for progenitor cell stimulation. Mo depletion abrogated HBsAg release in 9/10 patients, mo and T-ly depletion resulted in a total abrogation of HBsAg. Readdition of mo and T-ly was not able to restore HBsAg positivity. HBVDNA measurments were performed in progenitor cultures of 4 patients and were found to be positive in one MNC culture. More detailed analysis of the different cell populations and colonies for HBVDNA by PCR technique are performed. Our results suggest that HBsAg and HBVDNA is released from mo or T-ly and not by haematopoietic progenitor cells.

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HLA CLASS I AND II ANTIGENS IN PATIENTS WITH CHRONIC IDIOPATHIC AUTOIMMUNE THROMBOCYTOPENIA (c-AITP). ITS RELATION TO THE OUTCOME OF THERAPY.

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We studied MHC class I and II molecules associated with chronic idiopathic autoimmune thrombocytopenia (cAITP). Forty six patients (median age 51 years, range 20 to 78 years) with a disease duration of median 6 years (range 0,5 to 26 years) were phenotyped for HLA A, B, C by the standard lymphocytotoxicity test, HLA DR and DQ by restriction fragment length polymorphism (RFLP) and DP by oligonucleotide typing. Antiplatelet antibodies directed against glycoprotein Ib/IX and IIb/IIIa were determined by monoclonal antibody specific immobilization of platelet antigens (MAIPA). Patients were divided into those with antibodies (n=16) and those without detectable antibodies (n=30) and according to their response to therapy. The comparison of antigen frequencies of the whole group of patients revealed no significant difference for any of the MHC class I or II antigens (p> 0,05). The HLA DPB1*1501 phenotyp was only seen in patients with detectable antibodies. This result was significant after correction for the number of tested antigens (p< 0,01). There was no difference between patients with good (n=23) or poor (n= 17) response to steroids. HLA DPA* 0402 was significantly more common among patients with a poor (n= 4) response to splenectomy than among patients with good (n= 11) response (p< 0,01). This difference was also significant after correction was made for the number of tested antigens (p< 0,03).This study indicates that within the conception of chronic ITP subgroups can be defined, based on 1) the response to therapy, 2) serological findings. Within these subgroups association with certain HLA class II phenotypes can be found. For the evaluation of this data however, the confirmation in another independent patient group is needed.

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QUANTITATIVE PCR ANALYSES IN PATIENTS WITH CHRONIC MYELOID LEUKEMIA: INCREASE OF BCR-ABL SPECIFIC mRNA EXPRESSION DURING THE PROGRESSION TO BLAST CRISIS

A.Gaiger (1), T.Henn (2), K.Geissler (1), K.Lechner (1), T.Lion (2).

We have investigated 250 peripheral blood and/or bone marrow samples of 55 CML patients using qualitative and quantitative bcr-abl PCR. 46 pts were in chronic phase of disease, 9 in accelerated or blastic phase of disease. The median time after diagnosis was 25 months (range 2 to 126 months). A b3a2 mRNA was expressed in 37 pts, a b2a2 mRNA in 19 pts. 26 patients were monitored during their course of disease by serial quantitative PCR analyses (median number of samples analysed/patient: 7, median observation time: 16 months, range 3 to 42 months). All patients remained bcr-abl PCR positive. We could observe an increase of bcr-abl specific mRNA expression during the progression to blast crisis. Thus, indicating the possibility, that a molecular genetic acceleration might preceed the progression to the accelerated or blastic phase of disease.There was no association between fusion pattern of BCR/ABL mRNA and duration of chronic phase as well as response to IFN treatment. Although, recent reports indicated a possible correlation between fusion pattern of BCR/ABL mRNA and duration of chronic phase of disease and clinical response to therapy we could not confirm these observations in our group of patients.

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CYTOGENETIC AND MOLECULARGENETIC ANALYSES IN A PATIENT WITH M-BCR REARRANGED, PHILADELPHIA POSITIVE AML

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The Philadelphia chromosome (Ph) occurs frequently in CML but is less common in ALL and rare in AML. We report on a 52 year old male patient, who was diagnosed as Ph positive AML in october 1992. Karyotype analysis showed a Ph chromosome, which was confirmed at the DNA level by the presence of a rearrangement of M-bcr, PCR analysis showed that a b3a2 mRNA was expressed. Although these molecular features are identical to those seen in Ph positive CML, a detailed retrospective examination of clinical and labaratory data provided no evidence of an underlying myeloproliferative disorder. Following chemotherapy patient achieved complete hematological and cytogenetic remission. No bcr-abl rearrangement was detectable using Southern blot analysis, quantitative PCR indicated a decrease of bcr-abl specific mRNA. Only one month later karyotype analysis showed again Ph positive metaphases, Southern blot was bcr-abl positive and PCR showed an increase of bcr-abl specific m-RNA. During treatment with Alpha Interferone Southern blot analysis remained positive and quantitative PCR indicated a further increase of bcr-abl specific mRNA. The patient is still in complete hematological remission. Ph positive acute leukemia can present with either a p210 BCR/ABL as described in our patient, or with p190 BCR/ABL phenotype. It has been suggested that the p190 variant might represent de novo acute leukemia with the p210 form being an acute leukemia or blast crisis supervening on a prior covert or subclinical CML. The clinical course of the patient presented here is not in accordance with this speculation, but more patients need to be investigated to verify or refuse this association.

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A NEW CHROMOSOMAL BREAKPOINT t(14,18) IN A T-CELL CLONE DERIVED FROM A T-CLL OF A PATIENT WITH ATAXIA TELANGIECTASIA

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We report of a reciprocal chromosomal translocation t(14;18)(q11; q23) in a clonal T-cell line derived from a patient with ataxia telangiectasia (AT) and chronic lymphocytic leukemia of T-cell origin (T-CLL). The T-cell clone was clearly not derived from the major T-cell clone and is considered a minor T-cell clone within the fresh tumor cell suspension that remained undetected in the patient. Restriction fragment analysis of the T-cell receptor (TCR)-δ gene indicated that the breakpoint lies within the TCR-δ locus, directly upstream of the Dδ3 region. Rearrangement analysis exhibited that the breakpoint on chromosome 18q23 did not involve the myelin basic protein gene which is located on this chromosomal band. Hybridization of the RNA of the established cell line with a cδ-probe showed aberrant message sizes of 1.1 and 2.0 kb which are different from incomplete Tδ-transcripts. DNA cloning and sequencing of about 1.9 kb of the breakpoint region revealed 1.6 kb of Tδ germline DNA and 300 bp of an unknown gene on chromosome 18.
The specific chromosomal translocation was only detected in the established T-cell line. Further sequencing is needed to determine the genetic structure on chromosome 18 and the relevance of the translocation and the gene on chromosome 18q23.

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Use of IL-2 transduced tumor cells as a vaccine against cancer.
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There are several hypotheses why cytokine gene therapy could show same beneficial effects in cancer patients. First, it has been shown by P. Van der Bruggen et al. (Science, 254, 1643, 1991) that some melanoma cells express tumor specific antigens recognized by CTL. Second, melanoma patients do have circulating tumor specific CTL precursors in their blood (P. Coulie, et al, Int. J. Cancer, 50, 289, 1992). Third, in a mouse tumor model tumor specific CTL were enriched 2-4 fold by having the tumor cells secrete IL-2 (V. Ley, et al, Eur. J. Immunol., 21, 851 1991). Our working hypotheses was that in some HLA-A2 positive patients tumor cells express CTL defined antigens and tumor specific CTL precursors circulate. By injecting allogeneic HLA-A2 positive tumor cells that express the same antigen and secrete IL-2, clonal expansion of the tumor specific CTL should be induced. CTL do have access to systemic circulation and should reach residual tumor in this way. Our initial studies were done in a murine tumor model. We used recombinant retroviral vectors to introduce the IL-2 cDNA into the murine fibrosarcoma CMS-5. The resulting IL-2 secretion by these tumor cells had a potent effect on the host immune system inducing specific CTL's and memory (Gansbacher et al., J. Exp. Med., 172, 1217, 1990). Subsequent studies done in human melanoma and renal carcinoma cell lines showed that it was possible to use retroviral vectors to introduce and stably express cytokine genes in human tumor cells. The amounts of IL-2 secreted by bulk tumor cell population transduced with our retroviral vectors were in the range of 40-100 U/IL2/10^6 cells/24 hrs. In addition it was possible to irradiate the transduced renal carcinoma cells and they continued to secrete cytokine for several weeks (Gastl et al, Cancer Res., vol. 52, 6229, 1992). Irradiated transduced melanoma cells secreted cytokines for up to 35 days and were able to generate specific CTL's in vitro coculture (Gansbacher et al, Blood, 80, 11, 2817, 1992).
B-cell lymphoma cells are ideal targets of effector cells by expressing an idyotype specific for the maligant clone. A mouse model was developed to investigated that hypothesis. In summary, progress has been made in tumor vaccine development. Cytokine secreting tumor cells are now available for clinical use in vaccination protocols.

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ROLE OF HEMATOPOIETIC GROWTH FACTORS IN THE THERAPY OF MYELODYSPLASTIC SYNDROMES

A. Ganser

Myelodysplatic syndromes (MDS) are characterized by progressive refractory cytopenia with defective myeloid differentiation and cellular dysfunction due to an uncoupling between proliferative and differentiative programs in hematopoietic stem cells. In recent years clinical trials with hematopoietic growth factors have been performed to evaluate the potential of these cytokines to restore hematopoiesis. Both granulocyte colony-stimulating factor (G-CSF) and granulocyte-macrophage colony-stimulating factor (GM-CSF) have been shown to reverse neutropenia to normal in approximately 80%-90% of patients. Although the incidence of infectious episodes appears to be reduced in patients receiving G-CSF or GM-CSF, results of a randomized trial without crossing-over design are still pending. Reversal of thrombocytopenia has not been observed with G-CSF or GM-CSF, nor with interleukin-3, although improvement of low platelet counts has been seen in about 35% of patients receiving the latter cytokine. A stimulation of thrombopoiesis comparable to IL-3 has been seen in patients treated with IL-6, being however accompanied by worsening of anemia. Improvement of anemia by treatment with high-dose erythropoietin can be expected in 15% of patients, preferably in those with less severe anemia, no or mild transfusion dependency, and only moderately increased base-line serum erythropoietin levels. Combination of G-CSF and erythroietin apparently can increase the response rate to 40% with regard to improved erythropoiesis due to a still poorly understood synergism. Combinations of cytokines, especially of GM-CSF and IL-3, with the cytostatic agent ara-C, have failed to demonstrate a selective elimination of malignant cell clones and to be superior to cytostatic treatment alone. Cytogenetic analysis has equally failed to demonstrate a selective stimulation of either the normal or the abnormal cell clones by cytokine therapy. While treatment with GM-CSF, G-CSF, IL-3, and IL-6 can be associated with disease progression to acute leukemia, this risk appears to be minor in patients with a low leukemic blast cell burden (< 20% bone marrow blasts) and without the CMML subtype of MDS. Future trials will have to evaluate the potential of cytokine combinations and the concurrent treatment with cytokines and differentation-inducing agents.

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NO LARGE DELETIONS OF MITOCHONDRIAL DNA (MTDNA) IN ACQUIRED IDIOPATHIC SIDEROBLASTIC ANEMIA (AISA).

N. Gattermann, C. Aul and W. Schneider

It has been speculated that AISA might be caused by mutations of mitochondrial DNA because Pearson's marrow/pancreas syndrome, which is associated with sideroblastic anemia, characteristically shows large deletions (~5 kb) of mtDNA.
For Southern blotting of mtDNA, we obtained about 5ml of bone marrow from 10 patients with AISA who presented for regular control biopsies. The percentage of ring sideroblasts ranged between 15 and 85%. Total DNA from bone marrow cells was extracted by standard methods. Samples of about 5 µg were digested with restriction endonucleases PvuII, BamHI and SacI, respectively, separated by agarose (0,8%) gel electrophoresis, and transferred onto nylon filters. The filters were hybridized with a specific mtDNA probe kindly provided by Professor G. Attardi (California Institute of Technology, Pasadena, USA). The probe consists of an MboI fragment of mtDNA (positions 1-739), cloned into the BamHI site of pUC-9. This probe hybridizes with a region containing the origin of replication of the heavy strand (O_H). Deletion of O_H would render the mtDNA molecule incompetent of replication and thus prevent its propagation. Accordingly, to date all mtDNA deletions studied at the molecular level spare this origin of replication. Therefore, the mtDNA probe we employed can be expected to hybridize with any replicating mtDNA, regardless of the extent of possible deletions. Gel electrophoresis compared every digest with identically treated DNA obtained from a healthy control.
In none of our patients with AISA did electrophoretic mobility of linearized mtDNA suggest a major deletion or duplication. We always found a single band of 16,5 kb after BamHI and PvuII digestion, respectively, and two bands of 9,6 kb and 6,9 kb after treatment with Sac I (two restriction sites). Control experiments with deleted mtDNA from patients with mitochondrial myopathies, kindly provided by Professor A. Harding and Dr. M. Sweeney (Institute of Neurology, Queen Square, London) showed that we were able to detect deleted mtDNA even if its amount was only around 1% of the total mtDNA. Our findings do not exclude the possibility that mtDNA from patients with AISA contains small deletions or point mutations, which can be very important, as shown for several neurological diseases.

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ONKOLOG® – Aid the planning of therapy protocols

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MITOXANTRONE IMMUNOCONJUGATES ARE EFFECTIVE IN VITRO

M. Geiger, G. Eger, W. Baum, J.R. Kalden, M. Gramatzki

Mitoxantrone (MTO) is a potent drug in the treatment of hematologic malignancies. In order to potentiate specific effects on proliferating cells and to reduce toxic side effects, MTO- immunoconjugates were developed. MTO was coupled via a modified polysaccharide linker to several monoclonal antibodies directed against human T- and B- cell differentiation antigens. Optimizing the coupling procedure, a binding of up to 150 molecules per antibody could be achieved. Immunoconjugates proved to be stable at 4°C for at least several weeks.To evaluate the activity of the immunoconjugate, exponentially growing T-ALL cell line CEM was incubated for 30 minutes with the conjugate (drug-antibody-ratio 50:1) and proper controls. When cell line growth inhibition was analyzed 24 hours later, the immmunoconjugate at a MTO concentration of 0.2 ng/ml showed still 60% inhibition, while MTO alone had no activity. For similar antiproliferative efficacy at least a 1000-fold higher concentration of the unconjugated drug was necessary. Controls with the uncoupled antibody, the antibody-linker conjugate or a MTO-immunoconjugate with an irrelevant antibody (L227, directed against a framework HLA-DR structure) were inactive. In preliminary experiments, CEM tumor bearing nude mice showed visible tumor reduction upon injection of a single dose (20µg) of the conjugate. While preclinical studies in mice are still ongoing, the highly increased in vitro activity of the MTO-immmunoconjugate appears promising for the therapy of T-ALL tumors.

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BONE MARROW DERIVED FIBROBLASTS FROM HIV-INFECTED PATIENTS INHIBIT IN-VITRO HEMATOPOIETIC COLONY GROWTH

R.G. Geissler, A. Ganser, O.G. Ottmann, A. Maurer, D. Hoelzer

The pathogenesis of hematopoietic failure in patients with HIV-infection is still poorly understood. Own experiments have shown that bone marrow cells from HIV+ patients have a markedly reduced capacity to initiate long-term bone marrow cultures on less and later confluent stromal layers. Furtheron, bone marrow derived fibroblasts can be infected with HIV. To elucidate the changes of bone marrow stromal cells, the supportive effect of fibroblasts from patients with progressive HIV-1-disease on hematopoietic progenitor cells was investigated. Bone marrow derived fibroblasts from HIV+ and healthy persons were enriched by adherence on plastic dishes in RPMI/20% fetal calf serum and purificated by trypsination in 4 passages. Non-adherent bone marrow cells from the same individuals underwent immunoabsorption to enrich CD34+ cells. In serum-free suspension cultures containing kit ligand (100 ng/ml), GM-CSF (10ng/ml), and G-CSF (10 ng/ml) as stimuli, the progenitor cells were incubated on irradiated (20 Gy) fibroblast layers for 7 days. Thereafter, the remaining cells were added to a methylcellulose assay to enumerate the colony numbers after further 14 days of incubation. As results, fibroblasts from HIV+ patients needed significantly longer to grow confluent layers. While hematopoietic progenitor cells from healthy donors exerted a significantly higher colony forming capacity (CFU-GEMM, BFU-E, CFU-GM) grown on their own fibroblast layers, progenitor cells from HIV+ patients did not show a significant difference in this capacity when incubated with their own fibroblasts or without fibroblasts, respectively. In contrast, hematopoietic colony numbers from HIV+ persons were enhanced significantly after co-incubation with fibroblasts from healthy persons, while colony formation from the controls significantly decreased after co-culture with fibroblasts from HIV+ persons. In conclusion, these data clearly demonstrate that bone marrow derived fibroblasts from HIV+ patients have a decreased proliferative capacity and generate a reduced supportive effect on hematopoietic colony growth from HIV+ and healthy persons, respectively. Changed patterns of stimulatory or inhibitory cytokine production and alterations of adhesion molecule expression of fibroblasts are possible pathogenetic mechanisms. Further investigations have to clarify whether the fibroblasts are infected directly with HIV, affected indirectly by HI-viral proteins, or co-infected with other viruses.

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INCIDENCES OF LYMPHOCYTE SUBSETS AS PREDICTIVE FACTORS FOR CLINICAL COURSE IN APLASTIC ANEMIA

R.G. Geissler, U. Mentzel, R. Rossol, H.G. Vogt, A.B. Maurer, A. Ganser, J.P. Kaltwasser, and D. Hoelzer

T-lymphocyte subsets are suggested to mediate an important pathophysiological mechanism in aplastic anemia (AA). Immunosuppressive therapy with anti-lymphocyte globulin, cyclosporine A, and methylprednisolone leads to a complete or partial remission in 65% of the patients with AA within three months. Therefore, the proportions of T-lymphocyte subsets from AA patients with (R, n=8)/without (N, n=5) remission before, after 6 and after 12 weeks of therapy were analysed. Double cytophotometric fluorescence technique (FACScan) was performed on peripheral blood cells using monoclonal antibodies against the antigens: TCRαß, TCR δ, δTCS1, CD2, CD3, CD4, CD8, CD16, CD19, CD38, CD56, and HLA-DR. As result, the numbers of αß-T-lymphocytes from all patients with AA stayed within normal ranges before and under therapy as compared to healthy persons (HP), while initially .δ-T-cells were decreased in all patients reaching normal numbers during treatment.

Lymphocytes	HP	before therapy R	before therapy N	after 6 weeks R	after 6 weeks N	after 12 weeks R	after 12 weeks N
δTCS1 in CD3+ (%)	0.7	0.9	0.9	2.4	2.4	3.5	4.4
δTCS1 in .δ (%)	21.4	29.6	42.7	51.2	49.7	78.0	91.0
CD8+ in δTCS1 (%)	31.7	26.9	57.8	41.7	26.7	58.1	12.9
CD4/CD8-ratio	1.4	1.9	1.4	1.4	0.7	1.1	0.4

In contrast, the percentage of δTCS1+ cells in the CD3+ and in the .δ-T-cell population was elevated before treatment and markedly increased during therapy for R and N patients, respectively, as compared to HP. In N patients, δTCS1-cells frequently expressed CD8+ antigen before therapy which markedly decreased during therapy. In contrast, CD8+ δTCS1 cells in R did not differ from those of HP but increased during therapy. Before therapy CD4/CD8-ratio of all patients was similar to that of HP. After 6 and 12 weeks of treatment the ratio decreased moderately for R, but markedly for N patients. In conclusion, δTCS1-lymphoyctes increase in all patients with AA under therapy. Decreasing CD8-receptor expression in δTCS1-cells, and inversed CD4/CD8 ratio during therapy are suggested to be new predictive factors for bad prognosis in the clinical course of AA.

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Construction of a cDNA subtraction library from small amounts of globin mRNA using Oligo(dT)-beads and PCR

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Subtractive hybridization is a method to search for differetially expressed genes in two closely related cellular populations, e.g. normal and transformed cells. In order to start subtractive hybridization from low amounts of mRNA, we have developed a method using oligo-dT_{25} coated magnetic beads and subtracting adaptor ligated cDNA. First and second strand cDNA synthesis and adaptor ligation were performed at the beads. Second strand cDNA (tracer) was removed and hybridized to beads-coupled cDNA (driver) of a different sample. Non-hybridizing ss cDNA was recovered and amplified by PCR in order to achieve sufficient amounts for further analysis.

Methods: 2 µg of globin mRNA (Gibco BRL) hybridized to 1 mg of Oligo-dT_{25} coated magnetic beads (Dynal). Messenger RNA was reverse transcribed with RNAse$^-$-MLV RT (Superscript Plus; Gibco BRL) to achieve first strand cDNA covalently attached to the beads. Second strand cDNA synthesis was performed using RNAse H, E.coli DNA polymerase I and E.coli DNA ligase. Following T4-DNA-polymerase and T4-polynucleotidekinase treatment to gain blunt-ended ds-cDNA an estimated 10^4 fold molar excess of Xho I-adaptor (ClonTech) was used for the ligation reaction. The second strand of cDNA carrying the poly(A)-track at its 3'-end and the adaptor-sequence at its 5'-end was removed from the beads with alkali and neutralized. The cDNA second strand was spectrophotometrically quantified in a microcuvette and 100 ng were used for hybridization. After hybridization (3xSSC; 24 h, 68°C) to bead-coupled first strand cDNA derived from a 10 µg mRNA mix without globin mRNA (0.24-9.5 Kb mRNA; Gibco BRL), non-hybridized ss cDNA was removed from the beads with the supernatant. PCR was performed with an oligo-dT tailed Not I primer-adaptor (Promega) and a second primer complementary to the Xho I-adaptor sequence (ClonTech). Amplificates and plasmid vectors were cut with Xho I and Not I, then ligated. DH5α-cells were used for transformation.

This method has been transfered to more complex samples from bone marrow cells at different stages of disease. Inserts of transformation positive clones made from amplified subtracted cDNA are now sequenced and analysed.

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DO CYTOKINES IMPROVE THE RESULTS OF CHEMOTHERAPY IN HIGH GRADE NON-HODGKIN'S LYMPHOMAS ?

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Non-Hodgkin lymphomas (NHL) of high malignancy are usually treated by protocols employing the maximally tolerable chemotherapeutic (ctx) dose. So-called third generation regimen have attempted to increase dose intensity (D.I.) without much success regarding mean administered dose (due to clinically necessary modifications) as well as regarding survival prolongation (1). The availability of hematopoietic growth factors raises the possibility of reducing the risk of infections, of improving adherence to ctx and even of increasing the dose intensity. Unfortunately, not many controlled studies are available on these subjects. When G-CSF was added to the VAPEC-B ctx in a randomized trial of 80 pts. D.I. rose from 83% to 95% although this did not result in any difference regarding infections, response or survival (2). We employed GM-CSF as an adjunct to the COP-BLAM regimen in a placebo-controlled trial: GM-CSF reduced the incidence of infections and the demand for antibiotics and also prevented cycle delays thus increasing the D.I. from score 0.81 to 0.85. Despite this moderate difference the response rate was increased with GM-CSF in pts. with large tumor burden (48 vs 69%, P=0.04). Nevertheless the failure-free survival was not improved (3). It appears that the role of D.I. for survival has been largely overestimated. An analysis of 539 cases treated in a German multicenter trial with the COP-BLAM/IMVP-16 sequential protocol gave the surprising finding that - if any - those pts. receiving lower doses survived longer (P=0.037 in a multivariate analysis) while relapse-free survival was not influenced by D.I. at all (4). Ctx regimen of short duration and thus drastically increased D.I. may be better partners for hematopoietic growth factors to improve both remission rates and survival, e.g. the IEVM regimen (5). Randomized phase III studies are warranted to show the superiority of any dose intensified protocols over conventional ctx before such an approach should be employed outside studies.

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A PILOT STUDY OF INTERLEUKIN-3 PLUS LOW-DOSE CYTOSINE ARABINOSIDE IN MYELODYSPLASTIC SYNDROMES WITH HIGH RISK OF DEVELOPING ACUTE LEUKEMIA

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Patients (pts.) with myelodysplastic syndromes (MDS) and excess of blasts (>10%) (RAEB, RAEBt of the FAB classification) who were symptomatic (either transfusion-dependent or neutropenic below $1x10^9/l$ or thrombopenic below $50x10^9/l$) were treated with repetitive courses of low dose cytosine arabinoside (LD-AraC, 2-10 mg/m^2 s.c. d 1-14) together with interleukin-3 at various dose steps (IL-3: 1.0, 2.5, 5.0, 10.0 µg/kg b.w. s.c. day 8-21) in an attempt to find an optimal dose for this combination. Between 10/90 and 09/91 29 pts. were documented in this pilot study. They received a total of 104 cycles (1 to 6 per case), most of them on an outpatient basis. Toxicities were fever (92%), flu-like symptoms (41%), infections of WHO grade 2 or more (7%), bleeding (33%) and erythema (28%) of courses. While infections occurred less frequently in the higher dose groups (5-10 µg/kg IL-3) the subjective tolerability was better at the lower dose levels. Data on hematological and clinical responses are available for 27 pts.: there were 5 complete remissions, 1 partial remission and 3 pts. with minor response (9/27 cases). 9 pts. had stable disease, 4 progressed to AML and 3 died from hemorrhage and sepsis and 2 due to the MDS. These data show that LD-AraC plus IL-3 can induce responses in a considerable proportion of HR-MDS pts. with acceptable toxicity. The IL-3 dose of 2.5 µg/kg was chosen for a larger controlled phase III study.

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EFFECT OF CYTOSTATIC DRUGS AND OF IMMUNO-SUPPRESSIVE AGENTS ON THE LEUKOTRIENE PRODUCTION IN MURINE MAST CELLS.

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Mast cells are regarded as effector cells in allergic diseases, in host defense, tissue inflammation, growth, and repair. Suggestive of these roles are their capacity to produce a number of potent mediators and their localisation at exposed positions beneath epithelial surfaces, adjacent to blood and lymphatic vessels, and in bone marrow. Leukotrienes are 5-lipoxygenase derived eicosanoids and among the most potent mediators of mast cells.

We studied the cysteinyl leukotriene production in murine bone marrow-derived mast cells and the effect of some commonly used cytostatic drugs and immunosuppressive agents. Leukotrienes were determined by radioimmunoassay (RIA) or by combined use of high-performance liquid chromatography and RIA. Production in unstimulated cells and in cells stimulated with calcium ionophore were compared.

A number of structurally unrelated agents (Doxorubicin, Bleomycin, Asparaginase, Cyclophosphamide, Cisplatin, Methotrexate, Vincristin) modulated leukotriene production in mast cells in an apparently biphasic fashion. The stimulatory effect of most of these agents was more pronounced in the absence of calcium ionophore. Reduction of leukotriene production seemed to be associated with cytotoxic effects. Cyclosporine A inhibited leukotriene production in mast cells stimulated with calcium ionophore. Prednisolone was without significant effect in this model system.

From our data we conclude that mast cells represent a potential target for commonly used cytostatic or immunosuppressive drugs. Modulation of leukotriene production in mast cells may help explain some of the beneficial and adverse effects of these agents.

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Intracellular Pharmacokinetic of Anthracyclines in Hematopoietic Cells

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Background: Anthracyclines (ANT) are widely used in the therapy of leukaemia and lymphoma. These drugs exhibit multiple intracellular effects, such as direct membrane toxicity, liberation of free oxygen radicals, DNA intercalation and inhibition of topoisomerase II. Which of these are necessary for cell death is still under discussion. Cellular drug uptake, intracellular distribution and DNA binding have been studied in sensitive and resistant patient cells, cell lines and normal lymphocytes.

Methods: Cell viability was determined by MTT test, cellular and nuclear ANT uptake by incubation of cells with drugs, (isolation of nuclei), lysis, extraction of drugs, determination of concentration by fluorescence. DNA binding of drugs was determined by replacement of Hoechst dye 33342.

Results: HL-60 cells are 100-1000fold more sensitive to idarubicine and daunorubicine than normal lymphocytes.

Time (minutes) needed to reach peak concentration by incubation of HL-60 cells and normal lymphocytes in idarubicine 1µg/ml (1.873 µM) and daunorubicine 1 µg/ml (1.773 µM)

	cells	nuclei	DNA
HL-60 cells			
idarubicine	5-10	10	20
daunorubicine	5	10	45
lymphocytes			
idarubicine	5	5	30
daunorubicine	5-10	5-10	45

- Intranuclear factors determine the time which is needed to reach the DNA
- no correlation between total cellular drug uptake and sensitivity
- logarithmic correlation between drug uptake and DNA binding
- linear correlation between DNA binding and viability.

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RESULTS OF CHEMOTHERAPY IN FOLLICULAR LYMPHOMA - A SINGLE INSTITUTION ANALYSIS

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Aggressive chemotherapy is capable of achieving complete remissions in patients with follicular lymphomas. However, the long-term benefit of such an aggressive treatment strategy is a matter of debate. We analysed the outcome of therapy in 86 patients with newly diagnosed centrocytic (CC) or centrocytic-centroblastic (CB-CC) lymphomas who were admitted to our institution between 1981 and 1990. Of these 86 patients, 12 received neither chemotherapy nor radiation for various reasons and 17 patients received radiotherapy alone (all patients with localized CB-CC lymphoma). Chemotherapy was administered to 16 patients with CC and 41 patients with CB-CC lymphoma. We used two types of chemotherapy: aggressive protocols (CHOP, COP-BLAM, CHOEP) or those with palliative character (COP, Mitoxantrone / Etoposide / PRED , Chlorambucil / PRED). The rates of remission were as follows: CC: 56 % CR 31, % PR, CB-CC: 56 % CR, 31 % PR. The remission rates did not differ in patients receiving aggressive or palliative chemotherapy. For patients with CC lymphoma, the median time to treatment failure was 22 months, there were no patients with durable remissions and there was no impact of the treatment strategy on time to treatment failure. Patients with CB-CC lymphoma showed a different outcome. The median time to treatment failure was 28 months for all patients who received chemotherapy, 46% of the patients remained in remission after a median observation time of 41 months. There was a trend to longer progression-free periods for patients who received aggressive chemotherapy: 60 % of the patients who received an aggressive treatment protocol and 36 % who received a palliative regimen were free from progression after an observation time of 60 months. The achievement of a complete remission was followed by longer survival for patients with CB-CC lymphoma. For patients with CC lymphoma, there was only a marginal impact of complete remission on survival. We conclude that an aggressive treatment approach is reasonable for patients with centroblastic-centrocytic lymphoma but questionable for patients with centrocytic lymphoma.

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DISSEMINATED INTRAVASCULAR COAGULATION (DIC) AND HYPERFIBRINOLYSIS IN METASTATIC PROSTATE CANCER

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DIC and secondary hyperfibrinolysis are known as severe complications of prostate cancer, which may be difficult to diagnose and a challenge in therapy. We treated 2 patients with metastatic prostate cancer for DIC and hemorrhagic phenomena.

Patient 1: A 72 year-old-man with metastatic, hormone refractory prostate carcinoma was admitted to our hospital with recurrent hematoma and epistaxis. Laboratory values were as follows: platelets 83/pl, prothrombin time (PT) 34 %, partial thromboplastin time (PTT) 34 sec., fibrinogen 0,45 g/l, fibrin monomeres positive, fibrin split products (FSP) 259 %, antithrombin III (AT III) 78 %. The patient was treated with replacement of fibrinogen, fresh frozen plasma (FFP) and administration of heparin 12000 IU daily iv. Doxorubicin 20 mg/m^2 weekly was started. During this therapy routine coagulation tests normalized and prostate specific antigen decreased significantly. 3 weeks later the patient was discharged. He is still on heparin sc. and chemotherapy in an outpatient setting.

Patient 2: A 68-year-old patient was referred to hospital with multiple ecchymoses and gastrointestinal bleeding. Bone marrow and prostate biopsy revealed adenocarcinoma. Laboratory data were as follows: platelets 70/pl, PT 30 %, PTT 75 sec., thrombin time 18 sec., fibrinogen 0,7 g/l, FSP 26 µg/ml, AT III 32 %, fibrin-D-dimeres 11 µg/ml, plasminogen 44 %, fibrin degradation products > 40 µg/ml. This man received combination chemotherapy and replacement of FFP, AT III and prothrombin complex. Because of persisting hemorrhage aprotinin and epsilon-aminocaproic acid were administered. After having stopped bleeding, this patient unfortunately died of fulminant pulmonary embolism caused by tumor cells.

In summary, our 2 patients with prostate cancer associated with DIC and secondary fibrinolysis emphasize the necessity of differential therapeutic approach. Appropriate management of patients with excessive hyperfibrinolysis and life threatening bleeding remains difficult and controversial. In contrast, chronic DIC without significant bleeding may respond to anticoagulative and antineoplastic therapy. Furthermore, endocrine refractory tumors could be successfully treated with weekly administration of doxorubicin.

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REPOPULATING CAPACITY OF PERIPHERAL BLOOD STEM CELLS MOBILIZED BY CHEMOTHERAPY AND G-CSF TESTED IN VITRO AND IN VIVO USING A SCID MOUSE MODEL

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Up to now, there is only scarce information concerning the capacity of PBSC to repopulate the bone marrow and to replicate upon retransplantation. In order to characterize the replicative potential of the most primitive, undifferentiated subpopulation contained in PBSC preparations of tumour patients mobilized by chemotherapy (Dexa BEAM with subsequent administration of filgrastim = G-CSF) mononuclear cells separated from their cytapheresis products were seeded onto confluent, irradiated allogeneic bone marrow stromal layers. The clonogenicity of cells produced by these two-stage stromal cultures increased up to ten times within 2 to 3 weeks of cultivation, and multiple distinct haematopoietic foci (cobblestone areas) developed. Compared to freshly isolated PBSC or to those kept in simple liquid culture for several weeks yielding mainly GM-CFU, the spectrum of CFU produced by our stromal cultures predominantly consisted of early BFU-E types, while the proportion of GEMM-CFU increased remarkably. Considerable interpatient variations could be observed with respect to both the onset and duration of PBSC hematopoiesis in allogeneic stromal cultures and to the clonogenicity of their non-adherent subpopulations as well.

CD34$^+$ and CD33$^+$ PBSC depleted of peripheral blood lymphocytes and monocytes were transplanted into scid mice by different routes. Starting 3 weeks after transplantation, their peripheral bloods and sera were screened for the appearance of both mature human WBC and human immunoglobulins over a period of several month.

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POSITRON EMISSION TOMOGRAPHY (PET): A NEW METHOD FOR EVALUATION OF MEDIASTINAL RESIDUAL MASSES AFTER TREATMENT OF LYMPHOMA.

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While morphologic methods provide information about residual masses following therapy, problems still exist in the reliable differentiation of a benign tissue (e.g. scar) and a residual tumor lesion. Therefore, we used a new, noninvasive, functional method, PET with fluorine-18-fluoro-2-deoxy-D-glucose (FDG) to gain quantitative information about the glucose metabolism of a questional mass. All patients (5 Hodgkin's lymphomas (HL) and 5 high-grade non-Hodgkin's lymphomas (NHL) had received previous chemotherapy (frequently multiple regimens, containing at least one anthracycline), seven of them had also received radiotherapy and two had undergone surgical debulking. All patients had a mediastinal residual lesion of an initial bulk, still visible in other imaging studies. CT was used to localize the largest lesion diameter immediately prior to the PET study. Following the administration of 111-440 MBq FDG three cross sections were acquired simultaneously for one hour. The regional glucose metabolism was determined by a region of interest technique. We noted a low FDG metabolism in two lesions, which were classified ás scars due to an unchanged volume in follow-up radiological studies. In 8 patients an increased FGD-activity was observed. In 3/8 patients a control PET after high-dose chemotherapy (HAM or Dexa-BEAM) and in 1/8 patient with additional irradiation a response was noted in the follow-up PET study, demonstrated by a decrease in FGD metabolism. Another patient died after progression of the suspicious findings and 4 patients are still to be followed up after therapy. Increased FDG-uptake has previously been shown to correlate with proliferative activity of malignant lymhomas. In lymphomas pretreated with chemotherapy and radiation therapy inflammatory reactions may limit an accurate differential diagnosis due to a moderately increased glucose metabolism. Therefore, metabolic studies should be perfomed at least 3 - 6 months after the end of the radiation therapy. The preliminary results show, that PET is a promising method for the differentiation of residual lesions in lymphoma patients.

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T-ALL THERAPY IN A PRECLINICAL MODEL: THE CD7 ANTIBODY TH-69 IS HIGHLY ACTIVE

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While more sophisticated chemotherapy has significantly improved therapeutic outcome in T-acute lymphoblastic leukemia, still a significant number of patients eventually succumb to their disease. Thus, therapeutic strategies for residual tumor cells, more easily detectable recently, are needed. Here, we describe a CD7 monoclonal antibody, TH-69, raised in our laboratory, which is active in T-ALL tumors xenotransplanted into nude mice. Mice bearing T-cell tumors of approximately 2g were treated by single injection intravenously of 500µg TH-69 antibody. Immunoscintigraphy of radiolabeled TH-69 as well as immunohistology revealed excellent targeting of the antibody to the tumor. Tumor growth stopped immediately, and within 7 to 10 days the tumor vanished completely. This extraordinary anti-tumor efficacy was dependent on the Fc-portion of the antibody being present. Subsequently, possible mechanisms of Fc-mediated tumor destruction such as complement binding, macrophage mediated cytotoxicity and apoptosis were investigated, and antibody binding kinetics as well as antigen expression during therapy was monitored. The data favor a complex killing pattern. Differences in efficacy of TH-69 to other CD7 antibodies are at least in part due to immunoglobulin isotype and binding affinity. Since TH-69 appears to be unusually active in its native form, it may well be useful also for treatment of human T-cell tumors.

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ONCOGENE EXPRESSION IN MYELOMA CELLS

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The oncogenes *c-myc*, *p53* and members of the *ras*-family have been implicated in plasmocytomagenesis. While activation of *c-myc* may be an early event, expression of mutated *p53* and *Ras*-proteins may reflect unfavorable courses of the disease. In order to analyse the role of individual oncogenes, their role in the signal transduction pathway and their potential regulation by cytokines we are studying 7 myeloma and plasma cell leukemia lines and native myeloma cells covering a broad range of terminal B cell differentiation. Since at least a part of the cell lines has the prerequisites for an autocrine IL-6 loop (see abstract of A. Villunger), native oncogene expression patterns should also reflect the influence of IL-6.

Immunoblotting experiments demonstrate the presence of p21 *Ras* proteins and their degradation products in 5 native myeloma cell samples, as well as in all myeloma cell lines. Preliminary results suggest the presence of *K-* and *N-Ras* in the majority of cell lines, while *H-Ras* seems to be absent. Although the presence of *Jun-B* is in concordance to the murine plasmacytoma model, the expression of *c-Jun-* and *c-Fos* proteins in all cell lines is in remarkable contrast to IL-6-induced signal transduction in murine plasma cells. While PHA-activated T cells reacted positively with anti-*c-Raf* abs *in situ*, the protein could not be detected in immunoblots of five native myeloma cell samples. Using a cocktail of 2 anti-*c-Myc* abs, this protein could be detected in all myeloma cell lines (including the U-266). The results suggest that the molecular effects of *Ras* are neither induced nor mediated by the *c-Raf-1* protein. Since *c-Jun* and *c-Fos* were constantly present in our myeloma cell samples, and the *c-myc* gene has an AP-1 binding site, they might participate in the induction of the *c-Myc* protein expression, which was always observed in parallel. Finally, growth control in myeloma cells may not only result from deregulated oncogene expression and autocrine stimulation by cytokines, but also involve the repression of apoptosis. In fact, *bcl-2* expression was a constant feature of myeloma cells. Taken together, these results underline differences of the murine and the human plasmacytoma model, the complex nature of the multi-step carcinogenetic process in myeloma cells and point to the existence of several molecular levels where potential interference with pathogenetic mechanisms might be attempted.

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ALLOGENEIC BONE MARROW TRANSPLANTATION IN NON-HODGKIN LYMPHOMAS WITH T(14;18) TRANSLOCATION

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Chromosomal translocations such as the t(14;18) found in 85 % of follicular low or intermediate grade (centroblastic-centrocytic,CB-CC) Non-Hodgkin's lymphomas (NHL) as well as in 30 % of high-grade B-cell lymphomas provide a tumor-specific molecular marker. The t(14;18) breakpoints are focused at one of six immunoglobulin (Ig) heavy chain joining regions on chromosome 14 and a small major breakpoint region of the Bcl2 gene on chromosome 18. Patients with lymphomas carrying the t(14;18) have a poor prognosis because of high relapse rates. Attempts to cure t(14;18) lymphomas include aggressive conventional chemotherapy and myeloablative chemoradiotherapy with bone marrow transplantation (BMT). Since the reinfusion of autologous marrow in these patients may give rise to relapse,we performed allogeneic marrow transplants in five patients who had HLA-identical siblings as marrow donors available. Three patients suffered of CB-CC NHLs and 2 patients of high-grade NHLs in 2. remission or subsequent relapse. All patients had a detectable BCL2-Ig rearrangement in their peripheral blood before BMT as assessed with polymerase chain reaction (PCR) amplification. Conditioning therapy consisted of total body irradiation and cyclophosphamide. 4/5 patients had trilineage engraftment and are in complete hematological remission 3 to 45 months (median 21 months) after BMT. No t(14;18) cells could be detected in both peripheral blood and bone marrow by PCR at a level of 1: 10^6 indicating that allogeneic marrow transplantation has the potential to eradicate t(14;18) cells resulting in long-term disease free survival in patients with otherwise poor prognostic Non-Hodgkin lymphomas.

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DETECTION OF A HRX-FEL FUSION TRANSCRIPT IN PRE-PRE-B-ALL WITH AND WITHOUT CYTOGENETIC DEMONSTRATION OF t(4;11)

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The t(4;11)(q21;q23) characterizes a subset of childhood and adult ALL with a distinct pre-pre-B-phenotype, monocytoid features and a dismal prognosis. The molecular correlate of the t(4;11) has been identified as a fusion transcript of HRX, a gene on 11q23, homologous to drosophila trithorax gene, and FEL, a serine-proline-rich gene on 4q21. The aim of the study was to establish a RT-pcr approach for the rapid and sensitive detection of the HRX-FEL fusion transcript associated with the t(4;11). For this purpose, two groups of patients were studied: group A: three infant and 7 adult pre-pre-B-ALL with cytogenetically demonstrated t(4;11). Group B: 10 pre-pre-B-ALL with the identical phenotype as group A, in whom t(4;11) was not demonstrated by cytogenetic analysis. Using primers complementary to HRX and FEL c-DNA sequences 300 to 500 bp 5' and 3', respectively, of published breakpoints, specific amplification products were obtained in 10/10 ALL of group A. Of the 10 pre-pre-B-ALL expressing CDw65 and/or CD15 without demonstration of t(4;11) by gross cytogenetic analysis (group B), seven patients displayed a specific fusion transcript detected by RT-pcr. In this group, all HRX-FEL negative patients were detected by RT-pcr. In this group, all HRX-FEL negative patients were CDw65 negative. Sizes of amplification products varied between >650 and <400 bp, suggesting differential splicing of fusion transcripts and potentially different breakpoints. In summary, RT-pcr for HRX-FEL fusion transcripts was concordant with conventional cytogenetics in 10/10 patients and may be positive in the majority of patients in whom t(4;11) was suspected based on pre-pre-B-phenotype and CDw65-expression, but was not demonstrated cytogenetically. This method may lead to a better characterization of high risk ALL at diagnosis and will be useful for monitoring of minimal residual disease in t(4;11) ALL.

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INDUCTION OF APOPTOSIS IN STEROID RESISTANT MYELOMA CELLS BY NEW ANITMETABOLITES

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Steroids play an important role in treating B-cell tumors like multiple myelomas. Even in tumor cells insensitive to alkylating agents and anthracyclines, steroids may prove effective in at least 30% of advanced cases. Most likely, this effect is exerted by induction of apoptosis. Resistance to steroids often indicates the terminal phase of disease. Thus, alternative agents with apoptotic efficacy are of high interest for future therapeutic interventions. We studied 5 myeloma cell lines originating from different stages of differentiation and phases of disease. Apoptosis was analysed by demonstrating endogenous endonuclease activity as defined by the presence of DNA-ladders on agarose gels. All cell lines proved sensitive to 10^{-6}M Dexamethasone, while only the U-266 and IM-9 cell lines were resistant to the lethal effect of steroids.
The antimetabolite 2-chlorodeoxyadenosine (2-CdA) displays promising acitivity against many tumor types, among them B-CLL cells. *In vivo* achievable concentrations of 300 nM may induce apoptosis *in vitro*. After a 48 hr-incubation, the U 266 and IM-9 myeloma cells - both resistant to Dexamethasone – were killed by 2-CdA. A DNA degradation pattern typical of apoptosis appeared. Since the cytotoxic effects of 2-CdA on myeloma cells are disappointing *in vivo*, 2`,2`-difluorodeoxycytidine (gemcitabine), a new antimetabolite, was tested and shown to induce apoptosis. Our results suggest that 2-CdA and gemcitabine could play an important role in the treatment of steroid resistant malignancies and that they exert their effect via induction of apoptosis.

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COMPETITIVE PCR FOR QUANTITATION OF HUMAN *MDR1* GENE EXPRESSION

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Tumor cell resistance to cytotoxic drugs is considered as one of the major obstacles to successful chemotherapy. Multidrug resistance (MDR) describes the simultaneous expression of cellular resistance to a wide range of structurally and functionally unrelated drugs. The development of multidrug resistance is accompanied by multiple biochemical and morphological changes. Only one of these changes consistently occurs in all MDR cells: the increased expression of the (human) *MDR1* gene, which encodes a transmembrane efflux pump (P-glycoprotein). This protein leads to decreased intracellular accumulation and therefore to resistance to a variety of cytotoxic drugs.
Here we describe a competitive PCR system for absolute quantitation of *MDR1* mRNA. This assay makes use of an *in vitro* generated transcript as internal standard, which is later coamplified together with the *MDR1* cDNA. Both cDNAs exhibit the same *MDR1* primer sites, but differ in the length of the amplicon. This highly sensitive and specific diagnostic test for *MDR1* expression seems to be of great clinical relevance, to improve monitoring and design of chemotherapy.

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LONG-TERM FOLLOW-UP OF PATIENTS WITH HAIRY CELL LEUKEMIA - DETECTION OF MINIMAL RESIDUAL DISEASE

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Bone marrow biopsies were analysed in 6 patients with hairy cell leukemia (HCL) treated with 2`-deoxycoformycin (Pentostatin) according to a phase II trial of the EORTC Leukemia Cooperative Group. Within the observation period of 15,5-54,0 (median 47,0) months after discontinuation of therapy, bone marrow biopsies were analysed consecutively with three different methods including conventional histology, immunohistology and immunoglobuline gene rearrangement.
For immunohistological analysis as well as for DNA extraction and Southern blotting 47 bone marrow biopsies were available. Applying the MoAb B-ly7, HCs were found in all of these biopsies. Pretreatment values of HCs ranged from 50-80% of bone marrow cells. Nadir values accounted for 0,2-3% of bone marrow cells 7-15 months after start of pentostatin treatment. By last follow up after termination of treatment HCs had increased in all patients to 1,6-17,5% of bone marrow cells. Hybridized with a JH-probe the B-cell clone was detected in all pre-treatment biopsy specimens and in 27% of the 41 follow up samples. The percentage of HCs in these patients ranged from 3,5-45%. The 30 biopsies without detectable B-cell clones in Southern blot analysis contained 0,2-4,7% B-ly7 positiv HCs. In conclusion, this paper demonstrates that the application of immunohistology combined with immunoglobuline gene rearrangement analysis of bone marrow sections represents a powerful combination for studying the efficiency of the different treatment modalities in HCL.

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IMMUNOGLOBULIN GENE REARRANGEMENT ANALYSIS FACILITATES DIFFERENTIATION OF PRIMARY AND SECONDARY CUTANEOUS B-CELL LYMPHOMAS.

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The cutaneous involvement of malignant B-cell lymphomas has been regarded as a clinical sign of progression and dissemination of the disease. In contrast recent reports described a group of B-cell lymphomas of follicular center origin with disease restricted to skin and with a favorable prognosis. A strict discrimination between primary and secondary involvement of skin is necessary to project the most promising form of treatment.
In order to investigate if the detection of monoclonal B-cells in peripheral blood (pb) is of prognostic significance and to discriminate primary from secondary lymphomas we analysed skin, pb and bone marrow (bm) samples for Ig gene rearrangement. These results were compared to morphological, immunohistochemical and clinical data.
Three out of eight patients with "primary" cutaneous follicular lymphomas demonstrated a clonal rearrangement pattern in skin, pb and bm cells, detected with a JH-probe by Southern blot analysis. All patients with clonal B-cells in pb had a history of cutaneous relapses, in contrast to the patients without rearrangement in pb. Our study shows that the detection of clonal rearranged B-cells in pb runs parallel with bm infiltration by lymphoma cells. We conclude that the detection of clonal rearranged B-cell in pb enables to differentiate between primary and secondary lymphomas and is of great value for diagnosis, treatment and prognosis.

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CYTOGENETIC FINDINGS IN 533 PATIENTS WITH DE NOVO AND SECONDARY ACUTE NONLYMPHOCYTIC LEUKEMIA

H. Gudat, D. Haase, F. Hild, M. Kröger, H. Rieder, U. Kolbus, U. Koop, and C. Fonatsch

Chromosome analyses were performed in 533 patients with ANLL. In 70 patients the leukemia was secondary. Clonal karyotype abnormalities were found in 57.6% cases (n=307).The leukemias were morphologically classified according to the FAB-criteria. Aberration rates among the FAB-subtypes were as followes: M0(n=2) = 50%, M1(n=63) = 55%, M2(n=115) = 59%, M3(n=28) = 75%, M4(n=124) = 56%, M5(n=51) = 47%, M6(n=30) = 70%, M7(n=11) = 48%. The most common chromosomal anomaly was trisomy 8 with 67 cases (12.6% of all patients), followed by -5/5q- (10.1%) and -7/7q- (6.2%). The translocations t(8;21)(q22;q22), t(15;17)(q22;q21) and t(6;9)(p23;q34) occurred in 29, 18, and 5 cases, respectively. An inversion inv(16) was present in 28 patients. An inversion inv(3)(q21q26) or translocation t(3;3)(q21;q26) was found in 18 cases. The short arm of chromosome 12 and the chromosome band 11q23 were affected in 10 and 13 patients, respectively. Complex chromosomal abnormalities occurred in 76 patients (14.2%). The association of karyotypic changes with morphology, prognosis, and the primary or secondary character of the leukemias will be discussed in detail.

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Effectivity and toxicity of empiric interventional therapy for febrile neutropenic patients with hematological diseases.

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We investigated an antibiotic combination of beta lactams and aminoglycosides in regard to effectivity and toxicity which we used since 8 years. In a prospective unicentric study were 69 febrile episodes (duration of neutropenia 4-54 days; median 22) of 49 patients with hematological diseases eligible. 48/69 (69%) patients received antibiotic prophylaxis with trimethoprime/sulfonamide or ofloxacin/ciprofloxacin and a systemic antifungal prophylaxis with ketoconazole or fluconazole. All patients received penicillins (azlocillin n=44; piperacillin n=25) in combination with amikacin (loading dose 10 mg/kg BM; further 7,5 mg/kg BM twice daily). There were 30/69 (44%) microbiologically documented, 23/69 (33%) clinical infections and 16/69 (23%) FUO. We isolated 25/30 (83%) grampositive cocci (17/68% CNS) and 5 gramnegative pathogens. Treatment was succesfully in 56/69 (81%) periods. 7/69 (14,3%) patients died, 4 by leucemia and 3 by fungal pneumonia. The duration of therapy was 8 days (range 5-11). No new infections were seen by patients with ongoing neutropenia after withdrawal of antibiotic therapy (42/69; 61%). The therapeutic level of amikacin (20 µg/l) was mostly reached only with the initial loading dose. Adverse reactions were observed under azlocillin (8/44; 18%; allergic-toxic exantheme), under amikacin (4/69; 18%; nephrotoxicity) in patients with renal impairment but not under piperacillin.

Summary:

1. The combination therapy of penicillins and amikacin is effective even for years against infection in neutropenia.
2. Using prophylaxis and short course of interventional therapy may prevent superinfection and resistance.
3. A prompt clinical effect may be obtained by initial high doses of amikacin correlating with adequate but nontoxic serum concentrations.
4. A premature support by antifungal agents is necessary in pneumonias.
5. In the group of penicilllins piperacillin did not show adverse reactions.

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SYMPTOMATIC PANCREATITIS IN PATIENTS AFTER BONE MARROW TRANSPLANTATION

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Patients who undergo high dose chemotherapy and following bone marrow transplantation are often suffering from nausea, vomiting and abdominal pain of unknown origin. These side effects are mostly attributed to direct toxicity of the chemotherapeutic agents and toxic mucositis of the upper gastrointestinal tract during the aplastic phase. In a recent analysis of 102 consecutively transplanted bone marrow recipients (allogeneic 58, autologous 32, autologous PBSCT 9, syngeneic 2) we found increased levels of amylase and lipase consistent with pancreatitis (pancr.) in 22 of 102 patients. These enzymatic changes coincided with the above mentioned clinical symptoms. The incidence of pancreatitis was significantly increased in patients who had received a conditioning regimen consisting of busulfan, cyclophosphamide and VP-16 compared with other conditioning regimens (39,4% vs 10,6% in otherwise treated patients, p< 0.0001, see table). In addition we found that the mean duration of increased amylase and lipase levels was longer in patients with GvHD of the gut than in patients without GvHD (22 days vs 4,25 days, p<0,01).

chemotherapy	n	pancr.	%
BU/CY/VP16	33	13	39.4
CBV	20	2	10
TBI/CY/VP16	24	2	8.3
BU/CY	13	3	23.1
ATG/CY	5	1	20
CTM	6	1	16.6
TBI/CY/ATG	1	0	0
total	102	22	21.6

We conclude that acute pancreatitis may be a major cause of nausea, vomiting and abdominal pain after high dose chemotherapy and subsequent bone marrow transplantation. The etiology of the pancreatitis seems multifactorial. Early onset pancreatitis might in part be caused by chemotherapeutic agents used for conditioning. After engraftment GvHD of the gastrointestinal tract may be a predisposing factor.

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INVERSION OF THE PATERNAL-DERIVED CHROMOSOME 16 IN AML-M4EO

O.A. Haas and M. Neuwirth

Functionally equivalent genetic material can be marked and utilized differentially depending upon its maternal or paternal origin. This phenomenon is known as genomic imprinting and has been shown to play an important role in certain cancer predisposition syndromes and sporadic tumors characterized by loss of genetic material. Utilizing chromosome banding polymorphisms our group recently showed that in leukemias with a t(9;22) the translocated chromosome 9 is generally of paternal and the translocated chromosome 22 of maternal origin (Haas et al., Nature 359:414-416,1992).

The heterochromatic block of chromosome 16 varies also in size and location and these polymorphisms are inherited in a Mendelian fashion. We have therefore studied the track of inheritance of the inverted chromosome 16 in seven patients with acute myelomonocytic leukemia (AML-M4Eo) and their respective parents based on such heterochromatin banding polymorphisms. Four out of seven cases had an informative polymorphism and in all of them the inversion affected the paternal derived chromosome. Our data therefore provide the first evidence that imprinting phenomena may play an important role in the generation of this acquired leukemia-specific chromosome rearrangement.

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PERIPHERAL BLOOD STEM CELL TRANSPLANTATION (PBSCT) IN NON-HODGKIN'S LYMPHOMA (NHL)

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Since May 1985, 37 patients with NHL (18 high-grade / 19 low-grade) were autografted with PBSC following high-dose conditioning therapy. The median age was 38 (22-58) years. In the majority of patients, PBSC were mobilized with hematopoietic growth factors administered either during steady-state hematopoiesis or after cytotoxic chemotherapy. Compared with bone marrow, the blood-derived autografts were characterized by a significantly lower content of CD19+ B-cells, which may contain clonogenic tumor cells. High-dose conditioning therapy consisted of TBI (14.4 Gy)/cyclophosphamide (200 mg/kg) in 24 pts., whereas 12 pts. received the BEAM-protocol and 1 pt. high-dose Ara-C/VP-16. One treatment-related toxic death occurred.

At the time of PBSCT, 13 patients with high-grade NHL were in partial or complete remission. Six of them are disease-free with a median follow-up of 14 (8-99) months. The 5 patients with high-grade NHL autografted in progressive disease did not improve. Of the 19 patients with low-grade NHL, 17 are in continuing remission with a longest follow-up of 35 months.

13 of the 19 patients with low-grade NHL were autografted with G-CSF mobilized PBSC in an "up-front" approach (in first remission or first chemosensitive relapse). All of them received autografts containing more than 2.5 x 10^6/kg b.w. CD34+ cells and achieved rapid trilineage engraftment with a median time of 12 days to reach 0.5 x 10^9/l neutrophils and 10 days for 20 x 10^9/l platelets. No hematopoietic growth factors were given post-transplantation. Successful mobilization of hematopoietic stem cells and low treatment-related toxicity reflects recruitment of patients at a time when the hematopoietic reserve is not compromised by repeated cycles of chemotherapy. In heavily pretreated patients with poor prognosis the toxicity risks may outweigh the benefits of high-dose therapy. We conclude that PBSCT is more appropriate in patients who have been less extensively pretreated.

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CYTOGENETIC RESPONSE TO MYELOID GROWTH FACTORS IN MDS AND ANLL

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We studied the impact of G-CSF, GM-CSF and IL-3 on proliferation and clonal composition in MDS and ANLL. An assessment of mitotic responses to GM-CSF yielded an increased response in ANLL as compared to normal controls and a decreased response in MDS and AA. While bone marrow cultures from 2 MDS patients with 5q- showed no response to GM-CSF, we found a highly significant in vitro growth advantage for monosomy 7 cells under GM-CSF incubation in a patient with RAEB-T. In a recent sequential cytogenetic study of 13 patients with MDS treated with GM-CSF, we observed one patient with monosomy 7 who displayed a rapid clinical and cytogenetic progression. Recently, a Japanese group observed a high rate of transformation to MDS in pediatric patients with AA after treatment with G-CSF. All patients with transformation to MDS showed monosomy 7 (Kojima, 1992). We observed a comparable association of G-CSF therapy, monosomy 7 and leukemic transformation in a patient with Kostman's syndrome. To examine the influence of IL-3 on cytogenetically defined cell populations, bone marrow cultures from 7 patients (4 MDS, 3 ANLL) with a mosaic of normal and abnormal cells were analyzed. In all 7 patients, independent from diagnosis and chromosomal abnormality, normal cells seemed to gain a proliferative advantage by stimulation with IL-3.

Cytogenetics might help to identify patients with specific CSF-response especially, when the problem of leukemic stimulation and priming strategies are addressed.

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Improved Quality Control, Intra-Hospital Communication and less Manpower Requirement with "Oncobase" in a Central Hospital

Habertheuer KH, Kier P, Ruckser R, Höniger S, Mandl A, Sterz M, Hinterberger W

Organization of chemotherapy administration to in- or out patients with malignancies requires EDV-support. "Oncobase", originally deviced by KH Habertheuer as a one-place-version (at the hospital of Bregenz) has been adapted under dBASEIV for VMS into the network of the newly built "Donauspital" on May 1st 1992. We report the one year experience with "Oncobase":

1. "Oncobase" properly supports communication between hospital pharmacy (where all cytostatics are prepared), ward, ambulance and doctor's rooms.
2. "Oncobase" provides written information exchange between doctors involved. Medical letters contain space for free text particles for, e.g., patient history and physical examination. Initial experience shows that physicians prefer self-input of history and physical exam instead of dictating to a secretary. Normal findings are automatically printed. Due to rapid access to patient's charts on ward, ambulance, secretary and doctor's room, the former time consuming "hide and seek" of medical charts, letters and results is no longer observed.
3. Since chemotherapies are increasingly given to outpatients, accompanying letters including all drugs prove helpful.
4. "Oncobase" informs to the Austrian "Krebsregistermeldeblatt".
5. "Oncobase" gives immediate information on cumulative drug doses, side effects and blood cell kinetics after previous therapies and allows planning of further chemotherapy.
6. Oncobase" permits assessment of costs of chemotherapy.

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TREATMENT OF MULTIPLE MYELOMA WITH VAD IN HEMATOLOGICAL OUTPATIENTS

U. Hahn, T. Munk, R. Haas and H. Goldschmidt

Patients and Methods

Between January 1992 and March 1993 16 patients with multiple myeloma (MM) stage III (Salmon & Durie) were treated with VAD. 6 were female and 10 were male. The median age was 56 years (range 44 - 68 years). 11 had disease refractory to alkylating agents, 4 had severe neutropenia (<0.5/nl), 1 developed BCNU pneumonitis, another a severe allergic rash following cyclophosphamide. The 16 patients received a total of 73 cycles of VAD. 9 were treated as outpatients from the outset of therapy. VAD was administered according to the protocol first published by Barlogie et al. A solution of vincristine and doxorubicin was filled into the reservoir of battery-driven infusion pump. The administration was strictly via a central venous catheter. In the majority of the patients the internal jugular vein was used, due to the very low complication rate following sonographical marking of the puncture site.

Results

According to the remission criteria of the "German Myeloma Treatment Group" 50% of the patients achieved CR after a median of 4 cycles of VAD. The median duration of remission was 6 months. 20% had PR, 10% had stable and 20% progressive disease. We did not experience severe hematological toxicity. Neither diabetes mellitus requiring insulin during high-dose dexamethasone therapy, nor major complications due to chatheterization were observed.

Discussion

As outlined by different authors a response to treatment with VAD was seen in 70%. However the main progress lies in the possibility of administering VAD on an outpatient basis by using an infusion pump. Consequently patients were less hospitalized and had a better quality of life. In order that a weekly physical examination and routine laboratory tests can be performed, patients should live in the same geographical area.

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CHRONOPHARMACOLOGY OF MITOXANTRONE (MX): EVIDENCE FOR A CIRCADIAN RHYTHM OF ITS MYELOTOXICITY FROM AN ANIMAL MODEL AND A CLINICAL PILOT TRIAL

M. Hallek, F. Lévi°, I. Langenmayer, I. Kammermeier, and B.Emmerich

The dosing time of anticancer agents largely affects both the tolerance and the antineoplastic activity of cytostatic drugs. Considerable circadian variations of these drug effects have been reported for anthracylines such as daunorubicin, doxorubicin, epirubicin and 4'-0-tetrahydropyranyladriamycin. This led us to investigate the chronopharmacologic properties of MX, an anthraquinone with similar properties. Our investigations were conducted in an animal model and a clinical pilot study. 144 $B_6D_2F_1$ mice which were synchronized by standardized 12h:12h light-darkness cycles, were injected a single, potentially lethal i.v. dose of MX (13, 14.5, or 16 mg/kg) at six different time points (8 animals per time point). All mice receiving 13 or 14.5 mg/kg MX survived, if the drug had been injected at 3, 7, 11, or 19 hours after light on (HALO), but 25% died if the same doses had been given at 23 HALO. The administration of 16 mg/kg MX at 3 or 7 HALO respectively killed 100% or 80% of mice, as compared to none (!) following drug dosing at 11 or 15 HALO (X^2= 40; $p<0.001$). In subsequent experiments, 99 $B_6D_2F_1$ mice were injected 14.5 mg/kg at four different time points, and the body weight, WBC, or spleen weight were determined as compared to baseline levels after 5, 9, 14 or 21 days. On d 5, leukopenia varied from -84% at 4 HALO to -34% at 22 HALO, and spleen weight loss varied from -34% (22 HALO) to -83% (14 HALO) ($P<0.01$). On d 9, where the maximal body weight loss occured, this variable was least at 16 HALO (-28%), as compared to -36% at 22 HALO ($P<0.01$). We then conducted a clinical pilot study in 9 cancer patients receiving a combination chemotherapy with MX (8 mg/m^2/d IV, d 1+2) and prednimustin (PM; 100 mg/m^2/d PO, d 1-5). While PM was constantly given at 8h00, the dosing time of MX was changed after each treatment course in each patient (2h00, 6h00, 10h00, 14h00, 18h00, 22h00). Again, a small, but significant circadian variation of the leukocyte nadirs was observed with highest counts at 22h00 and lowest counts at 6h00 (difference 20%; $P<0.05$; Wilcoxon). Taken together, both preclinical and clinical data suggest that the toxicity of MX may be significantly reduced by correct circadian timing of its administration. MX seems to be best tolerated in the second half of the activity span.

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SERUM THYMIDINE KINASE (S-TK) LEVELS ARE ELEVATED IN PATIENTS WITH OVARIAN CANCER AND EXHIBIT IMPORTANT CIRCADIAN VARIATIONS

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Deoxythymidine kinase (TK) is a cellular enzyme involved in a 'salvage pathway' of DNA synthesis. s-TK levels have been shown to reflect the proliferative activity of a variety of tissues and neoplasias including acute and chronic leukemias, Hodgkin's disease, small cell lung cancer, and brain tumors. Elevated s-TK levels seem to predict a poor prognosis in patients (pts) with non-Hodgkin's lymphoma. The value of s-TK in ovarian cancer has not been investigated so far. We therefore investigated this proliferation marker in 14 pts (mean age 56.1 ± 8.0 yrs) with ovarian cancer (stage IC-IV). Because preliminary analyses of cancer pts with different neoplasias had shown that s-TK levels may exhibit considerable diurnal fluctuations, we additionally monitored s-TK and serum CA 125 levels by repeated determinations every 4 h over a 24-h time span.

S-TK levels were elevated at least once over the 24-h period in 10, and CA 125 levels in 11 of 14 cases. All 4 pts with normal s-TK values (≤ 4.7 U/L) had elevated CA 125 levels indicating that these two parameters may reflect independent biological characteristics of the tumor. Both s-TK and CA 125 levels showed considerable diurnal changes over the 24-h period in some pts. For s-TK, the individual peak-trough differences ranged from 0.1 to 8.5 U/L or from 8% to 268%, and for CA 125 from 4 to 125 U/mL or from 11% to 130%, respectively. Peak-trough differences of s-TK were ≥100% in 5 pts. Retrospectively, a single daily determination would have given a "normal" result in 8 of 14 pts for TK, and in no pt for CA 125. The circadian variations of s-TK and CA125 did not show any correlation, nor could any regular pattern (e. g. sinus-wave form) be observed. These results demonstrate that s-TK levels are elevated in the majority of pts with ovarian cancer. Both s-TK and CA 125 levels may exhibit considerable, irregular diurnal fluctuations. Repeated determinations should therefore be performed in situations where these markers are relevant for patient monitoring.

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ADDITIONAL MODULATION OF 5-FLUOROURACIL (5-FU) BY INTERFERON (IFN) α-2B (INTRON A®) IN ADVANCED COLORECTAL CANCER (CRC) REFRACTORY TO 5-FU AND FOLINIC ACID (FA)

E.Hansmann, G.Grüntjens-Prinsen, K.Bremer, J.Kemper

IFN α-2b is added as 2. step of combined treatment of CRC-pts refractory to 5-FU and FA alone. We are giving 3-5 x 10^6 IU IFN s.c., 400 mg FA i.v., 750-1000 mg 5-FU 1 hr later as 2 hr infusion, d 1-5, q d 28. From 11/89 to 4/93 49 CRC pts with progressive visceral metastasis refractory to 5-FU and FA alone were enrolled in an ongoing pilot study. Pt characteristics include: median age at time of diagnosis 61 (39-79) y; male/female=30/19; baseline PS (WHO) - 0:6 pts, 1:37 pts, 2:4 pts, 3:2 pts. Primary sites of disease: colon: 31 pts, rectosigmoid: 4 pts, rectum: 12 pts, synchronous CRC:2 pts. Sites of metastatic disease -liver (39), lung (11), locally unresectable or recurrent (9), lymph nodes (16), bone (1), soft tissue (1), peritoneum (6), other sites (5). Number of metastatic sites-1:20 pts, 2:14 pts, 3:12 pts, 4:2 pts, 5:1 pt. Time from diagnosis to metastasis (DFI) 0 (0-64) mos. 25 pts presented metastatic disease at time of diagnosis. Time from detection of metastasis to onset of FA/5-FU treatment - 1(0-29) mos. Response to FA/5-FU alone - 3 PR, 22 NC, 24 PD. TTP to FA/5-FU=onset of FA/5-FU/IFN α-2b treatment - 4 (1-29) mos. Most common toxicity in FA/5-FU/IFN treated pts is grade 2 diarrhea in 21 pts treated by loperamid-hcl or tincture opium. Stomatitis seems to be diminished in many pts by allopurinol mouthwashing. Acute IFN related flu-like symtoms are abolished by paracetamol. Median CEA (35 ng/ml)-, CA 19-9 (64 U/ml)- and LDH (198 U/l) levels did not change significantiy during FA/5-FU containing treatment protocols. The overall response in 49 pts progressive to FA/5-FU alone to additional IFN has been 25 NC and 24 PD. Progressive at once were 14 pts to FA/5-FU and following FA/5-FU/IFN. Median time to progression (TTP) is 2 (1-17) mos. In 37 pts with progressive disease Mitomycin has been added +/- IFN. 19 pts refractory to i.v. chemotherapy received hepatic artery infusion (HAI) in case of dominant liver metastasis (17) or bilateral iliac artery infusion in 2 pts with recurrent rectal cancer. 39/49 pts died because of tumor progression, 2 of them caused by cardiac infarction. Median survival time is 17 (3-68) mos. Though there are only a few objective responses, median survival time of 17 mos did not differ significantly from recently reported survival times of other i.v. or i.a. treatment modalities of advanced CRC pts. Only 11/42 pts with advanced CRC are alive after 2 years and only 6 pts for more than 3 years. The impact on survival prolongation of all palliative chemotherapeutic regimens for advanced CRC is marginally. In further studies we have to consider by quality of live assessments, if there is a real benefit in live gained for our pts.

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INTERFERON (IFN) α-2B (INTRON A®) TREATMENT IN ADVANCED RENAL CELL CARCINOMA (RCC)

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Between 12/88 and 4/93, 21 histologically proven RCC pts with progressive metastatic disease received IFN α-2b. IFN was given at weekly doses of 70 (35-110) x 10^6 IU s.c. adjusted according to individual tolerance and response. Median duration of IFN treatment is 4 mos (1-21). Pt characteristics include: median age at time of diagnosis 56 (36-76) yr. Male/female = 17/4. 19 with prior nephrectomy. Time from diagnosis to metastasis (DFI) 3 (0-185) mos. 10 pts presented metastatic disease at time of diagnosis. Sites of metastatic disease - lung (11), locally advanced with involvement of juxtaregional nodes (4), liver (2), bone (8), soft tissue (3), asynchronous bilateral RCC (1). Number of metastatic sites - 1:11 pts, 2:8 pts, 3:2 pts. 2 pts were with sarcomatoid-type RCC. Baseline PS (WHO) - 1:7 pts, 2:12 pts, 4:2 pts. In 21 RCC pts evaluable for response we observed only 3 (14%) partial responses (5, 16 +, 35 +). 2 pts showed a minor response and another 2 pts mixed response with more than 50% reduction in lung metastasis for 4 and 20 mos respectively, with progressive bone metastasis in the first pt. The 2. pt with mixed response developed brain metastasis 14 days after complete disappearance of lung metastasis, successfully treated with palliative brain irradiation. 7 pts have been withdrawn from IFN treatment because of asthenia. 10 pts had stable disease and 3 pts progressive disease on IFN therapy. Median time to progression is 7.5 (0-35 +) mos. 14 pts died. The median survival since onset of IFN treatment is 13 (4-35 +) mos and the MST since detection of metastatic disease is 17 + (3.5-43) mos. Since the median survival in pts with metastatic RCC is approximately 10 mos (Figlin et al.: Semin Oncol Vol 18, No 5, Suppl 7, 1991 : 102-107), IFN treatment may marginally improve survival of pts with metastatic RCC.

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CHROMOSOMAL ABNORMALITIES AND PROGNOSTIC MEANING OF HYPERDIPLOIDY >50 IN CHILDHOOD ACUTE LYMPHOBLASTIC LEUKEMIA (ALL)

A cooperative study of the International BFM Study Group (I-BFM-SG)

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Hyperdiploidy with more than 50 chromosomes is known as a specific chromosomal abnormality of childhood ALL, indicating a good prognosis. As different types of aberrations (special combination of trisomic chromosomes or structural aberrations) in this group might also be of prognostic value, a cooperative study of five European cytogenetic centres was initiated.

Up to now the data of 148 children with a completely banded karyotype were collected and the comparison showed a high degree of concordance for all types of data: The modal chromosome number ranges mainly between 52 and 57, and the chromosomes #4, #6, #10, #14, #17, #18, #21, and the X-chromosome are most frequently found to be tri- or even tetrasomic. Most of the patients are 1 - 4 years old, have a low WBC (<15,0 x 10^9/l), and have an immunophenotype of c-ALL. The risk factor, however, is higher than 0.8 in $^2/_3$ of the children, and the DNA-index is less than 1.16 in 25 %.

As hyperdiploidy >50 may play an important role in new therapy protocols, the prognostic value of this type of chromosomal aberration and all methods to detect this group of patients have to be evaluated.

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PHASE II TRIAL OF A DOUBLE BIOCHEMICAL MODULATION OF 5-FU BY PALA AND MTX IN ADVANCED PANCREATIC CARCINOMA.

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Phosphonacetyl-l-aspartate (PALA, US Bioscience) and methotrexate (MTX) are both capable to increase the cytotoxic potential of 5-FU, mainly by facilitating the incorporation of 5-fluorouracil triphosphate into cellular RNA. Based on these rationales we initiated a phase II-trial of the combination of PALA, MTX and 5-FU in advanced pancreatic adenocarcinoma. The treatment schedule was: PALA 250 mg/m^2 i.v. day 1; methotrexate 200 mg/m^2 i.v. day 1, followed by oral leucovorin rescue (15 mg/m^2 x 8, days 2 and 3); 5-FU 600 mg/m^2 i.v., day 2. Cycles were repeated every two weeks until progression; dose escalation of 5-FU up to 800 mg/m^2 was attempted as permitted by toxicity. PATIENTS CHARACTERISTICS: 29 patients with advanced or metastatic adenocarcinoma of the pancreas have been entered, 23 are evaluable. 19 male, 10 female; median age 58y (38-72), median PS 1 (0-2).

RESULTS: The median number of cycles per patient was 4 (2-16). Toxicity was moderate with mucositis ≥ WHO grade 2 in 6 patients, diarrhea ≥ WHO grade 2 in 7 patients, mild nausea and vomiting in 13 patients. 1 patient had WHO grade 3 renal toxicity following methotrexate. No significant hematological toxicity and no infections were seen. 23 patients are currently evaluable for response (2 too early; 4 received less than 1 complete cycle). There were 2 PR (9%), 13 MR/NC (56%) and 8 PD (35%). Median time to progression was 12 weeks (4-32 weeks).

CONCLUSIONS: The double modulation of 5-FU by PALA and MTX was well tolerated with no unexpected toxicities seen. The anti- tumor activity of the three drug combination used in this dose and schedule was not significantly different from 5-FU alone. The development of new treatment strategies for advanced pancreatic carcinoma is clearly needed.

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HIGH ACTIVITY OF N-BENZYLADRIAMYCIN -14- VALERATE (AD 198), A NEW ANTHRACYCLINE NOT TRANSPORTED BY P-GLYCOPROTEIN, IN MULTI-DRUG RESISTANT HUMAN OVARIAN AND BREAST CARCINOMA CELL LINES.

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Multidrug resistance (MDR), caused by an overexpression of P-glycoprotein, a drug efflux pump, confers resistance to a variety of natural cytotoxic agents and constitutes one major obstacle to successful cancer chemotherapy. N-benzyladriamycin -14-valerate (AD198) is a new, highly lipophilic anthracycline analog, specifically designed to circumvent MDR. Activity of AD 198 was demonstrated in MDR-human and murine leukemias, however no data are available about the efficacy of this new drug in multiple drug resistant solid tumors.

MATERIALS AND METHODS: Multiple drug resistant sublines were derived from the human breast carcinoma line MCF7 and the human ovarian carcinoma line A 2780. All resistant lines overexpress the P-glycoprotein as shown by immunocytochemistry and Western blot; the two multidrug resistant ovarian carcinomas additionally have a marked increase in cellular glutathione and glutathione-S-transferase activity. Cytotoxicity was assessed in vitro by the sulforhodamine -B-assay; all drugs were given continously for 96 h.

RESULTS: The cytotoxicity, expressed as the IC 50 (concentration to inhibit cell growth by 50%) is shown below:

IC 50 (µM; 96h continous exposure) *RF = resistance factor

	Doxorubicin	RF*	AD 198	RF*
MCF7	0.02	-	0.06	-
MCF7AD	2.5	125	0.15	2.5
A 2780	0.009	-	0.008	-
A 2780 Dx1	0.03	3.5	0.03	4
A 2780 Dx5	0.6	66	0.07	9

Coincubation with the P-glycoprotein blocking agent cyclosporine A (2 µg/ml) significantly increased the activity of doxorubicin in MCF7 AD (dose modifying factor of 10) whereas no effect was seen for AD 198 (dose modifying factor 1,2).

CONCLUSIONS: AD 198 shows high activity in multidrug resistant human ovarian and breast carcinoma cell lines. Obviously it is not a substrate for P-glycoprotein. Increased levels of glutathione or glutathione-metabolising enzymes as expressed in A 2780 DX1 and DX5, might still confer some resistance to AD 198. This new agent might be an attractive way to clinically circumvent the problem of multiple drug resistance.

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ERYTHROPOIETIN THERAPY IN MYELODYSPLASTIC SYNDROMES (FAB-TYPE I AND II): A PHASE II STUDY

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Anemia in patients with myelodysplastic syndromes (MDS) is usually referred to ineffective erythropoiesis, although the contributory role of other mechanisms so far is not sufficiently understood. Evidence has been found, that erythropoietin in some cases of MDS is able to restore anemia to a certain extent. Especially in cases of MDS with a relatively good prognosis such as Refractory anemia (RA) and Refractory anemia with Ringsideroblasts (RARS) a benefit may be expected for patients treated with erythropoietin since erythrocyte-transfusion and its associated risks could be spared or at least markedly reduced.

In a phase II-study a total of 21 patients was recruited, 19 were evaluable. 8 patients with RA and 11 patients with RARS were initally treated with 5000 IE erythropoietin s.c. for 4 weeks daily. 8 patients were male, 11 female, median age was 73 years. In case of response treatment was continued up to 24 weeks, in case of nonresponse the erythropoietin-dose was augmented to 10000 IE for further 4 weeks. If there was still no response treatment was stopped, otherwise continued up to 24 weeks. Inclusion criteria were among others anemia below 10 g/dl, no evidence of bleeding and a life expectancy above 6 months. Treatment response was defined as an increase of haemoglobin for at least 2 g/dl or a major reduction in transfusion rate. Two patients, one with RA, the other with RARS, responded to treatment with 10000 IE s.c. per day. No major side effects were seen. In the responding patients endogenous erythropoietin levels prior to therapy were markedly increased (up to tenfold = +/- 200 mU/ml). Thus in some MDS-patients with good prognosis erythropoietin might serve as a save treatment option for anemia.

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NEUROLOGICAL AND PSYCHIATRIC ADVERSE EVENTS IN IFN-α TREATED PATIENTS WITH CHRONIC MYELOGENOUS LEUKEMIA - RESULTS OF THE GERMAN CML-TRIAL

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The most common adverse events under IFN-α treatment are flu like symptoms. In the last time some severe psychatric symptoms (e.g. depressions) have been reported by different working parties.

The German CML-trial is a multicentre three-arm randomized clinical trial. 622 patients from 60 centres in Germany and Switzerland were randomly allocated to either IFN-α (164), Busulfan (226) or Hydroxyurea (232).

Since there was only very limited experience in the treatment of CML-patients with IFN-α, particular attention was given to the documentation and analysis of adverse events (AE) in the group of IFN-α treated patients. This was achieved using a modified and extended WHO grading system for recording treatment-related toxicities (41 symptoms in 7 groups). The occurrence of adverse events was routinely checked at standardized time intervals. Analysis included descriptions of type and severity, incidence and time of onset. Ten neurological and psychiatric symptoms could be recorded.

Depression was rated as "severe" (grade 4) if a hospitalisation was necessary. Depression was reported in 36 patients. None of the 36 patients had a depression rated as grade 4. Seven patients had a depression grade 3. 5/7 patients had in addition to depression other adverse events (e.g. agitation, concentration deficite) and discontinued treatment (mean treatment duration 15 months). Most patients however continued IFN-α treatment.

Detailed results of this analyses will be presented including some clinical case reports.

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LOCAL TREATMENT OF THROMBOCYTOPENIC MUCOSAL HEMORRHAGE

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Diffuse mucosal hemorrhage due to thrombocytopenia is a common complication in the course of hematological disorders. The management of patients with epistaxis or diffuse gut bleeding presents a problem, as platelet transfusion is usually not effective due to platelet antibodies. We have tested the local administration of platelet concentrates in 8 thrombopenic patients with epistaxis and 1 patient with diffuse colon bleeding. The lavage was performed with random-donor concentrates. In patients with epistaxis 10 ml of random concentrate were drawn into a plastic syringe and applied to both nostrils in several 1 ml doses. A sufficient effect was also achieved, if the platelet-filled syringe was stored refrigerated at -20° C. In the case of gut bleeding 1 platelet concentrate was diluted in 500 ml 0,9% NaCl and administered by an enema. In this way the hemorrhage was successfully treated and prolonged freedom from bleeding was achieved. We conclude that local application of platelet concentrates is a useful treatment in diffuse mucosal hemorrhage due to thrombocytopenia.

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SIMPLE METHOD FOR CEREBROSPINAL FLUID CELL PREPARATION

J. Hastka, R. Hehlmann

Cytologic evaluation of cerebrospinal fluid (CSF) is important to diagnose central nervous system involvement by hematologic malignancies. The diagnostic value of this method is limited by preparatory difficulties, if standard methods are used. Methods which use a filter to remove the fluid suffer from low cell yield. Using a cytocentrifuge, the cells are mostly distorted and the yield is still insufficient. The long air drying of the cells after removal of the supernatant, which lasts about 10 minutes at room temperature, leads to insufficient cell preservation in all standard methods. We describe a simple procedure which offers a relatively high cell yield and good cell preservation. We use a special sedimentation chamber which contains a sucction nozzle to remove CSF after sedimentation. The chamber is attached by magnetic force to a polycationic coated slide and CSF is added. After 15 min, CSF is slowly removed via the sucction nozzle using a small plastic syringe and can be re-used for other tests. The suction nozzle is then connected with a vacuum pump to dry the cells. The drying procedure requires about 30 sec. The slow removal of the supernatant fluid and the extremely short air-drying are important for the high cell yield and good cell preservation. In our experience, the described procedure was superior to other preparatory methods as Sayk-type chambre or the cytocentrifuge.

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MONITORING OF PHLEBOTOMY IN HEMOCHROMATOSIS

J. Hastka, J.J. Lasserre, R. Hehlmann

In hemochromatosis, systemic iron overload leads to organ damage. Treatment is by venisection. The aim of such a treatment is to remove the excess of iron without altering the hemoglobinsynthesis. When iron supply to the erythropoietic marrow is inadequate, zinc instead of iron is incorporated into protoporphyrin IX and zinc protoporphyrin (ZPP) is produced instead of heme. ZPP-concentration (normal ≤40 µmol/mol heme) is increased in iron deficient erythropoiesis. We used ferritin and ZPP in 3 hemochromatosis patients for monitoring the phlebotomy therapy. Repeated phlebotomies led to a continuous decrease of ferritin. After 12 months ferritin levels were subnormal in 2 patients, whereas ZPP remained within the normal range, indicating still sufficient iron supply to the erythropoietic marrow. Hemoglobin and the red cell indices were still normal in these patients. In the 3. patient, an iron deficient erythropoiesis developed, as diagnosed by continuously increasing ZPP. At this time ferritin was still normal (75 µg/l). ZPP turned to normal after the phlebotomies were discontinued.
We conclude that ZPP can be used in combination with ferritin to optimize the phlebotomy therapy in hemochromatosis patients. An iron deficient erythropoiesis can be avoided by ZPP-measurements in patients with inflammation when ferritin is falsely normal.

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ZINC PROTOPORPHYRIN IN STAGING OF IRON DEFICIENCY

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In iron deficiency (ID) zinc protoporphyrin (ZPP) is produced instead of heme and ZPP concentration is increased (normal ≤ 40 µmol/mol heme). We measured ZPP in 102 patients with ID, grouped according to the commonly used iron parameters into the three stages of ID: iron depletion, iron deficient erythropoiesis and the iron deficiency anemia.
=>In iron depletion (n=19) ferritin was < 20 µg/l, ZPP and hemoglobin (Hb) were within the normal range.
=>In iron deficient erythropoiesis (n=11) ferritin was < 20 µg/l and ZPP was increased (67 ± 12 µmol/mol heme, range 48-83). Hb levels were within normal range (13,2 ± 0,8 g/dl, range 12,1 - 14,9).
=>In iron deficiency anemia (n=72) ferritin was < 12 µg/l. In mild anemia (Hb between 10 and 12 g/dl, normal red cell indices), mean ZPP was 100 ± 16 µmol/mol heme. In severe anemia (Hb < 10 g/dl, decreased red cell indices), ZPP values were significantly higher (265 ± 109 µmol/mol heme).
We conclude that ferritin, ZPP and Hb can reliably be used to classify the different stages of iron deficiency. Stage I (iron depletion): ferritin decreased, ZPP ≤ 40 µmol/mol heme, Hb normal; Stage II (iron deficient erythropoiesis): ferritin decreased, ZPP > 40 µmol/mol heme, Hb normal; Stage III (iron deficiency anemia): ferritin decreased, ZPP > 40 µmol/mol heme, Hb < 13 g/dl in men or < 12 g/dl in women.

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THE GERMAN CML-STUDY GROUP EXPERIENCE: PROLONGATION OF SURVIVAL BY HYDROXYUREA AND BY INTERFERON ALPHA IN CHRONIC PHASE CML

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In a randomized multicenter study the influence of hydroxyurea (HU) vs interferon alpha (IFN) vs busulfan on the duration of the chronic phase and on survival of CML is determined. Further goals of the study are the examination of cross-resistance and adverse reactions of the drugs; the development of a prognostic score on the basis of prospectively documented parameters; the comparison of the terminal phases of CML under different treatment modalities; and the determination of the outcome of bone marrow transplantations after the different drug therapies.
622 patients were randomized, 232 to receive HU, 164 IFN, and 226 busulfan. 89,5% were Philadelphia-positive (Ph+). HU-treated patients show a survival advantage over busulfan-treated patients of about 1 year. The median survival of Ph+ CML patients in the busulfan group is 45,4, in the HU group 58,2 months (p=0,008). IFN-treated Ph+ CML patients also show a significant survival advantage over busulfan-treated patients (p=0,01). The median survival has not been reached after 5 years. Cross-over of busulfan and HU after drug resistance prior to blast crisis showed an impact on survival by secondary busulfan after primary HU therapy. Over-all toxicity was lowest in the HU and highest in the IFN group. 68 patients were transplanted, 24 of these after IFN pretreatment. 5 years survival after BMT remains at 60% independent of pretreatment. In addition, a new prognostic score (Score1) was evaluated. Score1 comprises prospectively documented age, organomegaly related symptoms, Karnofsky index, extramedullary manifestations, erythroblasts, and circulating blasts. Comparison with Sokal's score in 450 Ph+ CML patients showed its superiority in the study population.
We conclude that HU and IFN are superior to busulfan in chronic phase CML, but that busulfan may have a role after HU resistance. The optimal treatment of newly diagnosed chronic phase CML will include HU, IFN, and/or allogeneic bone marrow transplantation. The optimal choice in the individual patient remains to be determined.

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ROLE OF P53 IN TUMOUR PROGRESSION OF COLORECTAL CANCER

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Mutations of the p53 tumour suppressor gene appear to be a major determinant in many forms of human cancer. The mutant protein adopts a characteristic conformation, which lacks the growth suppressor function of wild-type p53 and accumulates in the nuclei of transformed cells due to a prolonged half-life of the protein. In order to investigate the role of p53 in tumour progression and metastasis we analyzed 22 liver metastases of colorectal carcinomas with respect to mutational changes, loss of heterozygosity and expression of the p53 gene. Direct sequencing of PCR products corresponding to the coding region of p53 revealed that 17 of the 22 liver metastases (77%) had mutations in the p53 gene. Interestingly, the distribution of mutations along the coding region of p53 differed in the liver metastases compared to the data published for primary tumours. In metastases the data suggested a preferential occurence of mutations in the conserved protein domains C and D, when compared to the distribution of mutations along the four domains in primary colon cancer. Thus, cells carrying mutations in the domains C and D might have a selective advantage in the metastasizing process. We are currently sequencing p53 in primary colorectal tumours of the same hospital in order to account for epidemiological differences due to exposure to different carcinogens. Loss of heterozygosity at the p53 locus was detected only in cases with p53 mutations which indicates the importance of total loss of wild-type p53 function for tumourigenesis in these tumours. Furthermore, tumours carrying p53 mutations showed significantly higher p53 mRNA levels compared to those without p53 mutations. Thus, regulation of p53 mRNA levels seems to be also subject to selection processes in tumourigenesis.

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LONG TERM CULTURE-INITIATING CELLS (LTCIC) IN PERIPHERAL BLOOD PROGENITOR CELL (PBPC) POPULATIONS.

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Long term bone marrow cultures (LTBMC) have been demonstrated to be most valuable in vitro assays indicating the presence of pluripotent hematopoietic stem cells[1]. Limiting dilution analyses of progenitor cells have further defined that the frequency of long term culture initiating cells (as assayed by LTBMC) and the frequency of pluripotent hematopoietic stem cells (as assayed functionally by bone marrow repopulation of lethally irradiated mammalian organisms) are almost identical[1,2].
We employed human long term bone marrow cultures to study the ability of chemotherapy plus hematopoietic growth factor mobilized PBPCs to initiate LTBMC. The ability of various unmanipulated and ex vivo manipulated PBPC populations to maintain hematopoiesis on preformed, irradiated stromata was followed over periods of 6 to 12 weeks. Stromata were generated in 25cm^2-culture flasks from bone marrow aspirates of normal healthy donors to reproducibly provide an optimal microenvironment for stem cell growth.
We find, firstly, that PBPCs isolated by leukapheresis from patients receiving VIP chemotherapy in combination with G-CSF give rise to continuous progenitor cell production in LTBMC at levels and over periods comparable to or even exceeding those of normal bone marrow mononuclear cells. Secondly, PBPCs undergoing large scale (>10^{10} cells) enrichment for surface CD34 expression by immunoaffinity columns (CellPro Inc., Seattle, Wa., USA) fully preserve LTCIC during processing for patient use. Thirdly, we have evidence that LTCIC are maintained during short term ex vivo expansion of CD34+ cells in liquid culture in the presence of interleukin (IL)-1, IL-3, IL-6, erythropoietin and stem cell factor, indicating that ex vivo expansion of PBPCs does not result in depletion of early hematopoietic progenitors.
We consider that LTCIC evaluation of PBPCs manipulated ex vivo by CD34+ enrichment or liquid culture expansion is mandatory before extending the use of these cells from dose intensified chemotherapy regimen towards fully myeloablative protocols.

[1]: N.G. Testa and T.M. Dexter, Current Opinion in Oncology 3, 272 (1991).
[2]: R.E. Ploemacher et al, Blood 78, 2527 (1991).

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FLUDARABINE PHOSPHATE IN THE TREATMENT OF ADVANCED-STAGE CHRONIC LYMPHOCYTIC LEUKEMIA

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Fludarabine phosphate (FAMP, 2-fluoro-ara-AMP) is a fluorinated adenine analogue that has been shown to be highly active in chronic lymphoid neoplasms. From 11/89 to 3/93, 46 patients (pts, age: median 62 yr, range 37-77 yr, male/female 36/10) with relapsed or refractory chronic lymphoid neoplasms (B-cell chronic lymphocytic leukemia = 45 pts, Waldenström's macroglobulinemia = 1 pt) were treated with FAMP. 14 pts had one prior chemotherapeutic regimen, 13 pts had two, and 19 pts had more than two. FAMP was administered at a dosage of 25 mg/m^2 for 5 consecutive days at a 4 week interval. Pts achieving a complete remission (CR) after 6 courses received two additional courses, and pts achieving a partial remission (PR) or stable disease (SD) received additional treatment up to a total of 12 courses. Responses were seen in 13 of 46 (35%) pts, including 2 CR (4%) and 14 PR (30%). The time to best response for PR ranged from 3 to 9 courses (median 6 courses). The two CR were achieved after 6 and 12 courses. The median duration of remission was 12 months (range 1-37 months). Major hematologic toxicity was thrombocytopenia (grade III = 8 pts [17%], grade IV = 3 pts [7%]). Most important nonhematologic toxicity was infection (WHO grade III = 6 pts [13%], WHO grade IV = 2 pts [4%]). One pt with progressive disease died from sepsis after the third course. In four pts treatment was stopped because of severe infection: pneumocystis carinii pneumonia (after 11th course); candida pneumonia (after 3rd course, pt with concomitant prednisone/cyclosporine treatment because of severe hemolytic anemia); atypical pulmonary mycobacteriosis (after 3rd course); and generalized herpes-zoster-infection (after 3rd course). Our data confirm that FAMP is an active agent in relapsed or refractory B-cell chronic lymphocytic leukemia. Immunosuppression with the risk of severe and atypical infections seems to be the most important toxicity.

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PATTERNS OF EPSTEIN-BARR VIRUS (EBV) LATENT GENE EXPRESSION IN AIDS-RELATED LYMPHOPROLIFERATIVE DISEASE

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NHL and atyp. lymphoproliferations arising in immunocompromised patients are often associated with EBV. Three different forms of latent EBV infection have been documented in vitro.
We have testet 23 paraffin-embedded lymphoma specimens from HIV-infected patients for their expression of EBER, LMP, and EBNA-2 by in situ hybridization and immunhistology. The data were correlated with the previously determined morphology and immunphenotypic characteristics.
21 lymphomas were of B-cell, 2 cases of T-cell type. The morphology of the B-cell lymphomas was heterogeneous, ranging from a Burkitt-like to bizarre large cell morphology, most cases displaying a polymorphic mixed centroblastic-immunoblastic picture. In six cases the diagnosis of atypical lymphoproliferation was considered. 19 of 23 cases, including both T-cell lymphomas, proved to be EBV-positive. Cases with Burkitt-like morphology most frequently displayed absence of LMP and EBNA2 (type I latency), whereas many polymorphic mixed centrobl.-immunobl.lymphomas showed LMP expression, but absence of EBNA2 (type II latency).4 out of 6 cases considered as atypical lymphoproliferations were EBER$^+$,LMP$^+$,ENA2$^+$ (type III latency).
Our results indicate that EBV is frequently associated with lymphomas in AIDS patients, and that alle three forms of latent infection are represented. Furthermore, lymphoma morphology and EBV latent gene expression appear to be correlated.

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CONVENTIONAL CHEMOTHERAPY IN RESISTANT AND RELAPSED HODGKIN´S DISEASE (HD)

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Between 1982 and 1988 we treated 50 heavily pretreated patients with mainly advanced stages of HD with a combination conventional chemotherapy program (CCNU 60 mg/m^2 p.o. day 1, Chlorambucil 12 mg/m^2 p.o. day 1-5, Methotrexate 5 mg/m^2 p.o. day 1-5 and Prednisolone 50 mg p.o. day 1-5, given q 4 weeks).
Chemotherapy was planned for 6 cycles and a additional consolidation of 2 cycles for complete responders. 12/50 pts.(24 %) reached a complete remission, 7/50 pts.(14%) had a partial remission and 31/50 pts. (62%) failed to treatment. At present 5/12 complete responders are still in CR after salvage treatment. 5-years RFS-rate is 39% and overall-survival-rate 57%(median 29 and 59+ months respecively) for CR-patients. For the entire group of pts.median survival after salvage therapy is 18 months,the 5-years-survival-rate is 25%(Kaplan-Meier-estimation).The toxicity of the program was moderate;but nearly half of the pts. suffered from MTX-related mucositis (WHO-grade I-III). The results presented are well comparable to those of other investigators reported recently. The advantages of our program are the p.o.-administration and the relatevely low toxicity. Summarizing the reports from the literature and our own experiences standard-dose salvage programs do not solve the problems of resistant and relapsed HD, but it may be an acceptable palliative treatment approach in certain groups of patients.

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The Role of Cytokines in the Pathobiology of Multiple Myeloma

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Production of cytokines as well as responsiveness to these polypeptides was assessed on purified populations of plasmacells and long term bone marrow stroma layers cultured in the presence or absence of autologous peripheral blood mononuclear cells obtained from patients with multiple myeloma (MM) by RT-PCR, specific ELISA, ^{3}H-thymidine incorporation assay, and measurement of MM cells in S-phase of cell cycle. Cytokine production and cytokine responsiveness assayed for include IL-1-β, IL-3, IL-6, IL-7, IL-8, IL-11, GM-CSF, G-CSF, TGF-β, TNF-α/β. Based on the results obtained a scenario is being proposed which explains characteristics of in vivo growth, differentation, progression, and spreading of MM. MM cells and more importantly their circulating precursors express a variety of surface adhesion and extracellular matrix structures which enable them to be captured by the bone marrow microenvironment (BMM). Upon contact of MM precursors with BMM cells a large array of locally autocrinously or paracrinously produced cytokines (IL-1-β, IL-6, IL-11, IL-7) are being released to amplify the bone marrow myeloma precursor compartment. IL-3, being produced by activated T cells in response to stroma-derived IL-7, might then stimulate terminal maturation of the myeloma precursor cells into plasmacells requiering the presence of IL-6 which is mainly produced by bone marrow macrophages and fibroblasts. Since stroma layers established from multiple myeloma bone marrow represent a potent source of IL-8, this molecule might act by attracting and capturing myeloma precursors at the bone marrow site. The interaction and crosstalk of MM cells with their surrounding stroma not only results in self-perpetuation of myeloma growth but also in the development and activation of osteoclasts via plasmacell-derived IL-1-β, TGF-β, TNF-β, M-CSF, and thus explaining why the expansion of the plasmacell compartment is associated with an activation and numerical increase of the osteoclast population. The view of MM as a disease of unbalanced cytokine production also bears clinical implications. In vitro growth of MM cells in culture can be relieved by interfering with paracrine stimulation via IL-6 by the use of anti-IL-6 neutralyzing monoclonal antibodies, by prostaglandin antagonists or by IL-4 which acts by suppressing stroma cell production of IL-6. IL-1-β being produced by the plasmacell population itself might act to stimulate IL-6 release by stroma cells and is therefore a possible therapeutic target as well. Data are presented to suggest that IL-1 receptor antagonists may also be a potential therapeutic tool for the treatment of this disease.

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DIFFERENT PATTERN OF CYTOMEGALOVIRUS (CMV) REACTIVATION EARLY AFTER BONE MARROW TRANSPLANTATION (BMT) IN PATIENTS RECEIVING IN VIVO / EX VIVO T CELL DEPLETION OR CYCLOSPORINE A / METHOTREXATE (CSA/MTX) FOR GVHD PROPHYLAXIS

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We evaluated CMV reactivation by virus shedding in urine and throat washings and antigenemia in the first 35 days after BMT in 43 recipients of a marrow graft from an HLA id. sibling donor. 21 patients (14 seropositive for CMV) received the monoclonal antibody Campath 1 G in vivo prior to transplantation of a T-cell depleted marrow, 22 patients (all seropositive for CMV) received CSA/MTX for GvHD prophylaxis. CMV reactivation was detected in 8 of the 14 seropositive patients in the T cell depletion group compared to 6/22 patients in the CSA/MTX group. Antigenemia was detected in 6/14 compared to 2/22 of the patients. A major difference was the timing of CMV reactivation: Whereas no patient of the CSA/MTX group had evidence of CMV reactivation prior to the 2nd week all cases of CMV reactivation in the T-cell depl. group occurred within the first 14 days after BMT ($p<0.05$, log rank). Two of these patients developed CMV antigenemia already 1 week prior to BMT indicating that not the use of the T-cell depleted marrow graft but the in vivo treatment with Campath 1 G caused the significant differences between both treatment groups. CMV reactivation in the T-cell depletion group was highly correlated with a different pattern in hemopoietic reconstitution characterized by an early increase in lymphocyte counts. This relative lymphocytosis with predominance of NK cells was observed in 6/8 patients with CMV-reactivation. One additional patient developed relative lymphocytosis shortly after day 35. It was not observed in patients without CMV reactivation or in any patient of the CSA/MTX group. Three patients in the T-cell depl. group developed radiologic and clinical signs of interstitial pneumonitis after CMV reactivation compared to none in the CSA/MTX group. All 3 patients were treated with gancyclovir and high dose immunoglobulins. In two patients symptoms rapidly resolved. The 3rd patient who was the only patient without relative lymphocytosis died on progressive IP although virus was no longer detectable after initiation of gancyclovir treatment.

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EFFECT OF CHRONIC GRAFT VERSUS HOST DISEASE (cGvHD) ON QUALITY OF LIFE AND SURVIVAL OF PATIENTS SURVIVING MORE THAN ONE YEAR AFTER ALLOGENEIC BONE MARROW TRANSPLANTATION (BMT)

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We analyzed clinical complications in 107 recipients (ALL 19, AML 32, SAA 19, CML 32, MDS 2, NHL 3) of an HLA identical, MLC negative marrow graft from a sibling donor with a relapse free survival of more than 1 year after transplantation: 17 of the 107 patients (16%) died later than 1 year after BMT at a median of 813 days (range: 473-2599). 9 of the 17 cases were due to relapse of malignant disease (CML 6, AML 2, ALL 1), one patient died of a metastatic cervix carcinoma 4 years after BMT. 7 of the 17 cases of death were due to infectious complications associated with cGvHD. CGvHD related death occurred at a median of 740 days (range: 575-1365) after BMT. CGvHD thus accounted for 41% of late treatment failures. CGvHD was the most frequent cause of requiring medical support more than 1 year after BMT. Treatment for cGvHD was necessary in 41/107 (38%) of the patients. 46% of these patients required repeated hospitalization for cGvHD related complications. CGvHD was observed in 9/17 patients who received methotrexate alone (MTX) for GvHD prophylaxis and in 28/60 patients who received Cyclosporine A (CSA) alone ($n=5$) or in combination with Prednisolone ($n=2$) or MTX ($n=53$) but only in 4 of the 30 recipients of a T-cell depleted marrow. Fatal cGvHD related complications occurred in 4/9 patients with cGvHD after MTX prophylaxis but only in 2/28 patients after CSA prophylaxis. The frequency of repeated hospitalization for cGvHD related complications however remained high (12/28) in patients after CSA prophylaxis. Our data indicate that chronic cGvHD contributes substantially to morbidity and mortality of BMT patients who survive more than 1 year. Although the use of CSA in GvHD prophylaxis has resulted in a decreased incidence of fatal cGvHD related complications, the occurrence of cGvHD substantially increases the need and length of medical support and reduces the quality of life after BMT. More effective measures for prevention of chronic GvHD such as potentially T-cell depletion are required.

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CARDIAC TOXICITY OF BONE MARROW TRANSPLANTATION: CORRELATION BETWEEN PRETRANSPLANT CARDIOLOGICAL EVALUATION AND POSTTRANSPLANT CARDIAC EVENTS.

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Cardiac toxicity induced by the high dose chemo-/radiotherapy used for conditioning in bone marrow transplantation (BMT) is considered to be an important contributor to treatment failure and many centers include cardiological examination in the prescreening program prior to BMT. Most data on cardiac toxicity of BMT however are limited to case reports or smaller patient numbers. To estimate the cardiac morbidity prior to BMT, the risk of cardiac complications and its individual predictability, we evaluated 170 patients undergoing allogeneic ($n=150$) or autologous ($n=20$) BMT by physical examination and history, rest and exercise ECG, chest X-ray, 2D-echocardiography and radionuclide ventriculography (RNV) prior to BMT and followed their clinical course over a 3 month period after BMT.
In 38 patients (22%) cardiological evaluation prior to BMT revealed pathological findings. In 22 of these patients left ventricular ejection fraction (EF) determined by RNV was reduced < 55%. Reduction of EF was the only abnormality in 17 patients but was generally mild with a lowest EF of 43%. Following BMT cardiac toxicity was observed in 8 patients (4.7%). 3 patients (1.8%) developed lifethreatening cardiac complications (Pericardial effusion and left ventricular failure $n=2$, sudden cardiac arrest $n=1$). There was no correlation between overall results of cardiologic evaluation prior to BMT and cardiac toxicity. Cardiotoxic events occurred significantly more frequently in patients with reduced EF ($p < 0.01$). This was however restricted to minor cardiac events. Lifethreatening cardiac toxicity was not significantly increased in patients with pathological results in cardiological evaluation prior to BMT. Moreover none of the patients with an EF <50% developed cardiac toxicity after BMT.
Our data indicate that lifethreatening cardiac complications are a rare event after BMT occurring in less than 2% of all patients. Although the occurrence of cardiac toxicity was correlated with a reduction of EF prior to BMT lifethreatening cardiac toxicity could not be predicted in our study.

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CHARACTERIZATION OF THE ERYTHROPOIETIN RECEPTOR SYSTEM IN PATIENTS WITH POLYCYTHEMIA VERA

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Polycythemia vera (PV) is a monoclonal disorder with extensive proliferation of hematopoietic cells associated with increased erythrocyte mass as well as increased polymorphonuclear cell and platelet production. Cytogenetic analysis revealed frequent deletions of chromosome 20 in patients with PV. In this study, we analysed (1) the chromosome 20 and (2) the erythropoietin receptor (Epo-R) system of peripheral blood and/or bone marrow samples of 27 PV patients - diagnosed according to the PV study group - by using polymerase chain reaction (PCR), reverse PCR, single stranded conformation polymorphism (SSCP), RT-SSCP and direct sequencing. To proof the hypothesis that loss of chromosome 20q is important for the development of PV, we performed PCR analysis using primers for genes located on chromosome 20q, e.g. ADA, NTS-1, src and GNAS. In all 27 PV patients, the expected specific DNA fragments were amplified. DNA-SSCP analysis revealed neither an allelic loss nor a point mutation in the DNA amplification products. These data suggest that structural alterations and partial deletions of the long arm of chromosome 20 are not a frequent feature of PV.
Autonomous proliferation of PV might represent a consequence of alterations of the Epo-R system. Therefore we characterized the expression patterns and structure of the Epo-R and its ligand. Using Northern blot and RT-PCR analysis no detectable level of Epo mRNA was observed in peripheral mononuclear cells of 27 PV patients. A RIA was performed for quantitative determination of Epo in serum from 13 of 27 PV patients. As expected, only marginal concentrations of Epo (0.7-5.0 mU/ml) were detected in all PV patients tested. Differential RT-PCR analysis demonstrated a heterogeneous expression of the Epo-R in all samples. In a murine model system, mutations of the Epo-R have been shown to lead to the development of PV. To test this hypothesis, RT-SSCP analysis and direct sequencing of the Epo-R in the 27 PV patients is in progress.

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PROGNOSTIC FACTORS IN DE NOVO ACUTE MYELOID LEUKEMIA (AML)

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Clinical and laboratory findings of 231 consecutive patients presenting with de novo AML in our institution from 1981 through 1991 were analysed for their predictive value regarding complete remission (CR) rate and continuous complete remission (CCR) rate. We employed the following statistical methods: Mann-Whitney U-test (U), Mantel-Haenszel-test (M) and Breslow-Gehan-test (B).
Unfavourable prognostic factors for achieving CR were: age > 40 years (CR rate 76 vs. 50%, p(U)=0,005); leukocyte count > 50000/µl (CR rate 62 vs. 42%, p(U)=0,008) and LDH > 600 U/l (CR rate 63 vs. 47%, p(U)=0,033). Unfavourable prognostic factors for maintaining CCR were: FAB-types M1 and M5 versus FAB-types M2, M3 and M4 (CCR rate after 2 / 3 years 15/11% vs. 28/25%, p(M)=0,005, p(B)=0,004); proportion of medullary blasts > 90% (CCR rate after 2 / 3 years 28/23% vs. 17/15%, p(M)=0,062, p(B)=0,043); leukocyte count > 50000/µl (CCR rate after 2 / 3 years 26/23% vs. 17/15%, p(M)=0,06, p(B)=0,01), LDH > 600 U/l (CCR-rate after 2 / 3 years 27/23% vs. 12/12%, p(M)=0,001, p(B)<0,0001) and AP > 200 U/l (CCR rate after 2 / 3 years 22/20% vs. 7/7%, p(M)=0,008, p(B)=0,003). Employing these five unfavourable prognostic factors, we were able to create a prognostic score for CCR rate. In a group of 56 patients uniformly treated with a double induction regimen (TAD - HAM or TAD - TAD), followed by TAD-consolidation and a cyclic maintenance therapy for three years, CCR rate after 2 / 3 years for patients with no risk factors (n=21) was 50/42% compared to 12/12 % for patients with one or more risk factors (n=35).
Our data may contribute to the development of a reliable prognostic scoring system for CCR rate in patients with de novo AML. Application of a reliable score might be able to improve treatment results by enabling stratification of patients to different consolidation therapies. Allogeneic BMT with related or unrelated donors may be beneficial for patients with a high risk of relapse, whereas intensified or conventional consolidation chemotherapy may be sufficient for patients without adverse risk factors.

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POSTREMISSION THERAPY IN ACUTE MYELOID LEUKEMIA - THE RELEVANCE OF CONSOLIDATION AND MAINTENANCE TREATMENT

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Substantial improvements have been achieved in the first line treatment of adults with acute myeloid leukemia (AML) and complete remissions are nowadays obtained in 53 - 71 % of patients. Still, the majority of cases experience a recurrence of their disease and more effective strategies of postremission therapy are deeply warranted. Recent results of a retrospective comparison of AMLCG postremission maintenance therapy versus IBMTR data for allogeneic bone marrow transplantation does show a significant advantage in leukemia free survival for the latter approach. However, most patients are not eligible for this modality due to restriction by age or the lack of a compatible sibling donor and must hence rely on alternative postremission strategies. A beneficial effect of consolidation therapy alone could be demonstrated by several randomized comparisons resulting in long term disease free survival in 10 - 16 % of cases. Further improvements may emerge from intensification of consolidation treatment with high dose AraC as suggested from a recent CALGB trial. This approach, however, is complicated by an increased rate of treament induced death in remission which is in the range of 10 -23 %. In a prospective randomized comparison the AMLCG could demonstrate a similar efficacy but substantially lower toxicity of prolonged monthly maintenance therapy with a 5 year relapse free survival of 25 % as compared to 6 % in patients with consolidation, only. A metaanalysis of 12 controlled trials on maintenance therapy confirms this finding and emphasizes the antileukemic effect of maintenance treatment. The combined application of intensive consolidation with maintenance therapy appears to offer the most effective approach to postremission therapy which may be further improved by more intensive induction therapy such as double induction and/or an increased sensitivity of leukemic blasts after priming with hematopoietic growth factors.

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TRANSMISSION OF SARCOIDOSIS VIA ALLOGENEIC BONE MARROW TRANSPLANTATION

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The etiology of sarcoidosis remains obscure. As some studies have postulated the pathogenetic role of a transmissible agent, organ transplantation may bear the risk of transmitting sarcoidosis.
Allogeneic bone marrow transplantation (aBMT) was performed in a 34-year old man because of malignant non-Hodgkin-lymphoma. Two years before bone marrow harvest, pulmonary sarcoidosis was diagnosed in the donor. After steroid therapy, disease of the donor was in clinical remission with only minor radiological residues at the time of bone marrow harvest.
On day 90 after aBMT, the recipient developed dry cough and retrosternal oppression. Active sarcoidosis was diagnosed by typical radiological signs with progressive diffuse reticulonodular pulmonary infiltrates and hilar lymphomas, characteristic histological changes in lung and liver biopsies as well as increased angiotensine converting enzyme levels. Immunosuppressive therapy was changed from high dose cyclosporin A to high dose methylprednisolone, and symptoms promptly resolved within 8 weeks.
This case indicates the possibility of transmission of sarcoidosis by organ transplantation. Interestingly, the onset of sarcoidosis was not prevented by continuous high dose CsA therapy, whereas the disease promptly responded to corticosteroids. This observation casts doubt on the beneficial effect of CsA in the therapy of sarcoidosis claimed by some authors. We recommend that persons with a previous history of sarcoidosis should be excluded from donation of organs as long as the etiology of sarcoidosis is unknown and the risk of transmission cannot be reliably predicted.

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TREATMENT OF HIGH-RISK MYELODYSPLASTIC SYNDROMES WITH SEQUENTIAL INTERMEDIATE DOSE CYTOSINE-ARABINOSIDE AND MITOXANTRONE (S-HAM) WITH OR WITHOUT GM-CSF: A PROSPECTIVE DOUBLE BLINDED RANDOMIZED STUDY.

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Myelodysplastic syndromes (MDS) represent a heterogeneous group of disorders with different biology and prognosis. Based on the percentage of blast cells and the degree of hematopoetic insufficiency a high-risk group of patients can be identified with an expected survival time of less than 12 month only. The outlook for these patients remains dismal in spite of therapeutic intervention with intensive or prolonged low dose cytostatic therapy. The discovery and characterisation of hematopoetic growth factors provides a new approach both by the ability to possibly increase the sensitivity of blast cells to subsequent cytostatic therapy as well as by the stimulatory effect on normal precursor cells shortening the period of treatment associated cytopenia. Both aspects are addressed by the current study. Patients with high-risk MDS (RAEB, RAEBT, CMML) are randomized in a double blind fashion to receive GM-CSF or placebo by 1x daily sc-injection starting 48 hours prior to cytostatic therapy with S-HAM. GM-CSF or placebo is scheduled to continue until the recovery of neutrophil counts. At the present time 13 patients have entered the study including RAEB (n=5), RAEBT (n=7) and CMML (n=1). Of 11 currently evaluable patients 4 achieved a complete remission while MDS criteria persisted in 4. 9 of 11 cases had a reduction of bone marrow blasts below 5 % at day 18. The time to the recovery of neutrophil counts to > 1500 was 34 days. Side effects consisted predominantly in nausea and vomiting, diarrhea and infection.
These data indicate that intensive chemotherapy may be applied to this high risk group of MDS patients. Further data and a longer follow-up are needed to finally judge the additional effects of GM-CSF.

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DELETIONS OF THE SHORT ARM OF CHROMOSOME 7 IN PH-POSITIVE CML: A SECONDARY CHROMOSOME ABERRATION WITH ADVERSE PROGNOSTIC IMPLICATION

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In myeloid leukemias and myelodysplasias loss of material of the short arm of chromosome 7 seems to be a rare event. So far, in CML it has been reported as a secondary chromosome anomaly in only five out of 1664 cases published worldwide.
We report the cytogenetic and clinical data of 6 out of 333 patients with Philadelphia-positive CML in which we observed a deletion of the short arm of chromosome 7.
Since five of these patients developed partial monosomy 7p during antineoplastic treatment, with four of them being treated with interferon, it may be suggested that del(7p) is a therapy-related chromosomal change which may particularly arise in IFN-treated patients.
Concerning the clinical significance, loss of 7p material was associated with an unfavorable prognosis, because all patients had rapid progression of the disease after detection of the anomaly.
7p is the site of a number of genes which may have considerable influence on growth and differentiation of hematopoietic cells (EGFR, PDGFA, IL6, TCRG, ARAF2, HOX1, ZNF12). Therefore, loss or rearrangement of any of these genes may contribute to the malignant transformation of the affected cells.

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SEMIQUANTITATIVE ASSESSMENT BY PCR OF CYTOKINE mRNA-TRANSCRIPTS OF HUMAN MONONUCLEAR CELLS AND CELL LINES IN VITRO

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We established a modified polymerase chain reaction protocol for the detection and semiquantitative assessment of mRNA-transcript levels for IL-2, IL-4, IL-5, IL-6, IFN-γ, TNF-α, GM-CSF, TGF-β and IL-2-receptor-α (IL-2R). The method was shown to distinguish 10-fold differences in template concentration after 4 rounds each of amplification in the range from 20 to 40 cycles; the lower threshold of sensitivity was at 10 copies per PCR-reaction. Reproducibility was >95% for a positive result after 32 rounds of amplification; it decreased to 80% after 36 rounds. This corresponded to the detection of 1,000 and 100 template copies, respectively, per PCR-reaction.
Human peripheral blood mononuclear cells (PBMC) and tumor cell lines were evaluated for mRNA-expression with or without stimulation and these results were compared to secretion of the corresponding cytokine. For PBMC, constitutive mRNA-expression was found positive for TNF-α, IFN-γ, IL-4, IL-6, TGF-β and IL-2R, whereas detectable expression of IL-2, IL-5 and GM-CSF was induced only after stimulation. Using β-actin as an internal standard, the PCR could demonstrate relative differences in cytokine transcripts after stimulation with (A) 100 IU/ml IL-2, (B) 10% lymphocyte-culture conditioned media (CCM) and (C) PMA (10ng/ml) plus A23187 (100ng/ml). For IL-2 transcripts no detectable expression was found without stimulus or after addition of IL-2, whereas CCM resulted in a 1,000- and PMA/A23187 in a 10,000-fold increase. Other mRNA-transcripts increased 10-fold (TNF-α) up to 10,000-fold (GM-CSF) with or without differences between the stimulating agents.
The cell lines CAKI-1 (renal cell carcinoma) and Daudi (Burkitt lymphoma) also expressed comparable levels for cytokine transcripts, with a strong induction after stimulation with PMA/A23187. The relative IFN-γ mRNA-content in CAKI-1 increased from 0 to 1,000, for GM-CSF from 0 to 10,000.

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IN VIVO KINETICS OF CYTOKINE mRNA-TRANSCRIPTS IN PERIPHERAL BLOOD MONONUCLEAR CELLS (PBMC) FOLLOWING SUBCUTANEOUS IL-2 ADMINISTRATION: THE USE OF SEMI-QUANTITATIVE PCR.

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We investigated the use of PCR for a semiquantitative estimation of cytokine expression patterns in PBMC before and after administration of IL-2 to patients with advanced renal cell carcinoma or malignant melanoma. mRNA of 9 cytokines was measured using a modified polymerase chain reaction protocol, which could detect 10-fold differences in mRNA-contents of stimulated PBMC *in vitro*.
Weekly RNA-samples of 7 patients receiving a total of 11 treatment cycles were examined for long term changes, in 2 patients frequent samples were taken immediately after IL-2-administration for transcript-kinetics. mRNA-expression for IL-4, IL-5, IL-6, IFN-γ, TNF-α, GM-CSF, TGF-β and IL-2-receptor-α was clearly detectable in most of the samples, including four healthy donors. However, our method could not detect significant changes in transcript-levels of PBMC during 3 days following injection of (A) 36 Mio.IU or (B) 2×18 Mio.IU rIL-2 daily.
This was in marked contrast to cytokine secretion assayed by ELISA. Thus, serum IL-2 peaked 2-4 hours after administration followed by secondary cytokines with a peak 2-16 hours later. Increases for TNF-α, IFN-γ, IL-6 and IL-2R serum levels were significant ($p<0,05$) with the highest response found for IL-6, increasing 35- (A) and 32-fold (B) at day 1, or 8- / 14-fold at day 2.
Comparing normal individuals to patients, only small differences in constitutive cytokine expression were seen (<10-fold) with no distinct pattern. During therapy, changes could be seen for all cytokines except for IL-2 and TGF-β. In one patient, a 100-fold increase for IL-6, TNF-α and IFN-γ transcripts was observed during week 4 of the second treatment-cycle, other changes were approximately 10-fold.

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ADVERSE EFFECTS OF HYDROXYUREA VERSUS BUSULFAN IN THE TREATMENT OF CHRONIC MYELOGENOUS LEUKEMIA (CML)

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435 consecutive patients (age between 8.1 and 86.9 years) with CML were followed prospectively from 1983 to 1992. The mean observation time under treatment was 2.1 years. The occurrence of adverse effects was routinely checked and documented at standardized intervals.
71 of 241 hydroxyurea-treated patients (29.5%) showed adverse effects at any time during therapy; 25 patients had gastrointestinal symptoms (gastritis, gastric and duodenal ulcers, nausea, vomiting), 14 had neurological symptoms (paraesthesias, headache, tremor, dizziness) and 13 had dermatological symptoms (hyperpigmentation, atrophy of skin, alopecia). In two cases the therapy had to be changed to busulfan due to drug fever 4 to 6 hours after the application of hydroxyurea. Serious side effects were not observed (2 transient depressions of the bone marrow). In comparison, 62 (32.0%) of 194 busulfan-treated patients of the same trial showed adverse effects. 22 patients had gastrointestinal symptoms, 15 had neurological symptoms, 14 had dermatological adverse effects and 17 patients had an increased activity of liver enzymes. Serious side effects such as long lasting bone marrow aplasia or lung fibrosis were observed in 14 cases. The frequency of adverse reactions is lower under hydroxyurea than under busulfan treatment (17.0 vs. 24.4 events per 100 patient years).
We conclude that in the treatment of CML adverse effects of hydroxyurea are less frequent and less severe than those of busulfan.

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LEAD POISONING CAUSED BY A POTTERY MUG - DIAGNOSIS BY ZINC PROTOPORPHYRIN SCREENING

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Two cases of chronic non professional lead poisoning are described in a couple: a 34 years old woman presented with a history of abdominal pain and anemia. No gastrointestinal and hematologic diseases were observed. The diagnosis of lead poisoning was made by an extremely elevated zinc protoporphyrin/heme ratio (ZPP), alterations in porphyrin metabolism and increased blood and urine lead levels, especially after edetate calcium disodium (CaEDTA). The ZPP was measured in a simple and cheap way by hematofluorometry.
Screening for lead poisoning of her immediate family members and neighbors identified the 32 years old husband to have a manifestation of plumbism, whereas a tenant was negative. The cause of the intoxication was attributed to an abnormal lead intake due a pottery mug which was used daily to brew a fruit tea regularly at least for the past two years. After 12 hours the acidic tea (pH 4.2) contained 318 mg/l lead. Cadmium levels in the tea were not increased. After CaEDTA chelating therapy, anemia and abdominal pain disappeared, the lead levels in blood and urine normalized and the ZPP decreased slowly.
The determination of erythrocyte zinc protoporphyrin with a hematofluorometer is a simple and cost-effective method not only to screen iron deficiency but also to diagnose lead poisoning.

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PROGNOSTIC PARAMETERS OF PHILADELPHIA - CHROMOSOME NEGATIVE CHRONIC MYELOGENOUS LEUKEMIA (CML)

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From July 1983 to July 1993 89 (49 male, 40 female) patients with Philadelphia (Ph) chromosome-negative CML were recruited for studies designed to compare the effects of hydroxyurea, interferon α (IFN), and busulfan on the duration of the chronic phase and on survival. Ph-negativity was observed in 10,5% of all evaluable CML-patients. The mean survival of Ph-negative CML by now is 1.4 years compared to 4.1 years for Ph-positive patients. Ph-negative CML is characterized by lower blood cell counts (WBC 106,000 vs 168,000/µl, platelets 337,000 vs 495,000/µl, hemoglobin 11.2 vs 12.1 g/dl). Ph-negative patients as a group are older (62.4 vs 47.7 years) and more ill (Karnofsky index 84% vs 88%, initial fatigue and general ill-feeling 79% vs 64%) than Ph-positive patients. In 16 (18.0%) out of the 89 Ph-negative patients a clonal cytogenetic aberration was observed, in 7 of these a trisomy 8. 6 of the 7 patients with trisomy 8 died in the first 13 months after diagnosis. 12 out of 35 examined patients showed the bcr/abl rearrangement. The Ph-negative, bcr/abl-positive cases have a disease that is virtually identical to Ph-positive CML and respond well to treatment. All but one bcr/abl-positive patients of our series are alive 3 to 80 months after diagnosis. The initial platelet number of the Ph-negative patients appears to be an important prognostic factor. Low platelet counts indicate a significantly worse survival, high counts a significantly better survival than normal counts. Histologically, Ph-negative CML is characterized by an elevated granulopoiesis on a lower level compared to Ph-positive CML, a more macrocytic erythropoiesis, a monocytosis and infrequently demonstrable pseudo-Gaucher-cells. Ph-negativity, bcr/abl-negativity, monocytosis, trisomy 8 and a low platelet count are markers for a worse prognosis and may be considered to be a typical constellation for the myelodysplastic syndrome. We confirm that Ph-negative CML may be a separate entity on the basis of survival, age maximum, clinical and hematological characteristics. In addition, it appears to be a heterogenous group, in so far as it includes some patients with an obscured Ph-chromosome, cases which obviously belong to the myelodysplasias, cases which are a myelofibrosis and a further group of not yet sufficiently characterized CML-like disease.

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HEMOLYTIC AND NON-HEMOLYTIC TRANSFUSION REACTIONS

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Transfusion reactions (TR) are immune-mediated adverse effects in causal as well as in temporal connection with blood product transfusion. First of all, hemolytic reactions due to confusion and errors must be named as the result of an antigenantibody reaction. Secondly, febrile non-hemolytic reactions (FNHTR) caused by preformed antileukocyte antibodies show increasing tendency. TR can also be induced by liberated products of lysed leukocytes like histamine, TNF or IL-6. Another type of TR with undervalued frequency is the so-called TRALI syndrome following transfusion of plasma products containing white blood cell antibodies.
One of the most intriguing TR is the allergic reaction which is presumably caused by hypersensitivity to foreign proteins. The most common cause is the presence of antibodies against IgA. Graft-versus-host reaction (GVHR) is a seriously immune-mediated delayed adverse effect of blood product infusion resulting from the introduction of non-histocompatible, immunologically competent lymphocytes into a host who is incapable to reject foreign cells. The list of reported TR in relation to leukocyte contamination of blood products which are now known to be stimulants of the immune system, vectors for viral diseases, and the cause of fatal transfusion-associated GVHR supports the necessity to improve the quality and safety of components prepared for transfusion therapy. Leukocyte removal has assumed a pivotal role to achieve this goal. Transfusion of leukocyte-depleted components and strongly indicated transfusion regimens provide state of the art treatment.
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CURATIVE TREATMENT IN PRIMARY BREAST CANCER

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Prognosis of primary operable breast cancer remained dubious until it became generally accepted that the disease must be regarded as disseminated at the time of diagnosis. Thus, surgery with or without irradiation of chest and locoregional lymphatic areas, can no longer be regarded as optimum treatment. For more than a decade, studies have been undertaken to define the role of of sytemic treatment measures in order to eradicate the micrometastases or at least to prolong the time until manifestation of overt metastatic disease. In parallel, attempts have been made to reduce radicality of local treatment modalities. Together with these investigations, the importance of irradiation had to be newly defined.
It is now widely acknowledged that primary operable breast cancer can be cured by a breast conserving surgical resection of the tumor, followed by irradiation of the remaining breast with or without the regional lymph node areas and by a defined systemic treatment. There are still many open questions as to time, dosage, quality and quantity of the systemic treatment.
The effect of sytemic treatment for increasing the cure rate of primary operable breast cancer is beyond doubt. However, the number of open questions warrants further treatment within controlled and randomized clinical studies. Moreover, recent results of preoperative systemic chemotherapy in breast cancer gives new hope to increase the number of patients amenable to breast conserving surgery.
In conclusion, only multimodal treatment with surgery, irradiation and systemic medical treatment offers a patient with primary operable breast cancer the optimum chance for cure which can be taken only at the time of diagnosis since an overt dissemination of the disease precludes curability.

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EARLY SECONDARY ANLL AFTER COMBINED MODALITY TREATMENT IN TWO PATIENTS WITH SOFT TISSUE SARCOMA.

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Besides the acute toxicity of chemotherapy in the treatment of solid tumors an increasing number of secondary malignancies have been stated since the establishment of combined modality therapy concepts.
Secondary malignancies tend to arise with a median latency of 5 to 10 years after treatment. Since the establishment of modern treatment concepts especially with combination of polychemotherapy regimens and radiotherapy there are increasing hints for an induction of early secondary hematologic neoplasias within months after treatment
We report two cases of ANLL 8 and 14 months after the treatment for soft tissue sarcoma.
Both patients were treated for intraabdominal soft tissue sarcomas with chemotherapy regiemens containing high doses of alcylating agents followed by radiotherapy of the tumor site with cumulated doses of 50 Gy and 40 Gy.
Secondary ANLL was diagnosed 14 and 8 months after treatment and was classified AML FAB 5b and FAB M4.

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CURRENT STATUS IN THE TREATMENT OF ADULT ACUTE LYMPHOBLASTIC LEUKEMIA IN ADULTS

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Recent approaches to improve the outcome for adults with acute lymphoblastic leukemia (ALL) include stratification by immunological subtype and cytogenetic/molecular aberrations into biological subtypes and the tailoring of therapy according to risk groups. For high risk patients in the 4 consecutive adult ALL trials the effect of intensification with different consolidation therapie was evaluated. The continuous complete remission (CCR) rate for these high risk patients could be substantially improved from 27% in study 01/81 without consolidation, 33% in 02/84 with VM26/AraC, 37% in 03/87 with high dose AraC (HD-AraC) (3 g/m²)/mitoxantrone to 42% in 04/89 with either HD-AraC (1 g/m²)/mitox randomised versus HD-MTX/L-asparaginase. Adults with Ph/BCR-ABL positive ALL have a very poor outcome with survival of 0 - 10% at 5 years. Since they constitute 25% - 30% of adult ALL they are probably the main reason for the inferior outcome in adults compared to children. In the ALL studies 03/87 and 04/89 HD-AraC/mitox and HD-MTX/L-asp may have brought about an improvement to about 20%. Allogeneic bone marrow transplantation (BMT) and autologous BMT with purging are further options for these high risk ALL patients. Particular attention was also given to elderly ALL patients (50 - 65 years) whose referral to the GMALL studies is increasing. Their initial very poor outcome with CCR rate of 19% in 01/81 could be improved to 37% in 04/89 by moderate intensification but not with highly intensive treatment (HD-AraC/mitox).

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The computer-based patient record - the communication standard of the future?

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A network supports decentralized and problemoriented processing of patient data. The next step is an integration of all data-structured, narrativ and pictorial data - in a computer-based patient record (CPR).
Features like mobilization, individualization, duplication of the DPR should be preserved in strong analogy to the paper record. They are advantagenous even in a hospital communication system with decentralized workstations. Such an approach is attractive if problem-, communication- and decision-oriented views can be defined by users.
The perspectives and chances of some thousands views for the support of the health care delivery process, for clinical studies and medical standards should concentrate the interest on the development of a CPR system.
A prototype of a CPR will be presented. Some characteristics are: UNIX, C; TCP/IP, X-Window, a simple datadictio-nary, an interpreter language for the definition of views. The prototype provides a lot of functions for modelling views and communication processes.

Hölzel D, Adelhard K, Eckel R, König A, Tretter W: Die elektronische Krankenakte - eine Perspektive für Klinik-Kommunikations-Systeme und die Gesundheitsversorgung der Bevölkerung, MMV, München 1993

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Importance Of Dose Intensity In The Treatment Of Metastatic Soft Tissue Sarcomas - No Relation To Response - A Retrospective Study

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The importance of dose intensity of drugs has been claimed in metastatic colorectal carcinoma, breast cancer and high-grade non-Hodgkin's lymphomas. There are reports suggesting that in metastatic soft tissue sarcomas, too, a correlation of response with dose intensity exists. In a retrospective study we have reevaluated our treatment results in metastatic soft tissue sarcomas with the special emphasis of dose intensity.
Patients and Methods: Between 1987 and 1990 we have treated 45 patients of whom 39 are evaluable for this study with a combination of adriamycin and ifosfamide. The planned dose intensity of adriamycin was 20 mg/m²/week and for ifosfamide 3300 mg/m²/week. Response to treatment was evaluated at day 21 after the second course.
Results: 15 patients achieved a PR or CR, 14 patients a SD, and 10 patients a PD. Dose intensity was: adriamycin 17,4 mg/m²/week and ifosfamide 2980 mg/m²/week for PR and CR; adriamycin 17,2 mg/m²/week and ifosfamide 2890 mg/m²/week for SD; adriamycin 17,5 mg/m²/week and ifosfamide 3000 mg/m²/week for PD.
Conclusion: This retrospective analysis does not support the concept that with increasing dose intensity of ADM/IFO response rates can be improved in metastatic soft tissue sarcomas. But this conclusion must be considered with caution for several reasons: it was a retrospective study, planned dose intensity was high, soft tissue sarcomas are a heterogenous group with several prognostic variables such as grading and histology.

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LOW-DOSE CYTOSINE-ARABINOSIDE-THERAPY IN PATIENTS WITH MYELODYSPLASTIC SYNDROME: THERE IS NO INFLUENCE ON THE GRANULOCYTIC FUNKTIONS

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Low-dose cytosine-arabinoside (LD-ARA-C) is an option to treat patients with myelodysplastic syndromes.
In-vivo-chemotaxis and phagocytic activity of neutrophils were evaluated in 22 patients with myelodysplastic syndromes before therapy (19 men, 3 women, mean age 66,1 [46-82] years), in 12 patients with myelodysplastic syndromes after therapy with LD-ARA-C (10 men, 2 women, mean age 64,7 [46-82] years) and in 20 normal individuals. To quantify the neutrophil function, the skin-chamber-technique by Ruffert and a phagocytosis test by Süss et al. was used.
We found that in patients with myelodysplastic syndromes the in-vivo-chemotaxis (total leucocytic mobilisation after 24 hours: TLM_{24}= 3,43 (±0,60) Gpt/l/cm^2) and the phagocytosis capacity of granulocytes (phagocytic index: PI_{blood}= 33,9 (±16,6) %, $PI_{skin\text{-}chamber}$= 34,1 (±13,3) %) were decreased (p<0,05). These functions were further impaired after LD-ARA-C treatment (TLM_{24}= 3,09 (±0,97) Gpt/l/cm^2, PI_{blood}= 35,6 (±24,1) %, $PI_{skin\text{-}chamber}$= 19,9 (±8,6) %, p<0,05).
The results show that the evaluation of leucocyte function in patients with myelodysplastic syndromes after treatment with LD-ARA-C can be used to define altered granulocytic infection defence.

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Data quality in computerized patient records

Analysis of a haematology biopsy report database

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Using one model electronic patient record - a computerized hematology biopsy report database - we analyzed five parameters needed to create the electronic equivalent of a traditional free text cytology report: organ biopsied, quality of specimen, cytological diagnosis including modifier code for the main diagnosis code (i.e. status post chemotherapy, Y-code) and an additional key describing the degree of remission obtained after chemotherapy of acute leukemias (R-code). From the multiple steps involved in generating the electronic record we selected two critical ones: a) encoding of free text by physician staff and b) entering of this code into a computer through lab staff. Data are presented to qualify and quantify degree and sources of errors involved in these processes.
Our findings indicate that in this model of an electronic minimal medical record a) there is significant inaccuracy during the process of encoding the free text report on the side of the physician staff involved b) once the reports are encoded lab staff enter these data into the computer with a lower but still significant error rate c) the choice of an appropriate coding system used is a significant parameter affecting error/omission rates of the users d) a significant source of error is the machine/user interface e)when a combination of various codes is necessary to generate a complete electronic record the end result will show much higher error and omission rates than those of individual codes used
We conclude that 1) there is evidence to suggest that coded data obtained through a multistep coding process from free text sources are not suitable for clinical purposes or as input for expert systems because of a significant error rate 2) only audit or statistical evaluation systems willing to tolerate 16 % erroneous and 38 % omitted information can take advantage of data stored in such a system 3) physicians are the weakest limb in the "coding chain"possibly due to the more complex task and other, more pressing responsibilities not analyzed here 4) coding systems developed locally will deliver significantly better results than systems taken from other institutions.

	Incorrect (%)		Missing (%)	
Kind of code	Physician	Lab staff	Physician	Lab staff
Site of biopsy	3.2	7.8	0.0	0.0
Quality	7.3	7.6	0.0	0.0
Y-key (modifier)	3.3	0.0	64.0	86.7
Diagnosis	16.0	1.6	38.2	0.9
R-key (remission)	4.9	0.0	30.0	91.7

Total of all correct cytology reports: 43 %
(i.e. those reports with neither incorrect nor missing codes/entries)

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PADS (Patient Archiving and Documentation System)

A Computerized Patient Record

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Our Patient Archiving and Documentation System (PADS) represents a computerized patient record presently used on a university hospitals' ICU, CCU and oncology ward. Taking full advantage of the user friendly graphical user interface and mouse as input device our system enables nurses and doctors to perform the following tasks: admission, medical history taking, physical examination, generation of problem lists and follow up notes, access to laboratory data and reports, semiautomatic generation of a discharge summary including full word processor capabilities. Furthermore, the system offers rapid, consistent and complete automatic encoding of diagnoses following the International Classification of Disease (ICD; WHO). Developing electronic form sheets for study protocols or customized patient profiles is possible. For educational purposes the user can also view disease entities or complications related to the diagnoses she/he encoded. The system has links to other educational programs such as cardiac auscultation. A MEDLINE literature search through a CD-ROM based system can be performed without exiting the system; also, CD-ROM based medical textbooks can be accessed as well. Any commercial Macintosh program can be accessed through this system without exiting the main program thus enabling users to customize their working environment.
Additional options include automatic background monitoring of users behaviour, analyses and graphical display of numerous epidemiological and health care related data.
This system represents the first of a line of modular models which will soon be integrated to form a decentralized hospital communication system in a major German university. We use a Ethernet based local area network (LAN) with Apple Macintosh workstations.

Keywords:
Computerized Patient Record, Intensive Care Unit, quality assessment, education/teaching, multimedia workstation, Apple Macintosh, medical record

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LIMITED SAMPLING MODEL FOR DRUG MONITORING OF ETOPOSIDE

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Limited sampling models (LSM) are able to estimate the area under the concentration time curve (AUC) from only a few timed plasma concentrations. This marks the possibility of an individual drug monitoring up to a large number of patients and it may facilitate population pharmacodynamic studies in conjunction with Phase II/III trials.
The AUC, a measure of total drug exposure, can be correlated with the extent of myelosuppression, furthermore it is related to anti tumour response and tumour cell lethality. The AUC is the best pharmacokinetic parameter for predicting anti cancer pharmacodynamic events, although its exact quantitation is inconvenient and costly, usually requiring the measurement of the plasma drug concentration at 8 to 12 time points. One method circumventing these problems is the LSM. This study employing etoposide demonstrates that the AUC can be accurately estimated from only 2 serum concentrations obtained at 4 and 8h after treatment. 30 patients (with mostly lymphomas) were treated with polychemotherapy including etoposide (80-150 mg/m^2), serum samples were obtained at 8 timepoints following drug administration. The serum etoposide concentration was determined by plasma desorption mass spectrometry and the pharmacokinetic parameters were calculated by standard compartmental methods. The first 15 patients formed the training data set for developing the LSM and the remaining 15 patients formed the test data set for model validation. Using the training data set LSM was developed by stepwise forward linear and multiple regression and the "best" model was validated on the test data set. For initial model validation the estimated AUC was correlated with the measured AUC of the test data and mean predictive error (MPE) and root mean square predictive error (RMSE) were also calculated as a measure of bias and precision. The following model was selected as "optimal":

AUC= 343 (min) * C_{4h} (µg/ml) + 650 (min) * C_{8h} (µg/ml) + 1252 (min µg/ml)
with multiple r = 0.93 , MPE < 0.5 % and RMSE < 5 %

Conclusion: This LSM is a very practicable and simple method to calculate the area under the concentration time curve (AUC) exactly for a large number of patients and useful as an instrument for prospective studies to reveal and quantitate correlations between drug dosage and response or toxicity, at least it might be possible to find an individual dosage scheme by drug monitoring for each patient.

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INDICATIONS FOR ABMT AND PSCT IN CENTROBLASTIC/CENTROCYTIC AND CENTROCYTIC NHL

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In comparison to high grade NHL which are potentially curable diseases, effective treatment for lymphomas of low malignancy such as CBCC and CC is still not available.Therapeutic possibilities ranging from wait-and-see, various chemotherapeutic regimens as well as irradiation are under discussion.New aggressive treatments such as ABMT or PSCT may increase curative success.
We evaluated retrospectively 95 pts.(73 CBCC and 22 CC) with Ann Arbor stage 3 and 4 and age < 60 years. First line treatment was with conventional chemotherapy +/- radiation or radiation therapy alone (extended field), as described below: (number of pts in brackets)

CHOP (50)	COP (17)	C-MOPP (10)	KNOSPE (5)
MOPP (2)	Leuk/Predni (2)	Sterecyt (1)	Radiation (5)
Bleo/Vinde (2)	No Treatment(1)		

No significant difference between response and survival rate among the different treatment groups was observed. 64/95 pts.(67%) responded (30 CR and 34 PR).Of the initial 64 responders 50pts relapsed (78%), the median remission duration was 17 months (range 1-119 months).
For the whole group disease free survival and overall survival calculated by Kaplan Meier method showed a constant decrease (median survival: 42 months, range 1-197 months).
We describe a new prediction model for patients suffering from advanced staged CBCC and CC who may profit from ABMT or PSCT .

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BLOOD TRANSFUSION AND CANCER: MODULATION OR TOLERANCE?

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Many retrospective studies show a detremental effect of BT on cancer prognosis. Most papers dealt with colorectal cancer and for this malignancy peri-operative BT seems a major factor determining long-term prognosis. Several authors, using multivariate statistic analyses, tested whether BT is a surrogate marker for other prognostic factors and found BT to be an independent, statistically significant, prognostic factor. A meta-analysis performed in our group confirms the negative effects of BT, although proof for prognostic factor independency - causal relationship - only can be concluded from clinical trials. In 1987 we started the CRAB trial that compares the prognosis of colorectal cancer patients who either received standard packed cells (PC) or leukocyte depleted blood (LD). LD was shown to lack the benificial immunosuppressive effects on renal allograft survival.

The intermediating mechanism of the BT effects on cancer prognosis is unsolved, but it is suggestive that the immune system (1) plays a central role. Alternative explanations comprise (2) BT effects on coagulation, thrombi-formation, and thereby on metastatic capacities, (3) direct BT effects on cancer cells or (4) on endothelium cell function and thereby on the chance for outgrowth of cancer cells and (5) selection for patients with bad prognosis.

Our triangular model requires interference by BT with the immune effector arm that lyses tumour cells, so that three effects have to be proven. (A) Immunosuppressive effects of BT. The impairment of T and NK cell functions, and suppression of antibody formation has been demonstrated by many studies mainly for alloreactivity. The immunosuppression seems donor-antigens specific. (B) The immunosurveillance hypothesis has not yet been proven for most human cancers. Cytotoxic T lymphocytes with tumour cell specificity, however, can by cultured out of Tumour Infiltrating Leukocytes (TIL) of melanomas and renal cell carcinomas. Some human Tumour Specific Antigens (TSA) have recently been defined; in general, however, the human CTL-TSA response is unclear. Furthermore, in case an anti-tumour response is established tumour cells have many mechanisms for escaping cytotoxicity and even might be able to induce tumour-specific tolerance. (C) The connection between (A) and (B) implicates that the tumour specific effector arms of the immune system must have been suppressed after BT, which postulates, in parallel with transplantation settings, common antigens on the blood donor cells and the patient's tumour. First, the existence of a relation between BT and cancer prognosis has to be confirmed on a clinical level. Our CRAB trial indicates that such association does not exist for colorectal cancer.

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HIV-ASSOCIATED MALIGNANT LYMPHOMA: SURROGATE MARKERS FOR STAGE AND PROGRESSION OF THE DISEASE AND RISK-ADAPTED THERAPY.

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Non-Hodgkin's lymphoma (NHL) develops in about 5 to 10 % of AIDS patients. The vast majority of AIDS-NHL are clinically agressive B-cell NHL that are histologically classified as immunoblastic, centroblastic and Burkitt-type lymphoma.
Methods. In a phase II study 107 patients from 15 institutions have been registered from 1/91 to 10/92. High-risk (HR) and normal risk (NR) patients were distinguished. The HR-group fulfilled two of three criteria: T4-lymphocytes < 50/µl; WHO-actitivity > 2; opportunistic infections. The NR-group did not meet HR-criteria nor stage I A or primary CNS-lymphoma. Biopsy material for histological evaluation was sent to two reference institutions. In all patients serial investigations of immunological parameters were performed.
Treatment. NR-group: 4-6 cycles of CHOP; CNS prophylaxis with MTX i.th.; maintenance treatment with IFNα and AZT. G-CSF was given in neutropenia according to a fixed scheme considering leucocyte values. Induction therapy for HR-patients: VCR and prednisone.
Results. Mean values (p.µl) of T4-, T8-; T4/T8-lymphocytes in HR-patients: 16, 314; 0,04 and in NR-patients: 193, 812; 0,2. The course of immunological values during and after treatment depended mainly on the state of remission that was achieved. Complete remissions were obtained in 64 % and partial remissions in 22 % of NR-patients. In HR-patients no complete remissions were observed. Median survival in NR-patients is 641 days, in HR-patients 82 days.

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PREOPERATIVE SYSTEMIC ETOPOSIDE/IFOSFAMIDE/DOXORUBICIN CHEMOTHERAPY COMBINED WITH REGIONAL HYPERTHERMIA IN HIGH-RISK SARCOMA: RHT-91 STUDY

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From November 1990 to November 1991 33 adults with high-risk, nonmetastic soft tissue sarcomas were entered in a protocol (RHT-91) involving regional hyperthermia combined with systemic chemotherapy followed by surgery. 16 had undergone previous surgery and/or radiation, 7 had received previous multidrug chemotherapy, and 10 were previously untreated. A tumor size of > 8 cm and/or extracompartimental tumor location (18 patients) or local recurrence (15 patients) were defined as high risk factors in addition to tumor grading (27 patients had grade 2 or 3 soft tissue sarcomas). Regional hyperthermia was produced by an electromagnetic deep-regional heating device. For systemic chemotherapy, the patients received etoposide/ifosfamide/doxorubicin [=adriamycin] (EIA) and mesna, with regional hyperthermia being given only on days 1 and 4 in repeated EIA/regional hyperthermia cycles every 3 weeks. Tumor temperatures (range 40°-44°C) were measured by invasive thermometry in all patients during each regional hyperthermia treatment. A total of 302 regional hyperthermia treatments were applied within the pelvic region (11 patients), trunk (6 patients), or extremities (16 patients) bearing relatively large tumors (mean volume 800 cm^3). By the cut-off date for this analysis (December 1992) 27 patients had undergone surgery after receiving 2-6 cycles of EIA chemotherapy combined with regional hyperthermia, all tumors except three were resected without disfiguration. In 33 evaluable patients (minimum 1 EIA plus regional hyperthermia cycle), the clinical response rate was 46% (2 CR, 5 PRa, 8 PRb). In addition, a pathologic response to preoperative thermochemotherapy was evaluable in 27 patients with 14 responders (52%) having >75% histologic necrosis or regression. Overall, 13 patients relapsed within 17.8 months of mean observation time. At the cut-off date, 17 patients show no evidence of disease, 28 patients are alive and 5 patients died. The study (RHT-91) is continuing as a multicenter phase II trial in patients with high-risk soft-tissue sarcomas to test further the potential of preoperative thermochemotherapy in regard to local control and survival. Supported by grant M12/857Wi1-M19/88/Wi9 from the Deutsche Krebshilfe, Bonn

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RADIATION-INDUCED LEUKEMIA
W.Jacobi

The available radioepidemiological studies agree in the conclusion that leukemia belongs to the most important carcinogenic effects of ionizing radiation. This is particularly valid for children and youths. The most reliable quantitative data result from the 'Life Span Study'(LSS) among the atomic bomb survivors at Hiroshima and Nagasaki. The findings of this study concerning the excess risk of leukemia as function of the bone marrow dose, the time since exposure and the age at time of exposure are summarized. For the extrapolation of the resulting risk coefficient to low doses or low dose rates the ICRP recommends a reduction factor (DDREF) of two.

Taking into account a DDREF=2 the LSS data enable an estimation of the possible age-specific leukemia rate in populations which might e caused by the exposure to natural and man-made radiation sources. In normal populations the highest contribution to the bone marrow dose results from the natural background radiation, in the average about 1.5 mSv per year. The risk analysis leads to the important conclusion that in the age cohort from 5-25 years up to about 20-30 percent of the observed total number of leukemia cases might be initiated by this average background radiation level. The influence of the local and individual variation of the natural background radiation is outlined. Finally, the possible role of ionizing radiation as a causative factor for the observed local clusters of childhood leukemia will be discussed.

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EFFICACY AND TOXICITY OF THE ORAL IRON CHELATOR L1 (DEFERIPRONE) IN THE TREATMENT OF SECONDARY HAEMOCHROMATOSIS
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Although secondary haemochromatosis can successfully be treated with desferrioxamine (DF), many patients are unable or unwilling to manage with the rigorous requirements of DF therapy. The full benefits of iron chelation therapy will therefore not be gained until orally effective and safe drugs are available. The new oral iron chelator L1 (1,2-dimethyl-3-hydroxypyrid-4-one) is such a promising agent.
We started a long-term clinical trial with L1 in patients with transfusion haemosiderosis. Up to now, six patients have been included. The mean age of our patients was 59 years (range 25-82). Serum ferritin levels varied from 2497 to 6324 ng/ml (mean 4117) after transfusion of 9 to 260 red-cell units (mean 120). Patients were treated over a period of 4 to 17 months, receiving L1 at a dosage of 15 to 65 mg/kg/day (corresponding to a total daily dose of 0.9 to 3.6 g). Urinary iron excretion varied substantially from day to day in each patient. In 3 patients, being treated for at least 8 months, mean iron excretion ranged between 13.5 and 26 mg per day. In these patients serum ferritin levels did not increase despite continued transfusion therapy, thus suggesting a beneficial effect of L1.
The following adverse effects were observed: Three patients experienced nausea which lasted for only a short period in two cases. Three patients developed zinc deficiency after several months of treatment, associated with skin lesions in one case. In two patients L1 had to be stopped after several months of treatment because of arthralgias. In two cases, L1 had a mild diuretic effect. One patient repeatedly developed urticaria, necessitating termination of L1-chelation therapy. Agranulocytosis, so far documented in 5 patients worldwide, was not seen among our patients.
According to these preliminary results, L1 appears to be an effective oral iron chelator. However, L1 therapy carries the risk of toxic side-effects. With regard to the potential adverse effects L1 should only be used in carefully controlled clinical trials primarily treating those patients who cannot tolerate DF or who are non-compliant with this therapy.

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PERSISTENCE OF t(14;18)-POSITIVE CIRCULATING BLOOD CELLS AFTER AGGRESSIVE CHEMOTHERAPY FOR LYMPHOMA
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We have studied the effect of aggressive chemotherapy on circulating t(14;18)-positive blood cells in 20 patients with non-Hodgkin's lymphoma (10 high-grade, 10 low-grade). One-stage PCR (detection limit $1:10^4$ cells) was performed on peripheral blood DNA before and after 3 or 6 cycles of therapy. 19 out of 20 patients (95%) remained PCR-positive throughout the course of chemotherapy despite adequate clinical response rates (CR=80%). Only 1 of 16 patients with complete clinical remission converted from positive to negative. Subsequent allogeneic bone marrow transplantation in two patients who had not responded to conventional chemotherapy resulted in the disappearance of t(14;18) cells from the peripheral blood at a level of $1:10^6$. We have also investigated the clinical outcome of 20 patients with t(14;18)-positive high-grade NHL. In comparison to t(14;18) negative lymphomas a BCL-2-Ig rearrangement was highly associated with centroblastic histology (75% CB-lymphomas in the positive group) and a higher rate of relapse (40% vs 25%). Our results indicate that (i) peripheral blood testing is a valuable tool for initial diagnosis of t(14;18) lymphomas, but its validity for monitoring of clinical remission is limited; (ii) aggressive chemotherapy fails to eradicate minimal residual disease while allogeneic bone marrow transplantation is able to eliminate the t(14;18) cells; (iii) t(14;18)-positivity in high-grade NHL is associated with adequate CR-rates but with a high rate of clinical relapses.

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COMBINATION OF 5-FLUOROURACIL (FU) FOLINIC ACID (FA) AND α-INTERFERON 2B (IFN) IN ADVANCED GASTRIC CANCER
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Based on encouraging treatment results with FU/FA or FU/IFN in gastrointestinal tract cancer, a pilot study was initiated to evaluate the effects and toxicity of combination FU/FA/IFN in patients (pts) with inoperable/metastatic gastric cancer. **Schedule:** IFN 6 M.U.s.c.1x/week, FU 500mg/qm bolus i.v.1x/week in the middle of a 2-hour infusion of FA 500mg/qm 1x/week. Of 55 treated pts, 53 (14 females, 39 males) are evaluable for response and toxicity (2 early deaths). Median age was 54 years (33-73), median Karnofsky performance status was 80% (60-90). Sites of tumor manifestation were inoperable primary tumors/local recurrences (18), liver metastasis (20), lymph nodes (30) and peritoneum (20). **Toxicity:** 1/53 pts had WHO grade 4 toxicity (diarrhea), 3/53 pts had WHO grade 3 toxicity (nausea 1, diarrhea 2). Except for 1 treatment limiting grade 4 toxicity, no modifications of dose or schedule due to toxicity were required. **Results:** 7/53 pts had complete response, 16/53 pts partial response, 12/53 pts minor response, 14/53 pts tumor stabilization and 4/53 pts had progressive disease. Median duration of response was 6 months, median progression-free intervals 4,5 months, median survival time of all treated pts was 9 months, of responding pts (CR/PR/MR/NC) 10 months. 31/39 pts experienced significant reduction of tumor related pain under treatment. **Conclusion:** Biochemical modulation of FU with FA and IFN is effective in locally advanced and metastatic gastric cancer. Moderate toxicity, treatment in an outpatient setting and high response rates of tumor related pain contribute to an effective palliation.

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ESTABLISHMENT AND CHARACTERIZATION OF A NEW PERMANENT BARRETT'S CARCINOMA CELL LINE: EXPRESSION OF EPIDERMAL AND MESENCHYMAL DIFFERENTIATION MARKERS AND SENSITIVITY TO CYTOKINES

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Barrett's carcinoma is a rare adenocarcinoma of the lower esophagus. Studies of its tumor biology have been hampered by a lack of experimental models. We report the establishment of a new permanent cell line from a patient with Barrett's carcinoma (MHH-167). Tumor cells proliferate as monolayers. Soft agar cloning of the primary tumor was unsuccessful, but up to 0.27% of MHH-167 cells form colonies. Subcutaneous and intramuscular transplantation of MHH-167 onto nude mice was successful. The modal karyotype of cultured cells was 46-48, XY with a derivated chromosome #1 and one marker chromosome mar1 (1;1)(p2.2 pter::p1.1 p2.1). Ck 18 cytokeratine staining was positive for the primary tumor, xenograft and cell line. Positive staining was also achieved with vimentin. Tumor markers CEA, EMA and 7A9 were not expressed. Cultured tumor cells expressed Epidermal Growth Factor receptor with a KD of 1.6 - 3.0 x 10^{-10} M. The number of binding sites/cell was 6100 - 8600. No significant amount of Epidermal Growth Factor activity was detected in conditioned media. Cell growth was stimulated by Interleukin-4 and Interleukin-7. We conclude that MHH-167 Barrett's carcinoma cells may be useful for further investigations into the biology of this rare tumor.

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IN VITRO AND IN VIVO DETECTION OF AN INTRACELLULAR SECOND MESSENGER OF IFN-α (THE HUMAN IFN-α REGULATED DNA-BINDING FACTOR ISGF-3) IN MNC

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The interaction of IFN-α with one or more specific cell-surface receptors on human cells results in the synthesis of intracellular IFN-induced proteins (proteinkinase, 2-5-A-Synthetases, Mx-proteins). A specific promotor sequence - a conserved regulatory element called ISRE - within the IFN-inducible genes allows the binding of a 336 kDa protein complex (ISGF-3) with a high affinity to the ISRE, which is responsible for the initiation of the transcription of IFN-inducible genes in human cell lines. To measure ISGF-3 in human mononuclear cells (MNC) exposed to IFN-α in vitro and in vivo 3x10^7 MNC from healthy donors were incubated with 1 to 10^4 I.U./ml IFN-α2 for the time periods indicated. Their nuclear extracts were prepared and analyzed by gel retardation employing an end-labelled synthetic ISRE oligonucleotide. Already 1 I.U./ml rIFN-α2 induced a measurable increase of ISGF-3 in MNC. Increasing doses of rIFN-α2 up to 10^3 I.U./ml rIFN-α2 led to rising concentrations of ISGF-3. Longer incubation times (2,4 hrs) led to subsequent decrease of ISGF-3.
Subsequently three CML-patients treated subcutaneously 3 x weekly with 10 Mill. I.U. rIFN-α2 were analyzed for the presence of ISGF-3 in their MNC. As expected, the kinetic of the emergence and decay of ISGF-3 in vivo differed from the in vitro kinetic. Upon subcutaneous injection of 10 Mill. I.U. rIFN-α2 ISGF-3 became detectable after 3 hours and increased until 9 hours. Subsequently ISGF-3 diminished and disappeared after 24 hours.
To our knowledge this is the first time that an IFN-induced nuclear factor was measured in IFN-treated patient.

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G-CSF AND GM-CSF ADMINISTRATION IN HEMATOLOGICAL PATIENTS - INITIAL EXPERIENCE

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Profound neutropenia represents a major problem of chemotherapy and a frequent cause of infectious complications. In recent years, recombinant human granulocyte-macrophage colony stimulating factor (rhGM-CSF) and recombinant human granulocyte colony stimulating factor (rhG-CSF) have been used either prophylactically, or therapeutically after chemotherapeutic regimens. Administration of rhGM-CSF and rhG-CSF to patients increases the number of leukocytes and progenitor cells in peripheral blood. We have treated 9 patients with profound neutropenia, mostly after cytostatic therapy, with 5μg rhG-CSF (Neupogen) per kg daily for 5 to 10 days. Additional 8 patients with profound neutropenia received 5μg rhGM-CSF (Leucomax) per kg daily for 5 to 10 days. Two patients treated by G-CSF subsequently died, in one patient, the death was caused by refractory bone marrow failure in aplastic anemia, the other death was connected with leukemia progression. In the remaining patients, we have observed stem cell mobilization in patients receiving rhGM-CSF or rhG-CSF, with the neutrophil counts recovered in 5 to 10 days in most cases.

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DIFFERENTIAL EFFECTS OF RED BLOOD CELLS ON PLATELET ADHESION AND PLATELET AGGREGATION IN PULSATILE SHEAR FLOW.

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There is considerable evidence that red blood cells (RBC) play an important role in the effective arrest of bleeding following small vessel injury. It has also been well documented that RBC support platelet adhesion to endothelial cell matrix and artificial surfaces as well as platelet aggregation in laminar shear flow in different experimental in vitro flow systems. We have previously reported that RBC-mediated potentiation of shear-induced platelet aggregation (SIPAG) in a Couette rotational viscometer is due to both physical transport by RBC of platelets to the surfaces of the flow chamber (platelet diffusivity) and the effects of ADP lost from RBC during shear (Reimers RC, Sutera SP, Joist JH. Blood 1984;64:1200). The studies reported here were designed to compare the effects of RBC on SIPAG with those on shear-induced platelet adhesion (SIPAD). Suspensions of human platelets labeled with Mepacrine and suspended in citrated plasma were exposed to pulsatile shear stress of varying amplitude (25-100 dyne/cm^2) in a computerized cone-plate viscometer in the presence or absence of intact RBC or Glutaraldehyde fixed (GTA) RBC for 120-300 seconds. The number of single platelets in the suspension before and after shear was determined and SIPAG was expressed as % loss of single platelets. SIPAD was assessed by determining the amount of Mepacrine-related, fluorescent material remaining on 4 glass disks inserted into the bottom plate of the viscometer after repeated washing with EDTA-saline. In keeping with our previous findings in the Couette viscometer, intact RBC were twice as effective as GTA-RBC (which are depleted of ADP) in potentiating SIPAG at all shear stress levels. In contrast, potentiation by intact RBC of SIPAD was markedly less than that observed with GTA-RBC and could be observed only at shear stresses above 50 dyne/cm^2. These findings and additional findings to be presented indicate that while RBC exert their potentiating effects on SIPAG by both physical and humoral (ADP) mechanisms, RBC support SIPAD largely, if not solely, by a physical mechanism, i.e., enhancement of platelet diffusivity.

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CHROMOSOMAL INTEGRATION OF EPSTEIN-BARR VIRUS IN A BURKITT'S LYMPHOMA CELL LINE: LOCATION NEAR A TRANSLOCATIONAL BREAKPOINT, LATENT GENE EXPRESSION AND CHANGES IN LONG TERM CULTURE

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Epstein-Barr virus (EBV) infection is associated with several lymphatic malignancies including endemic Burkitt's lymphoma (BL), immunoblastic B-Non-Hodgkin Lymphomas in immunosuppressed individuals and Hodgkin's disease. Usually infected cells harbour numerous copies of the viral DNA in an episomal state as covalently closed circles. Integration of EBV up to now has only rarely been reported. Using fluorescence in situ hybridisation (FISH) recently more cases have been described with integration of EBV into the host cell genome. The biological significance of this observation so far has remained unclear.

We have analysed EBV integration in the Burkitt's lymphoma cell line BL60 before and after cell fusion with autologous EBV-immortalized lymphoblastoid cell line (LCL) IARC 277. By FISH the integration locus was located near the breakpoint of a translocation (11;19), which is present in BL60 in addition to the BL specific translocation (8;22). In the BL/LCL hybrid cells BL derived integrated and LCL derived episomal EBV molecules show a different latent gene expression, i. e. downregulation of BL derived versus upregulation of LCL derived EBNA-1 and EBNA-2 genes. Furthermore in the BL60 cell line during long term cultivation episomal EBV is lost leading to the presence of exclusively integrated EBV. In contrast during long term cultivation of the BL/LCL hybrids selective loss of the integrated virus is observed and only episomal copies are retained.

This experimental model provides an example for a striking difference in the virus host cell interaction of integrated versus episomal EBV-molecules.

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cDNA SEQUENCE COMPARISON OF THE CD30 ANTIGEN EXPRESSED ON ACTIVATED T-CELLS AND A HODGKIN DERIVED CELL LINE, L540.

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The Hodgkin's disease associated activation antigen CD30 is a member of the growing family of cell surface receptors showing homology with the nerve growth factor receptor. In the hemopoietic cell lineage, expression of the CD30 antigen is predominantly found on Hodgkin and Reed-Sternberg cells (H&RS-cells), cells of anaplastic large cell lymphoma and activated or virus transformed lymphoid cells of either T or B origin. Recently it has been reported that ligand induced crosslinking of the CD30 antigen may lead to proliferation or apoptosis in different cell types expressing CD30. In order to investigate whether structural differences of the CD30 antigen between normal T-cells and neoplastic H&RS-cells are involved in the growth regulation of H&RS-cells we have compared the open reading frames (ORF) of the CD30 antigen expressed on normal activated T-cells and a Hodgkin derived cell line sharing features of an activated T-cell, L540. The ORFs were cloned by PCR and independent clones were sequenced. Comparison with the published cDNA sequence of the CD30 antigen from the HTLV-I transformed T-cell line HUT-102 revealed a silent mutation at position 771 of the ORF in both cell types (A→G). Beside this no significant mutations could be detected. We conclude that the primary amino acid sequence of the CD30 antigen expressed on the Hodgkin derived cell L540 is identical to that expressed on activated T-cells. Different functional properties of the CD30 antigen in these cells might be a consequence of different posttranslational modifications of the receptor or different postreceptor signal transduction mechanisms.

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IL-4 INHIBITS IL-2 INDUCED PROLIFERATION ON B-CLL-CELLS

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We used the BrdU-Incorporation method to show the effects of IL-2 (100, 1000, 3000 U/ml), IL-4 (0.1, 1, 10 ng/ml) and IL-2 (3000 U/ml) plus IL-4 (10 ng/ml) on B-CLL-cells. After Ficoll-separation, lysis of the erythrocytes (NH_4Cl) and lysis of monocytes (l-leucin-methyl-ester), cells were divided into two groups. Group 1 was cultured in a serum free medium (+BrdU +cytokine) without any T-cell depletion. Group 2 was cultured in a serum free medium (+BrdU +cytokine) after T-cell ($CD3^+$) elimination by the MACS (Magnetic Activated Cell Sorting). Samples were taken after 20h, 68h, 92h, 116h and 140h. After staining with anti-BrdU FITC and propidiumiodide (PI) proliferation was measured by flow cytometry (FACScan).

IL-2	G1	G2	IL-4	G1	G2	IL-2+ IL-4	G1	G2
100 U/ml	P=6 N=3 I=0	P=2 N=7 I=0	0,1 ng/ml	P=1 N=16 I=0	P=0 N=15 I=1	IL-2 3000 U/ml	P=10 N=0 I=0	P=10 N=0 I=0
1000 U/ml	P=7 N=2 I=0	P=7 N=2 I=0	1 ng/ml	P=1 N=12 I=4	P=1 N=13 I=2	IL-4 10 ng/ml	P=1 N=4 I=5	P=1 N=4 I=5
3000 U/ml	P=8 N=1 I=0	P=7 N=2 I=0	10 ng/ml	P=4 N=11 I=2	P=6 N=8 I=2	IL-2 + IL-4	P=0 N=3 I=7	P=1 N=1 I=8
	n=9	n=9		n=17	n=16		n=10	n=10

P=proliferation N=no effect I=inhibition

Conclusions: IL-2 shows a proliferative effect on B-CLL-cells independent of T-cells. IL-4 shows heterogeneous effects. By itself it has most often no effect on proliferation, but sometimes it inhibits or increases the proliferation. This effect does not seem to depend on T-cells. It could depend on the dosage or some unknown patients' characteristics. Further on IL-4 inhibits IL-2 induced proliferation in nearly all cases independent of T-cells.

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PHOSPHODIESTER ANTISENSE OLIGONUCLEOTIDES TO THE BCR-ABL JUNCTION CAN NOT SUPPRESS PHILADELPHIA-POSITIVE CLONOGENIC CELLS FROM PATIENTS WITH CHRONIC MYELOGENOUS LEUKEMIA

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Chronic myelogenous leukemia (CML) is a clonal disorder of hematopoietic stem cells. The reciprocal rearrangement of the two protooncogenes BCR and ABL is a genetic hallmark of the disease. The expression of two different BCR-ABL hybrid genes (b3a2 and/or b2a2) is specific for leukemic cells and therefore target for antisense oligonucleotides to suppress gene activity.

Mononuclear cells of freshly drawn or frozen bone marrow specimen from 5 patients with CML were cultured in semisolid medium with 30% fetal calf serum, GM-CSF, Il-3 and Epo for 7 days. Sister cultures with 40 or 60 ug/ml of either antisense oligonucleotide or without antisense were examined in parallel. The number of colonies was determined. RNA was isolated from single colonies and reverse transcribed. Actin and BCR-ABL specific cDNA was amplified, using a modified one-tube method for nested RT-PCR.

We examined 7 bone marrow specimen from 5 different patients in chronic phase and blast crisis. Without antisense there were 52 colonies (range 18 - 112) in average, 43% (range 20 - 66%) BCR-ABL positive. With b3a2 antisense oligonucleotide, the number of colonies was reduced to 25 (range: 1 - 85) with 36% (range: 0 - 100%) positive for BCR-ABL mRNA. b2a2 antisense oligonucleotide decreased the number of colonies to 23 in average (range: 0 - 73), 35% (range:0 - 80%) positive for BCR-ABL mRNA. Our results, although with a low number of patients, show a reduction of colony number by incubation with unmodified antisense oligonucleotides. But BCR-ABL specific colonies have not been reduced significantly. 35% and 36% BCR-ABL positive colonies compared to 43% without antisense. Further investigations with different modifications (e.g. phosphothioate, 3'-inversion, 3'-GC-clamp etc.) of the oligonucleotides are warranted. They may exert a higher efficiency. Antisense oligonucleotides may offer a new method for purging of autologous bone marrow for ABMT.

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NCAM: A POTENTIAL NEW PROGNOSTIC MARKER IN MULTIPLE MYELOMA

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Clinical staging and management of multiple myeloma (MM) represent a common clinical problem. The best parameter for monitoring the disease is quantitative measurement of the monoclonal immunoglobulin level. Recent *in vitro* data suggest an expression of NCAM (neural cell adhesion molecule), a membrane glycoprotein, by myeloma cells but not normal plasma cells (Barker et al. 1992). Analysis of NCAM values in patients' sera with multiple myeloma, initially determined as a control population to patients with bronchogenic carcinoma, indicates a potential value of NCAM as a prognostic parameter for MM : 36 patients with histologically proven MM were divided into three groups at the time of NCAM determination : stable disease (SD; n=20), minor and non response (MR+NR; n=5) and progressive disease (PD; n=11). Mean NCAM levels were 11.5 U/ml for SD (median 11, range 3.2-29.2) 32.9 U/ml for MR+NR (median 16, range 12.4-86.3) and 99.2 U/ML for PD (median 96, range 3.7-302) as measured with a specific chemiluminescence test. There was no difference between healthy controls (n=10), patients with monoclonal gammopathy (n=4) and with stable disease. There was no correlation of NCAM levels to immunoglobulin levels and to clinical stage according to Salmon and Durie. Interleukin 6 levels correlated with clinical stage but not with disease progression and NCAM levels. However NCAM values for SD and PD were significantly different in the Student's t test at the 0.05 level. NCAM may prove to be a relevant prognostic marker in MM.

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INCREASED RETROVIRAL MEDIATED GENE TRANSFER INTO HUMAN HEMATOPOIETIC PROGENITOR CELLS: EFFICACY OF A NOVEL VECTOR CONSTRUCT

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Bone marrow (BM) stem cells are optimal targets for gene therapy because of their long-term potential to generate a high amount of vector-containing progeny. Retroviral mediated gene transfer into BM cells has been hampered by the low percentage of progenitor cells that can be transduced with the available retroviral vectors. Recently, an amphotropic hybrid vector system has been described in which the envelope gene of the Moloney mouse leukemia virus (MmLV) has been replaced by the envelope construct derived from the gibbon ape leukemia virus (PG13/LN). We have tested the rate of transduction that can be achieved with this vector, both in non-enriched and CD34 enriched BM, as well as in G-CSF mobilized human peripheral blood mononuclear cells (PBMC). The percentage of neomycin resistance conferred by this vector was compared to that achieved with the standard MmLV-derived neomycin vector (PA317/LN). BM and PBMC from normal donors and solid tumor patients were exposed to high titer vector (2-3 x 10^6) containing supernatant and later assessed for neomycin resistant CFU-GM colony formation and presence of neomycin phosphotransferase (Neo^R) cDNA by polymerase chain reaction (PCR). Transduction of PBMC with the Neo^R gene was efficient in both BM (PA317/LN 10%, PG13/LN 17%) and PBMC (PA317/LN 8%, PG13/LN 25%). Transduction with the new vector system was significantly more efficient than with the purely mouse-derived vector ($p<0.05$). Transduction of progenitor cells was most efficient using a 5-day supernatant exposure without initial cocultivation on vector producing cell lines and with addition of interleukin-1 (IL-1), interleukin-3 (IL-3), interleukin-6 (IL-6), and stem cell factor (SCF). In both vector systems, CD34 enrichment of BM and PBMC increased transduction efficiency (5% to 14.5%, $p<0.01$). Conclusion: The PG13 gibbon ape-derived envelope vector transduces human hematopoietic progenitor cells more effectively than the standard MmLV-derived vector system. CD34 enrichment can enhance and simplify the transduction.

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CYTOKINE LEVELS BEFORE AND AFTER APLASIOGENIC CHEMOTHERAPY IN HAEMATOPOIETIC MALIGNANCIES

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Myelosuppression after chemotherapy requires intensive supportive care and often limits the possibilities of therapy in malignant disorders. Reconstitution of bone marrow function is dependent on cytokines. In 50 patients we investigated the endogenous production of cytokines by measuring serum levels of G-CSF and IL-6 24 hours before and after aplasiogenic chemotherapy, during the stage of leuko- (<1000/µl) and thrombocytopenia and after recovery of the bone marrow (leuko > 1500/µl). In almost all patients a more than 5-fold increase of serum G-CSF was found during leukopenia. IL-6 levels were found to be elevated about 4-fold in the aplastic phase.

	before chemotherapy	after th./leukopenia
Serum G-CSF levels (pg/ml, mean, range)		
AML (DAV)	25 (5-100,n=14)	2000 (50-8000,n=7)
ALL (Hoelzer)	100 (10-300,n=4)	1800 (150-4000,n=2)
NHL (CEVED)	120 (10-200,n=4)	nd
Serum IL-6 levels (pg/ml, mean, range)		
AML (DAV)	30 (5-90, n=14)	200 (20-700, n=7)
ALL (Hoelzer)	40 (20-70, n=4)	150 (20-300, n=2)
NHL (CEVED)	10 (5-20, n=4)	nd

Our results show that endogenous release of G-CSF and, to a lower extent of IL-6 after aplasiogenic chemotherapy is highly elevated above basic levels. These findings may have implications upon the therapeutic application of cytokines after intensive chemotherapy.

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C-MYC EXPRESSION CORRELATES WITH EXPRESSION OF MUTATANT BUT NOT WILD TYPE P53 IN METASTASES OF COLORECTAL CANCER

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Human colorectal cancer is characterized by multiple genetic alterations affecting oncogenes and tumour suppressor genes. Both, elevated expression levels of c-myc and mutations in p53 occur in up to 70% of primary tumours, which indicates the importance of these genes in colorectal tumourigenesis. However, little is known about the involvement of p53 and c-myc in the progression of colon cancer, finally leading to distant metastases. Using differential polymerase chain reaction we determined c-myc expression and gene amplification in 27 metastases of colorectal cancer. 50% of the probes showed an elevated c-myc expression level compared to normal colon mucosa. Two to four fold amplification of the c-myc gene could be detected in 16 of the 27 metastases analysed (59%) and occured with a significant higher frequency compared to primary colorectal tumours ($p=0.001$). Since the expression levels were not correlated with the amplification status of c-myc, the functional significance of this amplification in tumour progression remains obscure. However, a positive correlation could be detected between the expression levels of c-myc and p53 in those metastases, which carry a p53 mutation ($p=0.007$). Thus, mutation of p53 may be a prerequisite for deregulation of c-myc. The results suggest that p53 contributes to a negative feedback regulation of c-myc expression.

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DISSEMINATED GROWTH OF HODGKIN DERIVED CELL LINES IN SEVERE COMBINED IMMUNODEFICIENT (SCID) MICE

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Local tumor growth has been reported after subcutaneous and intraperitoneal injection of Hodgkin derived cell lines into different immunodeficient mouse strains. Since new immunotherapeutic strategies will be employed in patients with disseminated disease, an animal model with disseminated growth of tumor cells would be useful for preclinical testing. Therefore, the Hodgkin derived cell lines L540, L540cy, L428 and KM-H2 were injected intravenously into SCID mice. In contrast to L428 and KM-H2, widespread neoplasia occurred after a period of 4-6 weeks following injection of L540 and the subline L540cy. The lymph nodes were found to be the preferred site of tumor growth. The CD30 surface antigen on Hodgkin cells and the karyotype of the cells were preserved in the animal host. Thus, the SCID mouse model mimics to a large extent the dissemination pattern of Hodgkin's disease in man and may provide a useful tool for evaluation of the efficacy of conventional and newly developed therapies.

To evaluate the role of adhesion molecule expression in the dissemination of Hodgkin-derived cell lines, CD44 and members of the immunoglobulin, integrin, selectin and Fc receptor families were quantified by flow cytometry. CD30 expression was also measured. Although CD44 expression has been correlated with dissemination in non-Hodgkin lymphoma, this was not the case in the Hodgkin SCID mouse model. CD44 was not expressed on the disseminating cell lines L540 and L540cy.

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RECOMBINANT HUMAN ERYTHROPOIETIN (rh-EPO) IN THE TREATMENT OF ANEMIA IN PATIENTS WITH HEMATOLOGIC MALIGNANCIES

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Erythropoietin has been used successfully to correct anemia in patients (pts) with chronic renal failure. The drug has also been reported to be effective in some groups of pts with cancer associated anemia. We tested the efficacy and safety of treatment with rhEPO (Boehringer Mannheim, FRG) in 16 pts with anemia and hematologic malignancies. Six pts had multiple myeloma, 7 pts malignant lymphoma (5 non-Hodgkin lymphoma, 2 Hodgkin disease), 2 pts agnogenic myeloid metaplasia and 1 pt myelodysplastic disorder. With the exception of the 2 pts with agnogenic myeloid metaplasia, all pts underwent chemotherapy. Four out of 7 pts with malignant lymphoma had a bone marrow infiltration.

Response, defined as an increase in hemoglobin more than 2 g/dl and independence of blood transfusion, was observed in 10 pts (62.5%) within a median of 5 weeks. The calculated mean dose of rhEPO being successful was 65 U/kg of body weight given s.c. daily. rhEPO corrected anemia in 5 out of 6 pts with multiple myeloma and in 5 out of 7 pts with malignant lymphoma. The 2 pts with agnogenic myeloid metaplasia, and the pt with myelodysplastic disorder failed to respond. In pts with multiple myeloma, response did not appear to depend upon renal function, and there was also no general negative impact of bone marrow infiltration in malignant lymphoma. No severe adverse event was observed. Two (12.5%) pts had mild hypertension, 2 (12.5%) pts injection site reaction and 1 (6.3%) pt bone pain.

In conclusion, rhEPO appears to be a save drug to correct anemia in pts with hematologic malignancies, particularly in those with multiple myloma or malignant lymphoma. For final results, however, a greater number of pts is needed to define predictive criteria for response to rhEPO.

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SHEDDING OF THE C-NEU ONCOGENE PRODUCT INTO THE SERUM OF PATIENTS WITH PRIMARY BREAST CARCINOMA

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The c-neu oncogene (also designated as HER-2, c-erbB-2) codes for a 185 kD transmembrane tyrosine kinase commonly referred to as p185. Presently, there are contradictory findings concerning the prognostic relevance of the c-neu oncogene expression in breast cancer. Using a double monoclonal antibody capture enzyme-linked immunosorbent assay (ELISA, Dianova, Hamburg), we previously found a correlation between the c-neu protein serum expression and clinical outcome in patients (pts) with metastatic breast cancer (Annals of Oncology, 1993). We now prospectively investigated the levels of circulating c-neu protein in 50 pts with primary breast carcinoma. Medium age was 65 years (37-89 years). Before mastectomy the c-neu protein serum level was low in 41 pts (82%) and elevated in 9 pts (18%). No clear difference in age, tumor size, hormone receptor, and nodal status between c-neu protein serum positive and c-neu protein serum negative pts could be observed. Furthermore immunohistochemical c-neu protein expression did not correlate closely with c-neu protein serum expression since 12/50 pts showed differences in their serum and immunohistochemical c-neu protein expression. So far, monitoring of the c-neu protein serum expression did not contribute to clnical detection of relapse. In summary, while clinical outcome of breast carcinoma pts with advanced disease and an elevated c-neu protein serum level seems to be poor our preliminary data do not yet suggest that this assay contributes in pts with primary breast cancer to the determination of prognosis and treatment strategies.

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SEQUENTIAL CHEMOIMMUNOTHERAPY IN METASTATIC MELANOMA: IFNα/IL-2 FOLLOWED BY DTIC/IFNα, DTIC/IFNα/IL-2 OR CDDP/IFNα/IL-2

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This abstract summarizes our 3-year experience in the treatment of metastatic melanoma with sequential or combined chemo-immunotherapy. Patients with progressing metastatic melanoma have been treated with IFNα and IL-2. 10 mill U/sqm IFNα were given s.c. on days 1-5, and a new decrescendo regimen of IL-2 was used: 1mg/sqm/6hours, followed by 1mg/sqm/12 hours, and 1mg/sqm/24 hours, and 0.25mg/sqm/24 hours x 3. The current response rate is 33% (3 CR, 12 PR, 16 MR/SD, 14 PD). Patients not responding to IFNα/IL-2 (SD and PD) were eligible for subsequent chemotherapy with DTIC, 850 mg/sqm day 1, followed by IFNα, 3 Mill U/day 2-6. The response rate for this second line regimen is 18% (1 CR, 3 PR, 5 SD, 13 PD, n=22). Using this sequential approach, the overall response rate in this cohort is 51%, and the median survival is 17 months.

In preparation of a randomized trial comparing chemo-immunotherapy and immunotherapy a pilot study was performed. Patients not responding to the standard IFNα/IL-2 regimen received a single dose of DTIC, 850 mg/sqm (n=6) or CDDP, 100 mg/sqm (n=7) on day one, followed by IFNα/IL-2 according to the identical protocol as previously without chemotherapy. In the case of CDDP, grade 3 nephrotoxicity was observed in 2/7 patients. Pharmacokinetics of IL-2 was not influenced by previous chemotherapy, except in the patients with CDDP-associated nephrotoxicity. Induction of secondary mediators (TNFα, IFNγ, neopterin, sCD25) by IL-2 was not diminished by previous chemotherapy. 4 patients unresponsive to immunotherapy alone showed tumor regression upon chemo-immunotherapy.

Conclusions: With initial immunotherapy followed in nonresponders by chemotherapy a response rate above 50% is achieved. Combined chemo-immunotherapy is feasible, and the immunologic response to IL-2 is not diminished by previous chemotherapy. A randomised multicenter trial is currently being performed within the EORTC to determine, whether combined chemo-immunotherapy is superior to immunotherapy alone.

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A new Hodgkin cell line (HD Zi5) with phenotypic and functional characteristics of mononuclear phagocytes

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Hodgkin and Reed-Sternberg cells have been shown to bear features of both lymphocytes and mononuclear phagocytes (MNPs). Although phenotypic similarities of Hodgkin cells and MNPs have been demonstrated, functional properties of MNPs have not been examined in the few Hodgkin cell lines available. We here describe a new Hodgkin cell line with several phenotypic and functional characteristics of MNPs. The cell line HD Zi5 has been established in our laboratory from a pleural effusion of a patient with nodular sclerosing Hodgkins' lymphoma. The line continuously grows in RPMI1640, supplemented with 10% fetal calf serum, the doubling time is 2.8 days. On light and electron microscopy, 80% of the cells resemble Hodgkin cells, 20% show the typical features of Reed-Sternberg cells. All subclones of this line again consist of Hodgkin and Reed-Sternberg cells, suggesting differentiation of Reed-Sternberg cells from Hodgkin cells. Cytochemical examination reveals strong reactivity with nonspecific esterase, a MNP marker, but also with PAS. Sudan black, a marker for myeloid cells, is negative. On Southern blot analysis no T-cell receptor or immunoglobulin gene rearrangement was detected. FACS analysis shows high expression of CD71, HLA-DR, and the β-chain of the IL-2 receptor (p75). Expression of CD14 as well as CD13, CD33, and CD10 is also consistently found, and low density CD16, HLA-I, and CD54. CD71 can be upregulated by IL-1, GM-CSF, and G-CSF, p75 by M-CSF, and HLA-DR by IFN-γ. Constitutive secretion of IL-6, and IL-8 is present, and regulated by many cytokines and lectins. Secretion of Neopterin can be induced by IL-1, IL-6, LPS, and IFN-γ. Release of TNF-α is induced by IL-1, IL-6, and IFN-γ, not by LPS. Another striking feature of this new cell line is the ability for phagocytosis. Latex beads are incorporated, and numerous phagolysosomes can be demonstrated by electron microscopy. In summary, these characteristics suggest an origin of this Hodgkin cell line from the lineage of mononuclear phagocytes.

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STIMULATION OF HEMOPOIESIS BY INTERLEUKIN 4 IN HUMAN STROMAL CELL CULTURES.

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We investigated the influence of interleukin 4 (IL-4) on expansion of hemopoietic progenitor cells (HPC) in human stromal cell dependent long-term cultures of Dexter type. After confluent stromal cell (SC) layers were established from normal bone marrow, fresh nonadherent bone marrow cells were added to the cultures and incubated in presence of IL-4 (500U/ml) or in culture medium alone. After 2-4 weeks the number of CFU-GM was determined. In presence of IL-4 a dramatic increase of HPC was observed exceeding the control cultures with medium alone by a factor of 3-5. The further experiments were performed with a purified population of CD34+ cells enriched by positive selection with immunomagnetic beads. The enhancement of HPC number by IL-4 in stromal cultures was confirmed in these experiments, the total number of CFUs was doubled in presence of IL-4. Next we examined a putative costimulation of IL-4 with stem cell factor (SCF) in stromal free suspension cultures. In fact, in unseparated bone marrow cells, IL-4 in combination with SCF induced a 5-fold increase of CFU number as compared with SCF alone, whereas in pure CD34 cells this combination enhanced the CFU number only to 180-200%. Examining the influence on adherence of hemopoietic cells to the microenvironment, IL-4 caused a moderate, but significant increase of CD34+ cells adhering to the stromal cells.

We conclude that IL-4 appears to enhance the expansion of HPCs in stromal cell cultures by several mechanisms: 1) by costimulation with SCF and/or other stromal-derived factors; 2) by enhancement of direct cellular contact between stroma and HPC; 3) induction of stimulatory factors or downregulation of negative regulators of HPCs by IL-4 cannot be excluded and will be investigated in future studies.

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CISPLATIN PHARMACOKINETICS

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In spite of its broad clinical application in the treatment of malignant disorders the pharmacokinetics of Cisplatin and the causes of its nephrotxicity are not fully investigated yet. In the present study the plasma concentrations of both protein complexed and free Cisplatin were measured by atom absorption spectrography (AAS) in 8 patients undergoing two or three cycles of COSS, PEI or PEB therapy up to 120 hours after the end of a 120mg/m² 5 hours, 5x30mg/m² 60 min or 5x20mg/m² 45min Cisplatin infusion. Renal elimination was also analysed in these 8 patients. Additionally microproteinuria was measured in all patients, since it is believed to be a potential parameter predicting a later kidney damage. Fitting the results of the plasma concentration and renal elemination measurement to a two compartment model the following pharmacokinetic parameters for free Cisplatin were obtained (average and VC): $t_{1/2\alpha}$ < 30 min (33%), $t_{1/2\beta}$ = 34.4 hours (30%), V_c = 404 l (33%), $clearance_{total}$ = 331 ml/min (17%), $clearance_{renal}$ = 141 ml/min (23%), renal $elimination_{in\ \%\ of\ dose}$ = 45 (17%). There was neither substantial intraindividual nor interindividual variability in plasma and urine kinetic parameters. In contrast a significant interindividual variability in microproteinuria was observed whereas each individual patient showed microproteinuria in the same range over all cycles of his therapy. Additionally this microproteinuria did not correlate with the integral of the Cisplatin urine concentration curve and the time.

To investigate whether nucleophile substances could be used to prevent nephrotoxicity caused by Cisplatin the potency of α-liponic acid to complex Cisplatin was tested and the protective property of this complex against Cisplatin toxicity. Under incubation with Cisplatin at equimolar amounts (37°C, NaCl concentrations 0mM, 20mM, 100mM, 150mM, ph 7.3, analysis by HPLC, UV-spectrography and AAS) formation of a new substance was observed containing 70% of the originally used Cisplatin. Formation of this new substance was inversely proportional to the NaCl concentration. During incubations with Cisplatin and α-liponic acid alone no degradation of both substances was observed. In tests with K-562 and HL-60 cells the resulting substance is shown to be nontoxic. A possible nephroprotective effect of α-liponic acid will be investigated by in-vivo animal studies.

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EFFECTS OF EITHER RECOMBINANT HUMAN ERYTHROPOIETIN (rh-EPO) OR KIDNEY TRANSPLANTATION ON OXYGEN AFFINITY OF HEMOGLOBIN IN CHRONIC RENAL FAILURE

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Correction of anemia should normalize the shift of the oxygen dissociation curve (ODC). Therefore the behaviour of pH-corrected ODC after rh-EPO administration under regular dialysis treatment (RDT) was tested as well as after kidney transplantation (KT). In 7 patients of group RDT and 9 of group KT the p50 (half saturation pressure of the corrected ODC) was determined in a HEM-O-SCAN (Aminco) once a week after the start of rh-EPO or after successful kidney transplantation. Hemoglobin (Hb) and red blood cell (RBC) concentrations of 2,3-DPG (enzymatically), ATP (by HPLC) and blood phosphate (P_i) were determined simultaneously. Ten normal subjects served as controls.

	Nr. of exp.	Hb (g/dl)	p50 (mmHg)	P_i (mM)	2,3-DPG (mmol/L RBC)	ATP (mmol/L RBC)
control	10	14.8±1.0	26.48±0.56	0.80±0.5	4.90±0.46	1.01±0.55
RDT before EPO	7	08.8±1.2	27.36±0.89*	1.90±0.8*	4.69±0.76	1.47±0.31•••
RDT after EPO	7	11.8±1.4**	28.35±1.05*	1.72±0.5	4.83±0.34	1.67±0.28*
KT before EPO	9	09.3±1.1	27.76±0.25	1.72±0.6	4.57±0.79	1.56±0.11
KT after EPO	9	11.9±2.0	25.78±0.56*	0.75±0.2*	5.00±0.75	1.13±0.67*

RDT 111±52 days after rh-EPO; KT 63±30 days after rh-EPO.

Hence, at comparable regression of anemia, kidney transplantation in contrast to exogenous EPO supply at RDT appears to normalize the oxygen affinity of hemoglobin which was accompanied by decreasing concentrations of plasma P_i and red cell ATP. The elevated concentrations of P_i and ATP in group RDT could explain the further diminution of the low oxygen affinity in the persistent uremia of endstage renal disease managed by RDT and rh-EPO.

The increase in ATP concentration under rh-EPO traetment in group RDT might be due to the elevated portion of younger red cells. Another explanation could be the increase of plasma P_i in uremia which have been proven to effect the ATP level in red cells. Elevated ATP concentrations might exert their effects on the ODC either directly by binding to the ß-cleft of hemoglobin or more indirectly by increasing the free concentration of 2,3-DPG.

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EXPRESSION OF THE TRANSFORMING GROWTH FACTOR-α BY HUMAN BLOOD CELLS IS RESTRICTED TO THE EOSINOPHIL POPULATION AND IS REGULATED BY INTERLEUKIN (IL)-3, IL-5, AND GRANULOCYTE -MACROPHAGE COLONY-STIMULATING FACTOR (GM-CSF).

M. Kiehntopf, M. Azemar, F. Herrmann, and M.A. Brach

In the present article we report that unlike human neutrophils and mononuclear (MN) cells, eosinophils fractionated from peripheral blood cells express the TGF-α gene. TGF-α transcripts were found in blood cells highly enriched for eosinophils from ten of ten healthy donors investigated. Constitutive TGF-α mRNA accumulation was associated with the release of immunoreactive TGF-α protein that became detectable 1 hour after initiation of in vitro culture of these cells. Moreover, exposure of eosinophils to the leukocyte-activating cytokines IL-3, IL-5, and GM-CSF caused upregulation of both TGF-α steady state transcript levels and TGF-α protein release into culture supernatants, while neutrophils and MN cells did not respond to these factor by TGF-α gene expression. The activating cytokines IL-8 and G-CSF failed to induce TGF-α synthesis in neutrophils and MN cells as well, but were also unable to modify TGF-α expression in eosinophils. The upregulatory effect of IL-3, IL-5, and GM-CSF on synthesis of TGF-α transcripts by eosinophils took place at the transcriptional level and was sensitive to inhibition of protein synthesis by cycloheximide.

Our findings bear clinical ramifications considering the potency of TGF-α and the wide distribution of eosinophils in many tissues and organs. Likely in vivo targets for any TGF-α action are stromal fibroblasts, which might respond to TGF-α produced by neighbouring eosinophils with proliferation and more importantly with production of extracellular matrix. This may trigger development of excessive fibrosis. Eosinophils have indeed been associated with fibrotic conditions such as endomyocardial fibrosis in patients with hypereosinophilia, liver cirrhosis following parasitic infection, or pulmonary fibrosis in asthma patient. Also tissues involved in Hodgkin's disease which is frequently associated with abundant sklerosis formation, contain numerous eosinophils. The functional role of eosinophils in these disorders may thus be explained by the present demonstration of TGF-α production in these cells. Finally, given the angiogenic potential of TGF-α and the frequent occurence of eosinophils in inflammatory and tumor tissue, involvment of eosinophil-derived TGF-α may also be considered in vasculatory processes during these conditions.

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LONG-TERM RETROVIRUS-MEDIATED GENE TRANSDUCTION INTO CANINE PLURIPOTENT HEMATOPOIETIC STEM CELLS

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Pluripotent hematopoietic stem cells are attractive targets for gene therapy because successful gene transfer can result in the continued presence of the gene transduced in all hematopoietic lineages for the lifetime of the recipient. However it has been difficult to efficiently transduce hematopoietic stem cells in large animals. We have therefore studied gene transfer in the dog as a large animal model using retrovirus vectors. Retrovirus vectors containing the neomycin phosphotransferase gene (neo) were used to transduce canine marrow and peripheral blood stem cells applying different transduction protocols. We have five long-term surviving animals that received transduced bone marrow stem cells showing intermittently 1-20% G418 resistant marrow derived CFU-GM colonies and the persistence of the neo specific sequences in peripheral blood granulocytes and lymphocytes detected by polymerase chain reaction (PCR) for more than 4 years now. To study the feasibility of genetically marking peripheral blood repopulating cells, peripheral blood progenitor cells were mobilized by treatment with kit-ligand for 8 days, collected and enriched for class II antigen-positive cells by avidin-biotin immunoadsorption, thereby enriching for repopulating cells. 3/3 dogs engrafted showing up to 10% G418-resistant marrow derived CFU-GM colonies and neo-specific sequences in bone marrow, peripheral blood granulocytes and lymphocytes for now up to 5.5 months after transplantation. We have also begun to study transfer of the human glucocerebrosidase gene (hGC) into canine marrow. 3 dogs transplanted with a vector carrying the hGC gene have shown persistence of the gene for up to 6 weeks, and we are currently analyzing hGC protein production in these cells. Further development of this model system may provide a treatment for humans suffering from glucocerebrosidase deficiency. Our data suggest successful retroviral transduction into canine hematopoietic stem cells from bone marrow as well as peripheral blood. Further, our results indicate that our canine model can be used to test possible therapeutic genes for their suitability in future human gene therapy.

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ANALYSIS OF THE GENE ENCODING FOR LIPOPOLYSACCHARIDE-BINDING PROTEIN (LBP)

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We reported recently on the complete cloning of the cDNA for LBP (Science 249:1429, 1990). LBP is a 58 kD Glycoprotein that binds endotoxin, enhances its effects and thus appears to play an important role in the development of gramnegative sepsis. LBP directs LPS to the cell surface of responsive cells and the LPS/LBP complex is recognized by the cellular receptor CD14. Synthesis of LBP takes place in hepatocytes and during an acute phase response serum levels of LBP rise substantially. We are interested in the analysis of the regulation of LBP expression and report here on the analysis of the LBP gene with a focus on its promoter. An EMBL-3 library was screened with an LBP cDNA probe and a positive clone was analyzed by southern blot technique. Restriction fragments that hybridized of 3 to 8 kB length were subcloned, sequenced and analyzed. Parallely genomic clones of BPI, a structurally and functionally related protein found in neutrophils, were analyzed and homologies in the intron-exon patterns were detected. The detailed analysis of the promoterregion is underway and the existance of regulatory elements in the LBP promoter is expected. Upregulation during the acute phase in vivo and in vitro was shown to be IL-1, IL-6 and Dexamethasone-dependent, so analysis of the promoter will be helpful in explaining the cascade of events leading to the induction of LBP in hepatocytes that in turn contributes to the septic shock syndrome.

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SEQUENTIAL ANTIMICROBIAL THERAPY FOR THE TREATMENT OF INFECTIONS IN NEUTROPENIC CANCER PATIENTS

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The present study investigates a three step antimicrobial strategy in the treatment of neutropenic cancer patients (pts) with fever of unknown origin (FUO), clinically and/or microbiologically documented infections. Inclusion criteria: fever > 38.5°C and absolute neutrophil count (ANC) < 500/mm³. Between 1/91 and 1/93 90 pts aged 17-77 (median 43.5) years (yrs) entered the study. 85 out of 90 pts had hematological malignancies (AML 40, ALL 10, NHL 31 pts). At the beginning of treatment 60 pts (67%) received a selective oral decontamination with Ofloxacin (2x200mg/d) and Fluconazol (1x400mg/d) prophylactically. Our sequential antimicrobial strategy consists of 3 phases. Phase I: Piperacillin (3x4g/d) + Netilmycin (1x400mg/d). Pts with persisting temperature > 38.5 °C more than 72 h entered phase II and were treated with Teicoplanin (1x400mg/d) additionally. In phase III the current antibiotic regimen was substituted by Ceftazidim (3x2g/d) and Amphotericin B (0.5-1mg/kG/d). 24 pts (27%) had FUO (Response Rate RR 96%), 21 pts (23%) had pneumonia (RR 76%), 31 pts septicemia (RR 97%) and 14 pts (16%) - including three pts who died - had pneumonia and septicemia (RR 64%). In 75 events microorganisms were isolated: 56 (75%) gram+, 15 (20%) gram-, 4 (5%) Candida. 61 pts finished antimicrobial treatment within phase I with a RR of 68%. 29 pts entered phase II with a RR of 56% and 12 pts passed into phase III with a RR of 58%. At the end of sequential treatment 83 pts (92%) were healed, 4 pts (5%) suffered from persisting infection and 3 pts (3%) died. In responding patients body temperature returned to normal levels within a median of 3 days (1-20). Median duration of neutropenia was 9.5 days (2-23). Median duration of treatment was 10 days (4-51). This data underline the effectiveness and safety of the sequential therapeutic concept used.

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PILOT STUDY WITH WEEKLY HIGH-DOSE 5-FU/FOLINIC ACID (HD-FU/FA) IN HEAVILY PRETREATED BREAST CANCER (BC) PATIENTS.

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After prior exposure to anthracyclines, other single agents induce <15% and combination chemotherapy 20% of objective remissions (RR) in metastatic breast cancer. With conventionally dosed FU/FA, RR-rates of 30% were achieved in phase II trials. Based on these results, a dose finding pilot study with fixed doses of FA and escalated doses of FU was performed in heavily pretreated BC patients.
TREATMENT: FA/FU once weekly x 6 with 2 weeks rest. Number of cycles (1 cycle corresponds to 6 applications of FU/FA) depending on response and toxicity. FA 500 mg/m^2 2h inf, then FU (dose level (dl) 1: 1.5 g/m^2 ; dl 2: 1.8 g/m^2; dl 3: 2.1 g/m^2) as 24h inf.
PATIENT CHARACTERISTICS: f/m 25/1; age 53 yrs (28-71), WHO PS 1(0-2), pretreatment 2.5(1-5) regimens per patient.
RESULTS: 7 pts were treated at dl 1, 4 at dl 2, and 15 at dl 3. No dose limiting toxicities occurred at dl 1/2. During 40 cycles with dl 3 the following toxicities were observed in (n) cycles: leucocytopenia 2° (3), 3° (1); thrombocytopenia 2° (3); diarrhea 2°(4); mucositis 2°(2); hand-foot syndrome (HF) 2°(6); 1 patient had diarrhea, mucositis, and HF each of WHO-grade 4. Tumor response at dl 1 (N=7) 3 NC, 4 P; at dl 2 (N=4) MR/NC 4; at dl 3 (N=15) PR 6 (40%), MR/NC 8, PR/MR/NC 14 {90% (95% conf. interval 75-100%)}, PD 1. Remission duration (dl 3) 3, 3+, 4+, 5+, 5.5 8+ months and duration of MR/NC 2.5, 3+, 3+, 4, 5.5+, 6, 8 months; median survival time not yet reached.
CONCLUSONS: This pilot study shows that weekly FA (500 mg/m^2) and HD-FU (2.1 g/m^2) are highly effective and can safely be administered to intensively pretreated breast cancer patients. The doses of dl 3 are those recommended for phase II trials.

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LONG TERM THERAPY WITH G-CSF LOW-DOSE IN FELTY'S SYNDROME

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Felty's syndrome, consisting of the triad of rheumatoid arthritis, splenomegaly and neutropenia, is a rare complication of RA, which predisposes patients to recurrent bacterial infections. A specific treatment does not exist so far.
We report the case of a 65 year old farmer with Felty's syndrome and recurrent severe infections. These infections(rec. pneumonia, neck abcess) were only treated effectively if GM-CSF or G-CSF were administered in combination with antibiotics. After having finished the treatment of the last pneumonia, we decided to pursue the therapy with low-dose G-CSF in attempt to maintain WBC above 1500/µl. The patient was treated initially with 300 µg G-CSF a day sc for 6 months.The dose was then gradually reduced to a maintenance dose of 300 µg three times a week. The patient has been treated with G-CSF for half a year by now. During this time he has neither been affected by infection nor by recurrence of his arthritis.

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AIDS AND BURKITT LYMPHOMA OF THE APPENDIX

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We report the case of a 36 year old woman who developed a HIV infection six years ago. One year ago, she consulted for abdominal pain. The patient had still 380 T helper cells/µl and no history of opportunistic infections. She received zidovudin. The diagnosis of appendicitis was made and the appendix resected. The histology revealed a lymphoma of high grade malignancy (Burkitt lymphoma). After resection in sano, a staging was performed and did not reveal any further lymphoma manifestation . It was classified as stage IIA according the Ann Harbor classification. We were then faced with the problem of further therapy. As extranodal manifestation , an aggressive chemotherapy should be applied, and presented the problem to worse the immunsuppression. Furthermore, because of the localization, an adequate radiation seemed impossible. In this situation and because a resection in sano had been obtained, it was decided to wait and see. Appendectomy has been performed one year ago. No relapse occurred by now.

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PERLECAN IN THE HUMAN BONE MARROW: AN ANTI-ADHESIVE COMPONENT WITH GROWTH-FACTOR PRESENTING ACTIVITY

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Controlled release of maturing hematopoietic cells from the bone marrow can be regulated by adhesive as well as by counter-acting, anti-adhesive components of the microenvironment. We have analyzed the expression and possible functions of a defined extracellular matrix component from the proteoglycan family, perlecan, in the human bone marrow. Human perlecan consists of a large multidomain core protein (M_r = 460000) with three heparan sulfate side chains attached to the N-terminal domain. As shown by indirect immunofluorescence and immunoprecipitation, perlecan is synthesized by stromal cells in long term bone marrow cultures and is also strongly expressed in the native bone marrow. Using an adhesion assay, we can demonstrate that perlecan is a strong repelling component for various leukemic cell lines, mononuclear cells isolated from the bone marrow as well as for adult lymphocytes. In contrast, skin fibroblasts strongly adhered to the proteoglycan. Since heparitinase-digested perlecan still showed an anti-adhesive effect we suggest that the domain responsible for the repelling activity is located within the huge core protein.
To determine whether this repulsive molecule can bind growth factors and present them to progenitor cells we incubated plastic coated perlecan with GM-CSF for several hours. After removing the unbound factor bone marrow mononuclear cells were added in a semi-solid medium. After fourteen days of culture formed colonies could be only observed in those cultures where GM-CSF was added, perlecan alone had no growth factor activity. We suggest that anti-adhesive molecules like perlecan could serve for compartmentalization of the hematopoietic microenvironment by presenting growth factors only for short periods and forcing the maturing cells to different places within the marrow.

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Comparison of side effect rates in 3 different preparations of Amphotericin B

K.O. Kliche, M. Arning, A. Wehmeier, T. Südhoff, M. Reuter and W. Schneider

The clinical use of amphotericin B (Am B) is frequently accompanied by severe side effects such as fever, chills and hypotension. Therefore a liposomal formulation of Am B (AmBisome®) has been developed to reduce adverse effects. Recently the application of Am B in lipid emulsion has been recommended. The side effects of Am B are mediated by liberation of acute phase cytokines, e.g. TNF α. In contrast to clinical observation determination of cytokine plasma levels allows an objective documentation of adverse drug effects.
We treated 5 patients consecutively with conventional Am B, followed by AmBisome® and Am B in lipid emulsion. All of them had acute leukemia complicated by fungal infections in bone marrow aplasia after chemotherapy. We documented signs and symptoms of intolerance, i.e. fever, chills, nausea and vomiting. Plasma levels of Interleukin -1β (IL-1β), Interleukin-1-receptor-antagonist (IL-1-RA), TNF α, soluble-TNF-receptor (s-TNF-r), Interleukin-6 and Interleukin-8 were serially determined by ELISA before and up to 6 hours after Am B infusion.
All 5 patients showed severe febrile reactions accompanied by chills, nausea and sometimes vomiting after having received conventional Am B. Subjective adverse effects were reduced by Am B in lipid emulsion, although fever occurred in all patients. AmBisome® could be applied without side effects in 3 patients showing drug intolerance to conventional Am B. One patient had fever upon infusion of AmBisome® up to 39,0° C, another patient developed fever which could not be attributed to the study drug. All patients experiencing adverse reactions showed elevated cytokine plasma levels in a characteristic time dependent manner. Most pronounced increases in cytokine plasma levels were observed after Am B in lipid emulsion with a maximally 40-fold elevation of TNF α 90 minutes after start of infusion.
We conclude that Am B in lipid emulsion offers little advantage over conventional Am B with regard to clinical side effects and induction of cytokine activity. AmBisome® was tolerated best but was not free of subjective and objective adverse drug reactions.

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The role of cytokines in drug fever

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Febrile reactions are mediated by an array of cytokines. Determination of cytokine plasma levels promises insights into pathophysiological mechanisms as well as new therapeutical strategies. We chose acute toxicity after intravenous amphotericin B (Am B) application comprising fever, chills and severe hypotension as in-vivo-model of adverse drug reactions.
We analyzed 33 episodes of Am B-application in patients suffering from acute leukemia and fungal infection. In 10 patients Am B was well tolerated. These patients served as control group in order to study cytokine behaviour in the absence of adverse effects.
We determined plasma levels of Interleukin -1β (IL-1β), Interleukin-1-receptor-antagonist (IL-1-RA), TNF α, soluble-TNF-receptor (s-TNF-r), Interleukin-6 and Interleukin-8. Serial EDTA blood samples were collected every 30 minutes immediately before and up to 6 hours after start of Am B infusion. Samples were instantaneously centrifuged and stored at -40° C until analysis by ELISA (Medgenix and R&D Systems).
In patients experiencing fever a consistent pattern of acute-phase-cytokine liberation was observed. TNF α levels peaked first 90-120 minutes after Am B infusion and returned to normal within 1 - 2 hours.TNF α-peaks reached a 40-fold-increase in concentration with maximum levels of about 900 pg/ml and preceded fever peaks for about 60 minutes. Plasma levels of IL-6 and IL-8 rose up to 120 minutes later compared to TNF α. The kinetic of IL-1-RA and s-TNF-r was characterized by a delayed increase with maximum levels at 300-360 minutes and a slower decrease. Interestingly no IL-1β could be detected in the circulation. Patients without adverse reactions did not show significant elevations in cytokine plasma levels.
Several conclusions can be drawn from these results: 1. Cytokines are involved in the pathogenesis of drug fever, e.g. after Am B-application. 2. Adverse drug reactions induce secondary cytokines such as Il-6 and Il-8 which may mediate delayed toxicity. 3. Acute-phase-cytokines possess short half-lifes in circulation rendering their use in diagnostic purposes difficult.

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SUPPRESSION OF THE LEUKEMIC CELL CLONE OF PHILADELPHIA CHROMOSOME-POSITIVE CML BY INTERFERON: REMISSION QUALITY AND PROGNOSTIC IMPACT.

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Treatment of CML patients (pts) with interferon (IFN) alpha has been shown to lead to varying degrees of suppression of the leukemic cell clone characterized by the Philadelphia chromosome (Ph). In order to assess the prognostic impact of cytogenetic improvement, we have studied the outcome of 71 pts with chronic phase CML who were given IFN alpha as first-line therapy of their leukemia. Of 62 pts (87%) evaluable for cytogenetic response, 16 (23%) had no improvement, 28 pts (38%) showed a decrease in Ph-positive bone marrow metaphases to levels between 35% and 95%, and 9 pts (13%) to levels of 5-34%. In 9 pts (13%), Ph-positive bone marrow metaphases were no longer detectable. Cytogenetic improvement was found to translate into improved survival expectancy: The projected 5-year survival was 90% for pts attaining a Ph reduction to less than 35%, 55% for pts with a Ph suppression to levels between 35% and 95%, and less than 10% for those without any cytogenetic improvement. Pts demonstrating complete normalization of bone marrow karyotypes were further studied for the presence of residual leukemic cells. In some of these pts, cytogenetic analysis of blood cells still revealed varying numbers of Ph-positive metaphases. Polymerase chain reaction (PCR) also showed residual clonally derived cells in unfractionated blood and marrow samples. PCR analysis of single colonies derived from myeloid precursor cells, however, failed to detect leukemic progenitors in 4/7 pts tested. Thus, the nature of residual CML cells persisting within polyclonal hematopoiesis remains to be elucidated.

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THE BCL-2 BREAKPOINT BINDING PROTEIN INTERACTS WITH SINGLE STRANDED GG-SPACER-GG SEQUENCES

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The translocation t(14;18) juxtaposes the BCL-2 oncogene with the immunoglobulin heavy chain gene. Recent data (Wyatt et al., J.Exp.Med 175:1575) suggest that *chi*-like (minisatellite) sequences which occur around the breakpoints on both chromosomes 14 and 18 are involved in the mechanism of this illegitimate chromosomal recombination. We have previously identified a 45kDa nuclear protein (bp45) from early B cells which binds to these elements in gel retardation assays. We have now determined the binding specificity of this protein in competition experiments using a 20bp-fragment from the BCL-2 breakpoint region, mutated oligonucleotides and affinity purified fractions. bp45 interacts predominantly with the G-rich single-strand of the *chi*-like sequences. The minimal consensus sequence consists of variable spacers (2-18 nucleotides) flanked by two guanosine residues on either side (GG-spacer-GG). Since the BCL-2 major breakpoint region is S1-nuclease sensitive (Jaeger et al., Blood 81: 1833) and can therefore assume a single-stranded configuration in living cells, bp45 may play a key role in the process of chromosomal recombination.

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TRISOMY 12 IN B-CELL CHRONIC LYMPHOCYTIC LEUKEMIA DETECTED BY IN SITU HYBRIDIZATION: CORRELATION WITH ADVANCED STAGE DISEASE AND WITH REFRACTORINESS TO TREATMENT

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Trisomy 12 is known as a common chromosomal aberration in B-cell chronic lymphocytic leukemia (B-CLL). Its impact on therapy free survival and on overall survival has been discussed controversily. However, a proliferative advantage of trisomy 12 positive B-cells over trisomy 12 negative ones has been suspected. Therefore, in situ hybridization was performed to study incidence and clinical significance of trisomy 12 in 50 patients (pts) with B-CLL at various stages of disease. Trisomy 12 was detected in 12%-65% (median 53%) of the circulating neoplastic cells of seven out of 20 pts at Binet stage C, whereas 22 pts at Binet stage A and another eight pts at Binet stage B were found to be trisomy 12 negative ($p<0.005$). Moreover, trisomy 12 was associated with the presence of B-symptoms ($p<0.01$) and hepatosplenomegaly ($p<0.05$), thus further reflecting the correlation with advanced stage disease. No correlation with a lymphocyte doubling time of <12 months nor with a marked lymphadenopathy nor with prior treatment became apparent. Serum levels of CD8, CD23, and CD25 were found to increase with advancing stages of the disease. However, within the group of Binet stage C pts, those with trisomy 12 displayed higher serum levels of CD25 than pts without trisomy 12 ($p<0.05$), whereas no differences were detected in serum levels of CD8 and CD23. As elevated serum levels of CD25 corresponded to the presence of B-symptoms ($p<0.05$), again the linkage of trisomy 12 with symptomatic disease was evident. In addition, trisomy 12 was detected predominantly in pts refractory to treatment ($p<0.05$), suggesting an involvement of trisomy 12 in drug resistance. In conclusion, trisomy 12 in B-CLL appears to occur predominantly in advanced and symptomatic disease. It indicates a high risk for treatment failure and seems therefore to serve as marker of poor prognosis.

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EFFECTIVE TREATMENT OF MEDIASTINAL LARGE B-CELL LYMPHOMA WITH HIGH-DOSE METHOTREXATE BASED POLYCHEMOTHERAPY

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Primary mediastinal large B-cell lymphoma (MLCL) is a distinct rare entity among high-grade Non-Hodgkin's lymphoma, preferentially evolving in young female patients (pts). It is characterized by massive, infiltrating local growth, and is reported to have a poor prognosis after standard CHOP or irradiation therapy alone. Therefore, we examined efficacy and feasibility of a high-dose MTX (HD-MTX) based polychemotherapy in a phase II study. Eight pts (5 female, 3 male; 2 pts with stage II disease, 2 stage III, 4 stage IV) with a median age of 24 years (range 18-45) were treated with 1.5 g/m^2 MTX administered as a 24 h infusion and subsequent leucovorin rescue. In addition, an alternating combination of cyclophosphamide/ adriamycin and ifosfamide/Ara-C/tenoposide was given together with vincristine/ dexamethasone in 14 days intervals. The pts received 2-6 (median 6) courses of therapy. Five pts achieved a CR with a median duration of 40+ months (range 2-44), two pts attained a PR, and one was a non-responder. Toxicities were tolerable, and cytopenic periods were short (range 4-11 days). These results were compared with those obtained in another 16 pts with MLCL (9 female, 7 male; 9 pts with stage II disease, 2 stage III, 5 stage IV; median age 40 years, range 19-67) who were treated according the COP-BLAM/IMVP-16 protocol of the german BMFT study group. Medians of 5 courses (range 3-6) COPBLAM followed by 2 courses (range 1-5) IMVP-16 were given. Ten of these 16 pts received an additional irradiation therapy with 40-50 Gy. A CR was achieved in 11 pts, a PR in 4 pts, and one was a non-responder. Nine pts remained in CR with a median duration of 25+ months (range 6-60). In conclusion, multicomponent chemotherapy induces stable CR's in a substantial number of pts with MLCL. A shorter course, HD-MTX based alternating combination treatment may provide an alternative to conventional regimens.

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ANALYSIS OF CLONE-SPECIFIC T-CELL RECEPTOR (TCR) GAMMA/DELTA - CHAIN DNA SEQUENCES IN MALIGNANT LYMPHOMAS AND LEUKEMIAS.

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We have investigated the structure of rearranged gamma/delta TCR variable (V) - joining (J) - regions in DNA extracted from 17 patients (6 T-ALL, 9 peripheral T-cell lymphomas, 2 normal controls and the T-cell lines HUT 102 and Jurkat). Rearranged V gamma/delta - J gamma/delta TCR - gene segments were amplified by the polymerase chain reaction (PCR) with V - and J - region specific primers. Because most of the biopsy tissue or bone marrow samples, from which the DNA was extracted, contained significant amounts of admixed nonmalignant (polyclonal) gamma/delta T-cells, direct DNA - sequencing of the PCR products yielded unreliable sequence data due to coamplification of the polyclonal V-N-(D)-J junctions with the clonal TCR-gene segments. We therefore cloned the PCR-products after ligation in pUC 19 vector DNA and transformation in E.coli DH5a. The sequences of 4-10 cloned and separately analysed PCR products from each individual patient or cell line were determined. In the polyclonal controls all analysed PCR-products differed in their clone specific V-N-(D)-J- junctions, as expected. In the clonal controls (T-cell lines) and in the T-cell malignancies, several (30-100%) of the cloned isolates contained identical V-N-(D)-J- junctions which represent clone-specific identification sequences for individual T-cell clones. By sequencing a total of 104 TCR-gamma and 67 TCR-delta V-N-(D)-J- junctions, clonality could be demonstrated exclusively in the T-cell lines, in 6/6 of the ALL`s and in 7/9 of the T-cell lymphomas. The results were confirmed by temperature-gradient-gel-electrophoresis (TGGE) showing distinct DNA bands only with the PCR-products which contained clonal (i.e. identical) TCR-gamma/delta V-N-(D)-J- junctions. In summary we demonstrate that coupling of the amplification of TCR-gamma/delta V-N-(D)-J- junctions by PCR, identification of clonal PCR products by TGGE and DNA sequencing is the method of choice for the characterization of clonal TCR sequences as extremely sensitive and potentially useful diagnostic markers in T-cell malignancies.

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THE EXPRESSION OF THE P56^{LCK} PROTO-ONCOGENE IN B-CELL LINEAGE NEOPLASIAS

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p56^{lck} is a member of the src family of protein tyrosine kinases.
In T-cells p56^{lck} is known to be involved in the activation and signal transduction and can complex with CD4 and CD8 differentiation antigens or the ß-chain of the IL-2 receptor. In the mouse T-cell line LSTRA it was shown that the insertion of a retrovirus leads to an overexpression of p56^{lck} that results in the transformation of the cells.
We have shown that lck transcripts are detectable in 12 out of 16 analyzed cell lines derived from Burkitt's lymphoma (BL) as well as in lysates from patients with chronic lymphatic leukemia (CLL) [Leukemia, 1991, 6:528-530]. Our question was: is p56^{lck} expressed at the protein level in a catalytically active form ?
Using a polyclonal anti-human p56^{lck} antiserum by Western blotting we have found that p56^{lck} is expressed in BL's and CLL's.
Autophosphorylation and the phosphorylation of an exogenous substrate was demonstrated after immunoprecipitation which suggests that the protein may be catalytically active.
Stimulation of BL 2 with SAC/anti-Cμ and of Jurkat T-cells with anti-CD3/PMA leads to hyperphosphorylation of p56^{lck} shown by gel-retardation. The hyperphosphorylation is only detectable during the first six hours after stimulation, whereas in Jurkat cells the hyperphosphorylation is stable for 24 h after activation. Different kinetics may depend on differences in the serine/threonine protein kinase pathways.
After activation of a CLL with PMA a complete loss of p56^{lck} could be observed.
Addition of the tyrosine kinase inhibitor Herbimycin A results in BL 2 cells in an increase of expressed p56^{lck}, as determined by Western blotting in contrast to Jurkat cells, where the level of expressed p56^{lck} is not changed.
Thus we conclude that p56^{lck} may be involved also in B-cell transformation.

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MULTIMODALITY CONCEPTS IN THE TREATMENT OF SOLID TUMORS WITH CURATIVE INTENTIONS: COLORECTAL CANCER

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Colorectal cancer is the second most common malignancy in Germany with an incidence of 40,000 new cases per year. It is therefore a major public health problem. Over the past 30 years the mortality of colorectal cancer shows a declining trend despite a lack of major breakthroughs in therapy. Less than half of all diagnosed patients are cured by surgery alone. More recent studies of post surgical adjuvant therapy for stage II and III colon and rectal cancer document significant benefits with respect to disease free intervals and overall survival. Different multimodality therapy concepts are presently studied in trials to improve on published adjuvant treatment results. Pre- vs. postoperative radio- and/or chemotherapy in rectal cancer or postoperative immunotherapy with whole or modified cancer cell vaccines or monoclonal antibodies for colon cancer are tested. Defined surgical approaches with standardized TNM-staging procedures are tested for their prognostic value. The thorough planing of comprehensive multicenter trials is stressed, including reviewed documentation of alterations in newer genetic and molecular markers for colorectal cancer like allelic los variants oncogenes, tumor suppressor genes (ras, 5q-, 18q-, 17p-, nm23, p53 etc.) or thymidilate synthetase gene expression. Furthermore, these multicenter trials should be designed to possibly establish new markers of prognostic value. The medical community is urged to enter all eligible patients into studies for a better understanding of the disease and advancements in treatment. Finally, the declining trend for colorectal cancer mortality should be accelerated improvement of cure rates. A more thorough and intensified public health education conveying established facts of epidemiology, early detection, and prevention of colorectal cancer should contributed to further decline colorectal cancer mortality and to improve cure rates.

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PHASE II STUDY OF THE COMBINATION OF PALA/METHOTREXATE AND 5-FU IN ADVANCED COLORECTAL CARCINOMA (CRC).

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Modulation of 5-FU by methotrexate is clinically effective. PALA and methotrexate enhance the antineoplastic activity of 5-FU in experimental modes by increasing its incorporation into RNA via different mechanisms.

Treatment plan: PALA 250 mg/m^2 i.v. d1, methotrexate 200 mg/m^2 i.v. d1, 5-FU 600 mg/m^2 i.v. d2, folinic acid 15 mg/m^2 p.o. x 8 starting 24h after methotrexate. Cycles were repeated every 2 weeks until progression. If toxicity was acceptable 5-FU was to be escalated up to 900 mg/m^2.

Patients characteristics: 17 untreated patients with histologically proven colorectal cancer were enrolled. 10 male, 7 female, median age 59 y (32-71 y). Median PFS 1 (0-2). 75 % of pts. had liver involvement, 41 % lung, 30 % nodes.

Results: 121 cycles were administered. Median number of cycles per patient was 6 (2-17). Toxicity was mainly gastrointestinal with mucositis grade 2 (CTC) in 3 pts. (18 %), nausea grade ≥ 2 (CTC) in 4 pts. (24 %), and diarrhea grade ≥ 3 (CTC) 4 pts. (24 %). 1 pts. with diet controlled diabetes died of hyperglycemia and another insulin dependent diabetic had increased insulin requirement on days of therapy. Response: 1 CR, 1 PR, 13 NC and 1 pts. with disease progression. Progression free interval was 20 weeks (2.4-42+).

Conclusions: The combination of PALA, Methotrexate and 5-FU does not appear to be superior to other schedules in the therapy of advanced CRC. Investigators should be aware of the unexpected alteration in serum glycose levels, possibly due to PALA-treatment.

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RECOMBINANT HUMAN GRANULOCYTE COLONY-STIMULATING FACTOR (RHG-CSF) DOES NOT STIMULATE IN VIVO TUMOR GROWTH OF THE HUMAN COLON CANCER CELL LINE HTB 38 WHICH IS RESPONSIVE IN VITRO.

M. Koenigsmann, M. S. Topp, E. Thiel, and W. E. Berdel

The growth stimulatory effect of cytokines like granulocyte colony-stimulating factor (G-CSF) is not restricted to the hematopoietic system but also occurs in a variety of non-hematopoietic cell lines and in fresh tumor specimens in vitro. The clonal growth of the colon adenocarcinoma cell line HTB 38 was previously shown to increase 1.5-fold when incubated with 5 ng/ml rhG-CSF. In order to further study the implication of this finding for clinical trials with rhG-CSF in tumor patients, we have examined the effects of rhG-CSF on xenotransplanted HTB 38 cells in athymic mice. Recombinant human G-CSF (Amgen, Munich, FRG) was administered as a subcutaneous bolus twice daily from day 1 to 14 after tumor transplantation at a dose level of 312 µg/kg/day. Serum levels of rhG-CSF were within the range required for the in vitro effect. However, the cytokine caused no significant growth modulation of the tumor in vivo. This result suggests, with due caution, that the potential hazard of in vivo tumor stimulation may not be relevant for cancer patients treated with rhG-CSF in conjunction with cytotoxic chemotherapy.

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EARLY DOSE-INTENSIFICATION WITH AUTOLOGOUS HEMATOPOIETIC STEMCELL SUPPORT FOR POOR PROGNOSIS HIGH GRADE NON-HODGKIN'S LYMPHOMAS (NHL)

H. Köppler

Recently the intergroup study by SWOG, comparing standard CHOP with three intensive chemotherapy regimens, showed no advantage for any of these conventional protocols. However this study as well as several other large controlled trials demonstrate that the prognosis of patients with high grade NHL depends largely on initial prognostic features: The risk categories as proposed by the international prognostic factors project or risk models using only the initial LDH-levels are able to identify patient cohorts which will have a poor survival with currently available conventional treatment options. The major reason for treatment failure is relapse. A number of phase II-trials have shown that a fraction of relapsed patients can be salvaged by the use of high dose chemotherapy I TBI followed by autologous stemcell transplantation (ASCT). An analysis of the EBMT-registry data for patients, who underwent high dose chemotherapy + ASCT after having relapsed, indicate that especially patients who are still responsive to conventional chemotherapy may benefit from this approach. Based on these observations if seems rational to conduct trials which integrate high-dose chemotherapy followed by ASCT in first time treatment protocols for patients with risk factors that predict poor outcome.

In 1991 we started a randomized trial comparing 5 cycles CHOEP with 3 cycles CHOEP followed by high-dose chemotherapy (BEAM) with autologous bone marrow transplantation for patients ≤ 60 years and an elevated LDH-level at diagnosis. Based on our previous trials the predicted survival of these patients is 40 % at 3 years. Results of the present trial will show if this patient cohort will benefit from a high dose approach.

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HIGH-DOSE CVB OR BEAM FOLLOWED BY NON-CRYOPRESERVED AUTOLOGOUS BONE MARROW TRANSPLANTATION FOR POOR PROGNOSIS HIGH GRADE NON-HODGKIN'S LYMPHOMA

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24 patients with poor prognosis high grade non-Hodgkin's lymphoma (hg NHL) were treated either with high-dose CVB: cyclophosphamide (120 mg/kg), etoposide (2100 mg/m^2), and BCNU (300-600 mg/m^2) or BEAM: BCNU (300 mg/m^{2}g), etoposide (100 mg/m^2 x 8), cytosinarabinoside (200 mg/m^2 x 8), and melphalan (140 mg/m^2). All patients received an autologous stemcell rescue with bone marrow, which had been stored non-frozen at 4°C for 72-96 h. All evaluable patients (22/24) had a full haematopoietic recovery. Median time to achieve a neutrophil count > 500/µl was 17 days (range 12-27) and median time to achieve an unsupported platelet count > 20.000/µl was 21 days (range 16-55). 11 patients with risk factors were treated in first complete or partial remission, 12 relapsed patients were in second complete remission or had chemosensitive relapses, one patient had primary refractory disease. With a median follow up of 8 months (range 1-54), the estimated event free survival (event = death or progression) is 54 % at 2 years, with 39 % for high risk patients treated in first complete or partial remission and 54 % for relapsed patients in second CR or sensitive relapse. The patient with refractory disease died 2 week after transplantation from transplantation related toxicity. We conclude that high dose chemotherapy followed by non-frozen autologous bone marrow rescue is safe in terms of haematopoietic reconstitution and the follow up data suggest a useful efficacy.

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COST ANALYSIS OF G-CSF APPLICATION AFTER HIGH DOSE CYCLOPHOSPHAMIDE, ETOPOSIDE AND BCNU (CVB) AND AUTOLOGOUS BONE MARROW TRANSPLANTATION FOR POOR PROGNOSIS MALIGNANT LYMPHOMA

H. Köppler, K. Havemann

In a retrospective study the costs of G-CSF application in 24 consecutive patients with poor prognosis malignant lymphoma who were treated with high-dose cyclophosphamide (120 mg/m^2), etoposide (2100 mg/m^2) and BCNU (300-600 mg/m^2) followed by autologous bone marrow transplantation (ABMT) were analysed. The first 12 patients received no G-CSF after ABMT. The following patients received G-CSF 10 µg/kg sc/d from day +1. G-CSF dose was reduced to 5 µg/kg sc/d when neutrophil counts exceeded 1000/µl for three consecutive days and G-CSF was stopped if neutrophils stayed > 1000/µl for another three days. Results are shown below.

	no G-CSF n=12	with G-CSF n=12
days to PMN > 500/µl, median (range)	23 (15-39)	15 (11-20)
days with T > 38°C, median (range)	4 (0-9)	2 (0-5)
days with iv antibiotics, median (range)	16 (0-23)	6 (0-10)
days in hospital, median (range)	34 (23-44)	25 (21-36)
costs for iv antibiotics (DM)	45.836,-	15.280,-
costs for G-CSF (DM)	0,-	124.550,-
costs for iv antibiotics + G-CSF (DM)	45.836,-	139.830,-
hospital charge (sum of all pat. in DM)	205.500,-	161.500,-

We conclude that G-CSF reduces the rate of infections and thus the use of iv antibiotics due to an accelerated neutrophil-recovery. The significantly reduced number of days in hospital produces an unsatisfying development of costs for the hospital if charges are based on a fixed amount per day.

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MOLECULAR MONITORING OF MINIMAL RESIDUAL DISEASE IN APL PATIENTS

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Acute promyelocytic leukemia (APL) characterised by the chromosomal translocation t(15,17) is associated with a unique transcriptional product, PML/RARα. We investigated bone marrow and/or peripheral blood of 8 newly diagnosed APL patients during the course of disease by a method using RT-PCR. Patients were studied since April 1992, 8 patients received ATRA (45 mg/m^2), 6 of them in combination with high-dose chemotherapy for induction and subsequent consolidation therapy after 4-52 weeks in clinical complete remission, 2 patients were first treated with ATRA alone and received cytostatic therapy at relapse.
In concordance with previous reports we observed different types of alternatively spliced PML/RARα mRNA in the blasts of all investigated patients. All samples of both patients treated with ATRA alone were PCR-positive at 1st step (>1:10^4 cells) despite they achieved clinical and cytogenetic remission. Both patients relapsed within a few months. In all patients, who were treated with ATRA in combination with chemotherapy, PCR-negativity was induced. None of these patients relapsed so far after 6 - 13 months in complete remission.
In conclusion molecular monitoring in APL patients has significant clinical importance and the results may influence therapeutic management.

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INCREASED SENSITIVITY OF PURINE ANALOGUES (2-CdA, GEMCITABINE) FOR MYELOID PROGENITOR CELLS FROM PATIENTS WITH CML COMPARED TO NORMAL HUMAN PROGENITORS

G. Konwalinka, F. H. Geisen, M. Schirmer, U. Zilian, H. Braunsteiner

2-chlorodeoxyadenosine (2-CdA) and 2',2' difluorodeoxycytidine (gemcitabine) are new purine analogues with strong antineoplastic activity against various kinds of solid tumors and leukemic cell lines. The aim of this study was to test the growth inhibiting activity of these compounds against progenitors obtained from patients with CML in stable phase and normal human donors by using the clonogenic methylcellulose assay. The results show that both 2-CdA and gemcitabine inhibit the growth of CML as well as normal human progenitor cells in a dose dependent manner. This growth inhibiting effect correlated with the maturation stage of progenitor cells in that the more inmature progenitor cells are more sensitive to these drugs. Furthermore our in vitro results show that gemcitabine is a more potent cytostatic drug to CML myeloid progenitor cells with an inhibiting concentration of 50 % ranging from 2 to 3 nM for gemcitabine and from 10 to 20 nM for 2-CdA after continuous exposure. However, in comparison to normal human progenitor cells CML cells were markedly more sensitive to the inhibitory effect of these purine analogues; only half a concentration of these compounds was required to obtain a similiar inhibitory effect. This effect was time dependent, since preincubation studies showed that for 2-CdA and gemcitabine an exposure time of 48 and 144 hours, respectively, was required for normal human progenitors to obtain an inhibitory effect comparable to that found in cultures with continuous exposure. However, to obtain a similar growth inhibiting effect in CML myeloid progenitor cells a markedly reduced preincubation time was required for both purine analogues compared to that needed for normal human progenitor cells.
In conclusion our results show that both purine analogues may be promising drugs in the treatment of patients with CML in stable phase.

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PHASE I/II STUDY OF DEXVERAPAMIL (DVPM), EPIRUBICIN (EPI) AND GmCSF IN ADVANCED PANCREATIC CANCER. G.Kornek, J.Funovics, M. Raderer, J.Kastner, I. Virgolini, and W.Scheithauer.

Based on histological studies indicating that pancreatic cancer (PC) expresses high levels of P-glycoprotein that might be related to its inherent chemotherapeutic refractoriness and the favourable therapeutic index of the investigational MDR modulator DVPM, we have performed the present phase I/II study. Until today, 24 patients (pts) with previously untreated PC were entered; median age was 59 years, and the median WHO performance status was 0. Treatment consisted of oral DVPM 1000-1200 mg/d for 3 days, epirubicin 90mg/m2 on the 2nd day of DVPM with 15mg/m2 dose escalations in subsequent pt cohorts in the absence of WHO grade III systemic or grade IV hematologic toxicity, and GmCSF 400µg/d s.c. on days 5 through 14. Cycles were repeated every 21 days. Adverse reactions consisted mainly of myelosuppression (grade IV in 0/4, 4/8 and 3/8 pts at dose levels 1 to 3). Grade III nonhematologic toxicity was seen in 1/4 pts at dose level 1 (infection), 4/8 pts at dose level 2 (infection in 2, nausea and diarrhea in 1 pt each), and 2/8 pts at dose level 3 (stomatitis and nausea). DVPM related cardiovascular side effects, in particular hypotension, occurred frequently, but were generally mild. After a median of 3 (range 1-5) cycles,8/20(40%) evaluable pts had PR, 6(30%) had SD, and 6 had PD. According to these encouraging preliminary therapeutic results and the fact that the MTD has not yet been reached, pt accrucal is being continued at the current EPI dose level of 135 mg/m2.

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CYCLIC HIGH-DOSE REGIMEN OF HYDROXYUREA (CHD-HU) FOR INITIAL CYTOREDUCTIVE THERAPY IN CHRONIC MYELOGENOUS LEUKEMIA (CML) : PRELIMINARY CYTOGENETIC ANALYSIS.

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In a pilot phase and a multi-center randomized trial initiated by the EGSG in 1991, 84 patients were treated with CHD-HU (40 mg/kg / 8h 2 days weekly) to induce complete hematologic remission followed by maintenance therapy with Interferon alpha, low-dose Cytarabine or HU monotherapy. So far 26 patients have been evaluated and cytogenetic data about 4 weeks after achieving hematologic remission before starting maintenance are available. At time of diagnosis 24/26 patients exhibited exclusively Phi-chromosome positive marrow metaphases (median number of analysed metaphases 18, range 1 - 65) and a Phi-chromosome mosaicism was detectable in two Phi-positive CML patients (62 and 5% Phi-negative). After initial cytoreductive therapy and the following period of about 4 weeks with WBC 2.5 - 4.0 x 10^9/l cytogenetic evaluation was performed (median time after starting therapy 18 weeks, range 12 - 39). One patient achieved a complete cytogenetic remission and two patients showed a partial cytogenetic remission with 11% and 12% Phi-positive marrow metaphases, respectively. A minimal cytogenetic response occurred in five patients (2 - 18% Phi-negativity).

Conclusion : CHD-HU is an effective well tolerated initial cytoreductive therapy for CML. About one third of the patients shows a cytogenetic response. In 9% partial or complete cytogenetic remission was reached.

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APO-1 MEDIATED APOPTOSIS IN NORMAL AND MALIGNANT LYMPHOCYTES.

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In the context of negative growth regulation of normal and malignant lymphocytes, the monoclonal antibody anti-APO-1 was raised. Anti-APO-1 induced programmed cell death, apoptosis.
Anti-APO-1 defined the novel cell surface molecule APO-1. The APO-1 antigen was purified to homogeneity and found to be a transmembrane glycoprotein of 48 kD relative molecular weight, identical to the Fas antigen. Peptides of the purified APO-1 molecule were sequenced and the APO-1 cDNA was cloned. The APO-1 molecule is a novel member of the TNF receptor superfamily. The APO-1 mediated apoptosis signal required crosslinking of the APO-1 antigen. Two requirements for induction of apoptosis in the APO-1 system were identified: expression of the APO-1 antigen and an intact apoptosis signalling pathway. In human peripheral blood T and B lymphocytes, expression of the APO-1 antigen and susceptibility to anti-APO-1 induced apoptosis were dependent on the stage of activation and differentiation. Anti-APO-1 induced apoptosis was prevented by mechanisms of T cell activation, e.g. via CD3 of the T cell receptor. Heterogenous APO-1 expression was found on a variety of human tumor cell lines *in vitro* and on cells from various tumors taken directly from patients. These malignancies include pre-T-ALL, pre-B-ALL, CLL, various other tumors of the lymphoid series, B lymphoblastoid cell lines, glioblastomas, mammary carcinomas, colon carcinomas and soft tissue tumors. Susceptibility of corresponding *in vitro* cell lines to induction of anti-APO-1 mediated apoptosis was heterogenous. In contrast, cells obtained directly from ATL patients were almost completely sensitive.
These data may help to understand programmed cell death, apoptosis, on the molecular level, to assess the role of APO-1 expression in diagnosis and prognosis, and to test apoptosis as a concept of a rational intervention strategy in tumor therapy.

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PROGNOSTIC SIGNIFICANCE AND MOLECULAR PATHOLOGY OF THE HIGHLY-PROLIFERATING PHENOTYPE OF MAMMARY CARCINOMA

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Proliferative activity is a potential prognostic indicator of neoplastic cell growth. We have raised a monoclonal antibody, Ki-S1, suitable for the detection of proliferating cells in routinely processed and paraffin-embedded tissue specimens, thus making retrospective studies possible. In three retrospective studies on more than 400 mammary carcinoma patients with median follow-ups of up to 12 years, proliferative activity as determined by Ki-S1 was significantly correlated with the S-phase fraction, with recurrence and cumulative survival. Because little is known about the molecular mechanisms influencing the cell division rate in mammary carcinomas, we determined in 60 mammary carcinomas the copy numbers of the c-erbB-2 and c-myc protooncogenes that have been shown to be amplified in aggressive types of cancer and correlated them with the proliferation rate. It could be shown that amplification of c-myc but not of c-erbB-2 is associated with high-proliferative capacity in breast cancer. Furthermore, we analysed 223 nodal negative cases for over-expression of p53, indicating a defect within this tumor suppressor gene. A highly significant correlation between p53 overexpression and proliferative capacity of breast cancers could be demonstrated. We conclude that different molecular mechanisms give rise to the highly-proliferating phenotype of breast cancer that exhibits a particularly aggressive biological behavior.

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DNA ANALYSIS TO AID IN THE DIAGNOSIS OF CHRONIC MYELOPROLIFERATIVE DISORDERS

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Diagnosing myeloproliferative disorders (CMPD) can be difficult because of overlap and possible transitions between the different conditions and their similarity to reactive myeloproliferations. DNA analysis was applied to improve differentiation of CMPDs. All subtypes of CMPD analyzed, including chronic myeloid leukemia, agnogenic myeloid metaplasia, polycythemia vera, and essential thrombocythemia had in common that granulocytes and bone marrow cells were clonal in origin, as shown by X-chromosome-linked DNA polymorphism, in conjunction with methylation patterns. Reactive myeloproliferations, by contrast, showed polyclonal inactivation patterns. Clonality could not distinguish CMPD from cases of myelodysplastic syndrome, because the latter also exhibited clonal hematopoiesis. Because of their clonal origin, peripheral granulocytes were used in all cases to detect bcr gene rearrangement. Despite possible morphologic overlap between different types of CMPD, bcr gene rearrangement was specific for chronic myeloid leukemia, and could be applied to differentiate chronic myeloid leukemia from other CMPDs in cases of equivocal morphological diagnosis. A subset of agnogenic myeloid metaplasia exhibited point mutations of the p53 gene. We conclude that chronic myeloproliferative disorders represent clonal hematopoietic diseases that probably have specific underlying genetic defects.

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INTERLEUKIN-4 SWITCHES THE PATTERN OF INTEGRIN EXPRESSION ON HUMAN TUMOR CELL LINES AND CAUSES A SELECTIVE INCREASE OF ADHESION TO MATRIX PROTEINS

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Integrin receptors play a crucial role in cell-cell and cell-matrix adhesive function, and thus are supposed to influence invasion and metastasis. Very little is known about the impact of interleukins on integrin regulation in tumor cell lines. Therefore, we investigated the expression of 7α and 4ß integrin subunits on well (HT29) and poorly differentiated (SW620) human colon cancer cell lines using a panel of specific monoclonal antibodies and cDNA probes. HT29 and SW620 expressed similarly high levels of α_1, α_2, α_3, β_1, and ß4 subunits on the cell surface. No α_4, β_2, and β_3 was detected on either cell line. While α_5 was not expressed on HT29, SW620 showed higher levels of the laminin receptor $\alpha_6\beta_4$. The poorly differentiated cell line SW620 was resistant to IL-4, whereas HT29 was sensitive. Treatment with IL-4 induced a decrease in α_2, α_3, α_6, α_v, β_1, and β_4 integrin expression. However, α_1 subunit was markedly upregulated. In contrast to IL-4, there was no evidence that IL-1ß could modulate integrin expression on these cell lines. The function of integrin receptors was assessed by measuring adhesion to collagen, laminin, vitronectin, and fibronectin. IL-4 significantly increased the adhesion of HT29 to fibronectin, while attachment to collagen, laminin, and vitronectin remained unchanged. These results suggest differential integrin expression pattern on well and poorly differentiated tumor cell lines. We provide evidence that integrin expression may be selectively regulated by IL-4, but not by IL-1ß. Furthermore, IL-4 can alter adhesive behavior of tumor cells. Since IL-4 is currently studied in clinical trials, the metastatic potential of malignant tumors should be monitored thoroughly.

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DIFFERENTIAL DIAGNOSIS OF MYELODYSPLASIA (MDS) AND ERYTHROLEUKEMIA (FAB:M6): A MULTICENTER I.G.C.I. TRIAL.

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97 patients (53m, 44f; median age: 64 [20-88] years) from 14 hospitals were analyzed retrospectively in a multicenter study. Bone marrow smears were reclassified according to Bennett et al revised criteria of AML/M6. In 37 cases bone marrow erythropoiesis was less than 50% (group A/MDS), the remaining cases with > 50% erythropoiesis were reclassified as MDS (group B, n=31) or erythroleukemia (group C/M6, n=29) depending on blast cells of non- erythroid cells (NEC) < or > 30%. No significant differences were observed regarding sex, age, initial blood cell counts, bone marrow morphology, erythroblasts in blood or organ enlargement in the 3 groups. In 40 patients cytogenetic data are available, 24 (60%) of them had an aneuploid karyotype; only in 6/17 group A-pat. chromosomal aberrations were found, but in 18/23 pat. with a high amount of erythropoietic bm-cells. Regarding the incidence of major karyotype abnormalities (MAKA), there was no difference between the 3 groups.

Progression to AML(M1-5) occured in 24/97 patients (25%); in group B and C all blastic phases were observed within 12 months, whereas only in group A long lasting preleukemic phases occured. Treatment schedules were similar in all three groups with poor outcome. Survival time in group A was significantly longer (med.12 months, range 1-120) than in B and C (med. 8 months, range 1-87). The difference of survival between group B and C was not significant.

This analysis implies that erythroleukemia (M6) has similar characteristics and outcome to the remaining MDS-cases with bm-erythropoiesis >50%, but differs from cases with a low erythropoietic content; the number of myeloblasts of non-erythroid cells in bone marrow is of minor importance for the patients outcome.

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MONOCLONAL ANTIBODIES AGAINST HUMAN THIOL-PROTEIN-DISULFIDE-OXIDOREDUCTASE AS TOOLS IN B CELL IMMUNOPHENOTYPING OF LYMPHOMAS AND LEUKEMIAS

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Thiol-proteindisulfide oxidoreductase (TPO, EC 1.8.4.2., protein-disulfide isomerase, EC 5.3.4.1.), a luminal enzyme of the endoplasmic reticulum, is thought to be involved in the posttranslational processing of disulfide containing proteins. The enzyme is a multifunctional polypeptide, showing an amino acid sequence that is in a large degree similar to those of some other proteins (the ß-subunit of prolyl-4-hydroxylase, the thyroid hormone binding protein, thioredoxin, and ATL-derived factor, ADF, produced by HTLV-1 transformed T-cells).

Using monoclonal and polyclonal antibodies against human liver TPO we could show that this protein is also a new plasma membrane constituent of lymphocytes. Double staining experiments in flow cytometry and immunoprecipitation analyses revealed that this enzyme is mainly expressed on the plasma membrane of B lymphocytes. This is supported by the finding that B cells from patients suffering from chronic lymphocytic leukemia co-express this antigen with CD19. Moreover, immunohistological analyses of malignant lymphomas showed that monoclonal antibodies against human liver TPO react with a "pan-B" structure analogous to CD19. In recent experiments we could demonstrate that TPO as well as antibodies against this enzyme exhibit a growth-enhancing activity of mononuclear cells of healthy volunteers. Possibly, TPO acts as an autocrine growth factor, like ADF, and/or has a critical role in regulating the SH-/S-S-status of the cell membrane.

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PRELIMINARY RESULTS OF A MULTI-DRUG CHEMOTHERAPY IN HIGH GRADE MALIGNANT NON-HODGKIN'S LYMPHOMAS

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Between 1986 and 1993 64 patients (21 females, 43 males) under the age of 60 with high grade malignant NHL have been treated with either MACOP-B or MACYOP-B (49 and 15 resp.). MACOP-B was given according to the schedule of KLIMO and CONNORS and in MACYOP-B cyclophosphamid was replaced by an equipotent dose of bendomustive (60 mg/m^2). The histologicial subtypes were diagnosed according to the KIEL-classification and staging was made using the ANN-ARBOR staging system. The median age was 43 years (range 21-60). 11 pts had stage I, 13 stage II, 9 stage III, 31 stage IV disease. B symptoms were present in 35 patients, extranodal involvement in 25 pts. The median follow-up time was 26 months. In the MACOP-B-group 36/49 pts (73,5%) achieved CR, 4/49 (8,2%) were partial responders and 9/49 (18,3%) did not respond to the treatment, 8 out of these died. 7/36 CR patients relapsed, 4 of them died from their NHL.1 pt died from secondary AML. The probability of disease-free survival is 64% at 77 months in patients with MACOP-B chemotherapy. In the MACYOP-B-group 6/15 were complete responders, 2/15 achieved PR and 7/15 did not respond. The disease-free survival rate is 20%, because 2 pts had stage III and 13 pts had stage IV disease. In 15 cases did the expected toxicity of intensive chemotherapy reach WHO grade 3-4 including infections, septic complications, myelotoxicity and peripheral neuropathy. 3 pts had a cardiomyopathy induced by anthracycline. MACOP-B and MACYOP-B are effective but toxic treatment programms.

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HUMAN MILK-FAT GLOBULIN M-RNA IS TRANSCRIBED IN CELL LINES OF DIFFERENT ORIGIN AND IN NORMAL HUMAN BONE MARROW

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The human milk fat globule membrane (HMFG) has been used as a source of antigenic material to prepare polyclonal and monoclonal antibodies used for diagnosis and experimental therapy of breast cancer. It's components include glycoproteins of 150kD, 70kD, and 46kD molecular weight. Proteins of HMFG have been referred to as breast differentiation antigens. Patients with metastatic breast cancer carry high levels of HMFG-antigens in serum, while normal female controls do not. The 70kD component has been cloned and the base sequence shows no extensive homology to any other gene published. It is highly expressed in breast cancer and other cancer cell lines. Transcription is also detectable in Raji-cells, but at much lower level.

We detected by Northern-blot hybridisation with a specific oligonucleotide probe transcription in cell lines of different lymphoid and myeloid origin and in bone marrow of healthy donors. Breast cancer cell lines were used as positive controls and a mouse cell line was useful as a negative control. RNA-sequences were amplified by a reverse-transcriptase-PCR. The products of identical sizes were sequenced to exclude accidental amplification of unknown genes. No differences in base sequence were detected. 70kD-HMFG m-RNA could not be detected in mouse cells neither by Northern-blot analysis nor by PCR. These results show, that the transcription of the 70kD HMFG-component is not restricted to epithelial tissue or tumor cells and that the protein might have a function in non-epithelial cells.

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CERTAIN CYTOKINES ARE DIFFERENTIALLY EXPRESSED IN HUMAN LYMPHOMAS AND LEUKEMIA. THE REGULATION OF INTERLEUKIN 10 IN THESE NEOPLASIAS.

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Cytokines may regulate the growth and differentiation of normal hematopoietic cells and are possibly involved in the biology of malignant lymphoma and leukemia. The expression of cytokines is mainly regulated by extracellular factors via specific signal transduction pathways. The proto-oncogene p56lck for example is an protein tyrosine kinase involved in the transformation of lymphoid cells as well as in specific signal transductions from the cell surface, especially associated with IL2 and IFN-gamma.

To analyze the role of cytokines in human lymphoid neoplasia, we have studied the transcription of interleukins in acute (ALL) and chronic (CLL) lymphoblastic leukemias and Burkitt's lymphoma derived cell lines using the polymerase chain reaction. The results are summerized in the table:

	IL2	IL3	IL4	IL5	IL7	IL8	IL9	IL10	IL11	IL12
c-ALL	4/11	5/12	6/11	0/19	10/19	12/17	0/19	12/17	2/12	7/12
preB/B-ALL	2/6	0/6	2/6	0/6	3/13	9/10	0/13	7/9	3/6	2/6
preT/T-ALL	2/6	0/6	1/6	0/6	0/13	7/11	0/13	5/13	5/8	3/8
Burkitt's lymph.		0/18	6/18	4/18	2/18		0/18	12/18	9/18	
B-CLL								9/10		
activ.PBL	++	+	+	+	+	++	+	+	+	+
PBL	+	-	-	-	+/-	+	-	+/-	-	+/-

Our data demonstrate that certain interleukins are differentially transcribed in lymphoid neoplasia, especially IL10 in B-cell lineage leukemias.

Thus we have analyzed in more detail the transcriptional regulation of IL10 in cell lines derived from preT-ALL (Jurkat) and Burkitt's lymphoma (BL2) as well as in clinical samples of lymphatic leukemias, showing different kinetics of inhibition of IL10 transcription after cell type specific stimulation and activation with antiCD28 after crosslinking with antiCD3/CD19 antibodies.

We have cloned the IL10 promotor and now we are analyzing promotor fragments under different physiological conditions.

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ATTEMPTS TO PREDICT SENSITIVITY TO DOXORUBICIN AND ETOPOSIDE BY MEANS OF MOLECULAR RESISTANCE MARKERS IN AN *IN VITRO* LEUKEMIA MODEL.

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Resistance to doxorubicin (DOX) and etoposide (VP-16) may be conferred by a decreased expression/activity of topoisomerase II (topo II) as well as by changes in glutathione (GSH) metabolism. We investigated the usefulness of these factors as predictors for *in vitro* chemosensitivity studying a panel of 14 leukemia/lymphoma cell lines which were all negative for *mdr*1/P-glycoprotein expression. These cell lines displayed a variation of >250-fold in their sensitivity to DOX and VP-16 and were divided into a group of 6 sensitive and 8 resistant malignomas. Response to both cytotoxins was independent from cell type, doubling time, or intracellular accumulation of VP-16. Activity of topo II was assayed by formation of cleavable complexes, which did not predict for tumor response. In addition, no correlation was found for topo IIα. For topo IIβ, the amount of detected protein (Western) was dependent from RNA expression (PCR) and expression tended to correlate with topo II activity. Expression of topo IIβ did allow to differentiate between sensitive and resistant cells. Analysis of subtypes of glutathione-S-transferases (GSTs) revealed the absence of GST-α. Contrary to common assumptions, resistant cell lines were characterized by significantly decreased protein levels of GST-π. Intracellular GSH levels correlated highly with cellular resistance to DOX and VP-16. Accordingly, cell lines could be sensitized to VP-16 by pre-treatment with buthionine sulfoximine which causes GSH depletion in cells. The above results indicate 1) a potential clinical relevance of GSH as a predictive marker in leukemias/lymphomas, 2) the possibility to replace enzymatic measurements by quantification of expression, and 3) the necessity to determine various resistance markers for more accurate predictions.

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Tissue Levels of 5,10 Methylenetetrahydrofolate in Pts. with Colorectal Carcinoma with or without Preoperative Application of Folinic Acid

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The modulation of 5-fluorouracil (5-FU) with folinic acid (FA) has been established in vitro and in various clinical studies for the treatment of advanced colorectal carcinomas. Binding of the folate cofactor 5,10 methylenetetrahydrofolate (mTHF) together with fluorodeoxyuridinmonophosphate (FdUMP), a metabolite of 5-FU, to thymidylate synthase (TS) increases dissociation half life of the complex compared to binding of FdUMP and TS alone resulting in a pronounced inhibition of TS. Although pharmacokinetics and metabolism of the parent compound FA in serum has been well investigated only few data are available about tissue levels of mTHF, the metabolite of interest with regard to TS-inhibition. Thus we used the "tritium-release-assay", a highly specific and sensitive enzymatic assay for the evaluation of reduced tissue folate pools with and without preoperative application of 300 mg FA. A standard curve was established with various concentrations of mTHF as rate-limiting substrate which could be used to detect unknown concentrations of mTHF in tissue lysates according to the tritium release after incubation with TS and 5-[^{3}H]-dUMP. FA was given i.v. as short term infusion 2 - 4 hours before surgery. After removal the tissue was frozen quickly in liquid nitrogen and stored at -80°C until used for analysis. So far, 10 pts. without and 7 pts. with pretreatment have been evaluated. In pts. without pretreatment, mTHF levels in tumor as well as in normal mucosa and liver were low. Only 4 pts. had levels above 100 pmol/mg protein. After pretreatment with 300 mg FA, mTHF tissue levels were significantly elevated up to tenfold compared to pts. without treatment. The study will be extended using different doses and schedules of administration in order to optimize the treatment regimen of colorectal tumors on the basis of a biochemical rationale.

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TRANSMISSION OF INFECTIOUS DISEASES BY BLOOD COMPONENT THERAPY

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Allthough over 400 different virus species may infect man and induce a broad spectrum of clinical manifestations, only a few cause problems in blood transfusion recipients of a magnitude which warrants the need for screening tests. Over the past decade, the risk of posttransfusion hepatitis and AIDS has been drastically reduced by sensitive and specific screening assays for hepatitis C-virus (HCV), HIV-1,-2 antibodies and p24 antigen. For the prevention of transfusion-associated AIDS (TAA), the *HIV-p24-antigen test* (sensitivity 7-12 pg/ml, ABBOTT) was introduced in the blood banks of Hamburg and the Bavarian Red Cross. Since June 1992, no p24-positive, anti-HIV-1,-2 negative specimen was identified among 300.000 donations. Only 18 TA-HIV infections by approx. $27x10^6$ screened blood units were officially reported since 1985. To further reduce the diagnostic window-period during DNA latency and the early viremic phase of HIV infection, a *PCR assay for the detection of HIV-1,-2* provirus genome (Amplicor, ROCHE) is evaluated.

With an *HTLV-1,-2 seroprevalence of 1:10.000* in Bavaria (Weise, 1993) and $4x10^6$ donations/ year in the FRG, 400 infectious units may lead to 100 seroconversions in recipients (30% transmission rate by blood stored < 14 days); at a disease manifestation rate of 2-4%, 2 - 4 transfusion-associated cases of TSPP or HAM (spastic paraparesis, myelopathy) or ATLL (leukemia) may occur. Although the seroprevalence is < 1/200.000 in Lower Saxony (Schmitt, 1993), and only 3 TSPP/ HAM cases among members of HIV-high risk groups have been reported, *HTLV-1,-2 EIA and PCR* assays are now evaluated in our donor population.

Today, the *risk of viral transmission* per transfused unit of blood component is estimated as follows (FRG, 1993): 1/ 500.000 - $3x10^6$ for HIV-1,-2; 1/ 30.000 for HCV; 1/ 50.000 - 100.000 for HBV; 1/ 10.000 - 25.000 for HTLV-1,-2; 1/ 4.000 for parvovirus B19. The HIV transmission rates by screened, antibody-negative blood units are estimated at $1:10^6$ in Germany, $1:10^6$ in USA, $1:10^4$ in Thailand and $1:10^3$ in Central Africa (Guertler, 1993). The *spectrum of screening procedures for infectious markers* in blood is determined by the local epidemiology, the clinical relevance of the virus, the public opinion concerning the "zero-risk blood supply" and the medical budget of the country.

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Computer-based structured data entry for abdominal ultrasonography Demonstration of a system and evaluation results

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Computer-based clinical systems have been shown to improve the availability of medical data and to reduce retrieval times, but direct data entry by physicians is still difficult and not generally well accepted. Therefore, the majority of clinical reports remains paper-based. Reports are routinely generated as free text, usually dictated onto audio tapes, or even written out in long-hand by the examiner. The advantages of the use of natural language lie in the strength and flexibility of verbal expression. On the other hand, computer-based systems with direct data input based on structured forms or menus can significantly improve data quality.

A system for structured data collection in abdominal ultrasonography has been created and brought into routine use. It uses a descriptive standard terminology and a graphical user interface which facilitates direct data entry by physicians. To date, more than 20.000 reports have been generated by use of the system, and have been stored in a departmental computer system. Several evaluations have been carried out. It can be shown that the completeness and correctness as well as the validity and objectivity of the entered data is high. The system is well accepted by physicians. The precise definition of a standard terminology helps to reduce ambiguity and inter-observer variation; it is especially helpful for less experienced users. Structured forms with adequate user guidance show an inherent "reminder effect".

Two modules have been added to the system: a component for "intelligent" user guidance and a prototypical image database to illustrate the terminology.

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COMPARISON OF DEXNIGULDIPINE, TAMOXIFEN AND VERAPAMIL AS MODULATORS OF MULTIDRUG RESISTANCE IN VITRO

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A number of structurally not related drugs including calcium antagonists such as verapamil (VER) and dexniguldipine (DEX) or the anti-estrogen tamoxifen (TAM) proved to be potent modulators (MOD) of multidrug resistance (MDR) *in vitro*. The modulation of vinblastine (VBL) cytotoxicity by VER, DEX, and TAM as well as their intrinsic toxicity were compared in the human lymphoblastoid cell line CCRF-CEM and its MDR-resistant subline CEM VLB100 by the MTT-assay. Furthermore, the influence of the MOD on the cellular efflux of rhodamine 123 (R123) was determined by flow cytometry. In both cell lines the intrinsic cytotoxicity of DEX, VER, and TAM as characterized by the 50 % inhibitory concentration (IC50) was 3-5 μM, 30-50 μM, and 15-20 μM, respectively. CEM VLB100 was 120-150fold resistant to VBL as compared to CCRF-CEM (0.2-0.3 μM vs 0,002 μM). Combinations of different concentrations (conc) of VBL with increasing MOD-conc were tested, the highest beeing only slightly toxic (≤ 10-15 % growth inhibition). With 0.1-1 μM DEX, 1-10 μM TAM, and 1-10 μM VER in CCRF-CEM only an additive effect was determined. In contrast the effect was synergistic in CEM VLB100. Increasing MOD-conc led to a sensitization against VBL in CEM VLB100 with a sensitization ratio (SR: IC50 in absence/IC50 in presence of MOD) in the range of 1 (no effect) to 35 at highest MOD-conc tested. Thus, no total reversal of MDR to the sensitivity level of the parental CCRF-CEM was achieved in VBL-MOD combinations with only weak MOD-intrinsic toxicity. DEX proved to be 8-12fold more potent than TAM and VER considering the conc tested. The R123-efflux was 50 % inhibited by 1 μM DEX. In contrast, 10 μM TAM and VER led only to a maximum efflux reduction of 30 %. Clinically achievable MOD-conc causing only tolerable side effects are 1-3 μM DEX, up to 6 μM TAM, and 3 μM R-VER, respectively. In conclusion, DEX seems to be the most promising MOD of MDR because of its higher SR and efflux inhibitory capacity *in vitro* at conc easily achievable in the clinics.

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MONITORING OF PATIENTS WITH *t(8;21)*-POSITIVE ACUTE MYELOID LEUKEMIA USING AML1/ETO RT-PCR

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The translocation t(8;21)(q22;q22) leads to a juxtaposition of the genes for AML1 (chr.21) and ETO (chr8). The AML1/ETO fusion RNA can be detected by Northern blot analysis and PCR (Nucifora et al., Blood 81:883; 1993). Using nested primers from both sides of the mRNA fusion product we have constructed a modified two step RT-PCR assay for the detection of AML1/ETO. The specificity of the PCR-products (1st step 166 nt, 2nd step 126 nt) was shown by an internal APA I restriction site as well as by hybridization with a breakpoint-specific oligonucleotide. With this assay we found that 7 out of 12 (58%) FAB-M2 leukemias had a detectable AML1/ETO fusion mRNA in their peripheral blood or bone marrow at diagnosis . The assay detected cytogenetically positive t(8;21) leukemia cells, but was also useful in cases where due to complex chromosomal events a t(8;21) could only be suspected by cytogenetic analysis. We have followed 4 patients who all achieved a complete clinical remission throughout the course of treatment: A transitory reduction of PCR-detectable fusion message (negativ in 1st step PCR) was found in one of two patients after conventional chemotherapy. Autologous bone marrow transplantation produced a transient PCR-negativity (in a two step reaction) of 5 months in one patient, while a second patient remained positive in the second step for 4 months and then progressed to first step positivity. Our data indicate that the AML1/ETO PCR is able to detect a t(8;21) at diagnosis, but that even patients in complete clinical remission remain PCR-positive at least on the two step level.

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ALTERATIONS OF THE HER-3 AND HER-2 GENES IN BREAST CANCER - POINT MUTATION IN THE TRANSMEMBRANE REGION

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Introduction: Amplification of the HER-2 oncogene and overexpression of its translational product p185 could be described not only as activating mechanism but also as a prognostic factor in breast cancer patients. In opposit to the homologous neu gene of the rat an activating point mutation in the transmembrane region has not been detected, since now. The HER-3 gene and its product p160 may be involved in tumorigenesis of breast cancer, too.
Material and Methods: 93 primary tumors of breast cancer were investigated concerning gene amplification of HER-2, in part HER-3 by southern blotting, RNA-overexpression of HER-2 by northern blotting, in part HER-3, overexpression of p185 and p160 by ICA, in part shedding fragment concentrations of p185 in serum by EIA, screening for point mutation in the transmembrane regions of HER-2 and HER-3 by direct sequencing and RLPP. Gene amplifiction and point mutation in primary tumors were confirmed by investigations of leukocyte DNA from the same patient.
Results: 1. Since now, we could find a heterozygous point mutation in 3 breast carcinomas at codon 661 of the HER-2 with a T to A transition from ATT to AAT and a consecutive change of amino acid sequence from Ile to Asn. In these cases no gene amplification of HER-2 but p185 overexpression was shown. 2. First data demonstrate in about 30% overexpression of the HER-3 translational product p160 in breast cancer. No HER-2 overexpression could be detected in these cases.
Conclusion: 1. The described point mutation in the transmembrane region may lead to an activation of the HER-2 proto-oncogene in cases without amplification. 2. An interaction between the HER-2 and HER-3 genes in oncogenesis of breast cancer could be discussed.

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THROMBOTIC MICROANGIOPATHY (TMA)

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TMA - a term that encompasses the thrombotic-thrombocytopenic purpura and the hemolytic uremic syndrome - is a rare disorder of unknown origin with a high mortality rate. TMA is characterized by hemolytic anemia, thrombocytopenia, fluctuating neurological symptoms, fever and renal dysfunction. TMA can occur idiopathically or as the consequence of another underlying condition such as infection, pregnancy, malignancy or tissue transplantation. The pathomechanisms of TMA resulting in multiorgan failure as a consequence of the deposition in the microcirculation of platelet-rich fibrin thrombi are not completely understood. It has been proposed that TMA is the consequence of the intrusion into the circulation of agents with platelet activating properties such as the platelet-agglutinating proteins P37 and P59, circulating immune complexes or abnormal von Willebrand factor (vWF) molecules. Various vWF abnormalities including absence from plasma of the large vWF multimers during the acute episode of the disease and the appearance in the circulation of larger than normal (unusually large) vWF multimers in acute TMA and/or during remission of relapsing TMA have been reported. A possible role of an abnormal vWF in the pathomechanism of TMA will be discussed in this presentation. On the other hand, endothelial damage of unknown cause resulting in platelet activation and formation of thrombi in the microcirculation has been postulated. Treatment with plasma exchange and administration of corticosteroids have been shown to be highly effective in patients with idiopathic TMA resulting in a remission rate of >80%. In patients with relapsing TMA, splenectomy in addition to the above therapy regimen has been proposed. In contrast, these treatment modalities are not effective in secondary TMA that is only responsive to treatment of the underlying disorder.

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RAPID ACHIEVEMENT OF PCR-NEGATIVITY BY COMBINED TREATMENT WITH ATRA AND CHEMOTHERAPY IN ACUTE PROMYELOCYTIC LEUKEMIA (APL)

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5 pts. with APL (2 with hyperleukocytosis) were treated with all-trans-retinoic acid (ATRA,45 mg / m^2) and induction Chemotherapy(CT) consisting of daunorubicine (45 mg / m^2,day 1-3) and cytosine-arabinosid (200 mg / m^2,day 1-7). The reduction of leukemic cells in peripheral blood and bone marrow was monitored with a two-step reverse-transcriptase-polymerase chain reaction (RT-PCR) which amplified the PML / RARalpha fusion transcripts generated by the chromosomal translocation t (15;17). The sensitivity of the assay as determined by dilution experiments was $1:10^{-3}$ - 10^{-4} cells in a one step reaction and 1: 10^{-6} in a two step reaction using nested primers. Hematological remission was achieved in all pts. after one course of ATRA + CT. One step PCR became negative in all pts. and two step PCR in 4/5 pts. Two step PCR negativity was achieved within 82,68,44 or 26 days, respectively. All pts. who became two step PCR negative are still in continuous complete remission after 14,13,3, or 2 months, respectively. The pt. who remained two-step PCR positive relapsed after 5 months.
Our data suggest that combined treatment with ATRA and CT is highly effective to reduce the tumor burden below the detection limit of 1/ 1.000.000 cells.The fact that PCR negative pts.remain in long term remission suggests that in APL eradication of leukemic cells below $1:10^{-6}$ should be the therapeutic goal.

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EXPRESSION OF RECOMBINANT HUMAN LIPOPOLYSACCHARIDE BINDING PROTEIN (LBP) IN A BACULOVIRUSSYSTEM

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Gramnegative sepsis is caused by the bacterial cellwall product LPS (Lipopolysaccharide or Endotoxin). In serum LPS is recognized by and forms a complex with a 58 kD Glycoprotein named LBP (LPS Binding Protein). LBP directs LPS to the cell surface of responsive cells and the LPS/LBP complex is recognized by a cellular receptor which has been identified as the CD14 molecule. To further test this "adapter" function of LBP we cloned the whole cDNA encoding for human LBP into a vector that is able to cotransfect the insect cell line Sf-9. Expression of LBP was achieved and the protein was purified using mono Q HPLC. It was recognized by anti-LBP antibodies and was active in different activity assays. Most likely due to a different glycosylation pattern, however, the recombinant protein comigrates on an SDS-PAGE at approximately 55 kD. Another expression system that was set up using E. coli cells, resulted in expression of a 50 kD non glycosylated protein, that was not active at all. To find out structural regions that carry the ability to recognize LPS we constructed mutated forms of LBP and expressed these in the Baculovirus system as well. Here we show that the Baculovirus system is able to produce large quantities of an active, at least partially glycosylated LBP protein. This system will be a powerful tool in studying structure-function relationships of recombinantely expressed LBP or other proteins. Early results of the deletion mutants of LBP showed that the N-terminal part of the protein carries the LPS-binding domain, and further mutation strategies will completely define the endotoxin- and possibly CD14-binding-site of LBP.

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ALTERNATING COPP+ABVD VERSUS RAPIDLY ALTERNATING COPP+ABV+IMEP FOR HODGKIN'S DISEASE.

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To investigate whether developement of tumor resistance might be prevented by rapid application of non cross-resistent drugs, a new 10 drug regimen COPP+ABV+IMEP (CAI), repeated every 6 weeks, was compared to conventional alternating COPP+ABVD (CA), given every 8 weeks. From 3/88 to 1/93 the GHSG conducted two randomized multicenter trials for pts. with first diagnosis of HD. 1318 pts. were randomized (HD5: 862; HD6: 556).

Eligibility criteria: HD5: CS/PS I and II with at least one of the following risk factors: massive mediastinal tumor, massive spleen involvement, extranodal disease, elevated ESR (≥ 50 mm/h or ≥ 30 mm/h with B-symptoms) or ≥ 3 lymphnode areas involved; and all CS/PS III A. HD6: CS/PS III B and IV.

Study design: HD5: Randomization to 2 courses of either CA or CAI followed by identical radiotherapy (30 Gy EF+10 Gy bulk).

HD6: Randomization to 4 courses of either chemotherapy regimen followed by IF irradiation in case of initial bulk, slow response, or residual nodal disease.

Feasibility: Comparing WHO grade 3/4 toxicity per cycle, emesis was less frequent (24% vs 9%) and leukocytopenia more frequent (31% vs 40%) in the CAI scheme. In HD6 the median duration of chemotherapy was 237 days for CA and 195 days with CAI. Similar for both regimens over 90% of projected total dose could be given, showing that rapid application was feasible.

Treatment results (12/92):	HD5		HD6	
	CA	CAI	CA	CAI
pts evaluable	202	227	155	156
CR-rate	93%	92%	76%	78%
FFTF-2yrs.	87%	86%	63%	67%
SV-2yrs.	96%	97%	85%	87%

To date no significant differences in treatment outcome are noticed in either studies. These data are preliminary, since many pts are still unter therapy and only ≤ 40% of the expected events have occured.

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EXPRESSION AND FUNCTION OF ADHESION MOLECULES IN NORMAL BONE MARROW

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Interactions between neutrophils or lymphocytes and endothelial cells could be shown to elicit an activation-dependent cascade of adhesion which may play a role in the adhesion of bone marrow colony forming cells (CFC) to the bone marrow stroma as well.- We have examined the adhesion of CFC to bone marrow-derived stroma grown under the conditions of long term culture. Mononuclear cells were incubated either with or without ligands or antibodies to block known adhesion receptors. The number of CFC was assessed in a colony forming assay. We found that 48,2% ± 12,5 (mean ± SEM) of non-treated CFC adhere to the preestablished stroma. Surprisingly, addition of the anti-L-selectin mab DREG 200 resulted in an enhancement of adherence and increased the binding to stroma suggesting that CFC adhesion is activated by the interaction of the DREG antibody with L-selectin and confirming the expression of this receptor in hematopoietic cells. Thus, L-selectin may be involved in CFC adhesion and migration. Furthermore, we examined the function of the fibronectin (FN) receptor 4ß1 in the adhesion process of CFC on stroma using the FN-derived peptide CS-1 as a ligand. We found a highly significant inhibition indicating that the integrin heterodimer 4ß1 is also involved in the adhesion of CFC to marrow stroma.- Our results suggest that adhesion of hematopoietic progenitors to stroma may be regulated in a manner similar to that observed in mature leukocytes, requiring more than one receptor and at least two sequential steps including attachment dependent on L-selectin and stable adhesion involving fibronectin via the 4ß1 integrin receptor.

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EVALUATION OF EARLY HEMORRHAGIC DEATH IN ACUTE MYELOID LEUKEMIA

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Between 1/1984 and 4/1993, 80 patients (P) with newly diagnosed acute myeloid leukemia (AML) were eligible for induction chemotherapy. Mean age was 51.4 (16-74) years. Induction chemotherapy uniformly consisted of cytosine arabinoside, daunorubicin and thioguanine. Cases of early death (ED), i.e. death within 6 weeks after start of chemotherapy, were analysed for laboratory values (white blood cell count = WBC, platelet count, LDH, clotting tests), interval time to death, cause of death.

	Total	WBC>100,000/µl	WBC<100,000/µl
ED/total pat.No	17/80 (21%)	6/16 (38%)	11/64 (17%)
Cause of ED			
Hemorrhage	6	5/6 (83%)	1/11 (9%)
Hyperleukocytosis	1	1/6 (17%)	0
Septicemia	8	0	8/11 (73%)
Cardiac Failure	2	0	2/11 (18%)

The 5 of 6 ED in patients with WBC>100,000/µl, due to cerebral hemorrhage, occurred 2.8 (± 0.45) days after start of chemotherapy. Patients with lower WBC died significantly later ($p<0.005$), mean 19.2 (± 11.5) days. Initial coagulation parameters (Quick, Fibrinogen) and platelet counts showed no significant difference in the 4 patient groups: high vs. low WBC with or without ED ($p>0.05$). In addition, fatal hemorrhage was not associated with a significant deterioration of clotting parameters. But the respective patients had extremely high LDH values (mean 2041 U/l) at diagnosis.

Conclusions: Patients with AML and WBC>100,000/µl had an increased risk of early fatal hemorrhage ($p<0.0005$). The coagulation parameters and platelet counts at time of diagnosis and time of death did not predict the risk of bleeding. But very high LDH values seem to indicate a high risk of hemorrhage.

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THE IMPACT OF SECOND-LOOK LAPAROTOMY ON THE OUTCOME OF CHEMOTHERAPY IN PATIENTS WITH ADVANCED EPITHELIAL OVARIAN CARCINOMA

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The role of second-look laparotomy (SLL) in the management of advanced epithelial ovarian carcinoma (OC) is a matter of controversial discussion. We retrospectively analysed the data of 29 patients (pts) with OC, who were in complete remission (CR) after primary surgery and cisplatin-based chemotherapy (CT). 19 out of the 29 pts underwent a SLL, while 10 pts did not. In 10 out of the 19 pts (53%) with a SLL, no residual tumor was found, and no further CT was given. These 10 pts were classified as pathological CR (PCR). In another 3 pts, there was a residual tumor, which could be resected totally. In these 3 pts, CT was continued for further 3 cycles. In the remaining 6 pts who had non-resectable tumor at SLL, CT was continued in 4 pts, and additional whole-abdominal radiotherapy was given in 1 pt. One pt refused further therapy.

For the whole group of the 29 pts, the estimated 8-yr disease-free survival (DFS) was 45%, and the probability of survival at 8 yrs 42%.

For the 10 pts with PCR at SLL, the estimated 8-yr DFS was 60% in comparison to 22% in the 9 pts without PCR. The estimated 8-yr survival was 59% in the first group of pts and 15% in the second. The differences between the two groups of pts were statistically significant ($p<0.05$).

However, there was no significant difference in DFS and survival between the 19 pts who underwent a SLL and the 10 pts who did not. For the first group of pts, the estimated 8-yr DFS and survival was 42% and 38%, respectively, compared to 50% and 48% in the 10 pts who did not undergo a SLL.

On the basis of these data, SLL appears to be of prognostic value, but it does not seem to influence the long-term results because of a lack of effective salvage therapy for pts who have residual tumor at SLL.

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CYTOTOXIC T LYMPHOCYTES AGAINST AUTOLOGOUS MALIGNANT PLASMA CELLS IN HUMAN MULTIPLE MYELOMA

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We demonstrated that the monoclonal immunoglobulin (mIg) concentration in the supernatant of cultured bone marrow cells from multiple myeloma (MM) patients is a specific marker for the number of tumor cells in the cultures. Using this system, we showed that the mIg production of MM bone marrow cells was suppressed by purified autologous T lymphocytes separated from peripheral blood. This effect was enhanced by addition of anti-CD3-antibodies or IL-2 to the cultures. The mIg production of MM tumor cells dramatically augments, when CD3+ bone marrow lymphocytes, contaminating bone marrow aspirates, were eliminated from the cultures.
To elucidate whether these effects were due to cytotoxicity, ^{51}Cr-release assays were performed using purified MM tumor cells as targets. Effector cells were generated by incubation of autologous PBMC for 4 days with IL-2 (10-100U/ml) alone or in combination with irradiated tumor cells. In 6/12 patients cytotoxicity was observed with interindividual differences between 10 and 65% specific lysis.
For further characterization of the effector cell population, we separated CD4+ and CD8+ subpopulations from the peripheral blood. Cocultivation experiments of these populations together with purified autologous tumor cells raised evidence that the suppressive effect on MM tumor cells is mediated by CD4+ as well as CD8+ lymphocytes.
Supported by Deutsche Krebshilfe/Dr. Mildred Scheel Stiftung, grant W25/90/Pe1.

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KINETIC OF HEMATOPOIETIC RECONSTITUTION AFTER MULTIPLE CONSECUTIVE COURSES OF INTENSIVE CHEMOTHERAPY SUPPORTED WITH GRANULOCYTE-COLONY STIMULATING FACTOR AND PERIPHERAL BLOOD PROGENITOR CELLS.

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Refractory or relapsed non-Hodgkin lymphoma (n=16) and relapsed or advanced non-seminomatous germ cell tumor patients (n=14) were treated according to the currently used chemotherapy (Ctx) protocols: G-CSF alone (2x12 μg/kg/die s.c.) for mobilization and apheresis of peripheral blood progenitor cells (PBPC) followed by 2 to 4 Ctx-courses with reinfusion of the previously collected PBPC (n=48) and application of G-CSF (5 μg/kg per die; n=64). PBPC-aphereses were also performed after Ctx when total leukocytes recovered above $1x10^9$/L. The CD3, CD14, CD19, CD25, CD33, CD34, CD56 and HLA-DR positive cells in peripheral blood (PB) were analyzed, using dual color fluorescence and flow cytometry (FACScan, Becton Dickinson). Fourteen days methylcellulose CFU-GM and BFU-E from the PB were used as control for the clonogenic capacity of $CD34^+$ cells. Samples were obtained before, immediately after Ctx and several times after the leukocytes rose above $1x10^9$/L. Positive correlations of the rising and decreasing counts of the subpopulations within the light density cell fraction were noticed ($r^2=0.65$-0.85). However, the $CD3^+$ were in inverted ratio to the $CD14^+$ cells ($r^2=-0.66$). The percentages of $CD3^+$ and especially of the $CD25^+$ cells showed an increment immediately after Ctx, where the proportions of $CD14^+$ and $CD34^+$ cells tended to fall. There was also a correlation between $CD34^+$ and $CD14^+$ cells ($r^2=0.76$, $p<0.001$) and between $CD34^+$ cells and colony growth ($r^2=0.82$, $p<0.001$). The Ctx, followed by G-CSF and PBPC-reinfusion contributed to substantially higher amounts of progenitor cells during the regeneration phase than G-CSF alone, by lower leukocyte values: 2.2 vs 5.4 CFU-GM, 1.9 vs 4.3 BFU-E, 21.8 vs 124.3 $CD34^+$ cells and 16,400 vs 54,700 leukocytes per μL PB ($p<0.001$; mean). Increased clonogenicity ($CD34^+$ cells - CFU-GM or BFU-E ratio) was associated with lower numbers of $CD34^+$ cells. Hematopoiesis recovered rapidly (median): 8 days to reach leukocytes > 1000 μL and 12 days - for platelets > 50000 μL; duration of leukocytopenia < 1000 μL lasted 5 days and 8 days to become platelet transfusion-independent. The CD25 and CD56 cells recovered simultaneously with granulocytes. Thus, the combination of G-CSF and PBPC should provide an option for escalating chemotherapy doses.

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RECOMBINANT HUMAN ERYTHROPOIETIN AFTER BONE MARROW TRANSPLANTATION: A PROSPECTIVE PLACEBO CONTROLLED TRIAL.

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The aim of this study is to evaluate the effects of recombinant human Erythropoietin (rhu EPO) on hematopoietic regeneration after allogeneic or autologous bone marrow transplantation. Patients were randomized to receive either 100 U rhu EPO/kg body weight or placebo as continuous intravenous infusion from day 1 after BMT until independence from erythrocyte transfusions for 7 days or until day 42. The randomization was performed per each center and strafified according allogeneic or autologous BMT and major ABO-blood group incompatibility. At the time of the planned interim analysis with 205 patients treated, the time to erythrocyte transfusion independence after allogeneic BMT was shorter in group A (n = 52) than in group B (n = 55). After autologous BMT no difference between group A (n = 49) and B (n = 49) could be detected so far concerning time to transfusion independence or the number of transfusions applied, considering either erythrocyte or thrombocyte transfusions. There were no major differences in side effects between groups A and B. As of October 1992, the study was finished with a total of 329 patients.

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FLOW CYTOMETRY AND IN SUSPENSION HYBRIDIZATION FOR DETECTION OF HUMAN CYTOMEGALOVIRUS DNA IN MONONUCLEAR BLOOD CELLS

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Human Cytomegalovirus infections (HCMV) are an essential cause of disease and death in transplant recipients. Rapid and reliable laboratory methods to diagnose HCMV infections are necessary, especially because effectiveness of specific antiviral drugs improves significantly by early application. The method of flow cytometry and in suspension hybridization (FLASH) has recently been described by us (H. Link, J. Med. Virol. 37: 143-148; 1992), which allows the quantification of Immediate Early Gene CMV-DNA carrying mononuclear blood cells (MNC).

The aim of this study was to correlate clinical data with FLASH analysis compared to shell vial culture, polymerase chain reaction (PCR) and antigenemia (CMV-Immediate Early Antigen expression - APAAP) from MNC. Ficoll separated MNC's of 31 patients (9 bone marrow, 13 liver, 9 kidney transplant recipients) were examined. 20 ml heparinized blood were taken at day fourteen and then weekly up to 8 weeks after transplantation. Defined amounts of MNC's were used for each test.

	FLASH	Culture	PCR	APAAP
No. samples	181	171	175	181
No. positive	140 (77%)	77 (45%)	131 (75%)	128 (71%)

Applied to PCR FLASH sensitivity was 94%, with a specificity of 83%. FLASH correlated well with antigenemia; r = 0.78 if CMV-IEA positive cells were >10 /µl ($p < 0.001$) and r = 0.978 if CMV-IEA positive cells were > 15/µl ($p < 0.001$).

9 out of 12 patients had > 10 CMV-infected cells/µl peripheral blood with FLASH and APAAP and developed symptomatic HCMV disease.

Conclusions: Specificity and sensitivity of FLASH is comparable with PCR. FLASH is more specific than culture and the results are available within 2 days. The detection of HCMV-DNA positive cells by FLASH and PCR was related to the development of HCMV disease. Flow cytometry and in suspension hybridization is a new quantitative and rapid method to detect relevant cytomegalovirus infections.

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INTENSIVE CONSOLIDATION TREATMENT IN ACUTE MYELOBLASTIC LEUKEMIA: ALLOGENEIC BONE MARROW TRANSPLANTATION VERSUS HIGH DOSE CYTOSINE-ARABINOSIDE/DAUNORUBICINE VERSUS UNPURGED AUTOLOGOUS BONE MARROW TRANSPLANTATION

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Different forms of intensive postremission therapy in AML-patients up to the age of 50 years were compared prospectively. The induction chemotherapy consisted of ARA-C 100mg/m² civi day 1-8; DNR 60mg/m² day 3-5, VP-16 100mg/m² day 4-8 (DAV I) followed by a second cycle with ARA-C 100mg/m² civi day 1-7, DNR 45mg/m² day 3,4, VP-16 100mg day 3-7 (DAV II). The first consolidation course in patients with complete remission consisted of DAV III identical to DAV II. Allogeneic bone marrow transplantation (BMT) was prospectively compared with either high-dose Cytosine-Arabinoside-/Daunorubicin (HD-ARA-C/DNR) or with high dose Busulfan/Cyclophosphamide (Bu/Cy) followed by autologous BMT after randomization or decision of the patient. From 4/1988 to 3/1991 148 evaluable patients with a median age of 35 years (range 16-50) were treated, 102 (70.9%) of whom reached a complete remission. 24 patients were treated with allogeneic BMT after conditioning with either 12 Gy fractionated total body irradiation day 1-4 and Cy 60mg/kg day 5,6 or Bu 4x1mg/kg day 1-4 and Cy 60mg/kg day 5,6. 44 patients were allocated for one to two courses HD-ARA-C/DNR: 1st course: 3g/m²i.v. over 2h every 12h, day 1-6, and DNR 30 mg/m² day 7-9; 2nd course: 3g/m²i.v. over 2h every 12 h, day 1-4, and DNR 30mg/m² day 5,6. 12 patients received unpurged autologous BMT after therapy with Bu 4x1 mg/kg day 1-4 and Cy 50mg/kg day 5-8. A high proportion of patients (n=25, 24,5%) did not receive postremission therapy, mostly due to early relapse. After 45 months the actuarial eventfree survival (EFS) following allogeneic BMT was 61%, after HD-ARA-C/DNR 33% and after autologous BMT 18%. The p-values for EFS and remission duration respectively by log-rank test were p=0.04, 0.002 for allogeneic versus autologous BMT, p=0.18, 0.02 for allogeneic BMT versus HD-ARA-C/DNR and HD-ARA-C/DNR versus autologous BMT p=0.17, 012.

Conclusions: The first postremission therapy should be performed as early and intensive as possible, in order to avoid early relapse. Allogeneic BMT provides the best chance for EFS probably due to the graft versus leukemia effect. High-dose ARA-C/DNR leads to 33% EFS after 45 months. Early high-dose Bu/Cy followed by unpurged autologous BMT yields no benefit in AML compared to high-dose chemotherapy.

Hannover[1], Nürnberg[2], Hamburg-St.Georg[3], Erlangen[4], Mannheim[5], Augsburg[6], Stuttgart[7], Wuppertal[8], München-Harlaching[9], Göttingen[10], Tübingen[11],

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LIPOSOMAL AMPHOTERICIN B FOR PULMONARY MYCOSES IN NEUTROPENIC AND IMMUNOSUPPRESSED PATIENTS

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The effect of liposomal amphotericin B (L-AmB, AmBisome®) on documented fungal pneumonia or presumed mycotic infection was analysed retrospectively. Twenty five patients were treated with L-AmB after bone marrow transplantation or during neutropenia after chemotherapy. They received L-AmB because of pneumonia refractory to standard amphotericin B, severe side effects from AmB or rapid deterioration despite adequate antibacterial therapy. The mean dose of L-AmB was 2.37 mg/kg body weight daily (range 0.7 - 4.2) for the first two weeks, then every other day for a mean duration of 22 days (range 6-95 days). In 13 patients the causative organisms were *Candida spp.*, in one patient *Aspergillus spp.*, in one *Mucor spp.* and in two *Aspergillus spp.* with *Candida spp.* Of the 25 patients treated with L-AmB, 13 (52%) responded completely and five (20%) partially. In microbiologically documented infections, 10 (58%) of 17 patients responded completely and four (23%) partially. If the 16 patients with mycologically documented pneumonia and the four with pulmonary infiltrates are considered separately, the complete and partial response rates totalled 17 (85%) of 20 patients. Two patients died of refractory mycosis. Besides a discrete rise of creatinine no side effects were observed. *Conclusion*: Liposomal AmB can be given in higher doses than conventional AmB. It is useful in patients with fungal pneumonia refractory or intolerance to conventional AmB.

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LONGTERM FOLLOW UP OF ULTRA- HIGH CARBOPLATIN, VP16, CYCLOPHOSPHAMIDE WITH ABMT IN REFRACTORY OR RELAPSED NSGCT.

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42 pts. with refractory or relapsed non seminomatous germ cell tumors (NSGCT) were entered in a prospective phase I/II trial of carboplatin 2000 mg/sqm, VP 16 1500 mg/sqm, cyclophosphamide 120 mg/kg BW rescued by bone marrow and/or peripheral blood stem cells (ABMT). All patients were deemed incurable with conventional therapy after second line cisplatin-based prior treatment. At time of ABMT 63 % of the pts. presented with advanced disease (Indiana staging >6). There were 7 pts. with heavily pretreated primary extragonadal germ cell tumors (EGCT). Regarding response to prior chemotherapy pts. were either absolute refractory (progressive disease, increase of markers < 4 weeks) or refractory (stable disease, marker plateau, never CR of PR marker neg.) or had relapsed (CR or PR marker neg > 4 weeks). 6 pts. with absolute refractory disease and 7 pts. with EGCT did not benefit from the procedure with a survival not longer than 5 months and 8 months respectively. 8/11 (72 %) pts. with refractory disease (EGCT's not included) were responders (3 PR, 5 CR lasting 46+, 45+, 27+, 26+, 25+ months). 11/16 (70 %) pts. transplanted in relapse responded (5 PR, 6 CR lasting 52+, 37+, 11, 13, 19+, 14+ months). After a median observation time of 31 (17 - 59) months, 26 % of all pts. are well and alive, 70 % of these pts. are still in continuous CR up to 52+ months.

Our longterm results demonstrate that ultrahigh-dose chemotherapy with ABMT represents a curative option in heavily pretreated pts. with NSGCT excluding pts. with absolute refractory disease and EGCT.

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DEVELOPMENT OF NEW RICIN A-CHAIN IMMUNOTOXINS FOR THE TREATMENT OF HODGKIN'S DISEASE USING HIGH-AFFINITY MONOCLONAL ANTIBODIES AGAINST THE CD-30 ANTIGEN

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Immunotoxins against the CD30 antigen constructed with either ricin A-chain or Saporin-6 have been desribed by our group and others to be effective against Hodgkin's disease. Since there is a clear correlation between affinity of the antibody moiety and potency of the immunotoxin, we have evaluated five new high-affinity monoclonal antibodies against the CD30 antigen for their potential use against Hodgkin's disease. O2-7.2, S9-13.1, 12-6-3, A14-20, and C7-18 recognizing three different epitopes on the CD30 antigen as demonstrated by FACS analysis were linked via the SMPT linker to deglycosylated ricin A-chain (dgA). The most effective immunotoxin, O2-7.2-SMPT-dgA, inhibited the protein synthesis of L540Cy Hodgkin cells by 50% at concentrations (IC_{50}) of 4×10^{-11} M. Under the same experimental conditions, O2-7.2-SMPT-dgA was 5-times more potent than the second most potent immunotoxin in this study, S9-13.1-SMPT-dgA, and other previously described CD30 immunotoxins. Since O2-7.2 showed very little crossreactivity with normal human tissue, O2-7.2-SMPT-dgA is the CD30-immunotoxin of choice for the therapy of Hodgkin's disease.

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EARLY DETECTION OF INCIPIENT RELAPSE AND MONITORING OF TREATMENT EFFICACY IN PATIENTS WITH CHRONIC MYELOGENOUS LEUKEMIA BY QUANTITATIVE POLYMERASE CHAIN REACTION

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The monitoring of minimal residual disease (MRD) in patients with leukemia after chemotherapy or after bone marrow transplantation (BMT) offers the possibility to study prospectively the correlation between the presence of residual leukemia and the risk of relapse. In certain types of leukemia which display specific genetic markers suitable for molecular analysis, the monitoring of MRD can now be routinely performed by the highly sensitive polymerase chain reaction (PCR)-technique. In patients with chronic myelogenous leukemia (CML) after BMT, it was shown that the employment of PCR for the detection of residual leukemic cells displaying the characteristic BCR/ABL rearrangement has a limited prognostic value. This fact is based on the observation that a proportion of CML patients have residual rearranged cells even years after BMT without progressing to relapse. We have therefore established a quantitative PCR-method which allows to monitor the proliferative activity of residual neoplastic cells. We have used chronic myelogenous leukemia as a model, and demonstrated by serial, quantitative PCR-analyses in patients after BMT that the detection of an expanding leukemic clone, which we termed "PCR-relapse", preceded a clinical relapse by up to 8 months. We have monitored over 30 patients during the course of disease post BMT by quantitative PCR and were able to correctly predict the later occurence of clinical relapse in all instances. In contrast, all patients in whom PCR-relapse was not detected remained in complete remission within an observation period of up to 9 years. The early detection of incipient relapse could provide a basis for the timely initiation of treatment directed at the elimination of a relatively small neoplastic clone. Alternatively, the employment of Interferon (IFN) at an early stage of relapse may help control the proliferation of the residual clone. Our preliminary results of serial, quantitative PCR-analyses in CML patients under therapy with IFN suggest that the efficacy of treatment can be assessed and monitored at the high level of sensitivity inherent in PCR analysis. This approach may allow to perform adequate adaptations of treatment and/or the respective dosage. Quantitative PCR-analyses in CML patients allow an early detection of incipient relapse and may facilitate the monitoring of both treatment-efficacy and quality of remission. The experience gained in CML as a model-disease provides a basis for similar investigations in other types of leukemia which display genetic markers suitable for PCR-analysis.

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G-CSF AND SUBSETS OF CD34+ CELLS IN PERIPHERAL BLOOD AND BONE MARROW AFTER PROGENITOR CELL MOBILIZATION OR ALLOGENEIC BONE MARROW TRANSPLANTATION.

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The study was designed to estimate the influence of G-CSF application on the distribution of CD34+ cells and their subsets in peripheral blood (PB) and bone marrow (BM). BM and PB samples were obtained as follows: (i) day 5 after onset of G-CSF application (2x12 µg/kg/die s.c.) for progenitor cell mobilization (G-CSF Group; n=6); (ii) day 14 or 18 after allogeneic bone marrow transplantation followed by G-CSF (5 µg/kg/die s.c.; BMT Gr.; n=6); and (iii) at BM-donation of 10 healthy individuals (Control Gr.). The percentages of CD34+ cells (PE MoAb) in the light density cell fraction (Ficoll-separation) were measured by flow cytometry. The parallel expression of CD15, CD19, CD25, CD33, CD38, CD45RO, CD45RA, HLA-DR (FITC MoAb) and c-kit (SR1, Amgen) was assessed by counting 500 to 2000 CD34+ in a live gate (SSC vs Fl-2). About 80% of the CD34+, in all samples tested, showed high fluorescence intensity: 10^2 to 10^3. The ratio of mean CD34+ cell percentages in PB vs BM of the Control Group was 0.09, as expected, and was significantly higher in the G-CSF (1.3) and BMT Group (0.4; p=0.01). Surprisingly, no notable differences of the CD34 subset distribution in PB vs BM could be established. The means of CD34 cell subset percentages are shown in the following table ($p<0.05$: *- G-CSF vs BMT Group, #- BMT vs Control Group, and ~- G-CSF vs Control Group):

CD34 and	G-CSF Group		BMT Group		Control Group	
	PB	BM	PB	BM	PB	BM
CD15	13.0	16.7*	14.0	30.7	16.0	20.8
CD19	6.0	7.0	6.5#	5.0#	15.0~	26.4~
CD25	23.0*	19.4	54.0#	34.7	16.0	10.4
CD33	83.6*	90.0	97.0#	96.0#	85.5	81.0
CD38	98.4	97.9	98.7	99.6	99.1	98.9
CD45RO	90.0*	86.0*	11.0#	18.0	40.0~	43.5~
CD45RA	7.0	26.7	9.0#	4.0	22.0~	26.5
HLA-DR	95.5	96.8	96.9	97.0	95.2	98.0
c-kit	9.5	8.0	-	2.5#	13.8	14.0

G-CSF recruits the CD34+ cells in the PB, leading to a relative BM depletion, as shown by the inversion of the PB vs BM CD34+-cell ratio. The highest proportions of uncommitted hematopoietic progenitor cells (CD34+/CD33-) in the PB of the studied groups are achieved after application of G-CSF alone.

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ONCOGENE MEDIATED SUPPRESSION OF MHC CLASS I EXPRESSION IS REGULATED BY POSTTRANSCRIPTIONAL MECHANISMS

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Downregulation of MHC class I expression may represent a common mechanism of cells to escape the surveillance of the immune system. Indeed it has been described, that regulation of MHC antigen expression by viruses and oncogenes, leading to either immune evasion or autoimmunity, is widespread and important for disease.
To proof the hypothesis that oncogene expression is inversely correlated with MHC class I antigen expression, we choose a model of inducible oncogenic transformation. Murine fibroblasts were transfected with oncogenes of different classes (ras, mos, fos) expressed under control of the dexamethasone-inducible MMTV promotor (MMTV-onc). In these MMTV-onc transfectants, we could show a time-dependent inverse association of H-2 and oncogene expression after dexamethasone stimulation. Both FACScan and Western blot analysis demonstrate that downregulation of H-2 antigen expression is preceeded by a dexamethasone-mediated induction of oncogene expression. These data were confirmed by Northern blot analysis using a β_2-microglobulin and H-2 specific cDNA probe. Treatment of the MMTV-onc transfectants with murine recombinant interferon-gamma (IFN) causes a reduction of oncogene specific mRNA levels, whereas the expression MHC class I heavy chain and β_2-microglobulin was induced under these culture conditions due to IFN-gamma mediated transcriptional regulation.
To investigate the level of regulation of oncogene-induced suppression of H-2 antigens, we performed H-2 promotor CAT analysis as well as nuclear transcription assays. MMTV-onc clones stably transfected with H-2 promotor CAT constructs were generated. Stimulation of these cells with dexamethasone causes no alteration of the H-2 promotor activity. This was in accordance with our results obtained by nuclear run on assays where the transcriptional activity of H-2 antigens was not affected by dexamethasone treatment. These data suggest that the oncogene-mediated inhibition of MHC class I expression is posttranscriptionally controlled.

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LIMITED EFFICACY OF INTERFERON-α AND VINBLASTINE AS SECOND LINE BIOCHEMOTHERAPY REGIMEN IN PATIENTS WITH PROGRESSIVE METASTATIC RENAL CELL CARCINOMA

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We report on thirty-four patients with metastatic renal cell carcinoma, who were treated with a combination of subcutaneous recombinant interferon-α and intravenous vinblastine upon progression after previous antineoplastic therapy. Pretreatment included chemotherapy (n=3), hormonal therapy (n=6) and immunotherapy (interleukin-2/interferon-α, n=25). In this study,treatment courses consisted of subcutaneous doses thrice weekly of recombinant interferon-α at 6 million U/m² (14 patients, group 1), and at 12 million U/m² (20 patients,group 2), respectively. Treatment was given over 8 consecutive weeks. Additionally, in all patients, vinblastine was administered intravenously at a dose of 6 mg/m² in weeks 2, 5 and 8. Of 14 patients treated in group 1, one had a partial response for 6 months (overall response rate 7.14%; 95% confidence interval, 0.18-33.87%), and four had disease stabilization (median duration, 5.0 months). Of 20 patients treated in group 2, there was one patient who achieved a complete response (response duration, 34+ months); in addition, two patients had a partial response (median response duration, 10.5+ months; overall response rate, 15%; 95 % confidence interval, 3.21-37.89%), and 13 patients exhibited disease stabilization (median duration 5.9+ months). Response rates showed no significant differences when comparing treatment results in patients in group 1 vs group 2. In contrast, significantly less patients treated in group 2 had progressive disease (p = 0.024), as compared to patients in group 1. This treatment combination was overall well tolerated with low to moderate systemic toxicity. In addition, there were no significant differences in frequency or intensity of therapy-related systemic toxicities when comparing patients in group 1 and group 2, respectively. We conclude that the combination of subcutaneous recombinant interferon-α and intravenous vinblastine has limited efficacy as second line biochemotherapy in pretreated progressive metastatic renal cell cancer patients.

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MULTIPLE MYELOMA - IMPROVEMENT IN PROGNOSIS AS A RESULT OF NEW THERAPEUTIC EFFORTS?

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Recent therapeutic efforts in the treatment of multiple myeloma involve the use of interferon (IFN), high-dose melphalan, autologous and allogenous bone marrow transplantation (BMT), cytokines as well as monoclonal antibodies against cytokines, and attempts to break through chemotherapy resistance.

The studies on combined IFN-chemotherapy, on induction treatment as well as on IFN-remission maintenance therapy all produce differing results, although in the majority of the investigations the use of IFN is reported to be of benefit.

Higher remission rates are achieved with high-dose melphalan therapy than with conventional chemotherapy; whether this therapy also results in longer survival is questionable at present. Expectations are higher for autologous and allogenous BMT, although a mortality risk of 30-40% is associated with the latter. Approximately 10% of patients treated with allogenous transplantation achieve a longlasting complete remission. Much the same is true for autologous BMT. For a definitive evaluation we will have to await the results of controlled studies comparing conventional chemotherapy and BMT.

Endeavors to break through resistance to cytostatic drugs by way of verapamil or cyclosporin lead to further short-term remissions in 30-50% of therapy-resistant patients . A significant increase in life expectancy is however not to be expected.

The use of monoclonal antibodies against Interleukin-6 (IL-6), the most important paracrine growth factor in multiple myeloma has led to a temporary inhibition of tumor proliferation in a small number of patients. IL-6 inhibition could also be achieved with other measures, as for example antisens-RNA, inactive IL-6 analogues, etc.

The first preliminary therapy results with IL-2 show a clear palliative effect in 30-40% of the patients. IL-4 can lead to tumor inhibition in individual patients, in others possibly to tumor stimulation.

In summary, recent years have brought only a marginal improvement in prognosis in multiple myeloma, despite extensive endeavors. However, innovative therapeutic measures have succeeded in effecting clear improvements in particular patients.

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SEVERAL MYELOID-SPECIFIC GENES ARE DIFFERENTIALLY EXPRESSED AND METHYLATEDIN CD34-ENRICHED PERIPHERAL BLOOD PROGENITOR CELLS DURING IN-VITRO CULTURING

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Tissue- and development-specific gene expression is coordinately regulated during maturation of normal bone marrow cells toward functional, peripheral blood end-stage cells. In order to study the expression of several myeloid-specific genes during maturation of normal hematopoietic progenitor cells, we used in vitro differentiation of $CD34^+$-enriched peripheral blood progenitor cells (PBPC). PBPCs from patients with various solid tumors were recruited by standard-dose ifosfamide-based chemotherapy and subsequent administration of recombinant Granulocyte Colony-Stimulating Factor (G-CSF). PBSCs were collected and $CD34^+$ cells selected by immunoadsorption columns using a biotinylated anti-CD34 monoclonal antibody (kindly provided by R. Berenson, CellPro, Seattle WA). Enriched cells contained between 85 and 90% $CD34^+$ cells as determined by FACS analysis. Cell preparations were cultured in the presence of Interleukin-(IL)1ß, IL-3, IL-6, stem cell factor/kit ligand, and G-CSF for up to 17 days. Expression of the genes for CD34, myeloperoxidase (MPO), lysozyme (LZM) and lactoferrin (LF) were examined by RNA analysis. A time-dependent downregulation of CD34 transcripts was observed, with concomitant upregulation of expression for LZM, MPO and LF. Analysis of the lysozyme gene methylation status by restriction enzyme analysis revealed a differentiation-dependent demethylation switch at several SmaI restriction sites. This study demonstrates that in early peripheral blood progenitor cells, several myeloid-specific genes are differentially regulated by G-CSF in conjunction with other cytokines during in vitro maturation.

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MALT-LYMPHOMA OF THE STOMACH IN A PATIENT WITH SJÖGRENS-SYNDROME

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Development of malignant lymphoma in patients with Sjögren$-syndrome is reported to occur in about 5-10 percent of patients. In most cases lymphoma affects the parotic glands. Here we report on a 61 year old female, who since 1985 suffered from Sjögren$-syndrome together with Raynaud$-phenomenon, sensible polyneuropathy and allergic vasculitis. In 1992 gastroscopy showed a MALT-lymphoma of the stomach, histologically confirmed, CS I_E. The patient underwent Billroth II operation and pathological stage I_{E1} could be confirmed. The resection-specimen in the submucosal vessels additionally showed marked signs of autoimmune vasculitis. This documents extraglandular manifestation of Sjögren$-syndrome with gastrointestinal involvement, which obviously may induce malignant lymphoma of the stomach. This case furthermore describes the close relationship of the different MALT-compartments.

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G-PROTEIN Gα-16 IS EXPRESSED IN HUMAN PRE-B-CELL LINES AND PROGENITOR B-ALL

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Recently a new GTP binding-protein G-α16 has been described to be specifically expressed in human hematopoietic cells. Expression of G-α16 could be reportedly observed in human cell lines of myelo-monocytic and T-lymphocytic origin. G-α 16 was not detectable in human B cell lines (*Amtruda. et al Proc. Natl. acad. Sci. USA 1991 88: 5587-5591*)

Using RT-PCR we studied the expression of G-α16 in human B cell lines corresponding to different states of human B cell differentiation and in other human cell lines. Expectedly G-α16 expression was observed in the human T-cell line Jurkat and the myelo-monocytic cell line HL60. The human Burkitt's lymphoma cell lines Raji, Ramos, BJAB, the lymphopblastoid cell lines LICR HMy2, the hairy cell leukemia derived cell line JOK-1 and the plasmacytoma cell line U266 were devoid of G-α16. In contrast the human pre-B cell lines Reh and Nalm-6 expressed transcripts for G-α16. The analysis of a broad panel of human neoplastic B lymphocytes ranging from immature progenitor B-ALL, cALL, mature B-ALL to low grade B cell lymphoma (chronic lymphocytic leukemia of B cell type, leukemic centrocytic NHL, hairy cell leukemia) disclosed that G-α16 expression is limited to early progenitor B-ALL cells. We therefore conclude that G-α 16 is expressed in early progenitor B cells and is downregulated during B cell differentiation. Thus G-α16 might be involved in the signal transduction processes of progenitor B lymphocytes.

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THE EFFECTS OF O^6-BENZYLGUANINE (BG) AND STREPTOZOTOCIN (STZ) ON BCNU CYTOTOXICITY AND ON THE INACTIVATION AND RECOVERY OF O^{6}METHYLGUANINE DNA METHYLTRANSFERASE (MGMT) ACTIVITY.

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This study was initiated to determine the interaction of two MGMT depleting agents, BG and STZ, in potentiating BCNU cytotoxicity. The inactivation and recovery of MGMT activity in vitro were used to assess the interaction. Pretreatment of HT-29 human colon carcinoma cells with BG (10μM) and/or STZ (1.0mM), prior to a 100μM dose of BCNU, showed that the combination of BG+STZ produced 1.5-3 logs greater synergistic cell kill than either agent alone. The combination of BG+STZ produced a more prolonged inhibition of MGMT activity than either agent alone. Utilizing doses of STZ and BG as single agents which deplete MGMT activity to below detectable levels, we studied the repletion kinetics of MGMT activity after repeated washing of cells. MGMT activity was not detectable for 24hr in HT-29 cells exposed to a 100μM dose of BG. However, cells washed four times with serum containing medium, subsequent to BG treatment, MGMT activity returned to near control levels by 24hr. Following STZ (2.5mM) exposure, the repletion of MGMT activity was not altered by media washes. MGMT activity was not detectable for 12hr, and recovered only to about 30% of control activity by 24hr. BG+STZ inactivated MGMT activity for 24hr irrespective of washings after drug treatment. Our observations suggest that prolonged depletion of MGMT activity may be required for optimal reversal of BCNU resistance. Because the combination of BG+STZ provides a prolonged inhibition of MGMT, the clinical use of multiple BCNU modulators might induce efficient depletion of MGMT.

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Lymphotoxin α and β are expressed on the cell surface of activated normal B Lymphocytes and leukemic hairy cells

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Cell surface expression of human Lymphotoxin (LT, TNF-β) was studied in human B cell lines as well as in normal and neoplastic human B lymphocytes. In the absence of TNF receptors the human hairy cell leukemia (HCL) derived cell line JOK-1 revealed constitutive cell surface expression of LT-α and -β but not TNF-α. Northern blot analysis of LT-α mRNA expression demonstrated one band in the expected range of 14S. Immunoprecipitation experiments with anti-LT monoclonal antibody (mAb) 9B9 from cell surface radioiodinated JOK-1 cells revealed that a cell surface lymphotoxin molecule (25kD) is expressed in association with a 33kD molecule, which has been recently designated LT-β.

Neoplastic B cells from chronic lymphocytic leukemia (BCLL) could be induced to express surface LT by in vitro stimulation with Staphylococcus aureus Cowan I (SAC). In contrast human HCL cells displayed constitutive cell surface expression of lymphotoxin. These findings suggest that cell surface LT is expressed on activated human B cells and neoplastic B cells representing an activated state. In addition these results indicate that cell surface expression of LT-α and β might be involved in the mediation of cell-cell interactions and thus might play an important role in the regulation of inflammatory processes and biology of B cell neoplasias.Thus one might speculate that surface LT could be involved in the processes leading to vasculitis in HCL.

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THE INVOLVEMENT OF P21RAS IN MITOGENIC SIGNALLING

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Experiments with neutralising antibody injection and dominant negative mutants demonstate that p21ras is required for mitogenic signalling by tyrosine kinase growth factor receptors and oncoproteins. We and others have shown that at least part of this requirement for signalling is reflected in the need for p21ras function for tyrosine kinases to activate the raf-MAP kinase kinase-MAP kinase pathway. Since MAP kinases can enter the nucleus and have been shown to phosphorylate a number of transcription factors the elements of one pathway from growth factor stimulation to changes in gene expression has been delineated.

Some of the signalling pathways of lower eukaryotes such as yeast appear to be homologous to the vertebrate MAP kinase pathway since the vertebrate MAP kinases have sequence homology to Schizosaccharomyces pombe spk1 and Saccharomyces cerevisiae FUS3, KSS1 and HOG1. The recent cloning of vertebrate MAP kinase kinase (MAPKK) extends this homology since the vertebrate MAPKK is homologous to S. pombe byr1 and S. cerevisiae STE7 kinases. Genetic analysis shows byr1 and STE7 to be upstream of the yeast MAP kinases. Attempts to show that the vertebrate and yeast pathways are functionally homologous will be described. Yeast strains expressing a mammalian signalling pathway would be useful for further dissection of the pathway and for screening compounds as potential inhibitory agents.

Previous work on the activation of MAPKK by immunoprecipitates of raf kinase left open the possibilities that there may be an intermediate between raf and MAPKK and that other kinases may activate MAPKK. Experiments will be discussed that strongly argue that raf directly phosphorylates and activates MAPKK and that raf plays a major role in the activation of MAPKK by growth factors.

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RECLASSIFICATION OF A PATIENT WITH PH-POS ACUTE LYMPHOBLASTIC LEUKEMIA AS RARE CASE OF CHRONIC MYELOID LEUKEMIA WITH *MINOR*-BCR/ABL-REARRANGEMENT.

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The Philadelphia-chromosome-positive (Ph-pos) leukemias comprise most cases of CML, about a third of cases of adult ALL, preferentially common-ALL, as well as a small minority of cases of AML. As corresponding molecular event the abl-gene from chromosome (chr.) 9 is rearranged with the bcr-gene from chr. 22. According to their position within the bcr-gene on chr. 22, breakpoints designated *Major (M-) bcr/abl* can be distinguished from breakpoints designated *minor (m-) bcr/abl*. While *M-bcr/abl* is found in CML and in one third of patients with Ph-pos ALL, *m-bcr/abl* is thought to be *confined* to the remaining two thirds of patients with Ph-pos ALL.
Here we report on a female 56-year-old patient presenting with Ph-pos acute leukemia, diagnosed as ALL by local and reference morphology and cytochemistry. The initial diagnosis ALL was substantiated by the characteristic finding of an *m-bcr/abl*-rearrangement by two molecular reference-laboratories (Maurer et al., Lancet 337:1055, 1991). The patient was treated according to the high risk stratum of the German multicenter ALL/AUL trial and scheduled for autologous BMT in CR1. However, due to morphological features resembling CML, the karyotype was reanalysed from remission marrow and revealed **30/30** Ph-pos metaphases. The molecular analysis confirmed the *m-bcr/abl* rearrangement, excluding a sampling error at presentation. If the patient had ALL, some metaphases in CR were Ph-neg, since in CR the majority of hematopoietic cells are not part of the Ph-pos clone, in contrast to CML in chronic phase. Consequently this patient had to be reclassified as a case of CML with atypical *minor-bcr/abl* rearrangement, initially presenting in lymphoid blast-crisis, and ABMT had to be cancelled.
We conclude that a *m-bcr/abl* rearrangement is not diagnostic for ALL in every case but may be a rare event in CML as well. Only very few other cases of CML with *m-bcr/abl* rearrangement have been reported previously (Bartram et al., Blut 55: 505, 1987). Cytogenetic analysis in complete remission may be mandatory in ambiguous cases to clearly distinguish between Ph-pos ALL and lymphoid blast-crisis of CML.

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DELAYED AND INCOMPLETE HEMATOPOIETIC ENGRAFTMENT AFTER AUTOLOGOUS BMT IS NOT DUE TO IMMUNOMAGNETIC BEAD PURGING BUT DUE TO PREVIOUS INTENSIVE CHEMOTHERAPY.

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Patients with high risk leukemia, such as Ph'-chromosome-pos.-ALL or ALL in ≥CR2 need highly intensified treatment strategies to improve in survival. We offered autologous BMT after marrow purging (pABMT) to patients with Ph-pos ALL in CR1 or ALL in later CR without histocompatible allogeneic marrow donor. Patients with Ph-pos ALL were initially treated according to the stratified German multicenter ALL/AUL trial. Autologous bone marrow was harvested after induction chemotherapy and consolidation with high-dose cytarabine (2 x 1g/m² x 4 days) and mitoxantrone (10 mg/m² x 4 d) (HD-AraC/Mitox) and purged in 2 rounds with a cocktail of anti -CD19, -CD10 and -HLA-DR (AB-4) IgM MoAbs directly coupled to immunomagnetic beads (MoAb-IMB, Dynabeads™). Despite posttransplant application of G-CSF (10 µg/kg s.c. daily), we found delayed and/or incomplete hematopoietic engraftment requiring prolonged platelet support until d +88, d >112 and d >196 in 3/3 evaluable patients autografted in CR1. In order to identify underlying factors we reviewed our precursor-B-ALL patients who were transplanted with MoAb-IMB-purged marrow and received postransplant G-CSF: Five of total 8 patients were previously treated with HD-AraC-containing regimens: 3/5 were autografted in CR1 as described, and 2/5 were in CR2 receiving either HD-AraC(2 x 1g/m² x 4d) and mitoxantrone (10 mg/m² x 4d) or HD-AraC (2x1g/m²x3d) and idarubicin (8 mg/m² x 3d) as relapse therapy. The remaining 3/8 pts. were autografted in CR2 and had previous relapse therapy similar to CR1-protocols (2/3 induction therapy phase I+II and 1/3 B-ALL protocol) (=noHD-AraC). The numbers of days (mean±SEM) to recover to postransplant neutrophil counts of 500/µl (ANC_{500}) and 1000/µl (ANC_{1000}) and the days of the last platelet transfusion (DLPT) were :

		ANC_{500}	ANC_{1000}	DLPT
CR1+HD-AraC/Mitox	(n=3) :	34,6± 8,1	>158 ±25	>132±33
CR2+HD-AraC/Mitox or Ida	(n=2) :	24,0± 2,0	26,5±2,5	> 55±10
CR2/noHD-AraC	(n=3) :	13,3± 0,9	16,6±2,9	43±11

The difference in $ANC_{500/1000}$ and DLPT between the 5 +HD-AraC patients and the 3 noHD-AraC patients is statistically significant (p=0.025), despite the low numbers of patients. We conclude that delayed and incomplete engraftment after pABMT with MoAb-IMB ist not due to the purging procedure but to myelotoxic pretreatment prior to bone marrow harvest including high-dose cytarabine.

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CLASSIFICATION OF PRIMARY MYELODYSPLASTIC SYNDROME (pMDS) IN BONE MARROW BIOPSIES AFTER EMBEDDING IN METHYL-METHACRYLATE: A RETROSPECTIVE STUDY OF 569 PATIENTS.

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From the Bone Marrow Registry of the Institute of Pathology, 569 patients with pMDS, of whom bone marrow biopsies had been referred in the years from 1980 to 1992, could be evaluated for this study. The evaluation of histopathology considered 28 parameters, which were determined semiquantitatively in each biopsy. Besides cellularity, the quantity and grade of dysplasia in each cell lineage of hematopoiesis as well as architectural changes and mesenchymal findings were evaluated. - The collective comprised 310 men (54.5 %) and 259 women (45.5 %). Median age was 71.4 years. The median follow-up amounted to 15.8 months. As follow-up closed (October 30st, 1992), 436/569 patients had died (77 %). FAB classification revealed 256 RA (45 %), 52 RARS (9 %), 133 RAEB (24 %), 52 RAEB-t (9 %), 53 CMMoL (9 %), and 23 unclassifiable cases (N-CLASS) (4 %). The median survival times (in months) and the transformation rate in ANLL were as follows: RA 26.5 (16.5 %), RARS 41.9 (3.8 %), RAEB 8.4 (42.1 %), RAEB-t 4.6 (57.7 %), CMMoL 12.5 (59.1 %), N-CLASS 22.4 (21.7 %). Hypoplastic MDS, which can be reliably recognized only in bone marrow biopsies, was found in 59 patients (10.4 %), and MDS with fibrosis in 99 cases (17 %). As published previously, the outcome of MDS patients with myelofibrosis is unfavourable. 11/28 histopathologic parameters proved to have significant impact on survival time in a univariate analysis, which, by using a simple scoring system, allows to differentiate 3 risk groups with conspicuously divergent survival times of 30.2, 10.7, and 6.0 months. - Conclusions: pMDS can be diagnosed reliably in plastic-embedded bone marrow biopsies according to the FAB system. Further advantages of this technique concern the identification of hypoplastic MDS and MDS with fibrosis. Subtle evaluation of histopathology in pMDS also allows the determination of risk groups with different prognosis.

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FREQUENCY OF BCR/ABL M-RNA IN ADULT LYMPHOBLASTIC LEUKEMIA (ALL) AS DETERMINED IN A PROSPECTIVE STUDY

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The Philadelphia-chromosome-translocation is the most frequent translocation in adult ALL. This chromosomal translocation leads to the fusion of the BCR and the ABL gene and results in an expression of a chimeric bcr/abl mRNA and the corresponding protein. In a retrospective study we have detected a remarkably high incidence of bcr/abl mRNA positivity in adult common-ALL. The presence of bcr/abl mRNA in those patients correlated with early relapse, poor overall survival and short remission duration. To confirm these data prospectively we have started to analyse patient samples belonging to the non T-ALL immunophenotype subgroup within the German ALL/AUL-BMFT multicenter trial. To avoid false positive results due to the high risk of accidental contamination during the PCR process, positive samples were checked at least two times in different labs. So far we have analysed 120 patients with non-T-lineage ALL for the presence of bcr/abl mRNA by PCR. 51 (43%) of them were positive. The breakpoint distribution in BCR was 36 (71%) for m-bcr and 14 (27%) for M-bcr. One patient showed both chimeric mRNAs.
The data at present confirm the previous results obtained retrospectively, showing further a high incidence of about 50% of bcr/abl-positivity in the c-ALL subgroup and a predominance of the m-bcr breakpoint. Only one bcr/abl mRNA positive leukemia was found in the pre-B-ALL subgroup. The clinical risk factors of bcr/abl-positive and negative patients as well as follow up data so far available will be shown .

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Prognostic significance of immunological marker analysis in acute myeloid leukemia

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Immunocytological analysis of leukemia has become an essential method to determine leukemic cell differentiation and maturation as defined by membrane surface, cytoplasmatic, or nuclear antigen expression. We investigated the prognostic significance of immunological marker analysis in 70 patients with de novo- and secondary acute myeloid leukemia (AML), starting in November 1990. Expression of the stem cell marker (CD 34) and of myeloid lineage associated markers (CD 11b, CD 13, CD 14, CD 15, CD 33), coexpression of lymphoid lineage associated markers (TdT, CD 7), and expression of C 219 antibody defining multiple drug resistance (MDR) antigen were measured in bone marrow or peripheral blood cells by immunofluorescence and flow cytometry. Moreover, cell cycle analysis was performed in samples containing at least 80% blast cells. Disease remission was defined according to the CALGB criteria. We found that CD 14 positive (monocytoid) AML had a higher rate (90 vs. 75%) but a shorter duration of complete remission (CR) as compared to CD 14 negative AML. In the latter subset CD 15 expression was associated with a higher CR rate (CRR) and a longer CR duration in contrast to CD 15 negative AML (CRR: 84 vs. 56%). AML characterized by TdT coexpression had a lower CRR as compared to those lacking TdT (67 vs. 84%). Expression of MDR antigen was found to be an unfavourable prognostic marker: patients with CD 15 coexpression achieved CR followed by early relapse, whereas patients with MDR positive, CD 15 negative AML achieved partial remission (PR) or were non-responders (NR). In AML with high proliferative activity (S phase > 6%) CR duration was shorter than in AML characterized by S phase < 6%, whereas CRR was found to be equal. In summary, certain immunocytological markers (CD 14, CD 15, TdT, MDR, and S phase) were found to have prognostic significance in AML. Longer observation time is needed to gain more detailed statistical data, and further patients will be investigated.

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SOLUBLE FACTOR SECRETED BY ALPHA-BETA AND GAMMA-DELTA T-LYMPHOCYTES INHIBITS HEMATOPOIETIC COLONY GROWTH IN SEVERE APLASTIC ANEMIA

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Aplastic anemia (AA) is associated with an immune-mediated inhibition of hematopoiesis probably caused by T-lymphocyte subpopulations. To clarify the role of αß- and γδ-T-cells in the pathogenesis of AA, the influence of these lymphocyte subpopulations on CD34+ progenitor cell derived colony formation from healthy persons was investigated. αß- and γδ-T-cells from patients with severe AA were incubated in serum-free liquid culture with CD34+ bone marrow cells isolated by an immuno-affinity column. Cell-cell contact was prevented by separating membranes. As stimuli, 2 cytokine combinations were used (I: MGF, IL3, EPO, GM-CSF, G-CSF, ±IL2; II: MGF, IL3, IL1, IL6, IL7, ±IL2). After 7 days of co-culture, the CD34+ fractions were plated into a methylcellulose assay to enumerate the colony forming capacity. As result, in cytokine combination I (±IL2) the presence of αß- and γδ-T-cells from patients with AA reduced numbers of colony forming units-granulocyte-monocyte (CFU-GM) to 64.4-73.6% as compared to controls without T-cells. γδ-T-lymphocytes from healthy donors also generated a reduction of CFU-GM growth to 74.3-76.6% of the control. As compared to experiments without T-lymphocytes, with cytokine combination II αß+ cells from patients with AA (54.1-56.6%) and γδ+ cells from patients (60.9-71.1%) and from healthy controls (70.1%) caused a decrease of CFU-GM numbers, respectively. In conclusion, these data suggest that under stimulation with T-cell activating cytokines (1) αß-T-lymphocytes from patients with AA suppress CFU-GM growth from allogenic CD34+ cells, and that (2) γδ+ lymphocytes from patients with AA and healthy controls play a suppressive role in the regulation of CFU-GM growth. Both effects may be generated by a soluble factor.

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MONOCLONAL ANTIBODIES (MoAbs) IN THE TREATMENT OF NON-HODGKIN LYMPHOMAS (NHL)

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MoAbs may kill tumor cells by activating host immune system (complement, ADCC), by triggering or interfering with physiologically important receptors, by targeting biologically active moieties to tumor cells (e.g. toxins, isotopes, drugs, cytokines) or by functioning as a biologic response modifier.
Although some obstacles (e.g. inability to deliver MoAbs to NHL or to kill all tumor cells, immune response to foreign proteins, toxic side effects) have generated solutions (MoAb 'cocktails', plasmapheresis of circulating antigens, anti-CD4 antibody and deoxyspergualin) therapies with MoAbs have not yet fulfilled their enormous theoretical promise.
Unconjugated MoAbs (Target antigens: CD4, CD5, CD10, CD20, CD21, CD24, CD33, CDw52, Ig): Over 100 patients have been treated in phase I/II trials but only single cases achieved complete or partial remission.
Immunotoxins (ricin, diphtheria toxin, pseudomonas exotoxin A): Preliminary data suggest that immunotoxins may have a role in combined modality therapy and in purging procedures.
Radioimmunotherapy (e.g. iodine-131, yttrium-90, rhenium-188, copper-67): Encouraging clinical results in over 100 patients cannot hide many unsolved problems (conjugation techniques, penetration into tumor sites).
At present MoAbs are inefficient in bulky disease. Thus, future trials should be directed at the treatment of minimal residual disease following standard therapeutic approaches.

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CYTARABINE AND IDARUBICIN (AIDA) AS INDUCTION THERAPY FOR PREVIOUSLY UNTREATED PATIENTS WITH ACUTE MYELOID LEUKEMIA

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In a phase II-study 53 patients (pts) with a median age of 37 (range 20-64) years and *de novo* acute myeloid leukemia (AML) were treated by AIDA: idarubicin (12 mg/m^2/d iv on day 1-3) and cytarabine (100 mg/m^2 iv bolus on day 2 followed by 200 mg/m^2/d continuous iv infusion for 5 days). A maximum of two induction courses was applied. The same regimen was used for consolidation. Pts in complete remission (CR) were then treated either by bone marrow transplantation, maintenance therapy or observation only. Up to now, 47 pts are evaluable for response and toxicity with a median observation time of 11 months. CR was induced in 32 pts (68 %), in 23 pts after the first (CR1) and in 9 pts after the second course (CR2). Treatment failure was due to persisting blasts in 11 pts (23 %) and early death in 4 pts (9 %), respectively. Actuarial median survival has not been reached with 58 % at 14 months. Actuarial median relapse-free survival of complete responders is 13 months without a difference between CR1 and CR2. Side effects were mainly due to myelosuppression. For pts in CR median time for reconstitution of leukocytes > 1.000/µl was 23 (range 15-34) days after first and 27 (19-37) days after second AIDA and median time for reconstitution of platelets > 50.000/µl was 23 (21-36) days after first and 28 (20-57) after second AIDA, respectively. In conclusion, AIDA is a well tolerated regimen for the induction therapy of *de novo* AML. With respect to the CR-rate, treatment results of AIDA are comparable to daunorubicin-based induction combination chemotherapy. However, the results of long-term follow-up are still pending.

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CLONING OF PERIPHERAL LYMPHOCYTES IN UNTREATED ACUTE MYELOID LEUKEMIA RESULTS IN PREDOMINANTLY CD3+CD4+ T-CELL CLONES WITH BLAST SPECIFIC AUTOLOGOUS CYTOTOXICITY.

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Induction of graft versus leukemia (GVL) like reaction by Interleukin-2 (IL-2) for elimination of minimal residual disease is a new therapeutic approach in treatment of acute myeloid leukemia (AML). The present study was designed to investigate mechanisms involved in specific T-cell interactions with AML blast cells in regard to cytotoxic effects. In a first step primary T-cell lines were established in an IL-2 driven coculture system and subsequently cloned by limiting dilution. Sixty three resulting T cell lines and clones were phenotypically characterized by mAbs (CD2, CD3, CD4, CD8, DR, CD56, TCR-alpha/beta, TCR gamma/delta). All cells stained positively for CD2, CD3 and DR. The vast majority of cells stained positive for CD4 (56/63) and a few for CD8 (5/63). In one patient 3 clones with TCR gamma/delta could be generated, two of them negativ for CD4 as well as CD8. Expression of CD56 was variable. Eight clones, including 4 CD4+, 2 CD8+ and 2 TCR gamma/delta+ clones from 2 patients were chosen for functional studies in regard to cytokine release and cytotoxic activity. Significant lysis of K562 (NK) was seen in one TCR gamma/delta+ clone, no Daudi cell directed activity (unspecific LAK) could be detected. However, all clones tested exerted a cytotoxic effect to autologous, as well as allogeneic blast cells. The data indicate that in vivo activation of blast specific cytotoxic lymphocytes might be one mechanism involved in immunotherapeutic approaches of AML with IL-2. For future aspects use of autologous cytotoxic T cells against AML blasts might be considered in adoptive immunotherapy and gene transfer models.

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MOLECULARBIOLOGICAL MONITORING OF BCR-ABL POSITVE ACUTE LYMPHOBLASTIC LEUKEMIA

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Using 1-step- and 2-step-PCR (nested primer) under highly standardized conditions we have measured minimal residual leukemia in 8 BCR-ABL positive patients with adult acute lymphoblastic leukemia (ALL). 7/8 patients had breakpoints in the minor-breakpoint cluster region, only one patient showed a breakpoint in the Major-breakpoint cluster region. These 8 patients were followed serially with 1-step- and 2-step-PCR after chemotherapy (Hoelzer et al, 1984) and bone marrow transplantation (BMT). BCR-ABL positivity was first detected in 4 patients at diagnosis and in 4 patients at relapse. 7 patients achieved complete remission, only one of these patients became BCR-ABL negative after chemotherapy and remained negative in 2-step-PCR for 4 years until hematological relapse. Six patients remained 2-step positive and 1-step negative for a median of 29 months (range 2 - 56 months). The time from 1-step positivity until hematological relapse was 4 weeks median (range 3 - 8 weeks). One patient who received autologous BMT in complete hematological remission, became 1-step-PCR negative after BMT, but remained BCR-ABL positive in 2-step PCR. Another patient in second hematological relapse underwent allogenous BMT; he became BCR-ABL negative in 2-step-PCR and remained negative for 4,5 month +.

Our data indicate, that the BCR-ABL positive clone could be suppressed below the detection level of 2-step-PCR only in 1/8 patients. All patients progressed to 1-step PCR positivity after varying time, but there was only a very short period until hematological relapse. With allogenous BMT the clone can be eliminated at least temporarily.

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EVIDENCE OF MIXED CHIMERISM WITH A XXY/XX/XY MOSAIC USING FLUORESCENCE IN SITU HYBRIDIZATION AFTER SEX MISMATCHED MARROW TRANSPLANTATION.

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A female patient with T ALL and t(4;11)(q21;q23) was grafted from his apparently healthy brother. She reconstituted well without any sign of a GvHD. However, a cytogenetic control after bone marrow transplantation (BMT) revealed cells with an abnormal karyotype (47,XXY) suggesting a Klinefelter syndrom of her brother. In PHA stimulated peripheral blood cells of the donor this abnormal karyotype could be confirmed. To study the pattern of hemopoietic reconstitution we analysed granulocytes and mononuclear cells (MNC) of peripheral blood (PB) and bone marrow (BM) using two colour FISH with a FITC-labelled Y-specific and a AMCA-labelled X-specific probe. It could be demonstrated that only few normal male cells (46,XY; <6%) were present in all specimens of PB and BM. MNC of BM exhibiting a normal female karyotype (46,XX) or an abnormal male karyotype (47,XXY) could be detected with equal frequency. Granulocytes showed in 61% a normal female karyotype whereas MNC of PB exhibited in 85% the abnormal male karyotype. Therefore, it can be concluded (1) that the donor is truly a XXY/XY mosaic with only few normal male stem cells, (2) that a patient can be reconstituted with a constitutively abnormal marrow, (3) that after transplantation the myeloid and lymphohemopoietic compartment show a different composition, (4) that recipient type cells survived the conditioning regimen (TBI-CY) in absence of a clinical GvHD. This complex pattern of mixed chimerism might influence the GvHD; on the other hand an altered function of the predominant abnormal donor hemopoiesis could influence chimerism. Long term follow up can demonstrate possible changes between the three stem cell compartments (47,XXY; 46,XX; 46,XY) favouring a polyclonal or oligoclonal growth of the hemopoiesis in this patient.

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EXPRESSION OF LFA-1, C-KIT AND DIFFERENTIATION ANTIGENS ON CYTOKINE-MOBILIZED CD34+ BLOOD STEM CELLS

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Using dual-immunofluorescence analysis, we evaluated the expression of the adhesion molecule LFA-1 (CD11a) on CD34+ cells of the leukapheresis products of 23 patients (LP CD34+) who had received G-CSF or IL-3/GM-CSF following high-dose chemotherapy. Furthermore, the expression of the commitment-related antigens CD33, CD38, HLA-DR and c-kit were analyzed. The results were compared with normal bone marrow (BM, n=6) and peripheral blood (PB, n=6) CD34+ cells. After mobilisation, LFA-1 was expressed on 60% of LP CD34+ cells at a low fluorescence intensity, whereas a high percentage and level of expression was observed on PB CD34+ (88%) and on BM CD34+ cells (75%). This finding implies that down-regulation of LFA-1 may facilitate the egress of CD34+ cells from the bone marrow and increase their circulation time. In the normal PB and after cytokine mobilization, only a small population (<20%) of CD34+ weakly expressed c-kit. On the other hand, a high proportion of CD34+ cells costaining for c-kit was found in the nomal BM (32%). Since c-kit positivity is related to a more primitive multipotent stem cell, the low proportion and level of c-kit expression may reflect the fact that the majority of cytokine-mobilized blood stem cells are committed progenitor cells. This idea is supported by the coexpression pattern of CD38 (>95%), HLA-DR (>95%) and CD33 (80%) on LP CD34+ cells. The low expression of LFA-1 on mobilized committed CD34+ cells is a surprising result, because during differentiation the level of CD11a expression is increasing. It is concluded that cytokine mobilized CD34+ blood stem cells differ in the expression of functionally important surface antigens from CD34+ cells of other sources.

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Evidence for a specific T-cell response by detection of preferential T-cell-receptor-(TCR)-Vαβ usage of tumorinfiltrating T-cells in melanoma metastases following immunotherapy with IFNα and IL-2.

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The identification and characterization of immunological effector cells mediating tumor regression in immunotherapy with IL2 is of great interest for understanding and further development of this therapeutic approach.
We therefore analyzed T-cell receptor V-Region distribution in tumor tissue from melanoma patients prior to and following immunotherapy with IL-2. We used a highly sensitive RNA-PCR method. After RNA-extraction from tissue and subsequent cDNA-synthesis semiquantitative PCR with different primers for all known Vα- and Vβ-T-cell receptor gene families (18 Vα and 20 Vβ) was performed.
12 tumor tissue samples were analysed including 6 samples of primary malignant melanoma and tumor samples of three patients after immunotherapy. The results were compared to control tissues (peripheral blood, unaffected skin, and liver tissue). The analysis of primary malignant melanoma tissue showed a weak overexpression of different Vβ-families. Analysis of metastatic lesions responding to immunotherapy revealed in comparison to control tissue of the same patient a more obvious predominance of different Va- and Vβ-genes. These results support the view of induction or enhancement of specific T-cells by immunotherapy.
Of special interest is a patient with a mixed response to immunotherapy with progressing and regressing skin metastases. In the regressing lesion we could demonstrate a predominant usage of TCR-Vβ11-Gene almost lacking in the progressing lesion. This suggests a role of Vβ11-expressing T-cells in mediating tumor regression in this patient. Cloning and sequenzing analysis are currently performed to assess wether this represents a true clonal T-cell proliferation.

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MODULATION OF DAUNORUBICIN AND VP-16 CYTOTOXICITY BY B 859-35 COMPARED TO VERAPAMIL AND CYCLOSPORIN A IN MDR$^+$ HUMAN LEUKEMIC CELL LINES

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Resistance to cytotoxic drugs, a major concern in the treatment of cancer, is correlated with the expression of a transmembrane 170 kDa glycoprotein (P-glycoprotein, Pgp). Pgp appears to act as an efflux pump encoded by multi-drug-resistance (mdr) genes. Since it was found that Ca^{2+} channel blockers modulate the Pgp we studied the effects of a new Ca^{2+} channel blocker, B 859-35((-)-3-methyl-5-3-(4.4-diphenyl-1-piperidinyl)-propyl-1.4-dihydro-2.6-dimethyl-4-(3 nitrophenyl)-pyridine-3.5-dicarboxylate-hydrochloride), verapamil and cyclosporine (CSA) on two common anti-neoplastic drugs, VP-16 and daunorubicine (DAUNO). In our study we used two human leukemic cell lines, CCRF ACTD 400 (mdr positive), and CCRF-sensitive (mdr negative), the blast progenitors of one newly diagnosed patient with acute nonlymphocytic leukemia (ANLL), and three normal controls. Cells were monoincubated with 500 ng/ml DAUNO, 1 μg/ml VP 16, 1 μmol B 859-35, 10 μmol verapamil, and 1 μmol CSA for 30 minutes at 37°C. Preincubation consisted of 1 μmol B 859-35, 10 μmol verapamil, or 1 μmol CSA for 30 minutes, followed by DAUNO and VP16 in the above concentrations. Cells were grown in liquid suspension culture and in CFU-GM assays. Our results indicate that B 859-35 and verapamil significantly increase cytotoxicity in CCRF ACTD 400 ($p<0.05$) when combined with DAUNO or VP 16 to the same degree with no effects in the mdr- cell line. In CCRF ACTD 400 treated with VP-16 preincubation with CSA proved to be significantly less toxic than preincubation with B 859-35 ($p<0.05$). B 859-35 should be discussed as an alternative to verapamil in combination with cytotoxic drugs, transported by Pgp.

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INITIAL THERAPY OF HODGKIN'S DISEASE - PROS AND CONS OF MOPP

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As the benefit of the MOPP schedule is being questioned, particularly regarding its late toxicity, 427 patients with Hodgkin's disease, diagnosed between 1970 - 1990 were investigated. 216 men and 211 women were staged as follows at the time of diagnosis: 12.2% stage I, 40.7% stage II, 28.3% stage III and 18.7% stage IV. The histological subtype was lymphocyte-predominant in 10.5%, lymphocyte-depleted in 6.6%, nodular-sclerosing in 38% and of mixed cell type in 32%. The median observation time of the patients was 82 months, whereby the longest observation time was 224 months. Overall response to initial therapy was 83% with 72% complete remissions and 11% partial remissions. In 17% of patients late relapse occurred more than 5 years after initial therapy. Of patients attaining complete remission at initial therapy, 20% received MOPP therapy alone, 42.5% radiotherapy alone, 26.7% MOPP combined with radiotherapy, 2.8% MOPP/ABVD + radiotherapy, 1.1% MOPP and ABVD alternately and 3.8% received various other forms of therapy with or without irradiation. Although MOPP was mainly applied as initial therapy, secondary malignancies were only observed in 3.9% of cases: 4 ANLL, 9 solid tumors, 6 non-Hodgkin lymphomas. Almost all these patients had received several cycles of chemotherapy and radiotherapy due to poor response of the primary disease before manifestation of the secondary malignancy. The MOPP schedule is an effective form of initial therapy. Its application appears to be justified considering the low rate of secondary malignancies in our patient group. In both men and women treated with MOPP fertility and pregnancy, respectively, were noted. Early decision for ABMT in the second sensitive relapse in high risk patients will avoid the need for salvage therapy in future thus reducing the cumulative cytostatic effect.

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LYMPHOCYTE-PREDOMINANT HODGKIN'S DISEASE: B-CELL LYMPHOMA OR SUBTYPE OF HODGKIN'DISEASE ?

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Lymphocyte-predominant Hodgkin's disease is often classified with other B-cell lymphomas. Both the presence of B-cell markers as well as the immunohistochemical behaviour show similarities with non-Hodgkin lymphomas. Among 426 patients diagnosed between 1972 and 1990, 46 (10.5%) were designated as lymphocyte-predominant Hodgkin's disease. The other subtypes were classified as follows: 6.6% lymphocyte-depleted type, 32% nodular-sclerosing type and 38% mixed type. The age of the patients with lymphocyte-predominant type was between 13 and 78 years at the time of diagnosis. The Ann Arbor stage at the time of diagnosis was: 10 patients stage I, 16 patients stage II, 14 patients stage III and 6 patients stage IV.
Generally, the lymphocyte-predominant subtype is considered to have a good prognosis. However, there is an increasing number of reports in the literature of transformation to non Hodgkin lymphomas which are difficult to treat. Of our 46 patients, 47% are at present in continuing complete remission, 26% have already died. Transformation to high grade non-Hodgkin lymphomas was not observed in our patients. In one of our patients a diagnosis of low grade non-Hodgkin lymphoma/CLL was made before Hodgkin's disease was diagnosed. The question now arises whether a difference in the clinical behaviour concerning prognosis and survival exists between lymphocyte-predominant Hodgkin's disease and the other subtypes of Hodgkin's disease, or whether similarities with other B-cell neoplasia exist in this respect.

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PRIMARY EXTRANODAL NON-HODGKIN'S LYMPHOMAS (NHL) OF THE GASTROINTESTINAL TRACT (GIT)

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Between January 1978 and April 1993 64 patients (38 males, 26 females) with primary GIT - NHL (6%) out of a large group of 1093 NHL-patients were examined and treated at our Department. The median age was 54 years (range 19-81). According to the ANN ARBOR staging system 27 patients had stage I_E, 22 stage II_E, and 9 stage IV. Histology (KIEL-classification): High grade malignant NHL was detected in 40 cases and 24 patients showed a low grade malignant NHL (centroblastoma 13, immunoblastoma 16, lymphoblastoma 7, centroblastic-centrocytic lymphoma 6, immunocytoma 16 and others 6). 36 were primary gastric lymphoma, 17 lymphomas of the small intestine and 11 of the large intestine. B-symptoms have been found in 24 patients.
After surgical treatment and/or polychemotherapy and/or radiation 44 patients achieved a complete remission. The median follow-up time was 49 months (range 1-166). Long-term survival (KAPLAN-MEYER) for the whole group of patients with B-symptoms was 42%, without B-symptoms 56%. The histological subtype alone was not a significant factor for the survival rate. After radical surgical treatment the long-term survival was significantly higher (61%) than in the group without the possibility of radical surgical resection (28%).
In conclusion the most important factor for long-term survival are dissemination, clinical activity and the possibility of radical surgical treatment of the disease. Our investigation suggests a modification of current histological classifications to include a separate category for mucosa-associated lymphoma (MALT-lymphoma).

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POLYMERASE CHAIN REACTION (PCR) AS A MONITORING TOOL IN AUTOLOGOUS BONE MARROW TRANSPLANTATION FOR LOW-GRADE NON-HODGKN'S LYMPHOMA (NHL)

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Twenty-six patients with advanced low-grade non-Hodgkin's lymphoma were autografted after high-dose conditioning therapy with purged bone marrow. The immunomagnetic bead purging was performed with the following monoclonal antibodies: CD19, CD20, CD22, CD23 and CD37. There were 6 toxic deaths, while 8 relapses were observed post-transplantation. Twelve patients are alive in remission with a median follow-up of 28 months (range 15 - 59). Bone marrow of 14 patients was evaluable for the assessment of the t(14;18) translocation by PCR. Using a primer pair covering an internal fragment of the MBR gene, we first demonstrated that purified DNA could be amplified. Standard method for all samples was a nested primer assay. For this retrospective analysis frozen material was used and PCR had to be performed with as little as 100 ng DNA. After one amplification round, bone marrow samples of 7 patients (50%) were found to be positive for t(14;18) prior to purging. In one case, insufficient purging was reflected by a positive signal after one amplification round, while in 3 cases the PCR signal after purging was only visible after a nested primer assay. Three patients were purged to PCR negativity. Of these, one patient relapsed 22 months post-transplantation at the site of previous disease, while the remaining 2 PCR-negative patients are still alive in remission. Even more important, the patients transplanted with PCR-positive bone marrow are in continuous remission with a longest follow-up of 32 months. Follow-up examinations showed bone marrow and/or peripheral blood samples to be positive. On the other hand, in the 7 patients with PCR negative bone marrow prior to purging 1 toxic death and 4 relapses were observed, while 2 patients are in continuous CR (+27/+35 months). In summary, immunomagnetic bead purging allows removal of contaminating tumor cells from autografts. However, the predictive quality of persistingly positive t(14;18) signal for clinical outcome after transplantation remains open.

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HLA-DP MATCHING IN BONE MARROW TRANSPLANTATION

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Long lasting remissions can be achieved in leukemia as well as in severe aplastic anemia by allogenic bone marrow transplantation (BMT). One of the main problems after BMT remains severe acute graft versus host disease (GvHD) (> grade II) occurring in 30 % of the patients after BMT with marrow from HLA identical siblings and in up to 50 % after BMT with marrow from unrelated donors or not completely HLA-identical family donors. Besides intensification of the immunosuppressive therapy during BMT, improving HLA matching of donor and recipient might diminish the incidence of GvHD. In a retrospective study we examined HLA-DP matching of donor and recipient in allogeneic bone marrow transplantation. The HLA-DPA and HLA-DPB genotype of 91 patients (44 with CML, 6 with SAA, 38 with acute leukemia, 1 with myelodysplasia and 2 with lymphoma) and their bone marrow donors were determined by oligotyping. 62 donor and recipient pairs were HLA identical siblings, in 7 transplants the donor was a not completely HLA-A,-B,-DR matched relative and in 29 transplants an HLA identical unrelated person. In 10 of the 62 (16 %) HLA-A,-B,-C,-DR identical siblings, differences in HLA-DP genotype could be detected (once only HLA-DPA, 3 x HLA-DPB and 6 x in both chains). In the other pairs HLA-DP differences were detected in 4 out of 6 (66.7 %) when transplants with not completely HLA-A,-B,-DR matched relatives were done and in 20 out of 29 (68 %) when matched unrelated donors were used. Mixed lymphocyte cultures had been performed in all patients and it was found that a significantly higher proportion of HLA-DP-matched than mismatched pairs yielded a low GvH index but only at the 0.1 % level, a very small difference difficult to detect reliably. Preliminary results indicate that patients with completely matched family or even unrelated donors might suffer from less severe GvHD. Therefore it seems advisable to do HLA-DP genotyping especially since this difference could not be easily detected by mixed lymphocyte culture.

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FREQUENT CMV-INFECTION OF THE SKIN AFTER BMT ASSOCIATED WITH CLINICAL, HISTOLOGICAL AND IMMUNOHISTOLOGICAL ALTERATIONS DESCRIBED AS TYPICAL FOR ACUTE GVHD

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To evaluate the interaction of CMV infection and acute graft-versus-host-disease (GvHD) presence of CMV-DNA was evaluated in 92 skin biopsy samples derived from 56 patients following allogeneic BMT and correlated with the development of cutaneous GvHD. Additionally skin biopsies of 45 of the 56 patients were screened for local dermal CMV infection prior to transplantation. Sensitive virus detection by PCR-amplification was used and correlated to immunohistological and clinical alterations of the skin. Nine of 45 patients (20%) revealed presence of the virus in the skin already prior to BMT. During the first 30 days after BMT a rise of dermal infection was observed to 27 of the 56 (48%) patients analyzed. CMV infection of the skin after BMT was exclusively observed in patients with clinically diagnosed severe acute GvHD (grade II-IV). Cutaneous GvHD was confirmed in these patients by typical histological and immunohistological alterations of the skin biopsies. PCR analysis of sequential skin biopsies and of simultanously obtained blood samples revealed that CMV infection was primarly loca-lized to the skin in 16 of 27 investigated patients shortly after BMT, whereas viremia was only diagnosed subsequently. In addition immunohistological staining in correlation to PCR-analysis of the sequential skin biopsies in 4 patients with clinical signs of acute GvHD after BMT revealed presence of CMV before the development of abnormal expression of HLA-class II-antigens on keratinocytes and of T-cell infiltrates representing established immunohistological criteria of dermal GvHD. Thus, local CMV infection may participate in evoking cutaneous lesions not only by augmenting, but also by inducing clinical signs of acute GvHD.

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EVALUATION OF FERTILITY OF PATIENTS WITH HEMOBLASTOSIS

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Between 1978 and 1992 1619 patients with the diagnosis of hemoblastosis were treated with chemotherapy. They were analyzed whether there are possebilities of having a normal parenthood. Out of them 217 female and 286 male patients were in an age of fertility (15-45 years). Altogether 19 women did become pregnant, some of them multiple, resulting in 15 sucessful deliveries and 8 induced abortions. Hodgkin's disease was diagnosed in 13 cases, acute leucaemia in 2 cases and CML in 1 case. The pregnancies were first detected as follows: 7 prior to the introduction of chemotherapy, 3 during and 13 after completion of chemotherapy. No pathological outcome of a pregnancy was found in patients who did not have any chemotherapy prior to there delivery. Patients who underwent chemotherapy during the pregnancy had 3 abortions and 4 succesful deliveries (2 pre term and 2 full term). Pregnancy after completed chemotherapy resulted in 7 full term deliveries, 4 induced abortions and 2 pathological outcomes (1 pre term twins, 1 cerebral palsy). 5 male patients fathered 6 children, 1 during chemotherapy and 5 after completed chemotherapy. In conclusion the application of antineoplastic chemotherapy in the first third of pregnancy contains the risk of teratogenicity and abortion. During polychemotherapy in late pregnancy there is not an increased risk of malformed off springs, low birth weight or pre term deliveries are possible. After complete chemotherapy of a hemoblastosis there is in case of pregnancy the probability of a normale outcome as high as in the normal population.

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DNA-repair, MDR expression and chemosensitivity profiles in haematological malignancies

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In order to facilitate the design of improved chemotherapy regimens for leukaemias on an individual patient basis, we related the expression of known mechanisms of drug resistance to chemosensitivity profiles of isolated leukaemic cells. A monoclonal antibody-based immunofluorescence assay combined with image analysis of amplified fluorescence signals was applied to evaluate DNA-repair on a single-cell level. The kinetics of elimination of the alkylation product O^6-ethylguanine from nuclear DNA were determined in leukaemic cells after pulse-exposure to N-ethyl-N-nitrosourea (EtNU) *in vitro*. The time required for repair of 50% of O^6-ethylguanine residues in nuclear DNA of single AML blasts varied by a factor of five (median 2.08 h, range 0.75 - 5.64 h; n=22). A wide range of interindividual DNA repair capacity was also observed in CLL (median 1.50 h, range 0.75 - 3.1 h; n=15) and normal lymphocytes (median 6.46 h, range 1.5 - 8.27 h; n=10). Repair time and *in vitro* resistance to mafosfamide, as determined by the MTT assay, were inversely correlated ($r = -0.84$, $p<0.001$; n=22), whereas no relationship was found to *in vitro* chemosensitivity to multidrug resistance (MDR) related drugs. P-glycoprotein (PGP) expression in leukaemic blasts was evaluated by a semiquantitative flow-cytometric procedure. Subpopulations of leukaemic blasts expressing PGP were detected in 39 out of 60 samples. In relation to clinical status, the median fraction of PGP-positive blasts was elevated 3.5-fold in relapsed AML patients (n=28) in comparison to patients at first presentation (n=29). In newly diagnosed patients, the median fraction of PGP expressing blasts was 4-fold higher in specimens from patients who failed to reach complete remission (n=11) in comparison to responsive patients (n=18). No obvious relationship was observed between PGP expression and DNA repair capacity. It is hoped that these studies will provide information leading to individualised chemotherapy based on profiles of drug resistance.

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DETECTION OF ANEUPLOIDY OF CHROMOSOME 17 IN BONE MARROW MICROMETASTATIC TUMOUR CELLS BY FLUORESCENCE IN SITU HYBRIDIZATION

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Detection of epithelial cells in the bone marrow of patients with histologically proven cancer has been shown to be of prognostic significance. To further characterize these cells we developed a method combining fluorescence antibody labeling with an anti-cytokeratin antibody as primary antibody and fluorescence in situ hybridization (FISH). To detect numerical chromosomal aberrations, we used an alpha satellite, chromosome specific DNA-probe for chromosome 17. Hybridization on actually diploid cells showed two signals in 88.1%, one signal in 8.5%, no signal in 2.2% and three signals in 1.2%. Previous experiments have shown that antibody labeling does not interfere with FISH. Twelve patients with breast cancer whose bone marrow has been shown by APAAP staining method to contain epithelial cells were screened by fluorescent antibody labeling. Ten of them were positive by these method and FISH was performed on the fluorescent labeled cells. Four patients showed only individual cells, three only aggregates of two and more cells and the remaining three patients individual cells as well as aggregates. Of the individual epithelial cells detected, only one of 62 cells showed five hybridization signals, whereas seven showed two signals, 51 one signal and three no signal. On the contrary were more than two hybridization signals visible in 103 of 135 cells, which formed aggregates of two or more cells. In these aggregates 20 cells showed two signals, eight one signal and four no signal. These preliminary data may indicate that during progression from individual cells to aggregates of tumor cells in bone marrow there is a trend from hypo- and euploidy to polyploidy of chromosome 17.

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SOFTWARE FOR PROTOCOL CONFIRMING IMPLEMENTATION OF THERAPY STUDIES IN PEDIATRIC ONCOLOGY

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In pediatric oncology about 70% of the malignancies are treated in therapy studies. These studies often use complex dosage schemes and sequences of the different therapy elements. They also include diverse strategies for different risk groups as well as randomized assigning to new therapy concepts. Exact performing of therapy instructions is necessary to fulfil the aims of the study. TheMPO (Therapy support and Management in Pediatric Oncology) is a computer program that supports all actions during a clinical therapy study in oncology:

1. Definition of the abstract therapy plans on the basis of the study protocols including dosage guidlines, chronological order of the therapy elements, as well as dosage modifications during the therapy course.
2. Management of all patients of different therapy studies in a database.
3. Assigning patients to risk groups depending on actual diagnostic findings at distinctive points of time. Requesting for all diagnostic procedures that are necessary to determine the risk group.
4. Randomized assigning of patients to different therapy concepts.
5. Calculating individual therapy prescriptions depending on stratum, randomisation, and actual situation (e.g. body weight, body surface) of each patient. Printing an individual prescription plan that includes the composition of all therapy elements (e.g. infusions) as well as a daily schedule for the nurses.
6. Documentation of all prescriptions, dosage modifications, and actually administered therapy elements.
7. Printing all documents which have to be sent to the principle investigator.

As an example the protocol of the ALL/BFM 90 study has been realized in TheMPO program.

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SURFACE EXPRESSION OF THE 72 kD HEAT SHOCK PROTEIN (HSP72) ON HUMAN MALIGNANT CELLS AFTER HEAT TREATMENT

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In an ongoing clinical phase II study (RHT-91 study), patients suffering from high-risk sarcomas are treated with a combination therapy consisting of regional hyperthermia and systemic chemotherapy. To study immunological aspects, we established an in vitro model to analyse the effects of heat exposure (41.8°C) on human Ewing's sarcoma (ES) cells. Using a HSP72 specific moAb (an indirect immunofluorescence assay), surface staining on heat treated ES cells could be detected. Pre-heat incubation of sarcoma cells with an inhibitor of protein synthesis (cycloheximide) blocks the cell surface expression of HSP72. By immunoprecipitation of membrane fraction of heat treated ES cells a single band of 72 kD was obtained after SDS-gelelectrophoresis. Using Western Blot analysis this 72 kD protein was recognized by HSP72 moAb. In contrast to malignant cells, PBL or fibroblasts derived from healthy individuals did not show HSP72 surface expression after heat shock.
Using a Cell Mediated Lympholysis assay (CML), we could demonstrate that the antigenicity of heat treated ES cells was much stronger compared to untreated ES cells. By the use of HSP72 moAb for CML blocking experiments the lysis of heat treated ES cells was inhibited, whereas MHC class I (W6/32) or MHC class II (L243) specific moAbs had no influence on the lysis pattern. CD3 negative and CD56 positive NK-like effector cells can recognize an antigenic HSP72 epitope on the cell surface of heat treated ES cells. These effector cells also show strong lysis for the NK target K562 cells, whereas the lysis of untreated ES cells and allogeneic EBV transformed B-LCL was low. Our results strongly suggest a role for the heat inducible HSP72 acting as an antigenic determinant on malignant cells after heat treatment. Supported by grant M90/91/is1 from the Deutsche Krebshilfe, Bonn

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BONE MARROW INFILTRATION IN MULTIPLE MYELOMA: CORRELATIONS OF QUANTITATIVE MRI WITH HISTOLOGY AND CLINICAL PARAMETERS

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Multiple myeloma is a neoplasm of plasma cells involving numerous skeletal sites. Using conventional radiographs, early or diffuse lesions are difficult to recognize. Magnetic resonance imaging (MRI) has enhanced the detection of osseous lesions in multiple myeloma. In this study, we examined 58 patients with biopsy-proved multiple myeloma in quantitative MRI +/- Gd-DTPA. The increase in signal intensity was compared with a group of patients without hematologic disorders. Gradients were calculated between vertebral marrow and disc and correlated with the degree of infiltration in bone marrow histology and clinical stage (Durie and Salmon). In 43 patients all gradients could be calculated. Based on these data we propose 4 separate types of involvement in multiple myeloma:
1) Diffuse infiltration (12 cases)
2) Diffuse infiltration with localized nodules (22 cases)
3) Localized nodular infiltration (6 cases)
4) Patchy involvement (pepper and salt) (8 cases)
Cases with minimal involvement proved histologically could not be reliably detected by MRI (2/3 false negative cases). We will follow our patients prospectively and try to establish prognostic correlations of the 4 types of multiple myeloma described here. Preliminary data show that the patchy subtype correlates with early suppression of hematopoiesis and occurs in the context of aggressive myeloma.

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BONE MARROW INVOLVEMENT IN HODGKIN`S DISEASE: AN ANALYSIS OF 135 CONSECUTIVE CASES *

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Among 2307 patients with Hodgkin`s disease (HD) treated according to the protocols HD1-3 and HD4-6 of the GHSG, 135 cases of primary bone marrow involvement (BMI) were observed between 1982 and 1991. The incidence of BMI was 4.8% if the HD4-6 study generation which includes all stages of HD was analysed. 31% of all stage IV patients had BMI. In 32.6 % of the BMI cases other organs (liver, bone, lung) were involved too. Compared with all non-BMI cases, a positive BM biopsy was significantly associated with B-symptoms, lymph nodes on both sides of the diaphragm, an unfavorable histological subtype (MC,LD), leukocytopenia, anemia, thrombocytopenia, LDH> 400 and ESR> 40. BMI was negatively correlated with the presence of a large mediastinal tumor (4% only as compared to 20% in non-BMI cases).
Patients were treated with either 3x (COPP/ABVD)± RT, 4x (COPP/ABVD)± RT or 4x (COPP/ABV/IMEP)± RT. 87 of 108 evaluable patients reached CR. This CR-rate of 80.6% compares favorably with the overall CR-rate of 78% in all stage IIIB/IV patients. Among all stage IV patients , BMI has no prognostic relevance with regard to Freedom From Treatment Failure, Relapse Free Survival, and Overall Survival.
21 patients with BMI relapsed after CR. Only 5 of these (24%) had again a positive bone marrow biopsy. Our results show that the prognosis for patients with BMI is not different from other advanced stage HD patients. In particular BMI does not define a special high risk group to be treated differently.

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* Supported by BMFT

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EXPRESSION OF TGF-BETA IN BENIGN AND MALIGNANT EFFUSIONS

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Transforming growth factor-beta (TGF-b) is a multifunctional polypeptide involved in the regulation of cellular growth and immune recognition. Among normal human tissues, bone, endothelial cells and platelets are major sources of TGF-b. Several tumor types like breast cancer, hepatocellular cancer and Hodgkin`s lymphoma were described to express isoforms of TGF-b. In order to delineate the expression of TGF-b, normal and malignant cells were isolated from ascites and pleural effusions. TGF-b was stained by indirect immunocytochemistry with a moAB directed against TGF-b. Acetone fixation without further treatment gave optimal specific results. We examined 5 benign effusions with only reactive cells and 21 samples from 18 patients containing variable numbers of tumor cells. Reactive leukocytes and mesothelial cells were negative or occasionally faintly positive (mesothelial cells, macrophages). In 7/11 cases with breast cancer, the morphologically identified tumor cells in ascites or pleural fluid reacted strongly with the moAB against TGF-b3, whereas reactive cells were negative. In other effusions containing tumor cells, 4/7 samples were positive for TGF-b (1 lung cancer, 3 gastric carcinomas, reaction fainter than in cases of breast cancer). We conclude from these preliminary results that TGF-b expression is common in tumor cells isolated from malignant effusions. It is tempting to speculate that the immunosuppressive properties of TGF-b enhance tumor progression.Further work will try to correlate TGF-b expression with soluble TGF-b and CEA positivity.

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AXL, a novel receptor tyrosine kinase, is expressed in myeloid leukemias.
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AXL has been isolated by means of gene transfection from cells of a patient with chronic myelogeneous leukemia in blast crisis (MCB 11:5016). The same gene was independently cloned by others and designated *UFO* (Oncogene 6:2113). Since its mode of activation is overexpression rather than point mutation, we sought to address the expression pattern of *AXL* in human leukemias. Blood / bone marrow from 114 patients suffering from different human preleukemias and leukemias was investigated for expression of *AXL* using a sensitive RT-PCR assay. *AXL* was expressed mainly in myeloid leukemias (39/67 cases), whereas lymphatic leukemias were preferentially *AXL* negative (1/40 cases *AXL* positive). Since leukemias display molecular features of disrupted differentiation, we analysed the role *AXL* may play in normal hematopoietic differentiation. Normal bone marrow (N=3) was analysed and *AXL* was expressed in every case; in contrast, normal peripheral blood cells did not show *AXL* message (N=11). We therefore asked if *AXL* may be expressed in normal CD34 positive progenitor cells. CD34 positive cells were analysed and found to express *AXL*. To further elucidate *AXL*'s role in hematopoietic differentiation, K562 cells were induced with TPA and showed a strong induction at the transcriptional level. Furthermore, *AXL* is expressed in mature peripheral monocytes treated with interferon-α. Thus, *AXL* is expressed in myeloid leukemias and may play a role in hematopoietic differentiation. Its role in malignant myeloid transformation remains to be determined.

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Maintenance therapy in acute myeloid leukemia (AML): Comparison of immunological and molecular markers when using interleukin-2 (IL2) alone or in combination with τ-interferon (IFN).
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Since interleukin-2 (IL2) has been shown to induce lysis of autologous AML blasts, maintenance therapy with IL2 could be of value in AML. However, IL2 normally is given at high doses, and side effects commonly occur. Low-dose regimens have been described (Lancet I:1590,1990). Cytokines are capable of inducing other biological active mediators, and it is not known whether the *in-vivo* effects of low-dose IL2 can be augmented by the addition of e.g.τ-IFN. We thus studied the biological effects of low-dose IL2 alone or in combination with τ-interferon in the maintenance phase. AML patients were first treated using idarubicin and ara-C (Blood 77:1666). 26 patients (24 *de novo*, 2 relapses) were enrolled in a prospective manner between November 1991 and January 1993. Median age was 52.5y (range 21-81). 14 (54%) patients entered CR, 9 (64%) of these after the first cycle. As maintenance treatment 4-week cycles of either low-dose IL2 alone or IL2 in combination with τ-IFN were alternated. Patients were randomized to start with IL2 cycles, or with IL2 + τ-IFN. After each cycle, patients were crossed over to the other arm. By this method, 23 immunological and 9 molecular markers of 11 cycles with IL2 alone and of 12 cycles of IL2 + τ-IFN could be compared. No side effects were observed. Immunological analysis using two-color flow cytometry showed activation of T-cells in single patients. Polymerase chain reaction using primers specific for various human cytokines and the respective receptors (IL2; IL2 receptor; IL4; IL6; τIFN; GM-CSF) revealed no clear cut correlation with treatment. In conclusion, induction of AML can be safely performed using ida and ara-C with CR rates comparable to own historical controls using daunorubicin. Maintenance with low-dose IL2 also seems to be safe and is well tolerated; however, no clear-cut difference when giving τ-IFN in addition can be demonstrated. Since most of the patients relapsed, other regimens such as high dose ara-C as consolidation and dose escalation of IL2 in the maintenance will be tested.

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THE SIGNAL TRANSDUCTION OF INTERLEUKIN-6 (IL-6) INVOLVES THE TYROSINE PHOSPHORYLATION OF AT LEAST FIVE CYTOSOLIC PHOSPHOPROTEINS.
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Binding of IL-6 to its receptor (IL-6R) induces the association of the IL-6R with gp130, a 130-kDa transmembrane glycoprotein which subsequently transduces a signal to the cytosol. The cytosolic signaling events following the activation of the IL-6R/gp130 complex are poorly understood. Because IL-6 has been proposed as an important para- or autocrine growth factor in plasmocytoma, the biochemical mechanisms of IL-6R mediated signaling may be relevant for the understanding and treatment of this disease. We therefore used the IL-6 dependent, human plasmocytoma cell line, B9, to characterize the biochemical mechanisms of IL-6 dependent proliferation. B9 cells were IL-6 deprived for 18 hrs and then stimulated for various times with 100 ng/ml recombinant human IL-6. Cells were lysed and the tyrosine phosphorylation of cytosolic proteins was assessed by SDS-PAGE and immunoblotting using an anti-phosphotyrosine antibody. IL-6 induced a rapid and transient tyrosine phosphorylation of at least five cytosolic phosphoproteins. Major proteins which were consistently and strongly phosphorylated upon stimulation with IL-6, had molecular weights (m.w.) of 80, 160, and 170 kDa (pp80, pp160, pp170), respectively. Minor phosphoproteins had m.w. of 93 and 140 kDa (pp93, pp140). Some of the phosphoproteins which were phosphorylated in response to IL-6 in the B9 cell line, were likely to be constitutively activated in the factor *in*dependent plasmocytoma cell lines, OPM-2 and U266, because the bands of pp93 and pp42/44 comigrated on SDS-PAGE. We are currently investigating the exact identity of the signal transducing phosphoproteins which may be of particular interest due to the nature of IL-6 as a plasmocytoma growth factor.

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IN VITRO RELEASE OF CYTOKINES IN WHOLE BLOOD SAMPLES FROM TUMOR PATIENTS UNDER CHEMOTHERAPY
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Whole blood samples from 51 patients with various malignant diseases were assessed for their ability to release Tumor necrosis factor-α (TNF-α, Interleukin-1-α (IL-1-α), IL-1-β, IL-2 and Interferon-γ (IFN-γ) in vitro. Serum concentrations and values after stimulation were determined. 50 µl of whole blood was stimulated with 7.5 µg/ml PHA and incubated in 5% CO_2 at 37°C for one day (TNF-α) respectively 4 days for all other cytokines. Concentrations were determined with a modified immune enzyme assay. Prior to chemotherapy compared to a group of healthy controls (n=58) IFN-γ concentrations were significantly lower ($p<0,05$). After a 4 months interval 10 patients who were resistant to chemotherapy had died. The remaining 41 patients proved to have had significantly ($p<0,05$) higher values of IFN-γ (31 ng/ml) prior to therapy compared to the patients who had died (8,5 ng/ml). IL-1-α, IL-1-β, IL-2 and TNF-α levels did not show significant differences after PHA stimulation. Serum concentrations of TNF-α however were significantly higher ($p<0,01$) in the patients with poor outcome (164 pg/ml) than in the patients still being alive after 4 months of chemotherapy (95pg/ml).

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MULTIMODAL APPROACH TO NON-SMALL CELL LUNG CANCER

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Non-small cell lung cancer (NSCLC) is one of the leading causes of cancer death around the world. While in clinical stage I and II patients (pts) with clearly operable disease are included, pts with distant metastatic spread (stage IV) are candidates for palliative treatment. In the majority of pts with locally advanced stage III A and B disease, however, there is still a controversy about optimal management. Several randomized trials have shown a better local and distant tumor control for combination chemotherapy followed by definitive radiation over radiotherapy alone resulting in a significant advantage regarding median and long-term survival. Moreover, some studies have suggested that concurrent Radio-/Chemotherapy can produce better results with respect to local tumor control, however, a significant elevation of the plateau phase of the survival curve in stage III NSCLC has still to be proven. During the last few years, therefore, the combination of preoperative chemotherapy plus/minus radiotherapy followed by surgery with curative intent has gained increasing interest. Preliminary data suggest that this multidisciplinary approach is feasible with tolerable side effects. Moreover, remission rates are improved pointing to more favourable median and long-term survival rates especially in selected pts population.

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INCIDENCE OF THE t(14;18) TRANSLOCATION IN MALIGNANT LYMPHOMAS

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The t(14;18) translocation fuses the bcl-2 gene located on chromosome 18 with a sequence on chromosome 14 coding for the immunoglobulin heavy chains. This translocation has been demonstrated in low-grade follicular and high-grade large cell Non Hodgkin lymphomas; its presence in Hodgkin's lymphomas is the object of controversal discussion. We investigated fresh frozen tissue of 61 lymphomas including 19 Hodgkin's lymphomas and a control group of 25 non malignant lymph nodes with the polymerase chain reaction for the presence of the t(14;18) translocation. The translocation was found in 4 follicular and 2 high-grade Non-Hodgkin lymphomas, but in none of the Hodgkin's lymphomas. 2 cases of chronic tonsillitis also had a rearrangement of bcl-2/JH. 5 of the positive cases were of B-cell origin, but also one case of lymphogranulomatosis X (T-cell lymphoma) was positive. The t(14;18) was detected not only in DNA of fresh frozen samples, but also after formalin fixation and paraffin embedding in 4 out of the 6 cases mentioned. By diluting t(14;18) positive DNA with -negative DNA we were able to demonstrate one positive among 10.000 negative cells, which is sensitive enough to detect a possible rearrangement in Reed-Sternberg cells of Hodgkin's lymphomas. These results, seen on the background of the current literature, do not provide evidence that the bcl2-/JH gene rearrangement plays a role in the pathogenesis of Hodgkin's lymphomas.

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EX VIVO ELIMINATION OF CHRONIC MYELOID LEUKEMIA (CML) CELLS FOLLOWING ACTIVATION AND TARGETING OF HOST T CELLS BY COMBINATION OF CYTOKINES AND CD3 MONOCLONAL ANTIBODIES.

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We have previously shown that acute myeloid leukemia cells can be eliminated by targeting autologous cytotoxic T cells with CD3 monoclonal antibodies (MAB) to the Interferon-inducible high affinity Fc receptor for IgG (Fcγ RI; CD64) expressed on malignant blast cells (Blood 78 No 10(1), 173, 1991). In this study, 10^7 peripheral blood mononuclear cells obtained in blast crisis or in the accelerated phase of 2 patients with Ph^1-positive CML and 1 patient with Ph^1-negative CML (WBC counts: 350 - 580 x $10^3/\mu l$) with constitutive expression of CD64 on 11 - 52% of leukemic blasts were exposed in vitro to permutated combinations of Interleukin-2 (IL-2), Interferon (IFN)-γ or -α, and CD3-MAB OKT3 under non-limiting culture conditions. Control cultures without additives contained >90 % $CD33^+$ tumor cells and < 2% $CD2^+$ T lymphocytes on day 0 and day 8, and cell number remained unchanged (10^7 vs. 11$\pm$1 x 10^6). In contrast, in day 8 cultures containing OKT3, IL-2, and IFN-γ, the total cell number was reduced (5$\pm$3,1 x 10^6), and activated T lymphocytes had completely replaced CML cells (CD2: >92%; CD25: 80-85%; CD33:<1%). These T cells efficiently killed autologous CML cells (31$\pm$4 % specific ^{51}chromium-release; effector target ratio 10:1). In Ph^1-positive CML cultures treated with IL-2, IFN-γ, and OKT3 for 34 days no 305 bp band indicative of the translocation t(9;22) was detected by nested polymerase chain reaction (PCR), whereas all other control cultures remained positive. OKT3-coated activated T cells of a healthy donor did not affect the number of hematopoietic colonies derived from autologous peripheral blood mononuclear cells, whereas they completely eliminated leukemic U937 colonies. This is consistent with absent CD64 expression on normal $CD34^+$ cells isolated from peripheral blood by immuno-magnetic cell sorting. In conclusion, activation of host T cells by IL-2, CD3 MAB, and IFN-γ is an effective ex vivo purging regimen to eliminate residual CML tumor cells as detected by immunofluorescence and PCR analysis. It has the dual advantage of no toxicity for non-malignant $CD34^+$ progenitor cells and of providing cytotoxic effector T cells potentially effective against residual CML cells in vivo. (Supported by Deutsche Krebshilfe W10/90 No1).

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INTENSIFIED SEQUENTIAL COMBINATION CHEMOTHERAPY (CEBOPP/VIML), G-CSF AND RADIOTHERAPY IN PATIENTS WITH HIGH GRADE MALIGNANT NON-HODGKIN'S LYMPHOMA (NHL)

MR Nowrousian, B Mengelkoch, R Kleine-Herzbruch, R Bauer, W Reiter, W Eberhardt, C Kasper, M Müller, L Schlenger, W Budach, LD Leder, H Sack, S Seeber

In patients (pts) with aggressive NHL, the outcome of chemotherapy (CT) appears to be related to the dose intensitiy of drugs. In the present study, an intensified sequential combination CT was used, and G-CSF (5 ug/d, days 11.20) was given additionally when severe and/or prolonged neutropenia, and/or infectious complication occured. In pts with stage I disease, and in pts with primarily bulky disease, additional radiotherapy (40 Gy) was given to the involved field after completion of CT. CT was started with a combination of Cyclophosphamide (400mg/m2/d, days 3,4), Epirubicin (40 mg/m2/d, days 1,2), Bleomycin (30 mg/d, days 1,10), Vincristin (2 mg/d, days 1,10), Prednison (100 mg/m2/d, days 1-10), and Procarbazine (60 mg/m2/d, days 1-10) (CEBOPP). Treatment was repeated every 3 wks. In pts with complete response (CR) after a maximum of 4 cycles of CEBOPP, this regimen was continued for a total of 6 cycles. In pts with progressive disease or with only a partial response, therapy was switched to a combination of VP-16 (130 mg/m2/d, days 1,3,5), Ifosfamide (1300 mg/m2/d + Mesna, days 1-5), Methotrexate (70 mg/m2/d, days 1,5), and Leucovorin (15 mg, 24, 30, and 36 h after each dose of Methotrexate) (VIML). In pts with Epirubicin contraindication, CT was started with VIML together with Vincristin (2 mg/d, days 1.10) and Prednison (100 mg/m2/d, days 1-10) (VIMLOP). Since 11/90, a total number of 50 pts (25 females, 25 males) were treated. The median age was 51 yrs (range 20-87). 17 pts had stage I, 15 stage II, 10 pts stage III, and 8 pts stage IV disease. B-symptoms were present in 16 pts, bulky disease (> 10 cm) in 19 pts, and extranodal involvement in 22 pts. Histologic subtypes of the lymphomas (Kiel classification) were: centroblastic 38, immunoblastic 2, lymphoblastic 1, and undifferentiated large cell 9.
Major toxicities (WHO grade III+IV) of therapy other than total alopecia were leukocytopenia in 45%, thrombocytopenia in 5%, and anemia in 3% of CT cycles. Infection occured in 50%, and peripheral neuropathy in 36% of pts. There was a toxic death rate of 4%. 89% of pts achieved CR. The CR rate was 100% in pts with stage I, and 85% in pts with stage II-IV disease. With a median follow-up of 15 months, the projected survival for the whole group of pts is 85% at 37 months, and 90% of pts with CR are predicted to be disease-free at 33 months. The probability of disease-free survival is 100% in pts with stage I, and 85% in pts with stage II-IV disease.
In conclusion, the therapeutic concept used appears to be highly effective in inducing CR. It also appears to be promising with regard to the long-term disease-free survival when the low rate of relapses during the first 2-3 yrs is considered.

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INTENSIFIED SEQUENTIAL COMBINATION CHEMOTHERAPY (CEBOPP/VIML), G-CSF AND RADIOTHERAPY IN ADVANCED-STAGE OR RISK-STAGE HODGKIN'S DISEASE

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To improve the results in patients (pts) with advanced-stage (IIIA2, IV) or risk-stage (II-IIIA1 with B-symptoms, high ESR, bulky tumor, extranodal site, involvement of spleen or more than 3 lymph node areas) Hodgkin's disease, an intensified sequential combination chemotherapy (CT) was used. G-CSF (5 ug/d, days 11-20) was given additionally when severe and/or prolonged neutropenia, and/or infectious complication appeared. CT was started with a combination of Cyclophosphamide (400mg/m2/d, days 3,4), Epirubicin (40 mg/m2/d, days 1,2), Bleomycin (30 mg/d, days 1,10), Vincristin (2 mg/d, days 1,10), Prednison (100 mg/m2/d, days 1-10), and Procarbazine (60 mg/m2/d, days 1-10) (CEBOPP). Treatment was repeated every 3 wks. In pts without residual tumor after a maximum of 4 cycles of CEBOPP, this regimen was continued for a total of 6 cycles. In pts with progressive disease or residual tumor, therapy was switched to a combination of VP-16 (130 mg/m2/d, days 1,3,5), Ifosfamide (1300 mg/m2/d + Mesna, days 1-5), Methotrexate (70 mg/m2/d, days 1,5), and Leucovorin (15 mg, 24, 30, and 36 hrs after each dose of Methotrexate) (VIML). In stage II-IIIA disease, an additional reduced radiotherapy (30 Gy) was given to the EF or IF dependent on number of involved sites (less than 3, 3 or more) when no residual tumor was present after completion of CT. In case of residual tumor, a higher irradiation dose (40 Gy) was given to residuum. Since 11/90, a total number of 28 pts (19 males, 9 females) with a median age of 30 yrs (range 18-67) were treated. 13 pts had stage II, 12 pts stage III, and 3 pts stage IV disease. B-symptoms were present in 12 pts, bulky disease (> 5 cm, mediastinal mass > 1/3 of chest diameter) in 20 pts, and extranodal involvement in 9 pts.
Alopecia, leukocytopenia, and peripheral neuropathy were the most frequent toxicities of therapy. Severe Leukocytopenia (WHO grade III+IV) occured in 26% of CT cycles, and 36% of pts developed infection. However, no therapy related death was observed. An overall response rate of 100% was achieved. The rate of remissions with no residual tumor or residual tumor of 2 cm or less was 89%, and the rate of remissions with residual tumor larger than 2 cm 11%. Residual tumors were mainly seen in pts with primarily bulky mediastinal disease. With a median follow-up of 17 months, the projected survival is 92% at 28 months, and 84% of pts with or without residual tumor are predicted to be in continued remission at 25 months.
The therapeutic concept used appears to be highly effective in inducing remission in advanced-stage or risk-stage Hodgkin's disease. For final conclusion, however, a longer period of observation is needed.

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PHASE–I STUDY OF I.V. DEXNIGULDIPINE–HCL (dex) PLUS VINBLASTINE (vbl).

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A bicentric phase–I study with the new drug resistance modifier dex in combination with vbl was performed. Patients with advanced cancer received dex as a 4–hour infusion for 4 days + 0.15 mg/kg vbl at day 3. The objective was to determine the maximal tolerated dose (MTD) of dex alone and in combination with vbl as well as to assess the serum levels of dex. A total of 40 courses was administered to 15 patients. Starting dose was 1 mg/kg/d dex, maximal injected dose was 11 mg/kg/d. Up to 7 mg/kg/d dex was tolerated by all patients without significant side effects. Out of 4 patients who received the next higher dosage of 9 mg/kg/d, in 2 patients the infusion was stopped because of a clinically relevant decrease in blood pressure. Thus 7 mg/kg/d dex i.v. as a 4 hour infusion is the recommended dose for phase–II studies. Maximal serum levels of dex at 5 mg/kg/d were 2,000 ng/ml (approx. 3 µMol). Only few adverse events caused by dex had been assessed: orthostatic dysregulation, peripheral thrombophlebitis (if infused in a peripheral vein), paresthesias at the fingertips and perioral, and locomotoric ataxia. No enhancement of the vbl toxicity caused by dex was observed. One patient with peritoneal mesothelioma achieved a partial response after 4 courses.

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MOBILIZATION OF CIRCULATING HEMOPOIETIC PROGENITOR CELLS WITH G-CSF AFTER CHEMOTHERAPY IN PATIENTS WITH MULTIPLE MYELOMA

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Due to the relatively low contamination of tumor cells in peripheral blood in patients (pts) with multiple myeloma, autologous transplantation of circulating stem cells may have theoretical advantages over autologous bone marrow transplantation. In four pts with multiple myeloma, who were considered as potential candidates for autologous stem cell transplantation, G-CSF (600 µg/die) was administered following chemotherapy in order to maximally increase the number of circulating progenitor cells during hemopoietic rebound and to facilitate progenitor cell harvest by leukapheresis. In two untreated pts, G-CSF (600 mcg/d) following chemotherapy according to the UVA protocol (Ultralan, Onkovin, Adriamycin) increased circulating CFU-GM from 247 to 7.552 in pt 1 and from 173 to 6.361 CFU-GM/ml in pt 2, respectively, which was much more effective than the increase of progenitor cells after chemotherapy alone (in pt 1 to 594 and pt 2 to 317 CFU-GM/ml). In two pts. having received multiple cycles of chemotherapy already, the combination of UVA and G-CSF was much less effective leading to progenitor cell increments from 144 to 735 CFU-GM/ml in pt 3 and from 222 to 232 CFU-GM/ml in pt 4, respectively. In both cases, however, mobilization of hemopoietic progenitor cells by G-CSF (600 mcg/d) following cyclophosphamide (4 and 5 g, respectively) was effective leading to CFU-GM peak values of 5.324 in pt 3 and 2.245 in pt 4, respectively, thus allowing harvest of mononuclear cell and $CD34^+$ cell numbers, sufficient to allow prompt and complete reconstitution of hemopoiesis in case of transplantation. The combination of UVA and G-CSF is an effective strategy to mobilize hemopoietic progenitor cells in untreated pts with multiple myeloma but seems to be uneffective in pts, who have received chemotherapy already. Due to its higher efficiency, G-CSF after cyclophosphamide should be prefered in such pts.

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IMMUNOTYPING OF BLASTS IN HUMAN BONE MARROW.

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Immunocytochemical investigation of human bone marrow is complicated by the presence of multiple cell lineages with many maturational stages. We developed a simple method for immunotyping of morphological identified blasts cells. After May-Grünwald-Giemsa staining of the bone marrow aspirates the cells were photographed, destained and investigated by an indirect immunoperoxidase technique. We identified 0.5 ± 0.2% blasts (according to blast I of the FAB classification) in human bone marrow aspirates from seven haematological normal volunteers. The immunotype was $CD34^+$ $HLA\text{-}DR^+$ $c\text{-}kit^-$. These cells showed a diffuse and a coarsely positive reaction with CD34. Strong expression of CD34 was also found in most megakaryocytes. Monocytes showed a low expression of CD34. 45 ± 7% of blasts I were CD36 positive and 11 ± 4% showed positivity with CD13.
We found 0.6 ± 0.3% blast-like cells. The nucleus was more irregular and the chromatin pattern coarserthan that of blast I. 73% of these cells showed a diffuse positive reaction with CD34. Most of these cells had a low expression of c-kit.
The proportion of $CD19^+$ lymphoblasts and $CD61^+$ megakaryoblasts was lower than 0.05% of the bone marrow cells. We did not find CD3 positive blasts. The percentage of c-kit positive lymphocytes was 0.02 ± 0.01%.
Our method allowed to immunotype the blasts and other cell types in human bone marrow aspirates. It was possible to establish morphological-immunocytological correlations.

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THERAPEUTIC FAILURE AND ANALYSES OF DRUG RESISTANCE
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In contrast to end stage cardiac, hepatic, renal, or pulmonary disease therapeutic failure has become the hallmark of advanced cancer with social, legal, psychological and scientific implications. Reduced host compliance may lead to delays in diagnosis or treatment and subsequent therapeutic failure. Equating treatments of unproven value with rational approaches on grounds of negative outcomes and other misconceptions about antineoplastic chemotherapy may contribute to such problems. The legal consequences of therapeutic failure and the costs incurred to medical care providers per year of life saved or lost are just beginning to undergo scrutiny.
The principles of antineoplastic chemotherapy are quite apart from general pharmacology with toxic side effects being the rule rather than exception. Reduction of unwanted side effects remains a major guiding principle of antineoplastic drug development and improved assessment of host tolerance should help to prevent irreversible toxicity. Choice of drugs, dose intensity, and timing of treatment are the major variables of treatment decisions. Subclassification of disease by morphological, immunological, cytogenetic, and genomic means should allow for risk adapted treatment. Information on adhesion molecules governing metastasis should aid in the diagnostic surveillance of anatomic failure patterns and use of preventive strategies in priviledged sites.
Many antineoplastic treatment strategies rely on dose intensification and broad coverage by complex patterns of combination chemotherapy similar to antimicrobial therapy rather than exploit individual profiles of drug resistance. A widening range of predictive tests for drug resistance is available but for any given antineoplastic agent several mechanisms of resistance may become relevant. Data on mechanisms of resistance still need to be reconciled with the overall pattern of prognostic factors. Assays of gene specific damage may help to unravel the intriguing relation between differentiation and drug resistance. Circumvention of resistance by introducing new treatment modalities rather than blockade of specific resistance mechanisms has prevailed in the design of most clinical trials. So far information on drug resistance has been of proven value only in retrospective analysis. Future use may entail transfection of resistance genes into normal stem cells and development of a coherent taxonomy (nosology) of neoplastic disease based on phenotypic and genotypic markers including mechanisms of drug resistance.

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DETECTION OF STROMA-DEPENDENT BLAST COLONY-FORMING CELLS IN PERIPHERAL BLOOD STEM CELLS PRIMED WITH G-CSF ALONE
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Peripheral blood stem cell transplantation (PBSC) using PBSC primed with G-CSF alone without preceding chemotherapy is an attractive new approach for the treatment of various malignancies. The direct quantification of marrow-repopulating stem cells in humans is currently impossible but their presence can be inferred from assays of more mature cells. Blast colony-forming cells (BL-CFC) are defined as primitive haemopoietic progenitor cells that bind to marrow-derived stromal layers and proliferate without the addition of exogenous growth factors. We tried to detect BL-CFC in PBSC primed with G-CSF alone using a combination of the long-term marrow culture system with a modification of the conventional BL-CFC assay. PBSC collected from 5 patients (2 NHL, 3 breast cancer) using G-CSF (5 µg/kg sc) for 4 days prior to leucophoresis were cultured on irradiated allogeneic stromal layers using long-term marrow culture conditions and assayed directly for the presence of stroma adherent BL-CFC. At day 0 of culture no BL-CFC could be detected, whereas starting at day 3-4 BL-CFC began to appear in the adherent layers of the long-term cultures. In parallel experiments these BL-CFC released secondary CFU-GM into the supernatant culture medium (delta assay) confirming the differentiation potential of the bound progenitor cells. In contrast no BL-CFC were detected in peripheral blood from normal subjects. Our results demonstrate the transitory status of mobilization of PBSC and indicate that PBSC primed with G-CSF alone have a marrow-repopulating capacity. This assay may be useful to assess the frequency of primitive haemopoietic progenitors cells in PBSC, to study their interaction with stromal elements and the feasibility of PBSC transplants between allogeneic subjects.

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LYMPHOCYTE SUBSETS IN CHRONIC AUTOIMMUNE THROMBOCYTOPENIC PURPURA (cAITP) : EVIDENCE FROM 52 UNTREATED PATIENTS.
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This study focuses on a possible role of diverse lymphocyte subsets in the pathogenesis of cAITP. Thus, the peripheral blood levels of total T (CD2+) and total B (CD19+) cells, CD4+T cells, CD8+T cells, interleukin-2 receptor positive T cells (CD3+CD25+), HLA-class II expressing T cells (CD3+DR+) as well as Leu8 (=LAM-1) positive CD4+T cells and NK cells (CD56+CD57+) were studied by two colour flow cytometry (FACScan) in 52 untreated cAITP patients and 40 normal controls. With regard to platelet counts (PC) the patients were grouped into severe disease (PC < 30 / nl, n= 8), moderate course (PC 30 to 150 / nl, n= 33) and complete remission (PC > 150 / nl, n= 7). Compared to controls, CD3+CD25+T cell levels were significantly elevated in all groups of patients ($p<0,01$), being most pronounced in severe disease ($p< 0,05$). The NK subset was expanded in severe disease ($p< 0,01$). As compared to controls, the Leu8+CD4+T cell subset was clearly augmented in complete remission ($p<0,01$), but reduced in severe disease ($p< 0,05$).
Based on these findings, 1) an activation of T cells might be involved in the pathogenesis of cAITP, but the precise role of CD3+CD25+T cells awaits clarification, 2) a complete remission of cAITP does not imply normal levels for CD3+CD25+T cells or Leu8+CD4+T cells, 3) a possible functional role of Leu8+CD4+T cells in complete remission remains to be established.

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PERIPHERAL BLOOD LEVELS OF CD5+ B LYMPHOCYTES DO NOT DIFFER BETWEEN PATIENTS WITH CHRONIC AUTOIMMUNE-THROMBOCYTOPENIC PURPURA (cAITP) AND HEALTHY INDIVIDUALS

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CD5+B cells, the precursors of autoantibody secreting plasma cells, are reported to be markedly increased in the peripheral blood of patients with various autoimmune diseases (e.g. rheumatoid arthritis, Sjögren´s syndrome, Graves´disease) and in HIV-related thrombocytopenia, a disease also revealing autoimmune features.
To evaluate their role in cAITP, levels of CD5+ and CD5- B cells as well as CD5+ and CD5-T cells were studied in the peripheral blood of 111 cAITP patients by two colour flow cytometry (FACScan).The results were correlated to the clinical course of cAITP and compared to 40 normal controls.
In untreated patients (n= 50) absolute and relative (percentage of mononuclear cells) levels of CD5+ and CD5- B cells did not differ from normal controls and showed no correlation to the platelet count. Absolute CD5+ B cell levels were not altered by glucocorticoid treatment (n= 27) or splenectomy (n= 25) alone but were obtained significantly decreased ($p \leq 0,001$) in splenectomized patients continuing glucocorticoid therapy (n= 9).
We conclude than an expansion of the CD5+ B lymphocyte subset of the peripheral blood is not involved in the pathogenesis of cAITP.

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BONE MARROW DONOR SEARCH FOR 1012 PATIENTS IN THE RELATED AND UNRELATED POPULATION: STRATEGIES, SUCCESS RATES, AND COSTS.

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From 1/1990 to 12/1992 a marrow donor search was run for 1.012 patients (pts) in whom allogeneic BMT was indicated. The types of searches offered were: CFDS (core family donor search), EFDS (extended family donor search) and UMDS (unrelated marrow donor search). CFDS was performed for 875 pts. A total of 1.825 siblings were tested (mean 2.1 siblings/patient, range 1 - 9). Serological HLA class I and MLC identity at least in GvH direction were the match criteria, but in 37 cases one HLA-A or -B mismatch was accepted. In 45% (394/875 pts) a matching sibling was identified. An average amount of 1.315 DM per patient and a mean sum of 2.921 DM per identified donor were spent. Within the EFDS program 1.369 parents and 1.472 other family members were tested. The search strategy was based on serological HLA class I and II typing results. The match criteria were the same as in CFDS. Father or mother were suitable donors in 5,3% (24/451 pts, who had no CFDS donor), while in 14 % (43/298 patients) the identified donor was another member of the extended family. An average amount of 822 DM and 3.068 DM were spent per patient for testing parents and other relatives respectively. The average costs for one matched donor (other than parents), generated by EFDS, were 21.261 DM. UMDS was run for 190 patients. Serological HLA class I and class II typings were performed in all pts and potential donors, confirmed by biochemical (1D-IEF) and molecular genetic (PCR-RFLP) analyses respectively. Match criteria were full identity for patients ≥ 35 years, whereas one minor mismatch was tolerated for patients < 35 years. For 45,8% (87/190 patients) a donor could be found. Since 1990, the mean duration of search shortened rapidly and was 144 days in 1992. At an average, 11.150 DM per patient and 24.350 DM per identified donor were spent in the case of UMDS. In summary, a suitable matched donor could be provided for 55% (557/1012) of all patients by either CFDS, EFDS or UMDS.

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ROLE OF CYTOKINES AND STROMAL CELLS IN GROWTH REGULATION OF BLAST CELLS IN T-LINEAGE ALL (T-ALL)

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We studied the capacity of lympho-hematopoietic growth factors (IL-7, IL-3, IL-1, IL-2 and IL-4) and homogeneous stromal populations (passaged bone marrow fibroblasts (Fb) and human umbilical vein endothelial cells (HUVEC)) to support the growth of T-ALL blasts in vitro. The proliferative response of blast cells from 8 pts. with T-ALL was assessed on the basis of cell number, tritiated thymidine incorporation, morphology, and immunophenotype. Apoptotic cell death was quantitated by flow cytometry. IL-7 alone provided a proliferative stimulus in one of 8 samples, resulting in a 4-fold increase of leukemic blasts after 14 days. In three cases, coculture of T-ALL blasts with Fb in the additional presence of IL-7 resulted in a 3-fold expansion (n=1) or maintenance at input levels (n=2) of leukemic blasts after 7-14 days. HUVEC were less effective in supporting proliferation of blasts than Fb. Neither IL-1, IL-2 nor IL-3 had significant stimulatory effects in addition to IL-7 and stromal cells. Conspicuously, IL-4 had a profound inhibitory effect on blast cell proliferation as determined both by thymidine incorporation and cells counts. The immunophenotype of the blasts remained unchanged throughout the culture period under the conditions examined, on the basis of FACS analysis of CD7, CD3, CD4 and CD8 antigen expression. T-ALL blasts were unresponsive to all tested stimuli in four cases. In conclusion, IL-7 is a potent stimulus of leukemic blast proliferation but not differentiation in a significant subset of patients with T-ALL, although this effect is dependant on stromal cells in some cases. The reasons for the differential growth requirements of phenotypically similar blast populations in T-ALL require further analysis.

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PARALLEL ADMINISTRATION OF R-metHuG-CSF (FILGRASTIM) AND INTENSIVE INDUCTION CHEMOTHERAPY IN ADULT ALL: A RANDOMIZED PHASE III STUDY

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This study was designed to determine whether recombinant G-CSF, administered in parallel with myelotoxic chemotherapy and irradiation during induction treatment of adult ALL, could reduce the incidence and duration of treatment-induced granulocytopenia. The effect of r-metHuG-CSF on febrile and infectious episodes and treatment delays due to neutropenia was also assessed. The feasibility of this treatment approach had been tested previously in two independant pilot studies (Ottmann et al. Exp Hematol 19:529 (1991), Scherrer et al. Annal Hematol 65:A114 (1992)).
All pts. were treated according to the protocol of the german multicenter ALL trial (Hoelzer et al. Blood 71:123; 1988). They were randomized to either concomitantly receive r-metHuG-CSF (5μg/kg/day s.c.) or no growth factor during the second half of induction therapy. Of 75 patients entered into the trial, 49 pts. have currently completed the study and are evaluable: 32 male and 17 female pts., with a median age of 34 years and a diagnosis of c-ALL (n=28), B precursor-ALL (n=6) and T-lineage ALL (n=14). Treatment is ongoing in 13 pts., three patients were withdrawn for reasons unrelated to G-CSF administration. The mean duration of severe neutropenia (ANC <500/μl) was reduced significantly in the pts. receiving concurrent G-CSF and chemotherapy (10 days) as compared with the pts. receiving chemotherapy alone (18 days). Potential clinical benefits of this still experimental treatment modality will be evaluated in the final analysis of this study.

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EXPRESSION OF HUMAN ENDOGENOUS RETROVIRAL SEQUENCE HERV-K IN NON-HODGKIN LYMPHOMAS

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Endogenous retroviral sequences are present in multiple copies in the human genome representing 0,1-0,6% of it. They show similarity to infectious murine, primate and human retroviruses. Their pathogenic potential has been shown for murine leukemia viruses, mouse mammary tumor viruses (MMTV) and intracisternal A-type particles. The human endogenous retroviral sequence HERV-K is a full-length provirus with homology to MMTV. HERV-K is until now the only endogenous retroviral sequence that contains an open reading frame large enough to allow synthesis of full-length polymerase proteins including reverse transcriptase. HERV-K is suspected to be involved in tumorigenesis.
RNA from 20 patients with non-Hodgkin lymphomas was transcribed in to c-DNA and analysed with PCR using primer pairs for the pol- (3937-4553), gag-(1866-2548) and env-region (6909-7690). For each sample ß-actin primers were used in parallel as a positive primer control. The PCR-products were verified with Southern blot hybridizations.
In two thirds of the patients HERV-K expression was present. No correlation between the expression of HERV-K and the type of lymphoma could be found. Moreover transcription of HERV-K was quite common among normal and other tumorcells.
In conclusion HERV-K expression is probably constitutive. In some lymphomas however a down regulation is possible. Further analysis of these transcripts is in progress in order to illuminate their physiological role and involvement in tumorigenesis.

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LONG-TERM SURVIVAL IN VITRO OF BCR/ABL-POSITIVE CML CELLS AS DETECTED BY REVERSE PCR

G. Pasternak and L. Pasternak

CML bone marrow or blood cells were cultured in vitro over periods of 50 - 245 days. Altogether, the cells from 20 patients were included in the experiments. At regular intervals of 10 or 20 days the cells were harvested and analysed for the following biological patterns: presence of Ph chromosome; morphology (Wright stain); expression of adhesion molecules such as β_1- , β_2-integrins, and of the immunoglobulin superfamily using immunocytochemistry; clonogenic activity in methyl cellulose; and the presence of bcr/abl fusion message.
The most interesting result is given by the fact that although cytogenetic conversion from Ph^+ to Ph^- may occur in vitro the PCR is still detecting bcr/abl-positive cells in the culture. Even in EBV-transformed CML long-term cultures maintained for 245 days in vitro a few admixed monocytic cells give rise to a positive PCR for bcr/abl.

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PSEUDOTHROMBOCYTOPENIA IN PATIENTS INFECTED WITH HUMAN IMMUNODEFICIENCY VIRUS TYPE 1

H.W. Pees, H. Hofmann*, and M. Pfreundschuh

Pseudothrombocytopenia is defined as a low platelet count resulting from a laboratory artefact, which may lead to erroneous diagnosis with serious clinical consequences. The phenomenon is caused by antibodies, present in the patients' sera, which react with platelets in blood anticoagulated mostly with EDTA, leading to agglutination and a spurious low platelet count. It can be readily recognized by inspecting conventional blood smears. We recently observed 5 HIV-positive patients with pronounced agglutination of platelets in EDTA (3 cases) or heparin. All had fluctuating platelet counts over time without any tendency of bleeding. The clinical relevance of this observation became apparent in a young woman during pregnancy when the platelets declined to 30,000 per cubic millimeter and the consulting physicians agreed in advising termination of pregnancy. Ultimately, a diagnosis of EDTA-induced pseudothrombocytopenia was made and the child was born without any bleeding complication a few months later. We do not know the frequency of this artefact in HIV infection; neither could we find any relation to CDC-status, CD4 counts, HIV-antigenemia or other clinical parameters. In any case, pseudothrombocytopenia should be excluded in HIV-counseling before embarking on additional costly examinations, and inappropriate medical or surgical therapy.

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NONTROPICAL PYOMYOSITIS IN ACUTE LEUKEMIA. A REPORT OF 3 CASES

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Pyomyositis, a suppurative bacterial infection of skeletal muscle, is most frequently observed in the tropics and most often caused by Staphylococcus aureus. The disease is characterized by localized muscle pain, swelling, and tenderness. In recent years, however, it is increasingly reported from temperate climate areas, predominantly in immunocompromised patients. The onset is usually insidious, and delay in diagnosis may lead to progression with large purulent collections, septicemia, shock, and death. Since the disease can mimic several other conditions, it may remain unrecognized for weeks. We report three cases of this entity in patients with acute leukemia. A common denominator in all three patients was a fulminant clinical course with severe local pain and fever during recovery from chemotherapy. Multiple imaging modalities including computed tomography and magnetic resonance aided in the accurate diagnosis. Thus, in two cases a combined approach with repeated drainage procedures and prolonged antimicrobial therapy eradicated the infection and chemotherapy could be continued resulting in complete remission of leukemia. So far nontropical pyomyositis has been associated mainly with HIV-infection. According to our experience, acute leukemia should be added to the list of prediposing conditions.

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REGULATION OF MALIGNANT B CELLS BY CYTOKINES AND CELLULAR INTERACTIONS

C. Peschel

Cytokines produced in an autocrine or paracrine fashion and direct cellular interactions are involved in regulation of the expansion of malignant lymphomas by influencing the proliferative capacity and programmed cell death of the malignant lymphocytic clone. Cytokines can modulate this regulatory network and interrupt stimulatory signals from accessory cells, such as T lymphocytes, monocytes, dendritic cells and stromal cells. Such interactions might lead to beneficial effects in novel therapeutic approaches for B cell lymphomas. We investigated the effect of cytokines with activities on B cells, including IL-4 and IL-10, on proliferation, cytokine expression, regulation of apoptosis and expression of adhesion molecules on B-CLL cells. Furthermore, the capacity of malignant and normal B cells to adhere to matrix proteins and bone marrow stromal cells was evaluated. The secretion of cytokines by non-malignant T lymphocytes from patients with B-CLL or normal controls was determined upon induction with various combinations of antibodies which stimulate TCR dependent or TCR independent pathways. In addition the role of B-CLL cells as presenting cells on secretion of T cell cytokines was compared with monocytic cells in this system. Functional studies of interactions by malignant B cells with cellular components forming the microenvironment in lymph nodes and bone marrow contribute valuable informations to elucidate mechanisms of tumor progression and should provide a rational basis for novel therapeutic strategies.

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Prophylaxis for pneumocystis carinii pneumonia with pentamidine in patients after renal and bone marrow transplantation

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Pneumocystis carinii pneumonia (PCP) is a common life-threatining opportunistic infection in the immunocompromised patients, preventable by either oral trimethoprim-sulfamethoxazole or by pentamidine inhalations.

We report on the results of a non-randomised multicenter study observing efficacy and side-effects of pentamidine (Pentacarinat) aerosol PCP prophylaxis in 64 patients after bone marrow transplantation (BMT) and 24 patients after renal transplantation (RT). Initial pentamidine dose was 200 to 600mg (median 300mg) for BMT patients, total dose averaged 1500mg. RT patients received 300mg pentamidine per inhalation, total dose averaged 2550mg.

Data of 42 (65,6%) BMT patients and 22 (91,7%) RT patients could be evaluated for efficacy. Study failures were caused by death (2), adverse events (6), non-compliance (12), underlying disease (2) and technical problems (1), in one case no reason was stated.

No PCP cases were reported.

During prophylactic pentamidine treatment (average duration: 12 weeks) there were three suspected cases of PCP. Diagnosis of PCP could not be confirmed in neither patient.

Adverse events were reported for 56,8 % of the patients. Cough occured in 39,1% of the BMT and 16,7% of the RT patients, bitter taste in 54,7% and 20,8% respectively. There were single reports about transiently elevated creatinine values, pharyngitis, nausea, and an upper respiratory infection.

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ADRIAMYCIN, CISPLATINUM, ARA-C AND METHYLPREDNISOLONE (ASHAP) COMBINATION CHEMOTHERAPY IN RELAPSED AND REFRACTORY LYMPHOMA AND HODGKIN'S DISEASE- PRELIMINARY DATA

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We have evaluated combination chemotherapy with ASHAP in 29 patients with refractory or recurrent Hodgkin's (HD) and non-Hodgkin's (NHL) lymphoma who where considered for autologous stem cell transplant. Pts were treated with adriamycin (40mg/m^2 continuous infusion (CI) over 96 hrs), with cisplatinum (100mg/m^2 CI over 96 hrs), methylprednisolone (=solu medrol, 500mg i.v. day 1-5) and Ara-C (2gm/m^2, day 5). Histology was as follows: 8 pts with HD, 16 pts with high-grade (HG) NHL (T-cell 4, „diffuse large cell" 2, Ki-1 1, centroblastic 6, lymphoblastic 3), and 5 pts with low-grade (LG) NHL (cb-cc 4, cc 1). Most patients were heavily pretreated--HD pts had received a minimum of 2, NHL a minimum of 1 therapy with curative intent.

Pts were re-evaluated after 2 courses for response. 9 pts with HG-NHL achieved a complete response, 1 pt achieved partial remission, 3 had stable disease and 3 progressive disease. 4 pts with HD achieved a complete response, 1 pt a partial remission and 2 had stable disease. 2 pts with LG-NHL had stable disease, 1 progressed, 1 pt died early due to underlying disease. Overall response rate was 54%. 6 responders received high-dose therapy and autologous stem cell transplants after remission induction, 5 of them are in CCR (2+,3+,6+,16+,18+), one relapsed and died 5 months post-transplant. Remission duration averaged 4 months in ASHAP-responders who did not receive high-dose therapy. Toxicity data will be presented.

We conclude that ASHAP is a very active salvage regimen for NHL and possibly HD.

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Effect of follicular dendritic cells on the proliferation and dissemination of neoplastic B-cells

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The enhancing effect of follicular dendritic cells (FDC) on the proliferation of germinal center B-cells in the normal lymphatic tissue has been described in detail. In vitro, FDC and B-cells from NHL spontaneously coalesce, forming small cellular clusters. Immunocytochemistry with Ki67 revealed that after a 24 hours culture period, a considerable number of lymphoma lymphocytes enclosed by the FDC processes were in late G1 to M phase of the cell cycle. Furthermore, the number of B-cells outside these aggregates staining positive for Ki67 was much lower as compared to the neoplastic B-cell population involved in cluster formation. These data were confirmed by autoradiography and suggest that FDC provide signals leading to the continued stimulation of lymphoma lymphocytes.

B-cells isolated from lymph nodes of patients with centroblastic-centrocytic lymphoma express LFA-1-alpha, LFA-1-beta, VLA-4 and ICAM-1, and FDC isolated from these tissues are strongly positive for ICAM-1 and C3bi. The LFA-1-alpha/beta = ICAM-1 and the ICAM-1 = C3bi receptor = ligand linkage enables neoplastic B-lymphcytes to aggregate spontaneously with FDC in vitro. Lymphocytes in the peripheral blood of patients with a leukaemic course of centroblastic-centrocytic lymphoma do not stain with anti-LFA-1-beta and only part of these cells stain with anti-LFA-1-alpha and anti-ICAM-1. The data indicate that the lack of LFA-1-alpha/beta and ICAM-1 surface molecules enables neoplastic lymphocytes to detach from FDC. The B-cells now invade new compartments.

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MOLECULAR BASIS OF GENETIC MYELOPEROXIDASE DEFICIENCY IN A PATIENT WITH CHRONIC OSTEOMYELITIS

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We have investigated a female patient with chronic osteomyelitis since the age of 13 for the presence of myeloperoxidase (MPO) deficiency as a potential cause of immune dysfunction.

Peripheral blood smears were prepared, stained and analysed by a manual differential procedure. For peroxidase determination the cells were stained cytochemically. For gene analysis genomic DNA was extracted and digested with Bgl II prior to electrophoresis into agarose and blotting to a nylon filter. The blot was probed with ^{32}P-labelled pMPO2 and washed under highly stringent conditions. For protein analysis white blood cell extracts were solubilized in SDS sample buffer, the proteins separated in 9% acrylamide gels and electroblotted to nitrocellulose paper. The blot was processed with monospecific rabbit antiserum to purified MPO followed by ^{125}I protein A.

Results: Cytochemical peroxidase analysis revealed a nearly complete MPO-deficiency. When compared to a normal control, Southern analysis showed an RFLP (2.1 kb in addition to the normal 2.6 kb fragment). Cloning and sequencing of exon 10 revealed a mutation in nucleotide 10595 causing an amino acid substitution. On Western analysis our patient had the 89 kDa-precursor (Pro-MPO), but lacked the heavy MPO-subunit (59 kDa).

Conclusions: The presence of MPO-deficiency (1% residual activity) in a female patient with a chronic infectious disease is associated with a mutation in exon 10 of the MPO-gene and the lack of appearance of the mature subunits of the MPO-protein. This indicates that a genetically determined disturbance of the proenzyme processing could be the cause of the functional MPO-deficiency.

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THE GROWTH-INHIBITORY EFFECT OF 2-CHLORO-2'-DEOXYADENOSINE (CDA) ON MYELOID PROGENITORS (CFU-GM) IN NORMAL HUMAN LONG-TERM BONE MARROW CULTURES CAN BE COMPENSATED BY THE ADDITION OF INTERLEUKIN-3 (IL-3) OR GRANULOCYTE COLONY-STIMULATING FACTOR (G-CSF)

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We often observed neutropenia and bone marrow suppression during the treatment with 2-chloro-2'-deoxyadenosine (CdA), a new promising substance especially developed for the treatment of lymphoid malignancies. In order to analyze the myelosuppressive effect of CdA, we performed Dexter-type human LTBMCs. In order to mimic the in vivo situation, where patients are treated with a continous infusion of CdA over a period of 7 days, LTBMCs were incubated with varying doses of CdA (5 - 20nM) during the first week. After week 1, LTBMCs were washed free from CdA and with weekly 1/2 medium change (MC) non-adherent cells were counted and analyzed for clonogenity.

At a low CdA-dose of 5nM no additional cell loss compared to untreated controls was found. However, the numbers of myeloid progenitors (CFU-GM) was already reduced to 50% at week 1, but recovered after 4 to 5 weeks of culture (Inhibition 0 - 20%). In contrast, at higher doses of CdA (10, 20 nM), the reduction in the number of myeloid progenitor cells was 60% and 85%, respectively during the whole observation period (7 weeks).

Concerning the composition of the adherent stromal layer, no difference between CdA-treated and normal LTBMCs was found. In order to exclude, that in CdA-treated cultures a functionally defective stromal layer was the reason for the reduced progenitor cell growth, we performed LTBMCs ± CdA on preformed irradiated stromal feeder layers. Similar results were obtained whether LTBMCs ± CdA were done on already formed stromal feeder layers or not.

As it is known that low doses of CdA reduce the release of IL6 from monocytes, and IL6 is secreted from the adherent layer after each weekly medium change to stimulate clonogenic hematopoietic progenitors with a high proliferative potential in LTBMCs, we analyzed, whether the strongly reduced progenitor cell growth might be a result of a possibly reduced secretion of IL6 from the adherent layer. Therefore, concentrations of IL6 were measured in the supernatant at certain points of time after the 1/2 MC. The results show, that the levels of IL6 investigated were similar in normal and CdA-treated cultures. Finally we tested, whether the addition of cytokines with stimulatory effect on myeloid progenitors can prevent the inhibitory effect of CdA on CFU-GM growth. We found, that the weekly addition of 100ng/ml of either IL3 and G-CSF can compensate this CdA effect.

We conclude that the myelosuppressive effect of CdA is mediated by a direct action on CFU-GM progenitor cells and not by a functionally defective stromal layer. Moreover, IL3 and G-CSF are able to compensate CdA-mediated myelosuppression.

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CYTOCHROME P-450 AND MULTIDRUG RESISTANCE

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Leukemia is one of the most effective targets for cancer chemotherapy. A major obstacle, however, is intrinsic or aquired drug resistance frequently observed resulting from a number of factors only partially understood. These include differential changes in drug metabolizing enzymes (cytochrome P-450, conjugating enzymes) as well as overexpression of a multidrug resistance (MDR) gene product involved in the energy dependent drug efflux. Otherwise, the P-450 system is known to be involved in the biotransformation of various substances as, for instance the cytotoxic and potentially MDR-inducing anticancer agents. Preliminary evidence indicates that the induction of selective members of both the MDR and P-450 gene families may depend on overlapping regulatory elements.

We chose the in vivo mouse model of P388 lymphatic leukemia and sublines made resistant to anticancer drugs like vincristine and adriablastine, to investigate the expression of P-450 protein(s) induced by these drugs in sensitive and in resistant cells. The P-450 contents were determined spectrophotometrically and, in addition, the affinity to bind each of these anticancer drugs has been tested. Further, the catalytic activities of P-450's were determined on the cellular and subcellular levels. MDR gene expression was checked by the uptake of the fluorescent dye rhodamine 123. Our results demonstrate clear correlations of P-450 expression and multidrug resistance dependent on the type of drug. In view to the clinical relevance of MDR development it is important to underline the necessity of gaining further insight into the regulation of P-450 and MDR gene expression in normal and tumor cells.

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Gα16 IS EXPRESSED IN NORMAL AND LEUKEMIC HEMATOPOIESIS

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G-proteins are crucial in signal transduction pathways in hematopoiesis. The Gα16 gene codes for a G protein α subunit expressed in a variety of hematopoietic cell-lines.

We have analysed Gα16 expression using a Reverse Transcription-Polymerase chain reaction (RT-PCR) approach in blood and bone marrow of Acute Leukemia (AL) and Chronic Myeloid Leukemia (CML) patients and of Peripheral Blood Stem Cells (PBSC) from patients prepared for autologous bone marrow transplantation.

Cells from 4/4 CML patients and 16/18 AL patients expressed Gα16. 7/7 AL patients in CR showed high expression of Gα16. PBSC were harvested from 10 patients (Lymphoma 8, Testicular cancer 2). High levels of CD34 positive cells and clonogenic cells correlated in PBSC with high Gα16 expression. In elutriation experiments Gα16 expression was found in the fractions containing predominantly monocytes and fractions with the highest progenitor cell content but was absent in T and B lymphocytes.

We conclude that (1) Gα16 is expressed mainly in cells of the granulocytic/monocytic lineage, that (2) normal and leukemic hematopoiesis express equal amounts of Gα16, that (3) Gα16 parallels progenitor cell content in PBSC and that (4) normal lymphocytes are negative in respect to Gα16 expression. Our data argue for a basic role of Gα16 in the regulation of early hematopoiesis.

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ANALYSIS OF PROLIFERATION AND DIFFERENTIATION OF LEUKEMIC CELL LINES IN DIFFERENT CULTURE CONDITIONS BY FLOW CYTOMETRY.

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A general feature of all cells of acute leukemia is a high proliferative potential combined with the inability to differentiate to mature cells of hematopoiesis. Under the influence of differentiation inducers some AML cells mature to granulocytes or monocytes whereas others do not respond. In addition to differentiation inducers the supply of growth factors and iron influences the ability of AML cells to differentiate or to proliferate. In the present study two permanent myelomonocytic cell lines - EW2 and GK37 - were analysed under specific culture conditions to measure simultaneously differentiation, proliferation and cell cycle status. After adaptation of the methods at different periods of culture a panel of differentiation markers (CD11b, CD13-16, CD33-34, CD71, HLA-DR, Glyc.A, IGF-1-R), BrdU - and propidium iodide staining were simultaneously determined by flow cytometry . HL-60 and KG1 served as control cell lines. The flow-cytometric results were controlled by morphology, cytochemical reactions, thymidine assay and cell numbers. TPA, ATRA, and IFN-gamma induced GK37 to differentiate to granulocytes and/or monocytes whereas EW2 cells exhibited no or only few changes of immunophenotype. Proliferation of these two cell lines was differently influenced. TPA stopped proliferation of both cell lines completely. ATRA had no effect on EW2 cell and only a mild antiproliferative effect on GK37. The iron chelator deferoxamin (DFO) induced differentiation of GK37 to monocytes and showed no reactions in EW2. Both cell lines were arrested by DFO in G1 phase of cell cycle. Antibodies raised against human transferrin and IGF-1-receptor had no differentiating effects but both were antiproliferatively active and arrested both cell lines in S-phase (>90 %). The different behavior of these cell lines agaist TPA and the possibility to arrest the cells in G1-or S-phase enable: 1 to study thoroughly the switch from proliferation to differentiation in these cells and 2 to test new therapeutic approaches.

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INTERFERON ALFA IMPROVES SYSTEMIC MASTOCYTOSIS AND DISEASE RELATED OSTEOPOROSIS

K.H. Pflüger, R. Weide, K. Ehlenz and W. Lorenz

Systemic mastocytosis is a myeloproliferative disorder characterized by an abnormal proliferation of mast cells, which infiltrate the skin, bone marrow, spleen, liver and lymph nodes. In a case report (NEJM 326; 619-23, 1992) good response of a patient suffering from aggressive systemic mast cell disease to IFN was reported. Here we report a patient with systemic mastocytosis and severe refractory osteoporosis who was treated with IFN- alfa-2b.

Case report: A 39 year old man developed in the age of 30 severe pain in the back caused by several vertebral body fractures and deformity of the spine. An advanced osteoporosis was diagnosed. By internal examination an urticaria pigmentosa and a nodular infiltration of bone marrow with mast cells without impairment of hematopoiesis were found. Treatment was initiated with sodium fluoride and calcitonin. As pain and restiction of movement were progressive medication was changed to indomethacin. Later on oral cromolyn sodium was added. Under this therapy the patient had pain relief and physical ability was sufficient. Mastocytosis seemed to be stable but osteoporosis progressed as determind by osteodensitometry (ODM). Since Sept. 92 we treated the patient with 5 million U IFN-alfa-2b three times a week for 6 months. Indomethacin and cromolyn sodium were kept unchanged. The patient was observed closely during the first week of IFN therapy. Prior to therapy and after 6 months bone marrow biopsies, osteodensitometry, quantitative computerized tomography, and histamin release after IFN injection were determined. Physical examination and laboratory studies were performed every 8 weeks.

Side effects of IFN were mild. The patient's condition improved and he terminated indomethacin therapy by himself after 5 mo. Urticaria pigmentosa and mast cell infiltration of bone marrow regressed. Maximal histamine release after first IFN injection was 2,07ng/ml blood and max. 0,35 ng/ml after 6 months. In addition objective parameters of osteoporosis improved, too:

ODM: Mineral content of vertebral body: 19,0 g to 29,09 g
average density of bone: 0,59 g x m^{-2} to 0,67 g x cm^{-3}

Q-CT: Hydroxylapatite-content of lumbar vertebra 2:
trabecular 59,2 mg x ml^{-1} to 74,0 mg x ml^{-1}
cortical 112,3 mg x ml^{-1} to 133,8 mg x ml^{-1}

In summary this case report indicates that IFN improves systemic mastocytosis as well as disease related osteoporosis.

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MOLECULAR BIOLOGY OF HODGKIN AND REED-STERNBERG CELLS: NEW ASPECTS FOR THERAPEUTIC CONCEPTS IN HODGKIN'S DISEASE?

M. Pfreundschuh

Investigations on the nature of Hodgkin's disease have been hampered by the fact that the neoplastic Hodgkin and Reed-Sternberg (H&RS) cells represent only a minority of the cells in a diseased tissue. We therefore developped a method for the micromanipulatory isolation of single H&RS cells to which PCR procedures were applied. At the *DNA level* our results show that H&RS cells harbour EBV more often than previously reported, while they rarely if ever show rearrangement of the IgH or TCR genes. Analysis at the *mRNA level* reveals that single H&RS cells of a given patient have a characteristic mRNA expression profile, while the inter-patient variation is rather high. At the *protein level*, the most specific and prominent immunological feature of H&RS cells is the expression of the CD30 antigen, a member of the nerve growth factor receptor family whose ligand has recently been identified as a TNF-related cytokine. Unstimulated human peripheral blood lymphocytes and bispecific monoclonal antibodies with reactivity to this growth factor receptor and T-cell or NK-cell activity triggering molecules, respectively, induce 100% complete remissions of Hodgkin's tumors established in SCID mice. Mutated forms of the CD30 ligand open an additional therapeutic avenue, as do anti-idotypic CD30 antibodies, which might be used as a vaccine in patients in remission with a high risk of relapse. Thus, while molecular biology and immunology are still unable to define the origin of H&RS cells, they can be successfully employed for the remaining therapeutic conquest of this elusive disease.

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DEXVERAPAMIL AS RESISTANCE MODIFIER IN ACUTE MYELOID LEUKEMIA (AML)

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The aim of this study was to determine both the clinical tolerance and the efficacy of dexverapamil (Knoll AG) as resistance modifier in AML. Eligible patients had to have relapsed or refractory disease and had to express the MDR1 gene in their leukemic cells. Chemotherapy consisted of daunorubicin (45 mg/m2 per d, d 1-3) and cytosine arabinoside (200 mg/m2 per d, d 1-7). Dexverapamil (4 x 300 mg/d) was added 36 hours before the 1st dose and until 24 hours after the last dose of daunorubicin. So far, 4 patients were admitted to this study and 6 treatment cycles were administered. No serious side effects did occur and all patients survived the treatment. A decrease of blood pressure was observed in all patients. Sinus bradycardia was seen in 2 patients and with first-degree atrioventricular block led to a dose reduction of dexverapamil to 4 x 250 mg/d in one patient. No signs of heart failure did occur. Two patients (1 early relapse, 1 second relapse) achieved complete remission (CR) and are in continuous CR at 3 and 6 months. One patient with refractory disease and 1 patient in 3rd relapse did show an improvement but did not achieve CR. In conclusion, dexverapamil was usually well tolerated and might improve outcome of chemotherapy. Thus further evaluation of dexverapamil as resistance modifier is warranted in patients with AML.

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A CONCEPT FOR DATA SECURITY IN CLINICAL APPLICATIONS

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Clinical application programs usually run on open systems that offer at most minimal security and data protection. Even if the application strictly controls data access everyone can see the data using an editor or other tools. Exposing patient data this way violates the regulations for data protection.

While devoloping a therapy management system for the pediatric oncology (TheMPO) we designed a security concept that prevents unauthorized data access from all levels of the operating system. It relies on cryptographic techniques that provide online encryption of data as well as electronic signatures of documents such as prescription of drugs. In this setting every user needs several keys. He stores them in his 'personal secure environment' (PSE) which should reside on a smart card but can provisionally be simulated by a floppy disk. The PSE is protected by a password (PIN); all the user has to remember is his PIN, so his inconvenience is minimal.

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FLUDARABINE IN A PHASE II STUDY OF A GERMAN LOW-GRADE LYMPHOMA STUDY GROUP

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After years of stagnation new perspectives in the treatment of low-grade Non-Hodgkin Lymphomas (NHL) have recently arisen through the introduction of new purine analogues like Fludarabine, Chlorodeoxyadenosine and Deoxycoformycine. While the latter two agents show a high activity predominantly in hairy cell leukemia, Fludarabine has been studied extensively in chronic lymphocytic leukemia. The current study aimed at exploring the anti-lymphoma activity of Fludarabine in relapsed low-grade NHL on the basis of a multicenter phase II study. 37 patients with relapsed low-grade NHL of stages III and IV entered the trial, compricing 17 cases with centrocytic-centroblastic NHL, 3 cases with centrocytic NHL, 16 patients with lymphoplasmocytoid immunocytoma and one patient with a T-cell lymphoma. All Patients had received prior chemotherapy with 1-11 (median 3) different drug combinations. Fludarabine was applied at a dose of 25mg/m^2/day over five days by a 30 minutes infusion at four week intervals. Patients received 1 to 8 (median 4) cycles of therapy and a total of 155 courses are evaluable for toxicity. 5 patients were not evaluable due to protocol violation. Of the 32 evaluable cases 11 patients (34%) responded, including 6 cases with complete remission and 5 with partial remission. Six patients did not show a significant reduction of the lymphoma cell mass and 13 patients had progressive disease, two patients died during treatment. Side effects were moderate, consisting mainly in myelosuppression. In 6 cases dose reduction was necessary due to leukopenia.
These data indicate a significant anti-lymphoma activity of Fludarabine in this heavily pretreated group of patients with a low treatment associated toxicity. Based on these results a follow-up study was initiated combining Fludarabine with Mitoxantrone and Dexamethasone in an attempt to further improve treatment results.

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Oncosys - Demonstration: Three years'experience with a computer based patient record

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Development: Since 1988 ONCOSYS has emerged from a minor laboratory data acquisition system within a local area network (HaemOncLan) to one of the most often used subsystems (GISNET) implemented in 1992. It now serves multiple functions: generating patients' records, scheduling daily work, planning therapy in close connection with the pharmacy and the blood bank.
Components: A DOS operated file server is connected to discless PCs. The system runs under Novell 3.11, its database being Dataflex. The host TANDEM computer of the hospital supplies the patient's identification number. On line entries from the laboratories or the clinical activities by all members of the staff, including data from physical examinations, vital signs, morphologic diagnoses and planning therapy and blood transfusions are converted into sets of useful lists.
Efficacy: In June 1993 ONCOSYS has generated more than 9500 letters to colleagues, more than 11.000 readings of bone marrow and other microscopic preparations, 1200 invitations for scientific meetings, and a great variety of lists for the facilitation of daily routine: nursing, laboratory, scientific work and even registration of (micro-)photographs.
Pitfalls: Until June 1993 the system failed twice with a major breakdown in 1991. Today the use of twin hard discs and daily duplicates on tapes secure the data. Authorized copies on WORM discs for offical use shall reduce hard copy need on paper.

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IN-SITU-HYBRIDISATION OF UVEAL MELANOMA

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We examined 10 uveal melanomas by comparative genomic hybridisation (CGH). With this method, unbalanced gains and losses of chromosome material are detectable. Some of the uveal melanomas were also studied by conventional chromosome analysis and/or by molecular analysis of DNA-polymorphisms of loci on chromosome 3 and 8q. CGH-findings of losses on the whole chromosome 3 and multiplication of regions on chromosome 8q were consistent with the molecular and cytogenetic data, thus confirming the specificity of these anomalies in uveal melanoma. Furthermore, CGH-findings of chromosome 1 were consistent with the cytogenetic data. Chromosome 6 anomalies, which are also frequent in this tumor, were detected by the CGH-method in cases where they were hidden in marker chromosomes which could not been identified by cytogenetic analysis alone. However, in some cases chromosomes containing additional material of unknown origin could not be identified by the help of CGH. Furthermore, In about 50% of the cases which were studied with the CGH-method, we detected anomalies of chromosome 9 and 16 which were not apparent by conventional cytogenetic analysis, and in one case multiplication of chromosome 7 was found by CGH but not in a number of 36 cytogenetically analysed metaphases. We conclude that the conventional cytogenetic analysis may not be representative for all genetic alterations of a tumor. A selection of subclones, caused by short-term culture of primary material or by the analysis of only proliferating cells might be the factor responsible for the differences between chromosome analysis and CGH-findings. In summary, monosomy of chromosome 3 and multiplication of 8q are consistently found using the different methods applied and thus are likely to represent early events in the formation of uveal melanomas.

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PHASE II STUDY WITH „SPLIT DOSE" CISPLATIN AND ETOPOSIDE IN ADVANCED ESOPHAGEAL CANCER (EC).

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Cisplatin (P) and etoposide (E) are active drugs for esophageal cancer (remission rate 20%). Both drugs have a different mode of action. In vitro and in vivo they act synergistic and are not cross resistant. Overlapping non-hematologic toxicities do not exist. Based on these data, pts with advanced esophageal cancer, PS <2, and age <70 years were treated with PE in a disease orientated phase II study.
TREATMENT PLAN: P 60 mg/m^2 1h inf, d 1 and 7; E 130 mg/m^2 1h inf, d 3,4,5; q d 22(28). Depending on response and toxicity up to 6 cycles were planned. Patients with locally advanced disease only and who responded to chemotherapy were candidates for surgery.
Since 10/91, 25 pts have been entered. THEIR CHARACTERISTICS m/f 20/5, age 56(43-68), PS 1(0-1), T3 N1-Nx 5, T4 N1-Nx 5, M1 15, SCC 22, adenoca. 3.
RESULTS: Evaluable for response 22 pts; 2 too early, 1 pt not evaluable (apoplectic insult during 1. cycle); PR 11 (52%), MR/NC 8(38%); P 2; PR in M1-patients 7/12 (58%), PR in T3/T4 N1-Nx M0 4/10 (40%), 1 pt underwent esophageal resection (NED); remission duration 6.5(3-16)months; median observation time 9 (2-16)months; median survival time of all pts 9(2-16+) months.
TOXICITY (WHO): Leukopenia 2°(32%), 3°(18%), 4°(18%); thrombopenia 3°(22%), 4°(18%); infection 2°(9%); nausea/vomiting 2°/3° (22%); diarrhea 2°(9%); neurotox. 2°/3°(18%); ototox. 3°(13%); nephrotox. 3°(13%); no treatment related death.
CONCLUSIONS: Cisplatin/Etoposide is an active regimen for far advanced esophageal cancer. Comparable to other intensive regimens used in esophageal cancer, myelotoxicity was the major side effect. Patients accrual is ongoing.

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CYTOKINE PRODUCTION IN MULTIPLE MYELOMA: TREATMENT-ASSOCIATED VARIATION IN THE RELEASE OF TNF-α, TNF-RECEPTORS, AND IL-1

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Tumor necrosis factor (TNF)-α and interleukin-1 (IL-1) are involved in the development of bone lesions in multiple myeloma (MM). We have followed serum and plasma levels of TNF-α, TNF receptors p55 (TNF-R55) and p75 (TNF-R75), as well as IL-1α and IL-1β in 50 MM patients (pts) at various stages of their disease . In parallel, cytokine and receptor production was evaluated in whole blood cultures stimulated with PHA, Con A, and LPS . Immunoreactive cytokines and TNF-R concentrations were measured with sensitive ELISAs and ELIBAs (TNF-R). TNF-α levels exceeded 5 pg/ml in 56/85 (66%) serum samples and in 10/30 (33%) plasma samples. Circulating IL-1α and IL-1β was rarely detectable (6/85 and 8/85 sera) and independent of disease stage and cytotoxic treatment. Serum levels of TNF-R55 (median, 1.90 vs. 1.32 ng/ml) and TNF-R75 (4.38 vs. 1.14 ng/ml) were elevated above normal in untreated pts and remained so during and after chemotherapy. TNF-α concentrations in supernatants of PHA- and LPS-activated cultures were normal in untreated pts and depressed during cytotoxic treatment, owing to reduced mononuclear blood cell counts. Chemotherapy impaired the PHA-induced release of TNF-R75 during and for 8 weeks after treatment. LPS-induced IL-1α levels were low (<100 pg/ml) but normal in culture supernatants of untreated pts and were reduced during and for 4 weeks after cessation of treatment. Elevated IL-1β levels were recorded in cultures from untreated MM pts (median, 5221 vs. 2475 pg/ml) as opposed to cultures from healthy individuals. Chemotherapy normalized the IL-1β production. In conclusion, 1. Cytotoxic treatment of MM pts transiently depressed TNF-α production in vitro and in vivo, impaired TNF-R75 and IL-1α release in vitro, and corrected elevated LPS-induced IL-1β release; 2. Enhanced serum levels of TNF-R55 and TNF-R75 remained unaffected by treatment, suggesting TNF-R release by non-circulating cells; 3. Persistently elevated serum levels of TNF-R55 and TNF-R75 in the context of therapy-mediated impairment of TNF-a production in vitro and in vivo and the correction of elevated IL-1β release in vitro suggested an impact of chemotherapy on TNF-α- and IL-1-stimulated cellular processes in vivo; 4. Serum levels of immunoreactive IL-1α and IL-1β did not reflect the capacity of the organism for IL-1 production, and TNF-α levels might have been erroneously high in serum as compared to plasma.

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INTERFERON-α TREATMENT OF CML: INDUCTION OF Mx-PROTEIN IN GRANULOCYTE LINEAGE CELLS AND IMPACT ON THE MONITORING OF IFN-α ACTIVITY IN VIVO

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Interferon-α (IFN-α) is an effective treatment for chronic myeloid leukemia (CML) and may suppress Philadelphia-positive hematopoiesis. If IFN-α is combined with cytotoxic chemotherapy the efficacy of the IFN component is no longer indicated by adequately adjusted blood cell counts. Mx-protein (Mx) induction in mononuclear blood cells (MNC) is a sensitive indicator of Type I IFN activity in vitro and in vivo and may be used for the monitoring of residual IFN activity in patients (pts) with IFN antibodies. Mx levels in whole blood cell lysates depend on the relative contribution of polymorphonuclear cells (PMN). We have quantified by ELISA Mx levels in whole blood cell lysates of 8 CML pts treated with IFN-α (2-9 MU/d) ± hydroxyurea on several occasions during their course. Mx levels varied considerably (median 1962, range 453-37,420 ng/ml) and were linearly correlated to the WBC count (median 7.2, range 2.0-70.8 x 10^9/l; $r = 0.93$). There was much less variation if specific Mx levels/10^6 WBC were calculated (median 377, range 57-1286 ng/10^6 WBC). In 8 experiments, WBC from 6 pts were separated by Ficoll-Hypaque, and Mx concentrations were assessed in whole blood (median PMN 67%, MNC 27%), in the cell pellet (PMN 89%, MNC 3.5%) and in interphase cells (PMN 1.0%, MNC 98%). The median Mx content /10^6 WBC was similar in all fractions: whole blood, 435 (57-1286) ng; cell pellet, 330 (74-955) ng; and interphase cells, 455 (210-1392) ng. In a pt in myeloid blast crisis, blasts were also Mx-positive. Gel formation sometimes hampered Mx determination if WBC exceeded 10x10^9/l. Thus, 1. Circulating granulocyte lineage cells are Mx-positive in IFN-α-treated CML pts and contribute substantially to the Mx signal in a whole blood ELISA; 2. The Mx content of 10^6 WBC is an adequate indicator for residual IFN-a activity in these pts; and 3. Predilution or alternative treatment of the whole blood sample may be required for Mx determination in the presence of high WBC counts.

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THREE COLOR IMMUNOPHENOTYPING OF ACUTE LYMPHOBLASTIC LEUKEMIAS: HIGH INCIDENCE OF ABERRANT AND ASYNCHRONOUS EXPRESSION OF DIFFERENTIATION ANTIGENS AS LEUKEMIA ASSOCIATED MARKERS

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Analysis of the composite immunophenotype plays a central role in the diagnosis, therapy stratification and in combination with molecular biology and cytogenetics in the definition of different biological entities of ALL. The aim of the present study was to establish the composite phenotype in ALL using sensitive multiparameter flow cytometry with directly FITC, PE or PerCP conjugated monoclonal antibodies and to define leukemia associated differentiation antigen expression patterns. Forty-nine leukemic samples were analyzed. The 22 adult and 27 pediatric leukemic bone marrow samples were 12 T-ALL (7 pre-T-ALL, 3 cortical thymocyte ALL, 2 T-ALL), 37 B-cell-precursor- (BCP-) ALL (29 c-ALL, 3 pre-B-ALL and 5 pre-pre-B-ALL). The following aberrant combinations were observed: coexpression of myeloid antigens (CD13, CD33, CD15, CD4 (only BCP-ALL)) on BCP- and T-ALL: 36 cases; coexpression of T-lineage antigens (CD2, CD7, CD5) on BCP-ALL: 8 cases with CD5 being expressed in 5 samples; expression of B-lineage antigens (CD19, CD22) on T-ALL: 2 cases. There was 1 c-ALL with loss of CD45 and CD45RO expression. Asynchronous patterns of expression of differentiation antigens defined as coexpression of antigens normally found on different stages of differentiation in the same cell lineage were observed in 35 cases. According to composite 3-color phenotype, 10 patients expressed an aberrant phenotype, 14 patients an asynchronous phenotype only, and 23 patients the combination of both. Currently, the significance of presence of residual cells with a leukemic phenotype in remission bone marrow for the clinical outcome is being analyzed. Furthermore, this method will allow to isolate cells with the leukemia associated phenotype and establish their clonal relationship to the initial leukemia.

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Hospital Communication System in a Medical Department as a model for the university wide Erlangen Hospital Communication System

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Aim: The Erlangen Hospital Communication System is intended to allow the various university hospitals and other medical departments to communicate electronically with each other and with the Medical Computer Centre. Standardized communication components will be used to support a closed system of requests and responses including the transmission of accounting data assigned to the patient data to the hospital administration.

Implementation: The physical connections between the Erlangen hospitals will use an FDDI glas fiber ring and the connections within each hospital or department will use a star shaped network of twisted pair cables. Digital switches can be used to create subnetworks independent from the structure of the physical networks. The analysis of the network structure is almost complete and its results are described in this paper. The communication between the hospitals and other organisations will be based on the electronic mail system X.400. As soon as European standards such as EDIFACT have been approved in Brussels these will be integrated into the system. The detailed requirements analysis of the hardware and software components is not jet finished. Currently the processing of outpatient and inpatient data is performed by the H90 computers in the Medical Computer Centre with BS2000 operating system, the ADABAS/NATURAL data base system and the patient administration system PATIK2. The program TRANSFER is used to transmit data from the computers of the central laboratory.

We envisage that the medical patient data will be processed using the programm MEDIK running under the operation system BS2000 and UNIX. This has suitable interfaces to the communication standards used in our Hospital Communication System and also interfaces to special documentation systems used in particular departments e.g. endoscopy, sonography, hematology and oncology. The convenient user interface of MEDICARE can be installed in PC´s and notepads to provide a front end system for MEDIK in the wards and other departments.

Perspectives: The findings at the Medical Department I should be trendsetting for the Erlangen Hospital Communication System.

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ACIDIFIED GLYCEROL LYSIS TEST - A SCREENING PROCEDURE FOR HEREDITARY SPHEROCYTOSIS?

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Several tests for the laboratory diagnosis of hereditary spherocytosis have been proposed.
We studied 50 patients with spherocytosis (21 non-splenectomized, 29 splenectomized) to compare the results reached with autohemolysis, osmotic fragility and acidified glycerol lysis test (AGLT).
Osmotic fragility is one of the basic methods and is routinely used in a lot of laboratories. In this study the test was normal in 14 cases of hereditary spherocytosis.
Autohemolysis was very sensitive in all 36 patients with hereditary spherocytosis. The test is not specific, takes a long time and a lot of blood is necessary, but it seems sometimes useful in confirming the diagnosis.
The results reached with AGLT indicate the test has many advantages over other tests. All 50 patients were positive. It is highly sensitive, quick, simple and unexpensive, but it is critically dependent on pH.
Positive results were also found in patients suffering on immunhemolytic anaemia, renal insufficiency and chronic myeloidleukemia.

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APLASTIC ANEMIA: A CLONAL DISEASE?

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Patients with aplastic anemia (AA) may develop clonal disorders of hemopoiesis, i.e., paroxysmal nocturnal hemoglobinuria (PNH), myelodysplastic syndromes or acute Leukemia. The present study addresses the question whether patients with AA have evidence of clonal hematopoiesis. We used restriction fragment length polymorphisms (RFLP) of the X-linked genes phosphoglycerate Kinase (PGK), hypoxanthine phosphoribosyl-transferase (HPRT) and the X-linked probe M27ß, to analyse patients granulocytes and lymphocytes. In some cases, cells were analysed on a FACSan for deficiency in glycoinositol phospholipid (GPI)-anchored surface molecules in parallel. 25 out of 60 healthy females showed a PGK RFLP and thus were suitable for clonaly analysis. 16% of these normals exhibited an imbalanced (oligoclonal) X-inactivation pattern. 15 out of 54 females with AA were informative, i.e., results in PMN, lymphocytes and DNA from mouth washes were interpretable. 10 patients exhibited a polyclonal pattern. An imbalanced pattern in 2 cases was due to extreme lyonisation. Clonal patterns were shown in 3 cases: 1 with a clonal pattern in a skin DNA sample, 2 cases with AA/PNH syndrome. Follow-up studies in 5 cases showed changing patterns in 3. Clonality patterns and deficiency in expression of GPI-anchored molecules were not coherent. Conclusions: Hemopoiesis is polyclonal in the majority of patients with AA. Interpretation of clonal patterns requires appropriate controls as well as longitudinal studies in the same patient.

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PHARMACOKINETICS OF YNK01 - THE ORAL DERIVATE OF ARA-C

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1-beta-Arabinofuranosylcytosine-5'-stearylphosphate (YNK 01, Fosteabine) is the orally applicable prodrug of the clinically widely used cytotoxic agent cytosine-arabinoside. During a recently started phase-one study the pharmacokinetic parameters of YNK 01 were determined by HPLC analysis. Patients suffering from low grade-NHL and AML were included into the study. So far six patients have been treated with 100 mg, three others with 200 mg. One patient was treated consecutively with 100 mg, 300 mg and 600 mg. Following a starting-dose the regular 14-days regiment with one dose YNK 01 daily was started after 72 hours. Plasma and urine concentrations of YNK 01 were measured for 72 hours following the application period. No YNK 01 was detected in urine samples (limit of detection = 2ng/ml). Fitting the results of the plasma concentration measurements of YNK 01 to a one compartment model the following pharmacokinetic parameters were obtained (median and range). YNK 01 dose independent parameters: Lag time = 1.10 h (0.24 - 1.98), t_{max}= 5.81 h (2.35 - 9.49), $t_{1/2}$= 8.9 h (6.3 - 16.7), $clearance_{total}$= 1780 ml/min (874 -2970). Dose dependent parameters: 100 mg dosage: AUC = 1054 ng*ml/h (646 - 1638), $concentration_{max}$= 55.5 ng/ml (37.9 - 69.3). 200 mg dosage: AUC = 2780 ng*h/ml (1860 - 3620), $concentration_{max}$= 174.0 ng/ml (75.3 - 215.0). The long lag time and late t_{max} can be explained by resorption in the distal part of the small intestine. Since YNK 01 causes intravascular haemolysis during i.v. application, it was not possible to determine bioavailability by comparing the AUC after oral and after i.v. application. Instead ARA-U, which as the main metabolite of ARA-C is nearly completely excreted by the kidneys, was measured in urine collected during the first 72 h after the starting dose and after the final application. It was thus possible to estimate the amount of YNK 01 that had been both absorbed and metabolized and is subsequently the crucial part of the absorbed dose. Concluding from the renal elimination of ARA-U a median of 12% of YNK 01 (range 9% - 18%) had undergone resorption and final metabolism to ARA-U. Whereas after i.v. application of ARA-C ARA-U has a half life of 5 hours, a much longer half life of about 40 hours was measured in our patients, presumably resulting from the slow hepatic metabolism of YNK 01. Moderate variability of AUC at the same dosage was detected, suggesting interindividual differences in resorption of YNK 01. On the other hand intraindividual variability was minimal. These data suggest that ARA-C concentrations as in low dose and in standard dose ARA-C therapy can be achieved by oral application of YNK 01.

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Karyotypic abnormalities and multidrug resistance in chronic lymphatic leukaemia.

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Up to this point in time there is no evidence whether P-glycoprotein (PGP) expression is linked to chromosomal aberrations in B-CLL. We studied 32 B-CLLs for glycoprotein (PGP) expression (MoAb C219), 21 of these B-CLLs were analyzed twice in a mean interval of 25 months. In 19 B-CLLs chromosome analyses were performed by routine G-banding (Cancer Genet Cytogenet 1991; 55: 49-56) at the first presentation. 21 of the 32 B-CLLs received chemotherapy before the second PGP analysis (Knospe, COP, CHOP or COP-BLAM). All B-CLLs studied expressed a multidrug resistance phenotype. One group with a low level, another with a high level of multidrug resistance, could be separated. During the observation period of two years intraindividual PGP levels did not increase significantly even if drugs were used which are known to induce multidrug resistance *in vitro*. PGP-mediated multidrug resistance was not associated with a rearrangement of the long arm of chromosome 7. Higher levels of PGP appeared more frequently in cells with clonal or non-clonal aberrations. However, chromosomal abnormalities were not associated with typical PGP levels. The following chromosomal aberrations were observed: Trisomy 12 (n=4), Trisomy 18 (n=1) and mar+ (n=1), complex aberrations (n=1), 5 of these aberrations were clonal, 2 non-clonal. 12 patients had a normal karyotype. Chromosomal abnormalities seem not to be the crucial event which determines the degree of multidrug resistance in B-CLL cells. Nevertheless, the data indicate multidrug resistance phenotype in B-CLL being intrinsic and frequently expressed. These results support the hypothesis, that PGP expression in B-CLL may be linked in the first line to the stage of maturation arrest. Chromosomal aberrations are later events in lymphoma progression and seem to exert - if at all - modulatory effects on PGP expression.

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THE REDUCED DNA SYNTHESIS AND IL-2 PRODUCTION OF MONONUCLEAR CELLS FROM PATIENTS WITH NON-HODGKIN-LYMPHOMAS IN VITRO IS NOT INDUCED BY TGF-ß

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Recent studies implicating a deficiency of interleukins in several diseases have underlined the importance of measuring in vitro the DNA synthesis and the cytokine production (IL-1, IL-2, IL-6, TNF-alpha) in the same cell system. Previously it had been found that normal peripheral blood mononuclear cells (MNC) of patients suffering from high-malignant Non-Hodgkin lymphomas showed a diminished capability to proliferate after mitogenic stimulation (PHA, Con A, PWM). Here we have studied the DNA synthesis and the production of different cytokines (IL-1, IL-2, IL-6, TGF-ß and TNF-alpha) by pokeweed mitogen (PWM) stimulated MNC from 15 healthy control subjects and from 14 patients with NHL. The IL-2 production of PWM-stimulated MNC of patients with NHL was found to be significantly decreased, wheras the IL-1, IL-6 and TNF-alpha release were not changed significantly. These data showed a good correlation with the reduced capability of MNC from patients with NHL to proliferate after mitogenic stimulation. The multifunctional cytokine Transforming Growth Factor-ß (TGF-ß) is known to inhibit the DNA synthesis, as well as the IL-2 production of mitogen-stimulated MNC. However, TGF-ß release was not significantly changed in cell culture supernatants from patients with NHL in comparison to healthy controls. We conclude that the suppressed DNA synthesis and IL-2 production of MNC from patients with NHL is not the consequence of a decreased TGF-ß level secreted by these cells.

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REGULATION OF SOLUBLE CD23 (sCD23) IN B-CLL

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Recently it has been shown that the low affinity IgE receptor (CD23) is overexpressed on B-CLL cells. To evaluate whether its soluble form (sCD23) reflects disease activity and tumor load in B-CLL, we studied serum levels as well as cellular membrane expression of CD23 under various conditions. The concentration of sCD23 was measured in the sera of 45 patients with B-CLL, 50 cases of other lymphoproliferative disorders and 41 healthy donors. The results indicate that sCD23 is highly elevated in B-CLL and immunocytoma patients. Furthermore, advanced disease stages and active forms of the disease are associated with higher serum levels of the molecule. There is a significant correlation between sCD23 and lymphocyte doubling time as well as serum thymidine kinase activity and total tumor mass score (Jaksic Index). The correlation between sCD23 and absolute lymphocyte counts, however, is poor. Repeated measurements of sCD23 over a period of two years enabled us to demonstrate the importance of sCD23 in monitoring disease progression. – While under *in vitro* conditions the density of membranous CD23 on CLL-cells correlated well with the concentration of sCD23 in the supernatant, there was no correlation *in vivo* between CD23 on circulating B-CLL cells and sCD23 levels in serum, thus pointing to additional sources of the soluble molecule.
The results indicate that sCD23 is a highly sensitive and specific marker for B-CLL. It has great prognostic potential and seems to be a useful parameter for monitoring disease activity and tumor load.

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CLINICAL OUTCOME OF SEVEN AML PATIENTS WITH TRANSLOCATION T(8;21)

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Specific chromosomal aberrations are associated with certain subtypes of acute myeloblastic leukemia (AML). The balanced translocation t(8;21)(q22;q22) is associated with AML with maturation and certain characteristic cytomorphological features: giant granules, single Auer rods, abundant basophilic cytoplasm and bone marrow eosinophilia. From 1/81 to 3/93 7/263 patients diagnosed with de novo AML in our department showed a t(8;21), 6/7 with additional cytogenetic abnormalities. According to the FAB classification 5 pat. had M2 and 2 pat. had M4. Immunologic phenotyping revealed high expression of HLADR, CD33 and CDw65. These pat. (5 male, 2 female) with a median age of 39 years (range 22 - 58) received induction chemotherapy according to standard regimens using cytarabine (100 mg/m^2/d x 5-7) and doxorubicin (45 mg/m^2/d x 3) or aclaplastin (30 mg/m^2/d x 3) combined with high dose cytarabine (2g/m^2/12h for 2 days). The 2 pat. with M4 were given additional etoposide (100 mg/m^2/d x 5-7). 6/7 pat. obtained complete remission (CR) after the 1st cycle, one pat. needed a second cycle. In CR, cytogenetics showed a normal karyotype. CR lasted 7 - 13+ months, overall survival was 11 - 23+ months. Pat. with loss of sex chromosome (2 pat.) and one congenital Rett's syndrome showed shorter remission (7, 9, 10 mo.) than the other 4 pat. (10, 13, 13+, 13+ mo.). 1 pat. underwent allogenic bone marrow transplantation and is still alive (18+ mo.). 2 pat. developed extramedullary infiltrations (meningeosis, skin), 3 pat. are alive (14+, 15+, 23+ mo.).
Our results confirm that CR rate is high (7/7), loss of the sex chromosome worsens prognosis and additonal aberrations are frequently found.

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LONG-TERM CLINICAL COURSE OF A PATIENT WITH B-PLL AND TRANSLOCATION T(8;14)

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**Chronic lymphocytic leukemia (CLL) and prolymphocytic leukemia (PLL) are distinct clinical entities distinguished by cytomorphologic, cytochemical and immunologic criteria. PLL compared to CLL is characterized by massive leukocytosis, splenomegaly without lymphadenopathy and poor response to chemotherapy. We report on a 61 year old female pat. with PLL diagnosed on admission in 3/83: WBC was 16 G/l, a few small collar lymphnodes and normal spleen were found. Bone marrow aspiration and blood smears were typical for PLL. The pat. needed no therapy till 9/84, when prednimustin and later chlorambucil p.o. were administered for increasing WBC. Because of progression, intermittend COP therapy was given from 12/86 till 2/88 resulting in stabilisation. Despite intensified chemotherapy skin infiltrations developed in 6/91; WBC was 240 G/l and LDH > 3,000 U/l. Lymphaphereses combined with multidrug chemotherapy were performed. Due to the refractory state of disease the pat. died in 7/91. In 12/88 cytogenetic analyses revealed 2 clones with t (8;14): (I) - 45,xx, der$_1$1,6q-, t(7;16),t(8;14), t(1;17); (II) - 45,xx,6q-,t(1;8;14), t(7;16),t(1;17). Immunologic analyses showed SIg, CD5, CD19 and CD24 positive cells, CD10 was negative. During progressive disease in 6/91 cytogenetics showed disappearance of clone II and development of another clonal aberration: (III) - 46,xx,der$_1$1,der$_2$1,6q-,t(7;16),t(8;14), t(1;17),-15. Immunologic analyses showed loss of SIg and CD5 expression, CD19 was unchanged.
Although the pat. showed typical cytomorphological and immunologic features of B-PLL there was an atypical long clinical course. Both change of marker expression and cytogenetic findings revealed high malignancy of this disease.**

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INFLUENCE OF IL-4 AND IL-10 ON THE IN VITRO DIFFERENTIATION OF BLAST CELLS FROM PATIENTS WITH ACUTE MYELOID LEUKEMIA

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IL-4 and IL-10 are anti-inflammatory cytokines which are produced by activated T-cells. The influence of IL-4 on the CSF-induced proliferation of AML cells has already been studied, however, the effects of these interleukins on differentiation of blast cells (AML) are unknown.
GM-CSF has been shown to markedly induce monocytic differentiation in human myeloid cell lines and in primary AML cells from patients. Here we studied the influence of IL-4 and IL-10 on GM-CSF induced differentiation in myeloid blast cells using the Nitro-blue-tetrazolium (NBT) assay.
Whereas IL-4 alone had no effect on differentiation it inhibited the increase of NBT positive cells by GM-CSF in a dose dependent manner. Similar results were obtained in blast cells from five patients with AML. In contrast, IL-10 had no effect on GM-CSF induced differentiation of U937 cells or primary blast cells, respectively.
Our results suggest that IL-4 may play a pathophysiological role in the disturbed maturation of leukemic cells in patients with AML.

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THE ROLE OF THE GLUTATHIONE REDOX CYCLE IN DRUG RESISTANCE OF AML.

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The different cell subpopulations (CSP's) in AML bone marrow and peripheral blood samples were determined by flow cytometry in 50 patients (pt's). The expression of CD34, CD38, HLA-DR, CD13, CD33, CD15, CD2, CD7, CD9, CD10, CD14, CD19 and CD41 was measured. For determination of MDR we examined the inhibition by verapamil of the rhodamine123 efflux (I-R123-E) of the different CSP's. R123 is a vital dye which is effectively pumped out of the cytoplasm by the gp170 protein. In 11 pt's the glutathione redox cycle capacity (GRCC) and the I-R123-E were measured. For quantification of reduced glutathione (GSH) ortho-phthaldialdehyde (OPT) was used. The GSH quantity can be measured because the OPT-GSH binding can be inhibited selectively by mercury chloride. The difference between the fluorescence of the cells without and with mercury chloride corresponds to the GSH-OPT quantity (GSHQ). The maximal velocity of the reduction of oxidated GSH (V-GSH) in the CSP's after exposure to oxidative stress with hydrogen peroxide was determined. The time to a 50% reduction of the oxidated GSH (GSH50), and further the maximal velocity of the recovery of the GSH content in relation to GSH content of the cells prior to the oxidative stress (V-GSH/GSHQ) were determined as parameters for GRCC. We found a correlation between high I-R123-E and the expression of antigens that indicate immaturaty. The CD34 strong positive and CD38 weak positive CSP had the highest and the CD34 negative and CD15 positive CSP had the lowest I-R123-E. A weak correlation was found between the maturity of the blasts on the basis of antigen expression and a high V-GSH, V-GSH/GSHQ and GSH50. But there were exceptions and also immature blasts may show a high I-R123-E and high GRCC.

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THE ROLE OF THE GLUTATHIONE REDOX CYCLE IN DRUG RESISTANCE OF MULTIPLE MYELOMA.

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The different cell subpopulations (CSP's) in myeloma bone marrow were characterized by flow cytometry using two-parameter analysis. The expression of CD38, CD56, CD9, CD10, CD19, CD20, CD24, and CD34 was measured. In 21 patients (pt's) the glutathione redox cycle capacity (GRCC) were also determined in the different CSP's. For quantification of reduced glutathione (GSH) ortho-phthaldialdehyde (OPT) was used. The GSH quantity can be measured because the OPT-GSH binding can be inhibited selectively by mercury chloride. The difference between the fluorescence of the cells without and with mercury chloride corresponds to the GSH-OPT (GSHQ) quantity. The maximal velocity of the reduction of oxidated GSH (V-GSH) in the CSP's after exposure to oxidative stress with hydrogen peroxide was determined. Further the maximal velocity of the recovery of the GSH content in relation to GSH content of the cells prior to the oxidative stress (V-GSH/GSHQ) was determined as second parameter for glutathione redox cycle capacity (GRCC). For determination of MDR we examined simultaneously the inhibition by verapamil of the rhodamine123 efflux (I-R123-E) of the different CSP's. R123 is a vital dye which is effectively pumped out of the cytoplasm by the gp170 protein. We found a high variability of V-GSH between the different pt's. Within the different CSP's the V-GSH increases generally in the following order : lymphoid, myeloma, and myeloid cells. In 30% of all cases the highest V-GSH was found in the myeloma cells. But overall the myeloma cells had never the lowest V-GSH. The order of the CSP's on the basis of V-GSH/GSHQ is different. In 15% of the examined cases the lymphoid cells had the lowest and in 30% the highest and the Myeloma cells had in 30 % the lowest and in 20% the highest V-GSH/GSHQ. In conclusion the lymphoid cells had the lowest GSHQ and lowest V-GSH but often they reached first their initial GSH level after oxidative stress. Therefore these lymphoid cells had a high GRCC. In contrast the myeloma cells had never the lowest V-GSH but reached often their initial GSHQ later. These myeloma cells had a low GRCC. The evaluation of a greater number of patients will show if there is a significance of these GRCC parameters.

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OnkoDat® - A documentation and information system to ease work and provide quality-control in a department of haematology and oncology.

M. Reng, S. Pabst, M. Burkert, J. Schölmerich, R. Andreesen

We developed the multiuser SQL-database-system OnkoDat primary to ease work and standardize diagnostic and therapeutic procedures in a department of haematology and oncology. OnkoDat consists of three integrated parts. The first is a global tool that offers helpful services such as calculation of body-surface-area, a personal adress-list and more. Secondly, there is an individually adaptable haematological-oncological information system. This information system focuses on generics, chemotherapy regimens and diagnostic procedures and finally offers therapeutic advice. The third part of OnkoDat, the patient-specific documentation, is closely linked to the other two parts in the system. So the information system can help to find the proper diagnosis for the individual patient. Furthermore - based on the chemotherapy-regimen information - the computer can automatically calculate the schedule of the chosen chemotherapy for this patient and print all necessary forms. Daily or cumulative maximal doses of generics can both be surveyed individually. The patient documentation system records case history, clinical and laboratory findings, and individually designable patient specific variables or comments. It generates various documents, and even supports data acquisition for clinical studies. OnkoDat is a tool for the use by clinician both in clinical and scientific work. Therefore it is necessary to maintain correct and complete data in the database at any given time. This leads to the concept of data acquisition where they are generated - the input of data into the computer is done by the clinicians themselves. Only if everyday work is rationalized and a unique benefit for work is provided by the system, a continuous and careful use can be ensured. Therefore the design of OnkoDat had to be different from common databases. OnkoDat represents everyday work on the screen in a simple format. It is the task of the machine to prepare the complex data in the background so that they are suitable for the database. The front-ends of the system, now designed under MS-Windows 3.1, are intuitive conceivable. As a lot of the conceptional work on OnkoDat was spent on connectivity, data-exchange with other disciplines or computer systems is easily possible. With additional tools, that have partially been designed, OnkoDat will be useful in the interdisciplinary treatment of patients.
The first prototype of OnkoDat has now been operating for more than two years. Next task to realize is the on-line connection of cooperating hospitals for exchange of global and - as far as possible and necessary - patient data. The integration of information system and patient documentation predisposes OnkoDat to be a powerful system for quality control and improvement of quality in clinical and scientific work in haematology and oncology.

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The Minimal Basic Data Set - A pragmatic software-interface for communication in medicine focussing on Haematology and Oncology

M. Reng, B.Tege, S. Pabst, J. Schölmerich, R. Andreesen

In haematology and oncology the trend is more and more towards out-patient treatment, due to the lower costs of treatment and due to an increase in the quality of life of out-patients. This leads to an increase of patient specific data acquired outside the hospital. Ever since a lot of information has to be collected about our patients from other disciplines like surgery or radiology. Thus a heterogeneous set of data on each patient exists as hand- or typewritten material, in text or database files.

Only the storage of a complete set of data concerning a single patient can give sufficient information about the patient's history, course of disease as well as incidence and mortality.

Usually computer-programmes in medicine are designed for documentation of a special set of highly structured data. An example is the design of any study-treatment-protocol. In contrary, the conventional way of data exchange between clinicians and the attending doctors is a low-structured heterogeneous report, which contains only a summary of individually preselected, relevant informations.

Several attempts have been made and to enable paperless communication in medicine. These communication protocols all consist of highly structured data and request an exactly defined software-design on both sides, the sender and the recipient, to make communication possible. Communication on a lower level, on the basis of merely unstructured data is usually not possible. Our attempt was to develop a basic software interface that should be able to transfer all different kinds of clinically and scientifically interesting patient-specific data from any system to the other. This led to the development of the so called 'minimal basic data set' (MBDS) that is required for all data exchange. All data that exist in a higher structured data subset are transformed into this low-structured data set by the sender. After the transfer, the recipient can either read the transmitted information as such without any further data processing or - if his software uses higher structured data sets - rebuild a high-structured data set from the MBDS.

With the help of this MBDS an exchange of information between all different sets of hard- or software can be easily performed.

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CURATIVE TREATMENT OF ESTABLISHED HUMAN TUMORS IN SCID MICE BY T CELLS AND A COMBINATION OF BISPECIFIC MONOCLONAL ANTIBODIES

Christoph Renner, Christoph Pohl, Wolfram Jung, Ralf Denfeld, Ugur Sahin, Volker Diehl and Michael Pfreundschuh

Crosslinking of tumour-associated antigens with the T cell associated CD3 and CD28 antigens can increase interleukin-2 secretion, proliferation and antigen-specific cytotoxicity in resting T cells. To investigate whether the enhanced activation of CD3-preactivated T cells by additional activation via the CD28 antigen can be exploited for the immunotherapy of human tumours, we generated bispecific monoclonal antibodies (Bi-MAbs) with reactivity to CD3 or CD28 and the Hodgkin's associated CD30 antigens, respectively. Using a combination of CD3/CD30 and CD28/CD30 Bi-MAbs, an antigen-dependent cytotoxicity was induced by targeting antigen presenting cells (APC) depleted peripheral blood lymphocytes (PBL) to $CD30^+$ Hodgkin's derived L540 cells. Human PBL T cells, activated by CD3/CD30 in the presence of CD30 antigen induced 100% complete remissions in human Hodgkin's derived tumors established in SCID mice if administered together with a combination of CD3/CD30 and CD28/CD30 Bi-MAbs. As human T cells, but no NK cells or granulocytes were detected in the tumours shortly after application and remained there for prolonged periods, the regression of the human tumours in SCID mice seems to be mediated by human T cells activated and targeted to the tumour by the combination of CD3/CD30 and CD28/CD30 Bi-MAbs. In contrast to gene-therapeutic approaches which also try to employ the T-cell stimulating activity of the CD28 antigen and/or its ligand, the Bi-MAb approach is simple, effective and readily applicable to the clinical situation.

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C-KIT POSITIVE ACUTE MYELOID LEUKEMIA IS NOT CORRELATED WITH A DISTINCT IMMUNOPHENOTYPE AND POOR PROGNOSIS

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Antigenic profiles in AML that have prognostic significance and allow treatment stratification have not yet been defined. In a previous report of Ashman et al. the expression of c-kit defined by binding of the moab YB5.B8 was found in about one third of AML cases, mainly of the undifferentiated FAB-subtypes and associated with poor prognosis and overall survival.

In this study the moab 17F11 directed against c-kit stained 33/38 AML and 4/6 CML-blast crisis samples whereas all 34 ALL specimens were c-kit negative. C-kit was not restricted to any particular, especially undifferentiated FAB-subtype, but found in 7/7 AML-M0/M1, 15/16 AML-M2, 10/12 AML-M4 and 1/3 AML-M5-subtypes. The precise immunophenotypical analysis showed no restriction of c-kit expression to immature, CD34+ precursors, but c-kit was also found on CD4+, CD34- precursor cells differentiating towards the monocyte lineage. Extraordinary heterogeneity of concomitant antigen expression on c-kit+ cells was shown by triple staining experiments. So 12/28 c-kit+ samples were CD56 and/or CD7 positive, 2 c-kit+, CD34+ specimens carried the B-cell antigen CD19. Furthermore, no significant difference in remission rate and survival in correlation to the percentage of c-kit+ cells and their strength of expression could be observed.

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FREQUENT OCCURRENCE OF DEL(5q) AND OTHER "MYELOID" CHROMOSOME ABERRATIONS IN ADULT ACUTE LYMPHOBLASTIC LEUKEMIA (ALL)

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Deletions of the long arms of chromosome 5, chromosome 7, chromosome 20, as well as the loss of an entire chromosome 7 or of the Y chromosome are well recognized chromosome aberrations in myeloid hematological malignancies, but have rarely been reported in lymphoid neoplasias. We found such "myeloid" chromosome abnormalities in 12 (8.2%) out of 146 adult patients with newly diagnosed ALL. A del(5q) was seen in 3 (2%), a missing chromosome 7 or a partly deletion of its long arm in 7 (4.7%) cases. Two patients demonstrated loss of part of the long arm of chromosome 20, and one showed not-age-related lack of the Y chromosome. All patients had additional chromosome changes, seven a Philadelphia-translocation. In two cases diverse "myeloid" chromosome aberrations were observed.

By morphological and cytochemical criteria all cases were classified as ALL. Immunophenotyping disclosed a common-ALL in nine patients. Deletions of part of the long arm of chromosome 5 were associated with pre-pre-B- in two, and pre-T-ALL in one case. Myeloid antigen expression was found in one of 9 cases investigated.

"Myeloid" chromosome aberrations may characterize a cytogenetically distinct subgroup of adult ALL, possibly of significance with respect to the biology of the leukemia and to the prognosis of the disease. These aspects will be discussed in detail within the presentation of the cytogenetic data.

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EFFECTIVE ADJUVANT TREATMENT OF RESECTED COLORECTAL CANCER DUKES C WITH MURINE MONOCLONAL ANTIBODY.

G.Riethmüller, E.Schneider-Gädicke, G.Schlimok, W.Schmiegel, R.Raab, D. Tübergen, K.Hoeffken, R.Gruber, H.Hirche

Numerous failed clinical trials with monoclonal antibody have led to a general demise of passive antibody therapy for solid tumors. One of the explanations for this failure has been insufficient accessibility and heterogeneity of tumor cells in advanced solid metastases. Therefore we have performed a prospective randomized clinical trial in minimal residual disease in colorectal cancer Dukes C after curative surgery, targeting 17-1A a murine IgG_{2a} antibody to dispersed epithelial tumor cells. 189 patients were assigned by a dynamic randomization procedure to postoperative treatment with antibody 17-1A (500 mg + 4x100 mg in monthly infusions) or to an observation regimen only. After a median follow-up of 5 years antibody treatment reduced the overall death rate by 30 percent (log-rank: $p = 0.05$, cox proportional hazard: $p = 0.04$) and decreased the recurrence rate by 27 percent (log rank: $p = 0.05$, cox proportional hazard: $p = 0.03$). As to the pattern of recurrences, the effect of antibody was most pronounced on the manifestation rate of distant metastases as the first event ($p < 0.002$) which was not seen in local relapses ($p = 0.57$). Toxic effects of monoclonal antibody 17-1A were infrequent and only minor, consisting mainly of mild general and gastrointestinal symptoms. Immunogenicity of 17-1A was low, inducing antibody titers in all treated patients. However, during 371 infusions only 4 anaphylactic reactions were observed, all controllable by intravenous steroids and not necessitating hospitalization. Thus, adjuvant treatment with 17-1A antibody extends life and prolongs remission in patients with colorectal cancer of stage C.

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DOUBLE TARGET IN SITU HYBRIDIZATION APPLIED TO THE STUDY OF NUMERICAL ABERRATIONS IN CHILDHOOD ACUTE LYMPHOBLASTIC LEUKEMIA (ALL)

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A hyperdiploid karyotype with more than 50 chromosomes in children with acute lymphoblastic leukemia (ALL) is well-known to be a parameter for good prognosis, and an even better outcome is described, when certain chromosomes are found to be trisomic in the same cell. Because of the poor metaphase quality which is often found in these patients, the feasibility of fluorescent in-situ-hybridization on interphase nuclei and metaphases of leukemic bone marrow cells was tested. Patients were selected on the basis of being trisomic or tetrasomic for chromosomes 6, 10, 17 and 18 by GTG-banding. We performed double target FISH using DNA probes specific for the centromeric regions of these chromosomes, i.e. #6 and #10 and #17 and #18 were applied in combination. The data obtained by FISH on metaphases corresponded with those obtained by FISH on interphase nuclei. Interphase FISH demonstrated the presence of one or more groups of cells with different combinations of trisomy and tetrasomy of the two chromosomes, which could not be detected in GTG-banded metaphases. These findings indicate that interphase FISH analysis could be a useful method to detect the presence of numerical aberrations, the combination of two trisomic chromosomes in one bone marrow cell, and sometimes also clones which cannot be found by classical cytogenetics. This technique can therefore be used as an additional tool for leukemia classification in children with ALL.

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MOLECULAR ALTERATIONS IN A PATIENT WITH TURCOT'S SYNDROME

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Turcot's syndrome is a rare hereditary disorder characterized by the association of colonic polyposis and colorectal carcinoma (CRC) with primary tumors of the central nervous system. No information on somatic or germline molecular alterations in Turcot's syndrome has been published so far. We evaluated malignant and non-malignant tissues of a 15-year-old patient with Turcot's syndrome and of her parents for the presence of molecular alterations in several oncogenes and tumor suppressor genes. Mutation-specific oligonucleotid hybridization and direct sequencing were used for the analysis of mutations of the Ki-ras and the p53 gene, respectively. Expression of the p53, the MDR1, the c-myc, and the nm23 gene was evaluated by differential PCR. Loss of heterozygozity (LOH) on chromosomes 9p and 17p was determined by differential PCR and by PCR amplification of highly polymorphic regions in tumors and normal tissues. A codon 175 point mutation of the p53 gene was found in a skin and a liver metastasis of the CRC, and a p53, codon 273 mutation was detected in an astrocytoma of the patient. Deletions on chromosome 17p, the locus of the p53 gene, were present in both the skin and the liver metastasis but not in the astrocytoma. In addition, a Ki-ras, codon 12 mutation was detected in a lymph node, the skin, and the liver metastasis of the CRC but not in the sigmoid primary or in the astrocytoma. A 9p deletion was not observed in any of the tissues analyzed. Overexpression of the c-myc, MDR1 and nm23 was found in the patient's diverse malignant tissues. No germline alteration of the genes examined could be detected in the patient or her parents. We conclude that genetic changes similar to those found in sporadic tumors were responsible for the development and progression of malignancy in this patient with Turcot's syndrome. Germline alterations different from the oncogene and tumor suppressor gene changes analyzed, however, must have been responsible for the genetic predisposition to tumor development in our patient.

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EXPOSURE PROTOCOL INFLUENCES THE MULTIDRUG RESISTANCE REVERSING CAPACITY OF CALCIUM/CALMODULIN ANTAGONISTS

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The occurence of multidrug resistance (MDR) in tumor cells is still a major problem in cancer chemotherapy. For restoring drug sensitivity several combination therapies using calcium/calmodulin antagonists together with cytostatic drugs have been tested. Dexverapamil and Dexniguldipine-HCL (B8509-035) are very potent chemosensitizers with lowest cardiovascular activity. To find an optimal clinical application protocol for these modulators, we examined the influence of exposure time and sequence of modulator administration on the active P-glycoprotein mediated transport of the fluorescence dye rhodamine 123 using flow cytometric analysis. In the case of Dexverapamil chemosensitizing capacity is lost if the drug is not present during the administration of R123. In contrast Dexniguldipine-HCL (B8509-035) was retained in the tumor cells, even after a longer incubation period in pure culture medium optimal R123 accumulation was achieved. Our results clearly demonstrate, that effectiveness of restoring sensitivity of MDR cells to cytostatic drugs depends on the administration schedule, which has to be consequently adapted to the used modulator.

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THE USE OF CYTOKINE/ANTIGEN-GENTRANSFER IN THE INDUCTION OF TUMOR SPECIFIC IMMUNE RESPONSES

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In syngeneic or autologous hosts, cancer cells are poorly immunogenic. The weak cellular reactions that occasionally can be demonstrated during early stages of tumor growth do generally not lead to tumor regression and become progessively impaired by tumor-induced immunosuppressive mechanisms. Local secretion of certain cytokines by cytokine gene transduced tumor cells, however, can induce potent tumor-specific cellular immune responses and result in inhibited tumor growth in vivo. We have studied the immunological consequences of retroviral transfer of IL-2, IFN-γ and GM-CSF in several murine tumor models and have reported that either IL-2 or IFN-γ secretion by CMS-5 murine fibrosarcoma cells induces specific antitumor immunity. To examine whether immunity induced by single cytokine secreting tumor cells could be enhanced by inducing the same tumor cell to provide two stimulatory signals, we used a retroviral vector carrying the IL-2 and IFN-γ cDNAs to transfect CMS-5 cells. Our data show a synergistic effect on induction of tumor-specific immunity and on inhibition of tumor growth. In vivo depletion of $CD4^+$, $CD8^+$ or NK cells suggests that $CD8^+$ CTL are primarily responsible for tumor rejection. To investigate whether a specific immune response could also be induced against mutated self proteins, we constructed retroviral vectors carrying both a mutated p53 gene and the IL-2 cDNA. Fibroblasts were stably transduced and clones secreting high amounts of IL-2 and expressing p53 were used to immunize mice. The ability to stimulate a CTL response was analyzed and effects on growth of p53 expressing tumors were studied.

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REARRANGEMENT OF THE IMUNOGLOBULIN HEAVY CHAIN GENE IS A RARE EVENT IN HODGKIN AND REED-STERNBERG CELLS AS SHOWN BY SINGLE CELL PCR

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The rearrangement of the immunoglobulin- and T-cell receptor gene locus offers a unique marker for origin and clonality of hematopoeitic cells. We describe a PCR-based method to amplify the rearranged VDJ-region of the immunoglobulin heavy chain gene (IgH-gene) from single lymphoma cells isolated by micromanipulation.

For detection of rearrangements of the IgH-gene six different "forward-primers" were constructed corresponding to the six known families of the IgH- variable region (FR I- region). A mix of two "reverse primers" was used corresponding to consensus sequences of the different J-regions. A PCR product of ca. 350 bp in length was obtained from cells that had rearranged their IgH-gene. If no rearrangement had taken place, the primer binding sites were too far from each other to yield a PCR product. The PCR-assay was tested using peripheral blood lymphocytes, where rearrangements of all six known V-families were detected. Similarly, rearrangements could be amplified from single cells of the Raji cell line (V_3DJ) and of a Non-Hodgkin lymphoma (V_6DJ). However, no rearrangements could be detected in single H&RS-cells isolated from biopsy tissue by micromanipulation of eight different patients (four mixed cellularity-, three nodular sclerosing- and one lymphocyte depleted-subtype), whereas sequences of the β-actin gene were amplified from single H&RS-cells in a parallel reaction. We therefore conclude that in contrast to previous results obtained with analysis of Hodgkin's disease tissue instead of isolated H&RS cells, a IgH rearrangement in the neoplastic cells of Hodgkin's disease is a rare event.

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A SIMPLE PCR-BASED TEST TO DETECT BICLONALITY IN B-CELL LYMPHOMAS

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Chronic lymphocytic leukemia (CLL) is associated with the development of secondary neoplasms, including B-cell malignancies. However, blastic transformation of CLL - Richter's syndrome - may also occur. To distinguish between these two possibilities in CLL patients presenting with a second lymphoma, a simple and reliable PCR test was developed. This test allows determination of V family usage and determination of bi- or monoallelic rearrangement of immunoglobulin genes. A 62-year old male patient presented with multiple myeloma (stage I A acc. to Durie and Salmon) and concomitant chronic lymphocytic leukemia of the B-cell type (stage II acc. to RAI). DNA was extracted from PBL and paraffin-embedded bone tissue from the site of a pathological fracture. PCR was performed employing primers to the six known variable gene families and the joining region of the immunoglobulin heavy chain gene, resulting in PCR products of about 350 bp length. PCR products were blotted and hybridized to an internal oligonucleotide probe. A monoallelic V3-D-J rearrangement was detected in the peripheral blood lymphocytes, whereas a V1-D-J rearrangement was present in the myeloma specimen. VH gene substitution was excluded by sequencing of the PCR products. Biclonality and therefore independent development of the two lymphatic neoplasms was proven by these experiments. We demonstrate the application of this rapid and simple approach to further cases of double lymphomas.

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A KNOWLEDGE BASED EXPERT SYSTEM SHELL USING A SEMANTIC NET OF ONCOLOGY AND HAEMATOLOGY (NOAH)

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A medical diagnostic expert system of haematology and oncology is presented. It works with a knowledge base which was developed from an algorithm of diagnosis of anemias. In this first step the functional scope of the decision making system contains hypo-, normo- and hyperchromic anaemias. The system is able to process history details, physical findings and laboratory data for supporting the physician in his diagnostic decision. Depending on introductory questions like sex, age and clinical signs ("fatigue, pale, jaundice" etc.) the system generates a few diseases that should be examined next. For this investigation the system asks questions which are necessary due to its inference machine. The user, however, is able any time to enter findings which seem important to him. This medical decision making system is based on a disease model of anaemia by looking not only for findings and final diagnoses but also for intermediate steps of diagnosis (semantic net). The goal is to set up some heuristical behavior features of the physician: Most frequent diseases, generating hypothesis depending on combinations of findings, early excluding a diagnosis or persuing a diagnosis when it is promising a good result. In future steps the system should be expanded by following features: The disease of anaemia will be supplemented by haematological neoplasias such as leukemias and malignant lymphomas as well as by solid tumors; the system should get the input data automatically from the laboratory devices and the diagnosis should be followed by recommendations of therapy as well as by an automatic generation of a summarizing medical outline report which may be refined by any word processor.

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Evidence of wild and mutant type p53 in the mucous membrane of bronchial carcinoma and pleuramesothelioma by histochemical staining

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Studies of brain, breast and testicular cancers showed, that in the majority of cases where p53 was deleted there was a detectable mutation in the remaining p53 allele. This causes tumor progression by loss of growth control by functional inactivation of p53 gene.
Patients and Methods:
Frozen specimens from 9 patients with various types of bronchial carcinoma and 2 patients with pleuramesothelioma were analyzed.
In addition, we examined mucous membrane specimens from seven healthy persons. Histochemical staining studies were performed to investigate the presence of wild and mutant type p53.
The following monoclonal antibodies were used:
Clone PAb 1801,derived by fusion of BALB/7c splenocytes with NS-mouse myeloma cells, clone PAb 240, derived by immunization of BALB7c mice with p53-B-galactosidase fusion protein and fusion of splenocytes with SP2 mouse myeloma cells, clone 1620, derived by immunizing BALB/c mice with VLM tumor cells and fusion of splenocytes with SP/0-Ag 14 mouse myeloma cells, and Clone DO-1 derived by fusion of BALB/c splenocytes with NS-1 mouse myeloma cells.
Results :
The p53 protein in the mutant conformation was localized in the cell cytoplasma in 6/9 bronchial carcinoma and in 2/2 pleuramesothelioma, whereas the wild type was less frequent in the cytoplasm but mainly located in the cell nucleus in numerous but not in all tumor cells in 6/9 bronchial carcinoma and in 2/2 pleuramesothelioma. Both protein types could be found in the same tumors.
Neither the wild nor the mutant type could be detected in the specimens of the seven healthy persons.
In the same tumors we also found EBV-DNA, HSV(I+II)-DNA, HHV6-DNA, CMV-DNA and Adenovirus 2 E1A (Types 2 and 5).
Discussion:
The evidence of wild and mutant type p53 in human bronchial carcinoma and pleuramesothelioma is consistent with the view, that alterations of tumor-suppressor genes play a role in the pathogenesis of this tumor type together with herpes viruses.

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HEMOSTATIC DISORDERS ASSOCIATED WITH ACUTE PROMYELOCYTIC LEUKEMIA

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Acute promyelocytic leukemia (APL) is a distinct variety of acute myelogenous leukemia characterized by the presence of a balanced reciprocal translocation between chromosomes 15 and 17, by the achievement of complete remission (CR) without obligatory bone marrow aplasia, and by the occurrence of potentially life-threatening hemorrhagic complications. Historically, the bleeding diathesis has been attributed to a particular coagulopathy which shares some biological features with disseminated intravascular coagulation, but the mechanisms responsible for hemorrhage are not yet completely understood. Bleeding results, at least in part, from the release of tissue procoagulant activity present in the azurophilic granules of APL cells. A fibrinolytic/proteolytic activity due to leukocyte proteases has been proposed as an important additional event. Aggressive chemotherapy leading to APL cell lysis may induce or amplify the release of both procoagulant and fibrinolytic activities but the optimal therapeutic strategy for the prevention and control of bleeding is currently not known. Recently, all-*trans*-retinoic acid (ATRA) has been reported to promote terminal differentiation of leukemic promyelocytes, resulting in CR rates of 65% to 95% with rapid correction of the coagulopathy. Despite these beneficial effects, ATRA therapy might not represent the optimal approach for control of hemostatic disorders in APL patients because of the frequent occurrence of ATRA-related hyperleukocytosis, which may be associated with fatal thromboembolic complications.

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STUDIES OF B CELL MARKERS IN PERIPHERAL BLOOD AND BONE MARROW IN CLL PATIENTS

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A comparative research on lymphocyte immunological phenotypes in peripheral blood and bone marrow was carried out on 16 patients with B-cell chronic lymphocytic leukaemia. Three types of B cells markers were estimated: 1-light chains/lambda or kappa/ on the surface of cells, 2-CD 5 antigen, 3-capability for forming mouse erythrocyte rosettes /MER/ the last two markers are characteristic mostly for leukaemic cells/. The patients were divided into 3 groups according to the localization of the main mass of tumorous cells: group I-the patients, whose clinical picture was dominated by the infiltration of lymphoid structures of the abdominal cavity and/or with massive infiltration of peripheral lymph nodes; group II-the patients, whose clinical picture was dominated by the infiltration of bone marrow; group III-th intermediate-patients with large mass of tumor in lymphoid structurs and with bone marrow insufficiency symptoms. In most of the patients of group I, the centage of *CD 5 + cells in peripheral blood was higher than the one in bone marrow /arithmetic means were 38,5% for blood and 26,7% for marrow/. It was the reverse in group II/the means were 44,8% for blood and 64,3% for marrow, P<0,01/. The percentage in group III was similar to that of group I/the means 48% for blood and 29,25% for marrow/. No correlation between the percentage of CD 5+ and MER+ cells was found in respective groups of the patients although the last marker behaved similarly to CD 5. The results obtained suggest that while estimating percentage of CD 5+ cells in peripheral blood and bone marrow, one can differentiate between chronic lymphocytic leukaemia /with domination of CD 5+ cells in bone marrow and leukaemic phase of small lymphocyte lymphoma/ with domination of CD 5+ cells in peripheral blood/.

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Clonogenicity of AML Progenitor Subpopulations Defined by CD34 and CD38

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The objective of this study was to further characterise subpopulations of AML progenitor cells with specific regard to their growth potential. CD34 positive/CD38 negative cells have been shown to contain normal progenitors with high growth potential. A similar pattern might be conserved in AML.

Bone marrow cells from 8 patients with AML, 7 newly diagnosed and 1 relapse, were isolated by Ficoll-Hypaque, doublestained with anti CD34 and anti CD38 and sorted. Using a light scatter gate for blast type cells, sorting into CD34+/CD38- and CD34+/CD38+ cells was performed. These subsets as well as stained/non-sorted samples were seeded into a CFU-L assay (methylcellulose, 20% FBS, rhGM-CSF 100U/ml, rhEpo, Pen/Strep, IMDM) and incubated at 37°C, 5% CO_2. Colonies were scored on day 14 at 40x.

6 samples were evaluable. Sorting deminished the clonogenicity of progenitor cells. 5 of 6 samples showed a high yet variable enrichment of clonogenic cells in the CD34+/CD38- subset, 1 sample had a higher enrichment in the CD34+/CD38+ subset.

We conclude that different subsets of AML progenitors resembling different maturation stages of normal progenitor cells in their surface markers display different growth potential.

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RADIATION TREATMENT OF CENTROBLASTIC CENTROCYTIC (cb-cc) NON-HODGKIN-LYMPHOMA IN EARLY STAGES - FIRST RESULTS OF A GERMAN MULTICENTRE TRIAL

H.Sack

From January 1986 till March 1992 171 patients from 44 institutions with cb-cc lymphoma were entered in a German multicentre trial. Minimal follow-up criteria were accessible for 151 (88%) patients. Median age was 51 yrs, sex ratio m = 78, f = 73. 68 pts were in stage I, 59 in stage II and 24 in stage III (not more than 5 involved regions, lymphoma not larger than 5 cm diameter). The patients were treated by extended field RT with 26 Gy to non-involved and 36 Gy to involved regions. The median time to relapse is 16 (2-46) months. The relapse-free 5 year survival rate (Kaplan-Meier) is 82% for stage I, 71% for stage II and 26% for stage III . The percentage rates of recurrences increased by stage up to 42% in stage III. 24 of all 34 recurrences were observed outside the irradiated areas. A detailed analysis of the local and distant treatment failures as well as the prognostic variables (age, stage, site, sex, dose) according to the proportional hazard model of Cox will be presented. The results suggest to apply total nodal irradiation even in stage I, to increase the dose up to 30Gy for non-involved regions and to have a better selection of patients who are treated by radiotherapy alone in stage III.

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AUTOLOGOUS STEM CELL TRANSPLANTATION (ASCT) IN PATIENTS WITH LYMPHOID MALIGNANCIES: A CASE FOR RISK ADAPTED PATIENTS SELECTION

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ASCT is an established post-induction therapy for hematopoietic malignancies like Non Hodgkin's Lymphoma (NHL) and Acute Lymphoblastic Leukemia (ALL), but the inclusion criteria remain controversial.
From 1984 to 1992 we performed 38/52 ASCT at our institution in patients (pts) with NHL or ALL. Inclusion criteria were: high grade histology, stage≥2B with bulky tumor, elevated LDH and slow response to therapy.
20/26 NHL and 11/12 ALL pts were in CR prior to ASCT. 2/7 pts not in CR died during aplasia, the other died of progressive disease in ≤ 9mo.
19/20 NHL pts and 4/11 ALL pts remain in CR from 3+ to 91+ mo. We found no effect of purging on the duration of CR. Conditioning regimens with or without total body irradiation gave similar results.
We conclude, that (1) ASCT is a very effective and safe remission consolidation treatment, (2) non remitting pts are not suitable for ASCT, (3) pts with ALL need different induction therapies to remove residual blasts.

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HYPOFIBRINOLYSIS IN PATIENTS WITH HEPATIC VENOOCCLUSIVE DISEASE AFTER BONE MARROW TRANSPLANTATION

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Hepatic venoocclusive disease (VOD) is a severe complication in patients after bone marrow transplantation (BMT). Injury of hepatocytes and endothelial cells is recognized as central pathogenetic step leading to an activation of the coagulation cascade. Because of the close connection of the endothelial cell system and the fibrinolytic capacity we prospectively investigated parameters of the fibrinolytic system in 32 bone marrow transplant recipients.
Materials and methods: Citrated blood samples were taken before (day -8,-5,-1,0) and weekly after BMT (from day 7 to 35). Tissue plasminogen activator (tPA) and plasminogen activator inhibitor (PAI-1) were measured by enzymimmunoassays (TintElize PAI-1, Imulyse tPA, Biopool, Umea, Sweden).
Results: VOD developed in 4 of 32 patients. The following mean PAI-1 levels (non-VOD versus VOD patients) were found after BMT: Day 7: 12.0 vs. 27.5 ng/ml; day 14: 16.7 vs. 177.3 ng/ml; day 21: 23.2 vs. 80.1 ng/ml; day 28: 28.7 vs 131.6 ng/ml; day 35: 32.5 vs. 98.2 ng/ml; tPA levels showed no significant difference between both groups.
Patients with the complication of VOD after BMT had an about fivefold increased level of PAI-1 antigen in the observed period between day 7 and 35. The only surviving patient showed increasing tPA levels from day 7 to 35. Nevertheless comparing the group of patients with and without VOD no significant difference in the levels of tPA was measured.
We conclude that endothelial cell damage in the course of BMT causes an imbalance of the fibrinolytic system. The resulting hypofibrinolysis seems to be of importance for the occlusion of liver veins in VOD and may explain the successful treatment with recombinant tPA.

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EARLY GANCICLOVIR PROPHYLAXIS OF CYTOMEGALOVIRUS (CMV) INFECTION AFTER ALLOGENEIC MARROW TRANSPLANTATION.

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In order to study the feasibility of early ganciclovir (GC) prophylaxis in CMV risk patients (recipient or donor IgG positive), we designed a protocol of 5mg/kg/bw GC i.v. for 21 days after engraftment (induction course), followed by an maintenance course of 5mg/kg/bw GC five times a week every other week until day+100. From 10/91 to 1/93 48 consecutive patients (pts.), who received an allogeneic marrow transplant for malignant diseases entered the protocol. Reasons for induction course exclusions were: graft failure (n=2), early death (n=2) and poor graft function (n=3). In 4/41 eligible pts. (9,8%) the induction course had to be temporarily interrupted due to grade III leukopenia, and in 2/41 pts. (4,8%) due to grade I renal toxicity.
Of the 40 pts. eligible for maintenance prophylaxis, 27 pts. (67,5%) received at least two weekly courses. Five (18,5%) of these pts. developed grade II thrombozytopenia, and in two pts. (7,4%) the schedule had to be changed due to grade III leukopenia. Eight of 40 pts. (20%) did not receive GC maintenance due to hepatic VOD (2 pts.), relapse (1 patient), poor graft function with GVHD (3 pts.) and compliance problems (2 pts.). Five pts. (12,5%) received only one maintenance course due to thrombocytopenia.
Within the first 100 days posttransplant, 2/48 pts. (4,2%) developed CMV pneumonia and both of these pts. had not received or completed GC induction. With a median onset on day+233 (range +120 to +390) 13/37 pts. (35,1%) surviving more than 100 days posttransplant developed 14 suspected or documented episodes of CMV disease (13 interstitial pneumonias, 1 hepatitis, 1 enteritis). Twelve of these 14 episodes were successfully treated with GC +/- immunoglobuline.
In conclusion the described GC prophylaxis is associated with significant, albeit reversible, marrow and renal toxicity, which appears justifiable by the very low rate of CMV disease within the first 100 days posttransplant. The delayed onset of CMV disease observed in this study suggests that the effect of GC prophylaxis results from suppression of CMV-replication in the early posttransplant course. This, in turn, may improve the prognosis of CMV disease due to the recovering host defense mechanisms.

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IN VIVO EFFECTS OF FLUDARABINE ON IMMUNOCOMPETENT CELLS IN PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKEMIA AND LOW GRADE NON-HODGKIN LYMPHOMA

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Severe infections have been reported in patients under Fludarabine (F-ara-A) therapy. To investigate the influence of F-ara on the immune system, we monitored the relative number of T-cells, NK cells, as well as the phagocytic activity of neutrophils and monocytes using multiparameter flow cytometry. 39 therapy courses were monitored in 17 patients with chronic lymphocytic leukemia and low grade Non-Hodgkin lymphomas. All patients were pretreated with standard chemotherapy and had relapsed or refractary disease. 12 CLL and 2 immunocytoma patients were treated with a five day regimen of F-ara-A 25 mg/m²/d. 3 further patients with immunocytomas received combination therapy with Mitoxantrone, dexamethason and F-ara-A. The absolute WBC decreased in two thirds of all courses. The percentage of T-cells decreased during 21 of 28 courses while it increased during 4 courses. The rate of CD 4 positive/CD 8 positive cells showed a significant increase during 10 of 27 and a decrease in 13 of 27 courses. The number of CD 16 positive NK cells dropped during 17 of 25 courses and increased in 3 of 25 courses. The number CD 56 positive NK cells showed a significant reduction during 11 of 22 courses while it increased in 3 of 22 courses. The phagocytosis test using FITC labeled E. coli showed a significant decrease of activity in neutrophils during 7 of 11 courses. The phagocytic activity in monocytes was reduced in 4 of 11 courses, while it increased in 1 of these courses.
Our results show a highly variable sensitivity of CD 4 and CD 8 positive T-cells to Fludarabine, while the number of NK cells decrease and the phagocytic activity diminished in the majority of patients. We conclude that F-ara-A can influence different components of the immune system which may predispose to an increased susceptibility to infection.

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GENETIC ANALYSIS OF p53 AND MDM-2 IN BLAST CRISIS OF CHRONIC MYELOGENEOUS LEUKEMIA

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The chronic myelogeneous leukemia (CML) is characterized by rearrangements of the c-abl protooncogene and the bcr gene. The molecular events leading to blast crisis have not been well established. Alterations of the p53 gene are absent during chronic phase, but have been frequently detected during blast crisis of CML patients. Based on the possible role of p53 inactivation for the transition from the chronic phase to the blast crisis, we used polymerase chain reaction (PCR) and single stranded conformation polymorphism (SSCP) analysis to detect p53 alterations in one patient during the progression of CML. RT-SSCP analysis demonstrated a band shift using primers for exon 5 to 6 of the p53 gene in the blast crisis, but not in the chronic phase of this patient. Furthermore, p53 mRNA expression was strongly reduced in the blast crisis as determined by differential and semi-quantitative RT-PCR analysis. Interestingly, RT-SSCP analysis using MDM-2 specific primers revealed a band shift in the sample harvested during blast crisis when compared to chronic phase and normal peripheral mononuclear cells. Sequencing of the p53 as well as the MDM-2 gene is in progress. These data confirm previous reports that mutations of the p53 gene occur in blast crisis samples and may contribute to the progression of CML to blast crisis. The role of MDM-2 in this context will be discussed. These results represent the first description that structural alterations of p53 during blast crisis can also be accompagnied by alterations of the MDM-2 gene.

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IMMUNOTHERAPY WITH IFNα AND IL-2: PRETREATMENT PROGNOSTIC PARAMETERS IN METASTATIC MELANOMA (MM)

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Immunotherapy with IFNα and IL-2 is an active regimen in malignant melanoma and has shown response rates of 20 - 30%. In previous studies no prognostic parameters for response could be identified. 64 patients with progressive metastatic melanoma have been enrolled in various immunotherapy trials including IFNα and high dose IL-2 since 1987 with an overall response rate of 31%. 59 patients with MM treated in our phase II trials could be analysed to identify possible prognostic parameters for response. Patients were divided into three groups: responder (3 CR/14 PR), stable disease (16 SD/2MR), and non-responder (24 PD).
We examined the following pretreatment parameters for prognostic relevance of response: age, sex, performance status, time from diagnosis to onset of first metastases/ to begin of immunotherapy, tumor load, number of metastatic sites, organ sites of metastases, LDH, AP, ESR, and HLA-type. Of these several variables were found to significantly correlate with response: tumor load (p=0.023), number of metastatic sites (p=0.045), serum LDH (p=0.005) and AP (p=0.013). Tumor load, LDH and AP are no independent parameters. While time from diagnosis to onset of first metastasis is of no prognostic significance for response, the time between first diagnosis and beginning of immunotherapy, reflecting metastatic disease necessitating systemic treatment, significantly correlates with probability of response (p=0.018). Since several HLA class I alleles have been shown to function as restriction elements for recognition of melanoma cells by specifiфc T cells in vitro, namely A1, A2, B44, and Cw7, we compared the frequency of these HLA antigens between responders and non-responders. We found A1 and Cw7 to be significantly increased in responders vs. non-responders (p=0.05 and 0.034, resp.). Our results indicate that in patients with MM tumor load, number of metastatic sites, LDH, and time from diagnosis to beginning of immunotherapy are prognostic parameters for response to immunotherapy. These parameters may be useful to determine patients with good and poor risk for response to immunotherapy and are of relevance for stratification in randomized clinical trials.

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THE IMMUNOLOGICAL COURSE OF HIV-INFECTION

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In a retrospective single centre study we evaluated the immunological und clinical course of HIV-infection in 507 patients between 1987 and 1992. Out of 1184 HIV-positive patients being treated during this period we included all patients with at least 2 (median=5) evaluations of lymphocyte subsets (CD4+ and CD8+ T-cells) and a minimum observation period of 6 months (med.20,8). (med.=median, mths.=months)

Results: Median duration and lymphocyte subsets in different WR-Stages

WR-stages	WR1	WR2	WR3	WR4	WR5	WR6
	N=23	N=152	N=86	N=61	N=135	N=182
lymphocytes/ul	2818	2478	1629	1440	990	899
med. CD4+cells/ul	805	531	281	222	166	56
med. CD8+cells/ul	790	980	573	654	569	401
Duration in mths.	17,3	24,2	18,6	10,0	22,3	18,8

Median duration of patients in stage WR5 receiving AZT (N=81) is 27.1 mths., the survival 31.6 mths.. The med. duration in WR5 without AZT (N=54) is 10.8 mths. with a survival time of 30.8 mths.. With primary pentamidin prophylaxis (N=65) the med. duration is 28,7 mths. without (N=70) 12.4 mths., the survival time with pentamidin is 34.2 mths. compared with 32 mths. in the group without pentamidin.

Conclusions: This study reveals the slow progression rate towards AIDS, which can be prolonged with antiretroviral medication as well as prophylactic medication. However, these treatment regimens do not seem to alter the natural immunological course of disease.

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5-FLUOROURACIL (5-FU) PLUS LEUCOVORIN (LV) VERSUS 5-FLUOROURACIL COMBINED WITH THE PURE (6S)-STEREOISOMER OF LEUCOVORIN FOR TREATMENT OF ADVANCED COLORECTAL CANCER:

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71 patients with advanced measurable colorectal cancer previously unexposed to chemotherapy were randomly assigned to treatment with either 5-FU (400mg/m2) and conventional (6R,S)-LV (100mg/m2) for 5 days, or the combination of 5-FU and the pure (6S)-stereoisomer of LV using the same dose schedule. In both treatment arms, courses were administered every 28 days if toxicity allowed for a total of 6 months or until evidence of tumor progression. The overall responses (complete and partial response) were 28% and 39% for the 5-FU/conv.LV and the 5-FU/(6S)-LV arm, respectively. Median time to progression or death as well as median overall survival have not been reached in either treatment arm. A comparative analysis of the toxicities experienced by the patients in the two treatment groups showed a comparable rate, though severe side effects were noticed more frequently in patients treated with 5-FU/conv.LV (28% vs. 11%). These results suggest that the therapeutic index of 5-FU/pure (6S)-LV in metastatic colorectal cancer may be superior. Definite conclusions from this phase III study, however, warrant additional patient accrural and follow-up time.

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EXPRESSION OF RETROVIRAL SEQUENCES IN HUMAN BREAST CANCER AND COLON CANCER

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The human genome contains numerous copies of retroviral sequences related to mouse mamma tumor virus (MMTV). Although in animal models the carcinogenic potential of retroviruses is well established, the biological activity of retrovirus related sequences in man is still unclear.
We are investigating the expression of human endogenous retroviruses (HERV) in breast cancer, colon cancer and in non-malignant tissues with reverse PCR. PCR-primers were derived from the gag-, pol- and env-region of HERV-K10 (Positions: 1875-2544, 3935-4545, 7263-7750). HERV-K10 is known to be transcribed in the human breast cancer cell line T47D.
Breast cancer biopsies (n=16), colon cancer (n= 5), blood-leukocytes (n=8) and various epithelial tissues (stomach, small intestine, thyreoid gland) have been analysed so far. In all tissues expression of HERV-K-sequences could be detected with various subsets of primers.
Semiquantitative estimation of HERV-K-expression in relation to ß-actin indicates a variable level of expression in breast cancer and colon cancer and a low level of expression in normal epithelial tissues.
The abundant expression of HERV-K related sequences in various tissues argues for an important role in physiological processes and possibly in pathological processes (e.g. carcinogenesis) as well.

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MODULATION OF THE EFFICACY OF DAUNORUBICIN PLUS HIGH-DOSE CYTARABINE BY DEXNIGULDIPINE IN PATIENTS WITH REFRACTORY ACUTE MYELOID LEUKEMIA

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The occurrence of multidrug resistance (MDR) may be one of the major obstacles to an effective chemotherapy of patients (pts) with acute myeloid leukemia (AML). It is associated with the overexpression of a membrane glycoprotein (Gp-170) acting as an energy-dependent efflux pump for anthracyclines and other xenobiotics. The dihydropyridine derivative dexniguldipine (DEX) has been shown to modulate MDR *in vitro* and was investigated in pts with AML in relapse. Treatment consisted of hAD (2 x 1,000 mg/m^2/d cytarabine i.v., d 2-5, 60 mg/m^2/d daunorubicin (DNR) i.v., d 1-3) followed by hAD & DEX (1,250-2,250 mg/d p.o., d (-2)-7) in case of blast persistence. Gp-170 expression was quantified in peripheral blasts by flow cytometry (FACS) by MRK16-immunostaining. Kinetics of cellular efflux of DNR and rhodamine (R123) was determined in peripheral blasts by FACS. DEX was measured in serum by HPLC. By the addition of DEX 3 responses were induced in 16 evaluable pts (19 %) resistant to prior hAD (group A: 1/7 pts on 1,250 mg/d, B: 0/4 pts on 1,750 mg/d, C: 2/5 pts on 2,250 mg/d). Toxicity of DEX was mild, except moderate cardiovascular side effects in 2 pts. Gp-170 was expressed by 4/5 blast populations in various amounts. Efflux of DNR and R123 was high in 9/11 blast populations and could be inhibited by DEX *in vitro*. Mean serum concentrations of DEX were 0.11 ± 0.052 µM in group A, 0.27 ± 0.11 µM in B and 0.20 ± 0.068 µM in C, respectively. In conclusion, DEX is a most promising drug for the modulation of MDR in refractory AML and needs further investigation when applied intravenously to achieve more effective serum concentrations.

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HEMOLYTIC ANEMIA AND FULMINANT HEPATIC FAILURE AS THE FIRST MANIFESTATION OF WILSON'S DISEASE

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Wilson's Disease should be considered in any young patient with non spherocytic Coombs negative hemolytic anemia of unclear etiology.
Most probably the rapid release of copper from necrotic hepatocytes into the circulation with resulting oxidative damage to red blood cells causes severe hemolysis which frequently occurs in the setting of fulminant Wilsonian hepatic failure together with a characteristic constellation of liver function tests exemplified below.
A 16 year old patient with a two week history of non specific epigastric discomfort and loose stools was admitted to another hospital because of jaundice. After a Coombs negative hemolytic anemia had been found and the serum bilirubin had risen from 12 to 40 mg/dl within 5 days, he was transferred to our department.
On admission physical examination showed deep jaundice but was otherwise unremarkable. His personal and family history were negative. The leading laboratory results were: Hb 7,7 g/dl, MCV 113 fl, Reticulocytes 20%, Leucocytes 23.500/ul, with a left shift, Platelets 170.000/ul, AST 69 U/l, ALT 7 U/l, LDH 579 U/l, Gamma-GT 58 U/l, Alkaline Phosphatase 38 U/l, Cholinesterase 0.80 KU/l, Bilirubin total 40.5 mg/dl, direct 27.5 mg/dl, Thrombin Time 25 sec, Partial Thromboplastin Time 97 sec., Prothrombin Time (Quick's method) 6%, Fibrinogen 190 mg/dl, FDP (D-Dimers) + positive, AT3 18%.
On abdominal ultrasound the liver appeared normal, the spleen was slightly enlarged (14 x 7 cm) and there was a discrete amount of ascites.
The slit lamp examination of the cornea disclosed bilateral Kayser-Fleischer rings. The serum ceruloplasmin level of 8 mg/dl (normal 20-40 mg/dl) and the excessive urinary copper excretion of 1780 ug/24 hours (normal < 70 ug/24 hours) further confirmed the diagnosis.
During the 12 hour hospital stay in our department the patient developed hepatic encephalopathy (grade II) and oliguria. The next day he successfully underwent orthotopic liver transplantation.

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CISPLATIN IN COMBINATION WITH ETOPOSIDE AND 5-FLUOROURACIL IN HUMAN GASTRIC CANCER CELL LINES.

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Cisplatin, etoposide and 5-Fluorouracil (5-FU) are the most active drugs in the treatment of gastric cancer. We studied the cytotoxicity of cisplatin alone and the interaction of cisplatin with etoposide and 5-Fluorouracil (5-FU) in two human gastric cancer cell lines (HM2 and HM51).
METHODS: Cytotoxicity of the individual drugs and the drug combinations were measured with the sulforhodamine B (SRB)-assay. A continuous drug exposure (72 h) was used; cytotoxicity was measured after 96 h. The concentration to inhibit cell growth by 50% (IC 50) was obtained from semilogarhytmic dose-response curves. The interactions of cisplatin with etoposide or 5-FU were assessed using the isobologram methodology (50% isobolograms) and classified as "synergistic", "additive" or "antagonistic". All experiments were done in triplicate. RESULTS: There was a significant difference in the cytotoxicity of cisplatin with a 8 fold relative resistance in the cell line HM2. The combination of cisplatin and 5-FU was highly synergistic in all drug-ratios studied in both cell lines. For the combination of cisplatin and etoposide, a synergistic interaction was demonstrated in cell line HM2, whereas this combination was less than additive in HM51.
CONCLUSIONS: The combination of cisplatin with either 5-FU or etoposide was strongly synergistic in the gastric cancer cell line HM2 which will provide a rationale for combination protocols of these agents in clinical studies. The marked difference seen between the two cell lines concerning the combination of cisplatin and etoposide mechanistic studies about the biochemical nature of the interactions between these cytotoxic drugs.

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EFFECTS OF FLUPHENAZINE ON HUMAN LEUKEMIC CELL LINES

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Earlier studies from our laboratory have demonstrated that lectin- or lymphokine-induced activation of human T-cells and thymocytes can be inhibited by phenothiazine derivatives, which exert their action by reducing the accumulation of lymphokine specific mRNA. The aim of the present study was to investigate the effects of the phenothiazine derivative fluphenazine on the human leukemic T-cell line H33-HJ JA1, which is a Interleukin-2 (IL-2) producing cell line derived from Jurkat cells. This cell line shows a highly proliferative activity in response to the autocrine produced IL-2. The phenothiazine fluphenazine (1-10 µM) inhibited this proliferation in a dose dependent manner, as evidenced by the incorporation of [^{3}H]-thymidine. Inhibition was maximal after 24 hours of cell culture and decreased with prolonged culture time. In analogy growth inhibition by fluphenazine has been investigated in the human myeloblastic HL-60 cell line. The spontaneous growth of this cell line was also inhibited by fluphenazine in micromolar concentrations and this growth inhibition was demonstrable between 24 and 96 hours of cell culture. These results suggest that the use of phenothiazines might be helpful in antileukemic regimens.

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INTERMITTENT SEQUENTIAL HIGH-DOSE CYTOSINE ARABINOSIDE AND MITOXANTRONE (IS-HAM) FOR REFRACTORY ACUTE LEUKEMIAS - A PHARMACOLOGICALLY DESIGNED PHASE II STUDY

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Pharamcokinetic analyses of the metabolism of cytosine arabinoside (AraC) in leukemic blasts and normal blood cells revealed substantial differences in the retention of AraC TP. After a 3 hour infusion of 3,0 or 1,0 g/m² AraC a rapid fall of AraC TP was observed in normal cells while high AraC TP levels were maintained in leukemic blasts for up to 3,5 hours. Based on these findings an intermittent schedule of AraC administration was designed which aimed at maintaining high AraC TP concentrations in leukemic blasts over a prolonged period of time while allowing an intermittent drop of AraC TP in normal blood cells. This schedule might therefore enhance the antileukemic activity of AraC without increasing the toxicity against normal hematopoetic cells and thus enlarge the therapeutic index. The current report summarizes the first clinical result of the IS-HAM regimen comprising 8 45 minute infusions of 750 mg/m² AraC per day separated by 135 minute treatment free intervalls on days 1 and 2. On days 3 and 4 10 mg/m²/day mitoxantrone is given as 30 minute infusion. After a three day treatment free intervall the same sequences is repeated on days 8 to 11. 13 patients with end stage AML (n=11) or ALL (n=2) entered the study. Patients were at late first relapse (n=1), first and second relapse refractory to salvage therapy (n=4), relapse after autologous bone marrow transplantation (n=1), second, third and forth relapses (n=4). 4 patients achieved a complete remission and 1 a partial remission, 4 patients were non-responders and 4 cases were early deaths. Treatment associated toxicity did not differ from the conventional S-HAM regimen and consisted mainly in nausea and vomiting, diarrhea and infection. These preliminary data indicate a high antileukemic activity of IS-HAM in this heavily pre-treated sub-group of acute leukemias.

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Molecular genetic and cytogenetic screening of bone marrow samples of children with acute leukemia

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Translocation t(9;22) is a rare chromosomal abnormality in children with ALL, with an incidence between 2.3 % and 6 %. Because of their very poor prognosis, it is important to identify all positive children.
As cytogenetic analysis sometimes fails, we started to screen all ALL-patients by PCR techniques to find out the real incidence of BCR/ABL-rearrangement in children and to diagnose high risk patients.
From March 1992 to April 1993 207 bone marrow samples of children with acute leukemias were analyzed prospectively by PCR techniques. A BCR/ABL-rearrangement was only found in children with common and pre-B-cell ALL, but never in pre-pre-B- or T-cell-ALL (n=17). The incidence was 6.2 % (5/81) in all newly diagnosed c-/pre-B-ALL, whereas in relapse patients BCR/ABL-rearrangement was found in 4 of 31 patients (12.9 %), confirming the poor prognosis. All but two patients had a breakpoint in m-BCR.
BCR/ABL-rearrangement positive patients were controlled by cytogenetics and/or a second analysis in an independent laboratory (Prof. Bartram, Ulm). All positive patients showed a Ph^1 chromosome in their karyotype. However, there was one child with AML who was Ph^1 negative (>100 metaphases analyzed), but BCR/ABL-rearrangement positive after several reinvestigations in both laboratories. This child is the second BCR/ABL-rearrangement positive and Ph^1 negative patient with acute leukemia.
In summary this study will help to find out the real incidence of t(9;22) in children with acute leukemia. This molecular approach may overcome cytogenetic failures and give quick results highly relevant for risk adapted therapy.

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IMMUNOLOGICAL CLASSIFICATION OF CHRONIC MYELOID LEUKEMIA DISTINGUISHES CHRONIC PHASE, IMMINENT BLASTIC TRANSFORMATION AND ACUTE LYMPHOBLASTIC LEUKEMIA.

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We have developed a double marker enzyme-immunoassay (DM-EIA) for investigating patients with CML (n=60) and acute lymphoblastic leukemia (ALL, n=21). Patients in clinical and immunological chronic phase (cP, n=14) of CML expressed late myeloid differentiation markers (CD15) at a high proportion (40-95%) but "early" antigens (CD10, CD20, CD34) at a low degree (<13%). These antigens were not coexpressed (0-4%). Some patients, however, were immunologically discordant (n=18) with an increased proportion of blast markers (25-48%) and double-labled cells (11-55%). These cases developed blast crisis (BC) earlier (median after 4.5 months) than immunological concordant cP-patients (76% in cP after 2 years). In patients with clinical BC the double markers allowed to distinguish myeloid blasts, characterized by a high degree of coexpression, from lymphoid blasts which typically did not label with CD15 and blast markers. Blasts from ALL patients, in contrast, had a high proportion of double-marked cells. Immunological findings were confirmed by Southern blot anayses (JH-, tcß- and bcr-probes). Our data show, that blast clones can be detected in CML-cP several months before clinical onset of BC. Moreover, the lymphoid "blasts" of CML-BC represent a relatively differentiated population of cells which can be distinguished from ALL blasts by lack of coexpression of foreign markers.

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EXPRESSION OF THE PROTEIN KINASE MEK IN ACUTE MYELOGENOUS LEUKEMIA BLAST CELLS

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The Raf-1 protein is a cytoplasmatic serine/threonine kinase which presumably plays a key role in signal transduction from the cell surface to the nucleus. Rapid activation of Raf-1 mediated signal transduction pathway has been demonstrated under mitogenic stimulation and growth factor treatment. There is accumulating evidence for important functions of this pathway in signal transduction in hematopoiesis as well. Treatment with granulocyte-macrophage colony-stimulating factor (GM-CSF), interleukin 3 (IL3), interleukin 2 (IL2), colony stimulating factor (CSF-1) lead to rapid phosphorylation and activation of Raf-1 kinase. Further, correlation of phosphorylation status of Raf-1 kinase with the spontaneous proliferation rate in acute myeloid leukemia cells could be demonstrated.
Whereas the precise localisation of raf-1 in signal transduction pathways ist not known, there is evidence that raf-1 activates mitogen activated protein (MAP) kinase either directly or via other intermediates. Recently characterization of a 45 kD protein (MEK) was described in mice which activates MAP kinase. To further investigate a possible role of this kinase in hematopoietic cells, we analyzed MEK mRNA expression in 21 AML blast cell cases (FAB M1: 7, M2: 5, M4: 4 and M5 5 cases) using a reverse transription PCR approach (RT-PCR). Specifity of the PCR products was confirmed by hybridization with an internal oligonucleotide. All 21 cases analyzed showed expression of MEK mRNA as determined by RT-PCR, with highest expression levels in FAB subtype M1.

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Detection of Cytomegalovirus after bone marrow transplantation by PCR, virus culture and antigen detection.

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We prospectively monitored by polymerase chain reaction (PCR) buffy coat leukocytes of 47 patients after 50 marrow transplantation (autologous n=18, allogeneic n=32 for the presence of cytomegalovirus (CMV). None of the 18 autologous graft recipients (9 seropositive, 9 seronegative) had positive PCR results nor CMV disease throughout the posttransplantation course. Six of 32 allograft recipients (19 seropopsitive, 13 seronegative) became PCR positive, four of whom developed CMV disease. PCR positive patients were found more often (5 of 10) in the group with aGvHD grade II-IV compared with 1 of 22 in the group without or with grade I aGvHD (p=.002). The comparison of PCR with antigen assay and virus culture showed an agreement in 90 of 96 (94%) samples. Discordant results were due to a higher sensitivity of PCR in comparison with antigen assay (n=4) and with virus culture (n=6).
In conclusion, PCR helps to define the patients who will not develop CMV disease and to narrow down the number of patients who will eventually get symptomatic CMV infection. Further, PCR is a useful tool to follow the posttransplantation course with respect to CMV and to judge the effect of antiviral treatment.

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OVEREXPRESSION OF THE RAF-1 PROTO-ONCOGENE IN ERYTHROLEUKEMIA

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The Raf-1 protein, a cytoplasmatic serine/threonine kinase, plays an important role in signal transduction pathways. Infection of murine hematopoietic cells with a v-raf containing retrovirus lead to erythroblastosis and erythroleukemias.
In order to examine the role of Raf-1 in human myeloid leukemia, we determined raf-1 mRNA expression by Northern blot analysis in blast cell samples from 27 acute myeloid leukemia (AML) cases and peripheral blood mononuclear cells from six healthy donors. A normal raf-1 transcript size was detected in all cases investigated. However, overexpression of raf-1 mRNA was found in two of 27 AMLs, both of which were erythroleukemias (AML, FAB M6). A sensitive cDNA-PCR assay was used to further determine raf-1 expression in normal erythroblasts. No altered raf-1 expression was observed in normal erythroblasts grown in vitro, while overexpression of raf-1 in the two leukemias was confirmed by this method.
We conclude that raf-1 overexpression occurs in human erythroleukemias and may have a role in the evolution of hematologic neoplasms. The biological significance of the observed findings remains to be determined.

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Molecular characterisation of AML with illegitimate TCR delta gene rearrangement - Mutations in the ras protooncogenes

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We previously described T-cell receptor delta (TCR-d) gene rearrangements in nine acute myelogenous leukemia (AML) cases with coexpression of T-lymphoid features (CD2, 4, 7) (Schmidt et al., Leukemia 1992). Expression of cytoplasmatic CD3 was not found in any of these cases. Rearrangements of the TCR-gamma gene were further observed in six of these nine cases. Seven of nine patients were children, two adults. In order to elucidate further molecular events in these cases, we sought to detect molecular alterations observed in AML, namely point mutations in the *ras* protoncogenes. Five ras mutations were observed in four of nine AML cases using single strand conformation polymorphism (SSCP). This incidence is higher than expected from published data. Mutations were found in Ki-1 and in Ki-2 in two cases each and in one case in N-1. Results of SSCP were confirmed by dot blot hybridisation and direct sequencing of PCR products in three cases so far. Whereas Ki-1 and N-1 mutations were found in all cells, both Ki-2 mutations were detected in a subpopulation of malignant cells. The biological relevance of this findings remains to be determined.

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DIFFERENTIAL REGULATION OF HLA-CLASS I GENES BY INTERFERON IN VIVO AND IN VITRO

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Expression of HLA class I antigens can be modulated in vitro and in vivo by interferon (IFN) α but also by IFN γ. A strong induction of HLA-class I antigens was found on both hematopoietic progenitors and normal peripheral blood mononuclear cells after one months of IFN treatment in 18 patients with myeloproliferative syndrome. By daily injections of IFN in the first month of therapy stimulation was continuously increasing suggesting a major effect of IFNα on hematopoietic progenitors with sustained enhanced expression of HLA-class I antigens during differentiation of myelomonocytic cells. Differential in vivo regulation of HLA-class I antigens by IFN was demonstrated by comparison of HLA-A2 with HLA-B antigen expression.

In vitro expression of the HLA-B7 and -Bw64 genes was significantly more inducible by IFN than the genes coding for the HLA-B27, HLA-B51, HLA-B38, HLA-B39, HLA-Cw3 and HLA-A2 antigens after transfection into mouse L cells. Modification of the 5' ends of the HLA-B7 and HLA-B27 genes before transfection in mouse L cells revealed the presence of enhancer sequences responding to interferon treatment in the 5' untranslated region of the HLA-B7, but not of the HLA-B27 gene and suggested further independently acting enhancer elements downstream of the transcription initiation site. When different fragments of HLA B7 or B38, including introns, 3' and 5'untranslated regions, were cloned in front of CAT genes IFN responding enhancers were only detected at the 5'end of the HLA-B7 gene. Further IFN independant enhanders could be detected within introns and at the 3' end of HLA class I genes.

These findings may indicate specific regulatory mechanisms of HLA class I antigen expression possibly influencing T-cell recognition in immune response.

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PROPAGATION OF LARGE NUMBERS OF T CELLS WITH NK CELL MARKERS

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Recently, a subset of T cells co-expressing the NK cell antigen CD56 has been described. Because of their scarcity these cells have been poorly characterized. Here, we present a protocol for the generation of large amounts of such cells. The protocol includes addition of interferon-gamma on day 0, IL-1, IL-2 and a monoclonal antibody against CD3 on day 1 to peripheral blood lymphocytes. Cells of the CD3+CD56+ phenotype increased more than 1000-fold using this protocol after 14 days in culture. These cells have been further characterized. It could be shown that cells of this phenotype have characteristics of both T and NK cells. T cell characteristics are the expression of the alpha, beta TCR, and co-expression of CD5 and CD8 antigens as well as their lack of co-expression of the CD16 antigen as determined by flow cytometry. Concerning distribution after gradient centrifugation and morphology these cells cannot be distinguished from NK cells. In respect to their behavior to lyse tumor cells these cells are intermediate between CD3-CD56+ NK cells and CD3+CD56- T cells. We conclude that these cells can be easily studied using the protocol described.

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HIGH-DOSE CHEMOTHERAPY FOLLOWED BY HEMATOPOIETIC STEM CELL RESCUE IN PATIENTS WITH HODGKIN'S DISEASE

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High-dose chemotherapy (HDCT) followed by hematopoietic stem cell rescue is increasingly being used as treatment for patients with relapsed HD. The success of such therapy is largely dependent on prognostic factors like performance status at BMT, bulk of disease prior to BMT, lines of prior therapy and - most importantly - on the question of remaining sensitivity of the tumor to conventional salvage therapy. At our institution none of 20 patients grafted for refractory relapse has remained disease-free more than 12 months after BMT. Thus, new strategies have to be followed for these patients as well as for those who are refractory to first-line therapy. For patients with sensitive HD results of HDCT are much better but formal proof of the superiority of HDCT as compared to dose-intensive salvage chemotherapy awaits the results of prospectively randomized trials. Results of allogeneic BMT have seldom been reported for patients with HD because of the procedure-related toxicity encontered with this approach. Nevertheless, allogeneic BMT in our hands was the only successful strategy for younger patients with refractory Hodgkin's disease. More recently, HDCT has also been proposed for patients with HD and poor prognostic features immediately after first CR is achieved. This strategy might become acceptable because the use of hematopoietic growth factor-mobilized peripheral blood stem cells has dramatically reduced the need for platelet transfusions, may have an impact on the frequency and severity of infections and thus further reduce toxicity of HDCT. However, it is not yet clear how such a poor-risk group might be defined. The prognostic indices proposed by Straus et al. (J.Clin.Oncol. 8:1173-1186, 1990) and Proctor et al. (Eur.J.Cancer 27:624-629, 1991) failed to identify a subgroup of patients carrying a prognosis deemed poor enough to justify HDCT in first CR when tested on the respective cohort of patients from the German Hodgkin Study Group.

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13-CIS-RETINOIC ACID AND INTERFERON-ALPHA-2A: AN EFFECTIVE THERAPY OF SQUAMOUS CELL CARCINOMAS OF THE HEAD AND NECK

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Cytokines are increasingly used in the treatment of different solid tumors. 9 patients with pretreated, advanced inoperable squamous cell carcinoma of the head and neck and especially of the oral cavity were treated with a combination of oral 13-cis-retinoic acid (1 mg/kg per day) and subcutaneous recombinant human interferon-alpha-2a (3 million units per day). This regimen was administered for a period of 6 months on an outpatient basis, only interrupted when life-threatening complications occurred. Out of 9 patients 5 (55%) responded. 1 (11%) had a complete, another (11%) a partial remission for 6 months duration. There were 2 (22%) minor remissions of 2 and 4 months duration, and 1 (11%) stable disease for 3 months. The median response duration was 4 months. On 1 patient the treatment was interrupted because of a severe bleeding from the tumor site after one week of treatment. No severe toxic side effects occurred, dose reduction was not necessary in any case. Most commonly seen was mild fatigue, dryness of the skin, hypertriglyceridemia and leukopenia. The combined systemic therapy with 13-cis-retinoic acid and interferon-alpha-2a is an effective messure in the palliative treatment of squamous cell carcinomas of the head and neck.

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RATIONAL DESIGN OF NEW CLINICAL TRIALS AFTER STUDYING THE PATTERN OF RECURRENCES OF COLORECTAL CANCER PATIENTS FOLLOWING ADJUVANT TREATMENT WITH MURINE MONOCLONAL ANTIBODY 17-1A

E. Schneider-Gädicke, G. Schlimok, R. Raab, W. Schmiegel, S. Said, K. Höffken und G. Riethmüller

In a prospective clinical trial, 185 patients with Dukes C colorectal cancer were randomized to be either treated with surgery followed by 900 mg of 17-1A or by surgery alone (control group). After a median clinical follow-up of 5 years, analysis of overall survival and disease-free interval of 166 eligible patients revealed a significant treatment benefit at minimal toxicity for patients treated with monoclonal antibody. In this analysis, 46 treated patients were found to be tumor-free as compared to 28 patients in the control group. Furthermore, a clear efficacy-profile of the antibody could be obtained when events were differentiated into local or distant recurrence. An excess of distant metastases as first event was observed among patients in the control arm. When plotted according to Kaplan-Meier, curves generated for treated and control patients differ significantly (p=0,002), showing a clear effect of 17-1A on distant metastases. No corresponding reduction of local recurrence was found among patients receiving monoclonal antibody. It is therefore interesting to combine the systemically effective antibody therapy with a locally restricted therapy such as radiotherapy. For such a combined approach, rectum cancer seems to be the ideal indication, since radiation therapy reduces the incidence of local-regional relapse but fails to affect overall survival in these patients (Fisher et al, J Natl Cancer Inst 1988; 80:21-29 and Boulis-Wassif etal, Cancer 1984; 53:1811-1818). We are therefore now engaged in a further study, treating rectum carcinoma patients with a combination regimen of postoperative radiation and therapy with monoclonal antibody 17-1A.

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17P ABNORMALITIES IN LYMPHOID MALIGNANCIES: DIAGNOSTIC AND PROGNOSTIC IMPLICATIONS

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Recently quite a lot of studies have been performed concerning mutations on the molecular level of the p53 gene which has been mapped to the short arm of chromosome 17, band p13. However, only little is known about cytogenetic abnormalities of 17p in lymphoid malignancies. In routine analyses we found abnormalities of 17p in tumor material of 18 patients with Non-Hodgkin lymphomas (1 Richter's syndrome (an immunoblastic lymphoma emerged from CLL), 1 centroblastic lymphoma emerged from a centroblastic-centrocytic lymphoma, 1 T-lymphoblastic lymphoma, 2 Burkitt's lymphomas), acute lymphoblastic leukemia (3 Burkitt's type ALL, 1 pre-B-ALL, 1 pre-pre-B-ALL, 1 T-ALL, 2 pre-T-ALLs, 2 ALLs with coexpression of myeloid antigens, 2 ALL without further classification) and plasma cell leukemia (1 patient). No 17p abnormalities were found in low grade lymphomas or chronic leukemias. A strikingly high proportion of Burkitt's lymphomas/leukemias (5/18) with one of the typical translocations involving 8q24 (locus of the c-myc oncogene) showed structural abnormalities of 17p. These cytogenetic data correspond well with molecular genetic findings of a high p53 mutation rate in Burkitt's lymphoma/leukemia and support the hypothesis of cooperation between myc and p53 shown in a mouse cell line. Remarkably, we did not find any rearrangement of 17p in the most frequent type of ALL, c-ALL, but in a relatively high percentage of the less widespread T-ALL. It has to be mentioned that in two of three T-ALL cases as well as in the T-lymphoblastic lymphoma and in one of the two ALLs with coexpression of myeloid antigens the 17p rearrangement was the sole cytogenetic abnormality, whereas all other twelve cases showed additional chromosomal aberrations. There is evidence that p53 mutations occur later in the course of a malignant disease and are associated with progression to a more aggressive form. Concerning the T-ALLs, a different (primary?) role of 17p anomalies and of p53 mutations could be discussed. Abnormalities of chromosome 17 in lymphoma are associated with poor clinical outcome, the special role of rearrangements involving 17p13 has not been analysed up to now. The diagnostic and prognostic implications of 17p abnormalities in lymphoid malignancies are discussed.

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Serum levels of IL-1, IL-6, IL-8, TNF alpha, G-CSF and IL-1-receptor antagonist protein during chemotherapy induced aplasia in patients with acute myelogenous leukemia

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Opportunistic infections are the most frequent complication of aggressive treatment of acute myelogenous leukemia. Despite extensive clinical, biochemical and microbiological examinations it is still difficult to discriminate between infectious and noninfectious causes of fever during neutropenia. As cytokines can be induced directly by components of infectious pathogens, serial determinations of cytokine serum levels might be useful for identification of patients at risk for severe infections.
Serum samples of 22 patients treated for acute myelogenous leukemia were analysed three times per week. A significant increase of the serum levels of IL-8, IL-6 and, to a minor extent, TNF alpha paralleled the occurence of fever. G-CSF levels increased significantly in all patients during neutropenia. The increase of G-CSF serum levels during aplasia was significantly higher in patients who experienced febrile episodes than in patients who remained afebrile. In patients suffering from major infections slightly higher serum levels of IL-6 and IL-8 were observed than in patients with fever of unknown origin. These differences, however, were not statistically significant.
We conclude that during neutropenia significant infection-associated production of IL6, IL-8 and G-CSF can be detected by measurement of serum levels. As only minor differences were observed between patients with severe infection and FUO, assessment of the serum levels of these cytokines cannot be recommended as a useful diagnostic tool for the differential diagnosis of febrile episodes in this patient population.

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NODULAR PARAGRANULOMA WITH COEXISTENT LARGE CELL LYMPHOMA OF B CELL TYPE. CLINICAL FOLLOW-UP OF THREE PATIENTS.

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Nodular lymphocyte predominant Hodgkin's disease (NLP HD) (nodular paragranuloma) is suggested to be of B cell nature. An association with large cell lymphomas (LCL) of B cell type - either simultaneously or subsequently - is rare but well known. We reviewed the case records of our patients with NLP HD. All were males, with a median age of 30 years. 3 out of 8 patients had B-LCL, two (patient 1 and 2) at diagnosis. They presented with stage IV NLP HD, localized in either splenic hilar (1) or axillary (2) lymphnodes and bone marrow (1 and 2). The site of involvement by B-LCL was the spleen (1) and a soft tissue mass of the back with an intraspinal tumor leading to an imminent transverse spinal cord syndrome (2). A third patient (3) presented with stage I disease in axillary nodes. He developed B-LCL of the stomach 6 months later. All large cell tumors were subclassified as centroblastic lymphoma, polymorphous subtype. Therapy consisted in surgery and chemotherapy in two patients (1 and 3), resulting in complete remission. Patient 3 had got extended field irradiation for stage I disease. Patient 1 developed liver involvement 49 months after diagnosis. Chemotherapy induced a second complete remission. Patient 2 underwent surgery for spinal decompression followed by combined modality treatment, which led to a complete remission. Now (04/93) all patients are alive 91 (1), 67 (3), and 7 (2) months after diagnosis. Patient 2 has no evidence of disease, patients 1 and 3 have abdominal nodes without any progression. Cure of advanced NLP HD seems to be difficult, but prognosis is favorable in persistent disease even if primarily high malignant.

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IMMUNOPHENOTYPIC AND CLINICAL FEATURES OF 19 CHILDREN WITH TCRδ+ T-CELL ACUTE LYMPHOBLASTIC LEUKEMIA (T-ALL)

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The development of monoclonal antibodies (mAbs) against the αß/γδ-chains of the T-cell antigen receptor (TCR) has facilitated the investigation of TCR protein expression in T-ALL, and more recent studies have suggested that TCRδ+ T-ALL represent an important subgoup of T-lineage ALL with distinct clinicopathologic features (e.g., Alfsen GC et al., Blood 1991, 77:2023). Very few studies, however, have yet evaluated the immunophenotypic profile and clinical behaviour of TCRδ+ ALL in a larger number of patients. We therefore characterized TCR protein expression in 109 children with T-lineage ALL (recruited from ALL-BFM studies 83, 86, and 90) by using mAbs to TCRβ- and δ-chains (βF1, TCRδ1, δTCS1). The expression of these molecules was investigated by flow cytometric analysis (TCRδ1, δTCS1) or by staining of fixed cytocentrifuge preparations (βF1). Leukemic cells from 19 children (17%) expressed the TCRδ-chain as detected by the mAb TCRδ1 (reacting with a framework epitope on the TCR δ-chain), and 7 of 19 disclosed reactivity with the mAb δTCS1 (indicating the usage of the Vδ1-Jδ1 gene regions). According to the maturational stages of normal thymocytes, 9 T-ALL expressed an intermediate (CD1a+) and 10 a mature (CD1a- mCD3+) phenotype. The phenotypic profile of TCRδ+ T-ALL (i.e., leukemic cells of 9 patients expressing both CD4 and CD8, 5 only CD4 and 5 none of these molecules) differed from the postulated phenotype of their normal counterparts. Analysis of clinical characteristics in 18 patients (no clinical data available in one patient) did not reveal any striking findings in the clinical behaviour of TCRδ+ ALL. 12 of the children with TCRδ+ T-ALL were boys (67%). 12 were 1-9 and 6 ≥ 10 years of age. High white blood cell counts (>50x10^9/L) were seen in 12 patients, lymph node enlargements in 11 (61%) and mediastinal tumors in 12 (67%). 10 children (63%; not evaluated in n=2) showed a good response to prednisone, and 16 achieved a complete remission (94%; not yet assessable in n=1). Immunophenotypic features and clinical outcome of TCRδ+ ALL will be presented in detail.

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INDUCTION OF APOPTOSIS BY FLUDARABINE IN CLL PATIENTS IN VIVO

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The purine nucleotide analogue 9-β-D-arabinofuranosyl-2-fluoroadenine phosphate (F-Ara-A) is a potent new drug in the treatment of low malignant non - Hodgkin's lymphomas. Its mechanisms of action are inhibition of DNA synthesis and, as shown in vitro, induction of apoptosis. We investigated apoptotic cell death in vivo in the peripheral blood lymphocytes of 22 patients with chronic lymphocytic leukemia (Rai stages III and IV) before and during a five day therapy with fludarabine. A total of 34 cycles was monitored. All patients had been pretreated with alkylating agents and / or anthracyclines. DNA was extracted on days 1 and 4 and run on a 1 % agarose gel. Ara - C treated HL-60 cells served as positive controls for the characteristic nucleosome fragmentation pattern of apoptosis . Only in one of the patients fragmented DNA was observed under F-Ara-A therapy. This was reproducible in a further therapy cycle. Degree of apoptosis estimated by band intensity increased up to day 3 of the five day treatment. DNA fragmentation was also observed in a second patient, but only 4 weeks after F-Ara-A application. It had disappeared after another 4 weeks. Induction of apoptosis did not correlate with immediate decrease in WBC. Our data confirm that F-Ara-A can induce apoptosis in vivo, but that this mechanism of action may be dominating only in a minority of patients.

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A HEMATOPOIETIC DEFECT IN APLASTIC ANEMIA (AA) ASSESSED BY LIMITING-DILUTION TYPE LONG TERM MARROW CULTURE (LTMC)

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In the past, the investigation of primitive human hematopoietic progenitor cells with repopulation activity was limited by the lack of appropriate assay systems (like the colony-forming-unit spleen (CFU-S) assay or marrow repopulation studies in mice). Recently it could be demonstrated that cobblestone area forming cells (CAFC) in long-term marrow cultures (LTMC) represent a population of pluripotent progenitor cells with long-term marrow repopulating activity (Ploemacher et al., Blood 70:2527, 1991). We established a microtiter limiting dilution (LD) type human LTMC system, that allows quantitative assessment of the CAFC. We used this assay system to characterize and to quantitate the hematopoietic defect in aplastic anemia (AA) on the level of primitive progenitor cells.

Bone marrow cells (mononuclear cells or rhodamine-dull cells) of healthy controls and AA patients were overlaid on preformed irradiated stromal layers in different concentrations with 12 to 18 wells per dilution. Micro-LTMCs were scored positive if at least one phase-dark hematopoietic clone (cobblestone area) was observed on day 35. The frequency of CAFC was calculated by Poisson statistics and the weighted mean method with iterative procedures. In BM-MNC of healthy donors (n=20) we observed a CAFC frequency of 1/1363 (median). The frequency of CAFC in BM of AA patients (n=23) was significantly lower (median 1/20123) compared to controls ($p<0.0001$). This 10.9-fold reduction of CAFC is less pronounced than the reduction of committed progenitor cells: CFU-GM: 4/10^6 BM-MNC in AA versus 174/10^6 BM-MNC in controls ($p<0.0001$); BFU-E: 5/10^6 BM-MNC in AA versus 170/10^6 BM-MNC in controls ($p<0.0001$). Compared to controls the frequency of CAFC was not only reduced in pancytopenic AA patients (n=16) (1/17597; $p<0.0001$ vs. control) but also in patients in remission after immunosuppression (IS) (n=7) (1/20381; $p=0.0004$ vs control; $p=0.94$ vs. pancytopenic AA patients). The CAFC frequency did not correlate with the severity of the disease nor did it predict response to IS.

In summary, the frequency of long-term repopulating cells is significantly reduced in AA. However, this reduction of CAFC does not completely explain the hematopoietic failure in AA since a significant reduction of CAFC is also present in remission patients. The low number of CAFC in remission patients is in keeping with other data pointing to a persisting defect of hematopoiesis even after response to IS.

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QUANTITATION OF PERIPHERAL BLOOD STEM CELLS BY LIMITING-DILUTION TYPE LONG -TERM MARROW CULTURE (LTMC)

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Peripheral blood stem cells (PBSC) are increasingly used as an alternative to bone marrow cells for support of high dose chemotherapy (CT) or radiation protocols and may even be used for allogeneic transplants in the future. Reliable assessment of marrow repopulating cells in PBSC harvests is very important if they are planned to be used after myelo-ablative regimens. The quality of PBSC harvests is usually measured by enumeration of CD34+ cells or granulocyte-macrophage colony forming units (CFU-GM). However, it is not clear whether these parameters accurately reflect the number of pluripotent progenitor cells that are a prerequisite for sustained reconstitution. We used a limiting-dilution type LTMC system for quantitative assessment of cobblestone area forming cells (CAFC) that are an in-vitro surrogate for repopulating cells (Ploemacher et al., Blood 78:2527, 1991).

Peripheral blood mononuclear cells (PBMNC) of controls (n=11) and patients recovering from CT (n=7) were overlaid on preformed irradiated human stroma layers at limiting dilution conditions. CAFC in the patients (5 high grade lymphoma, 2 AML) were investigated at various time points after CT (n=17, CAFC assessments). Micro-LTMCs were scored positive if at least one cobblestone area was observed after 35 days of culture. From the proportion of negative wells the frequency of CAFC was calculated by Poisson statistics.

In LD assays we observed single hit kinetics both in controls and in patients after CT. The frequency of CAFC in PBMNC of controls was 1/16257 (median). During recovery after CT CAFC in PBMNC increased significantly (median 1/638) compared to controls (p=0.001). The median number of CD34+ cells was 35/μl and the number of CFU-GM was 139/10^6 PBMNC in PB of the patients. CAFC correlated better with CFU-GM (r=0.31; p=0.03) than with CD 34+ cells (r=0.13; p=0.19). The lack of correlation of CAFC with CD 34+ cells might be due to a different time course of mobilization of these populations since we observed that the peak of CAFC in PB was reached earlier than the leucocyte or CD 34+ cell peak. We currently investigate the influence of G-CSF on the kinetics of CAFC mobilization.

Our results demonstrate that CAFC in PB can be enumerated by means of a LD-type LTMC. The assay is a valuable tool to asses the efficacy of mobilization protocols and the adequacy and timing of apheresis on the level of cells with marrow repopulating activity. It may be particularly useful for estimating the quality of PBSC harvested from patients heavily pretreated, since enumeration of CD34+ cells may grossly overestimate the number of true stem cells in grafts from these patients.

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IN VITRO MODULATION OF MULTIDRUG RESISTANCE BY DEXNIGULDIPINE AND VERAPAMIL IN BLASTS OF DE NOVO OR RELAPSED OR PERSISTENT AML

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The occurence of multidrug resistance (MDR) may be one of the major obstacles to an effective chemotherapy of patients (pts) with AML. It is associated with the overexpression of a membrane glycoprotein (Gp-170) acting as an energy-dependent pump for anthracyclines and other xenobiotics. We investigated the mode of action of dexniguldipine (DEX) and verapamil (VER) as modifiers of MDR in blasts of *de novo* or relapsed or persistent AML *in vitro*. The degree of MDR was estimated by flow cytometry (FACS) of uptake and efflux of rhodamine (R123), daunorubicin (DNR), and idarubicin (IDA). A total of 12 pts with AML, 6 *de novo* and 6 relapsed or persistent, were investigated. While only 2 out of 6 *de novo* AML blast populations showed moderate efflux of R123 and DNR, 5 out of 6 blast populations in relapsed or persistent AML had an efflux which was between 20 % to 44 % within 15 min. Efflux could be significantly inhibited by 1 μM DEX or 10μM VER. In contrast, the uptake of R123 but not of DNR was increased by MDR-modifiers. For IDA we found a MDR-independent effusion of 45 ± 9 % within 15 min. in all blast populations. All pts with relapsed or persistent AML were pretreated with IDA and cytosine arabinoside. The finding that IDA-effusion is not MDR-dependent does not exclude IDA as an inducer of MDR. Clinically achievable modifier concentrations causing tolerable side effects are 1-3 μM DEX and 3 μM VER, respectively. We conclude that the application of DEX might be a feasable approach to overcome MDR in pts with AML because it has a high efflux inhibiting potential *in vitro* at a concentration easily achievable in the treatment of pts with low toxicity.

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RESULTS OF PALLIATIVE ORTHOPEDIC SURGICAL TREATMENT OF BONE METASTASES

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Between 5/78 and 8/92 522 orthopedic surgical interventions were performed in our hospital for skeletal metastases caused by advanced metastatic malignancies.
140/522 (26.8 %) operative interventions were done to get histology in cases of an uncertain osteolysis (44/140) or an unknown primary tumor (96/140). In 23/522 cases (4.4 %) no malignant histology was found.
The charts of 359 patients admitted to the medical or orthopedic department with an initial primary diagnosis of pathologic fracture secondary to malignant disease were reviewed. All 359 operative interventions except 7 were carried out in palliative intention to improve the impaired mobility caused by metastatic lesions and/or to reduce pain.

In 33 % orthopedic surgical interventions of the vertebral spine and in 35.4 % of the extremities were done. The overall mortality within 4 weeks after operation was 5.8 % (21/359, pts) and only due to progressive disease. There was a high rate of benefit for those patients who achieved an ambulatory status (70 %), independently of survival time.

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RESULTS OF MULTIDISCIPLINARY TREATMENT OF PRIMARY GASTRIC LYMPHOMAS

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Gastrointestinal non-Hodgkin's-Lymphomas are the most common type of extranodal lymphomas and their incidence seems to be increasing for a long time in localized disease a curative role was demonstrated for surgery alone. But the follow up observation resulted in a changing of the therapy concepts which depend on stage and grade of malignancies.

21 pts. with primary gastric NHL were reviewed (male 9 / female 12, age 35-77 median 60 years).

Initial stage (Radaszkiewicz T.): E I_1 : 2, E I_2 : 3, E II_1 : 4, E II_2 : 6, E IV : 6

Histology: high grade 12 low grade 9

Procedure: 17 pts. underwent a primary surgical intervention: 4 in curative intention, 4 emergency surgery because of bleeding. 9 for staging and exact histology.

3 pts. had a delayed laparatomy because of persisting lesions of unknown dignity: 2 without activ tumor, 1 with residual disease.

1 pt. without surgery cause of initial stage IV.

Pts. with high grade lymphoma received postoperativly CT/+ RT.

Pts. with low grade lymphoma received postoperativly RT/+ CT.

Results: Till now 14/21 pts. (9 x high grade, 5 x low grade NHL) are in CR with a median time of 48 mos. range 6+ to 128+ mos.

4/21 pts. (2 x high grade, 2 x low grade) are still alive and undertreatment because of active tumor (6+, 14+, 25+, 34+ mos.)

2/21 pts. (low grade) died due to tumorprogression after 4 and 28 mos.

1/21 pts. (low grade NHL) in CR is lost to follow up after 64 mos.

2/14 pts. (high grade) in CR developed after 5 + 10 years a second malignancy (BC)

Summary: The follow up of gastric NHL shows individual courses. Individual treatment strategies according to the stage and grade of malignancy are necessary and a high rate of curation is possible. Secondary malignancies are not uncommon.

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PATHOMECHANISMS IN PAROXYSMAL NOCTURNAL HEMOGLOBINURIA (PNH)

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PNH is often manifest as sudden or chronic intravascular hemolysis and an abnormal susceptibility to venous thromboses. These clinical symtoms could recently been attributed to an abnormal activation of autologous complement due to the loss of negative complement regulators. These proteins belong to the group of glycosylphosphatidylinositol (GPI)-linked surface molecules which characteristically are missing on blood cells in PNH. Analyzing the cells phenotypically, the defect can be detected not only on erythrocytes and platelets but also on granulocytes, monocytes and lymphocytes. These deficient cells are supposed to arise as a consequence of clonally expanded bone marrow progenitor cells. In addition, phenotypic analysis of blood cells with respect to expression of GPI-linked proteins revealed a proportion of at least 33% of patients with aplastic anemia developing the GPI-anchoring defect after immunosuppressive therapy which appears to be related to an unfavourable outcome. The affection of lymphocytes by the PNH defect enabled us to establish GPI-deficient lymphocyte cell lines in comparison to normal lines from the same PNH patients in order to further investigate the biochemical and molecular basis leading to GPI-deficiency in PNH. This could be localized in the transfer of N-acetylglucosamine (GlcNAc) from UDP to Phosphatidylinositol (PI). Since a cDNA responsible for this enzymatic step was cloned recently our current investigations address the molecular mechanisms which may lead to the GPI-deficiency in PNH.

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Epirubicin (E) pharmacokinetics (Pk) - 1) in Serum (S) and Red Blood Cells (RBC) and 2) influenced by Quinine (Q).

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Interaction of Anthracyclines with RBC´s are known since years, but no data about RBC partitioning of E are available. Therefore PK of E and its metabolite E-aglycon (M) was investigated by HPLC over 4HR (detection limit for M) in S and RBC following 120mg/m² bolus inj in 6 pts (4 advanced breast, 2 ovar cancer)

n = 6	E (S)	M (S)	E (RBC)	M (RBC)
AUC ng/ml.h^{-1}	1363	257	1951	297
% total	35,2	6,6	50,4	7,7

RBC coefficient of partition (k) ranges from 1,28 to 1.82 for E not dependant on conc. time course, for M in contrary k decreases from 3.27 at 0.08H to 0.16 at 1H after E bolus suggesting further biotransformation of M within RBC. Only 35% of administered E is free bioavailable in S for pharmacological efficacy, hematocrit changes may influence toxicity and cytostatic action of E.

As Q has been shown to reverse M DR1 in vitro, in 5 pts resistant to E alone, Q 500mg TID p.o. d1-3 was added and E injected on d3 2HR after Q. As interference with biliary excretion of E was suggested, E-PK was analyzed over 24HR and compared to baseline without Q.

n = 5	E	E (Q)	p
c0	7395	4351	0,005
AUC	3405	2359	0,05
Cltot	45	148	0,08

Q related tox: nausea, tinnitus. In contrast to the expected higher E-bioavailabilty (AUC) under Q-modulation, Q causes a significantly reduced E-AUC by 30%, and 3xhigher total Clearance (Cltot), possibly due to a membrane effect preventing drug efflux.

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Pharmacokinetics (PK) of racemic or L-Leucovorin in Serum (S) and Red blood cells (RBC)

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As endogenous folic acid is located within RBC´s to more than 90%, the question arises whether Leucovorin (LV) behaves similar, as only the unbound fraction of LV may act therapeutically. Therefore in 10 pts with advanced G.I. Cancer PK-analysis of LV and its CH3 metabolite (MTHF) was performed by HPLC in S and RBC´s using monthly cross over of 200mg/m² (HD), 20mg/m² (LD) LV or 100 mg/m² L-LV, (all combined with 5-FU 370mg/m² daily x5) in order to compare common LV schedules and the pure L-enantiomer with regard to RBC binding and CH3 bioconversion. Results of bioavailability (AUC µg/ml.min):

AUC	LV(S)	LV(RBC)	MTHF(S)	MTHF(RBC)
HD	1425	639	406	80
LD	420	180	175	7
L-LV	472	23	197	1,5

K_{RBC} (ratio conc. RBC/S) for HD and LD nearly identical (mean 0.36), for MTHF declining (mean 0.2) and undetectable conc after 45min, for L-LV <0.1 therefore neglibile.

After bolus inj. of HD, LD, L-LV 45, 10, 30% were immediately cleared from the plasma. For the remaining amount AUC comparison in mol% xmin for HD,LD, L-LV was: S - 51, 45, 56% for LV and 24, 36 42% for MTHF, RBC - 20/17/1,5% for LV and 5/1,2/0.3% for MTHF. In RBC´s a dose independant depot effectof 20% for LV seems less important (probably d-related) as binding of the active forms L-LV and MTHF is minimal.

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ANALYSIS OF THE DEOXYCYTIDINE KINASE GENE IN PATIENTS WITH ACUTE MYELOID LEUKEMIA AND RESISTANCE TO CYTOSIN-ARABINOSIDE (ARA-C)

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Mutations of the deoxycytidine kinase gene (dCK) have been recently observed in vitro, and cellular deficiency of deoxycytidine kinase activity has been shown to represent one possible mechanism of resistance to ara-C in vivo .We therefore analyzed the dCK gene in 16 patients (pts) with acute myeloid leukemia and clinical resistance to intermediate/high-dose ara-C. At present, data from structural and functional analyses are available for 7 pts. Southern blot analyses using genomic DNA from peripheral blood or bone marrow material (containing ≥ 70 % leukemic blasts) and agarose gel electrophoresis of dCK cDNA obtained by RT-PCR using dCK specific primers did not reveal gross rearrangements of the dCK gene or aberrations of transcript size in any of these pts. Thus, sequencing of the dCK gene coding region was performed and revealed point mutations of the dCK gene in 4/7 pts. Besides one silent mutation (or RFLP) base pair mutations resulting in amino acid replacements were found in 3 pts affecting codons 20, 98, and 99, respectively. Bacterial expression in E. coli and analysis of enzyme activity showed normal dCK activity for 2 clones (codons 20 and 98) and no activity in one clone (codon 99). So far, we conclude that genetic alteration of the dCK gene may represent one possible mechanism for ara-C resistance in vivo, but further analyses are required to determine the incidence of such mutations and, thus, their clinical significance.

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ANALYSIS OF THE p53 GENE IN PATIENTS WITH ISOCHROMOSOME 17q [i(17q)] and Ph¹-POSITIVE OR -NEGATIVE LEUKEMIA

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Increased incidence of p53 gene aberrations or chromosome 17p monosomy resulting from an isochromosome 17q [i(17q)] has been observed with transition of chronic myelogenous leukemia (CML) to myeloid blast crisis (BC), and in some patients with poor risk acute myeloid leukemia (AML) progressing from myelodysplastic syndrome (MDS)[1-3]. These data suggested that disease progression may be linked to bi-allelic inactivation of p53. Here, we report on p53 gene analyses of nine patients with CML-BC and AML who showed an i(17q) as characteristic cytogenetic anomaly. Using Southern blots, agarose gel electrophoresis and single-strand conformation polymorphism analyses of PCR products from genomic DNA and cDNA, spanning exons 4 through 9, we did not detect any structural abnormalities of the remaining p53 allele. These findings question the hypothesis that p53 gene alterations are the principal molecular event responsible for progression of CML chronic phase or MDS to i(17q)-positive CML-BC or AML, respectively.

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RESULTS OF HIGH-DOSE THERAPY WITH AUTOLOGOUS BLOOD STEM CELL RESCUE (ABSCT) IN HAEMOBLASTOSES AND SOLID TUMORS

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Autologous transplantation (ABSCT) using peripheral stem cells may be useful in patients with high risk chemotherapy sensitive solid tumors and in some kinds of haematologic malignancies. Advantages of this method are the applicability in patients with bone marrow involvement of the underlying disease and in cases with earlier extensive irradiation of the pelvis region or if general anaesthesia for collecting bone marrow cells is impossible. Until April 1992 we transplanted 13 patients with autologous peripheral stem cells. Diagnoses: Hodgkin lymphomas (5), Non-Hodgkin lymphomas (4), CML (1), Multiple Myeloma (1), Testicular Cancer (1), Breast Cancer (1). There was 1 lethal complication: lung bleeding in ptn. with testicular cancer (7.6 %). Engraftment in all of the ptns. could be observed between day +7 and +11. Discharges from the unit were done always on day +14. 11/12 (92 %) of the ptns. are alive between 10 and 328 days. 4/12 (33 %) relapsed: 1 Myeloma, 1 Hodgkin lymphoma, 1 Non-Hodgkin lymphoma, 1 CML. Conclusion: APSCT is a valuable completition in order to improve the results in treatment of malignant diseases of unfavourable prognosis.

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PRETREATMENT OF ALLOGENEIC DONORS WITH G-CSF (NEUPOGEN 48^{R}) FOR REDUCTION OF EARLY TRANSPLANTATION-RELATED COMPLICATIONS

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Often early severe complications under bone marrow transplantation are connected with a prolonged grafting. In order to diminish such potentially fatal situations, since December 1992 we treated 4 healthy donors with sc. G-CSF before their allogeneic marrow donation. Donor characterization: 1 male, 3 femals, in age of 51, 57, 42, 30 years. Duration of G-CSF application 4, 4, 5, 5 days immediately before donation. Doses: 5.8, 6.8, 7.6 8.1 ug G-CSF/kg bw. daily. Peripheral WBC count before marrow sampling: 38.9 x 10^9/l, 28.9 x 10^9/l, 45.9 x 10^9/l. 43.7 x10^9/l. Graft characterization: MNC 6.5 x 10^9/l, 9.9 x 10^9/l, 13.3 x 10^9/l, not yet present. CFU-C (GEMM-techniques): not done, 7.2 x 10^4/kg bw, 3.94 x 10^4/kg bw, not yet present. Patients: Diagnoses (a) CML in 2nd blast crisis, 53 ys., male, (b) CML in CP, 44 ys. male, (c) Multiple Myeloma IIIA, female, 42 ys. (d) CML in CP, 28 ys., male. Because of the very early engraftment with a rapid peripheral cell increase - take in patn. (a) on day +17, patn. (b) +15, patn. (c) +16 - there were no serious complications in all of the patients. The transfusion frequency of blood and platelets were reduced and discharge from the unit could be done about 2 to 3 weeks earlier than in untreated grafts. Conclusion: The treatment with G-CSF of donors in allo-BMT should be discussed as an important method in order to overcome the early often life threatening phase.

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TISSUE DISTRIBUTION AND REGULATION DURING THE ACUTE PHASE RESPONSE OF THE NOVEL CLASS 1 ACUTE PHASE PROTEIN LBP

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Lipopolysaccharide Binding Protein (LBP) is produced in the liver and secreted into the bloodstream. It binds LPS at the Lipid A moiety and facilitates its binding and cellular uptake via the CD14 LPS receptor. During an acute phase response serum levels of LBP rise substantially, with the maximum being after 24 hours. Here we report on two different systems for elucidating the mechanism of LBP synthesis during the acute phase response: A rabbit model was used where an acute phase response was induced by stimulation with Silvernitrate. At different timepoints the animals were sacrificed and mRNA was prepared from different organs. Northern blotting revealed that the liver was the only source of LBP and that a maximum rise in LBP mRNA was at 24 hours. To further elucidate the mechanisms and possible mediators involved in LBP induction we established an in vitro system using the hepatoma cell line Hep-G2. After stimulation with IL-1, IL-6 and Dexamethasone the cells in vitro could be induced for LBP production as revealed by Northern- and Western-blotting. The fact that Dexamethasone and IL-1 act synergistically in enhancing the IL-6 mediated protein induction makes LBP a "class 1" acute phase protein. The proximity of the Kupffercells to the hepatocytes in the liver combined with our data give rise to the hypothesis that during gramnegative acute phase response LPS induces cytokines like IL-1 and IL-6 in the Kupffer cells that in turn induce hepatocytes for LBP production.

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DIFFERENTIATION ASSOCIATED REGULATION OF GENE EXPRESSION IN CHRONIC MYELOID LEUKEMIA

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CD34 positive cells were purified from peripheral blood mononuclear cells of patients suffering from chronic myelogenous leukemia by positive selection with CD34 antibody coated Dyna beads after removal of adherent cells and T cells. Normal human bone marrow cells were used as controls. Cells were grown in liquid culture for 14 days and stimulated either with SCF/G-CSF, IL-3/EPO or SCF/EPO. These culture conditions resulted in a predominant erythroid differentiation measured as expression of glycophorin A in presence of SCF/EPO, or myeloid differentiation measured as expression of CD15 in presence of SCF/G-CSF.
100000 cells were lysed before and after three, five, seven and 14 days of culture and RNA was isolated and analysed by reverse transcription PCR. Constant yield of RNA was controlled by comparing the amplification product of ß-actin after 25, 30 and 35 cycles of PCR. Results on the expression of c-abl, bcr, bcr-abl and abl bcr RNA during erythroid and myeloid differentiation will be presented.

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EXPRESSION OF ABL-BCR FUSION RNA IN CHRONIC MYELOID LEUKEMIA

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Whereas the role of bcr-abl fusion protein in the pathogenesis of chronic myelogeneous leukemia is well established, few data are available on the expression of the reciprocal fusion gene product abl-bcr. Nevertheless, functionally important domains such as a GAP site for p21 rac protein are located in the C-terminus of the bcr gene and might be deregulated by fusion to N-terminal sequences of the c-abl gene.
We have studied the expression of abl-bcr fusion RNA by reverse PCR in peripheral blood mononuclear cells of 30 CML patients. Primer oligonucleotides were prepared complementary to sequences of the 1a and 1b exon of c-abl and the exon 4 of the major breakpoint cluster region of the bcr gene. In 17 out of 30 patients (57%) 1b-abl/bcr transcripts were detectable, whereas only 6 out of 30 CML patients (20%) expressed 1a-abl-bcr RNA. In conformity with the fusion type shown in bcr-abl PCR we demonstrated amplification products corresponding either to a 1b-abl b3 or 1b-abl b4 fusion RNA. In one patients both types of fusion RNA were demonstrated. Correlation of the expression of abl-bcr fusion RNA to clinical stage, progression free survival and IFN response will be presented. Our results confirm that abl-bcr fusion RNA is expressed in peripheral blood cells of CML patients. Further studies have to clarify, whether expression of abl-bcr is an important event for the pathogenesis of CML.

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RECONSTITUTION OF THE OWN NORMAL HEMOPOIESIS AFTER TREATMENT WITH IFN α AND DONOR LEUKOCYTE TRANSFUSION IN RECURRENT CML AFTER ALLOGENEIC BMT

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Transfusion of donor leukocytes and IFNα can induce long-lasting remissions in recurrent patients with CML after BMT. We report on a 28 years old male patient who was transplanted in February 1989. A relapse was diagnosed in November 1991 by morphological and cytogenetic analysis of bone marrow. Thereafter the patient was treated in first line with IFNα resulting in a partial remission without any cytogenetic response. We transfused leukocytes of the bone marrow donor additionally to the IFNα therapy in July 1992. Subsequently the patient developed an increasing anemia and thrombopenia resulting in an increasing neediness of transfusions. The most serious complication of this treatment was a progressive paralysis (Guillain-Barré syndrome). After plasmapheresis and discontinuation of IFNα administration the symptoms were regressive.
Cytogenetic analysis showed an increasing Ph' negativity after leukocyte transfusion. We did not find any signs of CML in morphological smears, cytogenetic analyses, and PCR of RNA from bone marrow and peripheral blood in February 1992. However, PCR analysis of single progenitor colonies showed some bcr/abl positive lines.
Recent result of blood typing was identical to the recipient blood types (including all subtypes) before transplantation.
Conclusion:
1. The leukocyte transfusion led to a Ph' negativity in recurrent CML.
2. Results of blood typing indicate at least a subtotal reconstitution of recipients' normal hemopoiesis.

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COLONY SUPPRESSOR ACTIVITY AND DEFICIENCY OF HEMATOPOIETIC GROWTH FACTORS IN HCL.

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Bone marrow failure in hairy cell leukemia (HCL) has been attributed to a reduction of the hematopoietic progenitor cell compartment. To elucidate possible mechanisms responsible for this insufficiency we investigated in an autologous in vitro system the influence of either hematopoietic growth factors (CSFs), hairy cells (HCs) or T cells on the formation of hematopoietic colonies (CFU). Furthermore we measured the production of CSFs by MNCs isolation from HCL patients and healthy donors (HDs).
The data indicate a severe deficiency of hematopoietic progenitor cells in HCL. The removal of autologous HCs, but also of T cells resulted in a significant increase in colony formation (BFU-E, CFU-GM, CFU-mix). In none of the experiments, however, the colony numbers were within the normal range. This was only achieved by supplementation of the culture medium with CSFs (IL-3, rh GM-CSF). Since a clear correlation existed between the numbers of circulating progenitor cells in HCL patients and HDs and the monocyte counts in these groups, we tested whether purified monocytes are able to produce CSFs *in vitro*. In 6 out of 8 HCL patients the *in vitro* release of GM-CSF, G-CSF, IL-6, IL-3 and TNF-α was heavily reduced as compared to HDs. Only in 2 patients with complete hematological remission almost normal values were obtained.
These results suggest that the hematopoietic failure observed in HCL is probably due to an insufficient supply of CSFs as well as to an inhibitory activity of HCs and T cells which might exert their effects in a synergistic fashion. There is also evidence that the lack of monocytes play a role in the development of bone marrow insufficiency in HCL.

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INTENSIVE COMBINATION CHEMOTHERAPY OF HIGH GRADE MALIGNANT B-CELL LYMPHOMAS WITH HIGH-DOSE METHOTREXATE (MTX) AND LEUCOVORIN - PRELIMINARY RESULTS OF A SINGLE CENTER STUDY

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Nine adult patients aged 17 to 59 years with high grade malignant B-cell Non-Hodgkin`s lymphomas (4 pleomorphic centroblastic, 2 Burkitt-type lymphoblastic, 1 mixed centroblastic-lymphoblastic and 2 clear-cell mediastinal lymphomas with sclerosis) of stages IB to IVB were treated with high dose methotrexate (1.5 g/m^2/24 h) plus leucovorin rescue (beginning 36 hours after start of MTX infusion) in combination with vincristine, ifosfamide, teniposide, cytosine arabinoside and dexamethasone (Block A) and vincristine, cyclophosphamide, adriamycin and dexamethasone (Block B) for first remission induction. Two patients had previously undergone surgical intervention with abdominal tumor resection. All other patients received a five-day pretreatment regimen of cyclophosphamide and prednisone before entering their first course of high dose MTX. In four patients, prophylactic cranial radiotherapy was implemented. After one to three blocks A plus B, seven patients achieved a complete and two a good partial clinical remission. Three patients were given involved field irradiation for consolidation, two patients received no further treatment, one patient was autotransplanted after conditioning with chemotherapy plus total body irradiation, and in one patient, IF irradiation for consolidation is intended. Two patients, both stages IVB with multiple involved sites, who received no consolidation radiotherapy, relapsed one to three months after completion of MTX chemotherapy. All but one patients experienced toxicity of WHO grade IV with dominating hemocytopenia requiring blood cell support and prophylactic oral antimicrobial therapy. A life threatening infection was not observed. Mucositis grade II to IV was present in all patients requiring parenteral nutrition in four. All adverse events were completely reversible. With a follow-up of 3 to 13 months, all other patients are in continuous complete remission.
These preliminary results indicate a high efficacy of combination chemotherapy with high dose methotrexate plus leucovorin rescue in patients with high grade malignant B cell Non-Hodgkin`s lymphomas.

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SYSTEMATIC ANALYSIS OF MONONUCLEAR CELLS (MNC) AND HEMATOPOIETIC PROGENITORS GRANULOCYTE-MACROPHAGE (CFU-GM) DURING AUTOMATED BONE MARROW PROCESSING (BMP)

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Autologous bone marrow transplantation (ABMT) has been introduced in the treatment of several malignant diseases. Reduction of the initial bone marrow (BM) volume and removal of polymorphonuclear cells and red blood cells are important prerequisites for cryopreservation. We evaluated recoveries of MNC and CFU-GM using an automated program (3 BMSC) of the Fresenius AS 104 cell separator. Bone marrow processing (BMP) was done in 10 patients, 2 females and 8 males, median age 24 years (range: 16-56), suffering from germ cell tumors (n=8), acute lymphocytic leukemia (n=1) and lymphoma (n=1). MNC (n=10) and CFU-GM (n=7) were analysed in the following compartments: BM concentrate ready for cryopreservation (**a**), unprocessed BM fat (**b**) and residual processed BM suitable for autologous transfusion (**c**). The recoveries were: **a**: 36% MNC and 39% CFU-GM, **b**: 8% MNC and 5% CFU-GM, and **c**: 28% MNC and 24% CFU-GM, related to the MNC and CFU-GM yield of unprocessed BM (100%). Adding BM fractions **b** and **c**, not available for cryopreservation and ABMT, losses for MNC and CFU-GM are 36% and 29%. The remaining 28% MNC and 32% CFU-GM, not demonstrable in the BM fractions **a**, **b** and **c**, are probably unspecific detriments due to the cell separator device. We conclude that the automated BMP tested is not optimal for harvesting BM progenitor cells, at least in patients with germ cell tumours. Improvements in minimizing losses into compartments not utilized are needed.

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EFFECT OF ALL-TRANS RETINOIC ACID (ATRA) AND G-CSF ON PROLIFERATION AND DIFFERENTIATION OF MYELOID PROGENITORS AND PRIMING OF ACCESSORY CELLS.

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A combination of ATRA and G-CSF has been used in 10 patients (pts) with myelodysplastic syndromes (MDS) to reverse cytopenia. ATRA was given at 45 mg/m^2/day PO from week 1-12 and G-CSF at 5 µg/m^2/day SQ from week 5-12. During the course of therapy the granulocytes increased in all pts, hematocrit in 1 pt, and platelets in 2 pts. 10 pts were investigated for changes in serum cytokine and cytokine receptor levels (IL-6, IL-8, TNF-α, sTNF-R, sIL-2R, sICAM-1) by ELISA during ATRA/G-CSF therapy. sTNF-R increased 1.5-fold (p<.01) and sIL-2R 2.7-fold (p<.01). The serum levels of IL-6, IL-8 and sICAM-1 remained unchanged during therapy. 7 patients were investigated for changes in cytokine secretion (IL-1ß, IL-6, IL-8, TNF-α) from plastic adherent monocytes/macrophages (MΦ) after LPS stimuation. IL-6 IL-8 and TNF-α secretion doubled in all patients (p<.01) during therapy and remained increased even 4 weeks after cessation of therapy. Using purified CD34+ cells (>95% purity) from normal donors we investigated the effect of ATRA on progenitor cells in vitro. CD34+ cells were stimulated with IL-3 (20 ng/ml) and SCF (50 ng/ml) for 5 days in suspension culture and then assayed in methylcellulose for colony growth (CFU-GM). Addition of ATRA led to a dose dependent colony growth reduction. Coculture with MΦ or fibroblasts (Fb) significantly improved ATRA-induced decrease in colony growth (with Fb 128% ± 9% of control without ATRA).
These data suggest, that the effects on hemopoiesis seen during ATRA therapy, are due to activation and cytokine secretion of accessory cells, such as MΦ and fibroblasts.

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MOLECULAR ANALYSIS OF p53 AND MDM-2 IN HUMAN ACUTE MYELOGENEOUS LEUKEMIA

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In order to define the significance of the tumor suppressor gene p53 and the MDM-2 gene wich encodes a protein associated with p53 in the pathogenesis of human acute myeloid leukemia (AML) , the expression as well as the structure of both genes were examined in samples of bone marrow and/or peripheral mononuclear cells of 50 patients using Northern blot analysis, immunohistochemistry, polymerase chain reaction (PCR), single stranded conformation polymorphism analysis (SSCP) and direct sequencing. Cytogenetics were available from 20 out of 44 patients (46%). One patient showed a deletion of chromosome 17p. However, none of the patients exhibited intragenic deletions of the p53 gene. Only 1 out of 44 AML patients showed a point mutation of the p53 gene. This missense mutation occured in the evolutionary highly conserved region of p53 at codon 255 (ILE to PHE). Distinct levels of p53 mRNA and protein expression were observed in AML patients of the various FAB classifications, although in patients with AML M4 and M5 p53 expression was more pronounced. Since inactivation of p53 due to point mutations was a rare event in our AML samples examined, the role of MDM-2 was determined in myeloid leukemogenesis. Parallel to p53 a heterogeneous MDM-2 mRNA expression was detected in AML patients. Furthermore evidence suggested close correlation between p53 and MDM-2 mRNA expression, p53 overexpression was accompagnied by an enhanced level of MDM-2 mRNA in AML samples of FAB M4 or M5 classifications when compared to normal control. These data suggest that structural alterations of the p53 gene play not an important role in initiation and/or progression of AML. The hypothesis is discussed but abrogation of p53 tumor suppressor function due to MDM-2 overexpression may lead to a selective advantage of clonal outgrowth during progression of disease.

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p53 AND MDM-2 EXPRESSION IN HUMAN RENAL CELL CARCINOMA

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Inactivation of the tumor suppressor gene p53 by various distinct mechanisms, e.g. allelic loss, point mutations, association with viral and cellular proteins, has been demonstrated in a variety of human tumors. We examined 48 human renal cell carcinomas (RCC) and their corresponding normal renal tissue for structural changes and expression of the p53 and MDM-2 gene, which encodes a protein associated with p53 using Northern blot, polymerase chain reaction (PCR), single stranded conformation polymorphism (SSCP) analysis and immunohistochemistry. Neither allelic loss nor mutations in the hot spot regions from exon 5 to 9 of the p53 gene were detected in any RCC examined. Northern blot as well as differential RT-PCR analysis showed a heterogeneous p53 mRNA expression, but revealed no specific differences between RCC and normal kidney tissue. This was confirmed by p53 immunostaining. In a number of sarcomas amplification and/or overexpression of MDM-2, which is known to inactivate p53 function has been described. Northern blot and semi-quantitative RT-PCR analysis demonstrated MDM-2 overexpression in renal cell carcinoma when compared to normal kidney. MDM-2 immunostaining was observed in all RCC cells. Interestingly, a relation was found between MDM-2 immunostaining, the grading of the tumors and the proliferative capacity of the cells. However, the overexpression of MDM-2 mRNA and protein was not due to amplification of this gene as determined by semi-quantitative PCR analysis.

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Treatment of Metastatic Renal Cell Carcinoma with Vinblastine, Verapamil and Interferon alpha (VVI)

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There is still no standard therapy for patients with metastatic renal cell carcinoma (RCC). Interferon was of limited success. Chemotherapy showed disappointing results, probably caused by the multiple drug resistance of the renal cancer cell. However verapamil has been proven to inhibit p 170-glycoprotein, one of the possible drug resistance mechanisms.
We report results of palliative treatment of 14 patients with metastatic RCC receiving chemo-immuno-therapy with a combination of vinblastine, verapamil and Inferferon.
All patients had undergone radical nephrectomy. Some (7 of 14) had different chemotherapeutic regimens (5-flurouracil, dex-verapamil and vinblastine) before starting VVI protocol.

In VVI-protocol patients were treated as follows: vinblastine 6 mg/m^2, given as iv-bolus. From 48 hrs before until 24 hrs after vinblastine application, verapamil (80 mg p.o. 4 times daily) was given. Protocol was repeated on day 15. To prevent hypotensive or bradycardiac side effects, an oral sympathomimetic agent (etilefrin 5 mg) was given with each dose of verapamil. In combination with chemotherapeutic treatment, patients received interferon alpha-2a (Roferon A.Roche company) 6 million units s.c.three times per week. Clinical staging was performed every 2 courses. Vinblastine was postponed in case of prolonged myelosuppression or severe polyneuropathy . VVI protocol was stopped if progressive disease was observed. All patients received at least 2 courses of vinblastine and 3 months of interferon treatment. Minimum follow-up so far is 4 months.

Preliminary results of the VVI protocol show 35 % (5 of 14)progressive disease, 14 % (2 of 14) stable disease, 21 % (3 of 14) partial remission, and 29 % (4 of 14) complete remission. VVI protocol was generally well tolerated. No serious hypotensive or bradycardiac side effects were observed.

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Inv (3)(q21; q26) in AML: a new subtype?

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Inv (3)(q21; q26) was identified in 7 patients with AML and one patient with megakaryocytic blastic phase of CML. In patients with AML inv (3) was associated with monosomy 7. Clinically and hematologiccally most cases were characterized by MDS preceding AML, normal or increased platelet counts, short duration of remission or primary chemotherapy resistance. Immunophenotype could be analysed in 6 cases; 4 of them revealed CD7 +, CD 34 +, CD13 +, CD33 +, CD38 +, CDw65 +, CD2 -, CD3 -, CD4 -, CD8 -, CD19 -, CD20 -. Colony assays could be performed in 2 cases. Both cases showed increased spontaneous colony formation in unstimulated cultures. Stimulation with G-CSF and GM-CSF did not increase the number of colonies but colony size and all differentiation. In such cultures a high percentage of eosinophilic colonies were seen some of which showed macrophage or granulocytic differentiation in addition to eosinophilic differentiation.

We conclude that our karyotypic and immunologic findings may characterize an new AML-subtype.

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LIPOSOMAL AMPHOTERICIN B (AMBISOME) IN NEUTROPENIC PATIENTS WITH PULMONARY ASPERGILLOSIS

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The incidence of disseminated fungal infections in patients (pts) suffering from hematological malignancies with severe neutropenia has increased within the last years. Aspergillus spp are a major problem and treatment with Amphotericin B (AB) is often limited by severe side effects. The liposomal preparation (AmBi) is tolerated without any pretreatment and higher doses can be applied. 12 pts with evidence of pulmonary aspergillosis received 3 mg/kg AmBi for 24 days (2 - 42 days). All pts were pretreated with conventional AB. Change of the preparations was due to toxicity in 4 or/and persistent fever in 4 or/and progression of pulmonary infiltrates in 10 pts. **Diagnosis** was primarily made when typical signs of aspergillosis were found by high resolution computer tomography such as angiotropic lesions, infarctions and halo sign or X-ray. The diagnoses were confirmed in 5 pts by bronchoalveolar lavage, biopsy or postmortal examination. **Results**: 10/12 pts were evaluable, the other 2 died after 2 and 4 days of AmBi treatment. Defeverescence was seen in 9/10 pts, 6 pts were still neutropenic. One patient was not feverish when she changed to AmBi. Response of pulmonary infiltrates was achieved in 9/10, 5 pts were still neutropenic when regression occured. 2 pts additionally needed resection to be cured. The nonresponder died of disseminated aspergillosis (lung, lever, intestine, CNS). **Conclusion**: In 7/12 pts AmBi alone was effective in pulmonary aspergillosis, 2 pts needed additional resection and 3 pts expired. We conclude that the liposomal preparation seems to be at least as effective as the conventional preparation in the treatment of pulmonary aspergillosis.

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Expression of HERV-K Sequences in Hematological Disorders

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The human genome contains sequences that are related to retroviral *gag, pol, env* and *LTR* sequences, and termed human endogenous retrovirus (HERV). They are estimated at up to 1% of the genomic DNA. The HERV-K family has sequence homology to the B-type mouse mammary tumor virus (MMTV). HERV-K10, a full-length provirus, is particularly well characterized and contains long open reading frames in the viral genes. RNA expression of *gag* and *pol* genes has been shown in placenta and cell lines.

The goal of our study was to assay specimens of various hematological disorders for expression of HERV-K genes, searching for biological activity of these retroviral sequences.

The expression of HERV-K sequences was studied by reverse PCR in bone marrow or blood of various hematological disorders, including acute leukemias, myelodysplastic and myeloproliferative syndromes, as well as normal bone marrow. The primers used were derived from the sequences published by Ono (J.Virol. 1986). In 20 samples assayed so far by primers derived from the *pol* region (position 3935 and 4545), we found uniform expression of these *pol* sequences.

Thus, preliminary results support a constitutive expression of this gene, argueing for a physiological role of this gene. A specific pathogenetic involvement in carcinogenesis is not evident. But expression of other HERV-K genes (*gag, env*) has to be evaluated by further primer sets.

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LEUKEMIA INDUCED BY CHEMICALS

R. Snyder

Many chemicals which are known to produce bone marrow depression leading to aplastic anemia, are recognized as leukemogens or as potential leukemogens. Among the chemicals discussed will be benzene, a ubiquitous environmental chemical; drugs such as chloramphenicol, nonsteroidal anti-inflammatory pyrazoline derivatives such as aminopyrine and phenylbutazone, phenothiazines such as chlorpromazine, thiourea analogs such as propylthiouracil and anticancer alkylating agents such as mechlorethamine and busulfan. A more extensive discussion will be devoted to the mechanism of benzene-induced bone marrow damage and will include a review of benzene metabolism and the production of biological reactive intermediates, effects of benzene metabolites on bone marrow functions, suggestions regarding target cells within bone marrow that may be affected by benzene metabolites, and intracellular targets for benzene metabolites. A review of benzene-induced chromosome damage will be included. The association between the development of aplastic anemia and leukemia following benzene exposure will be discussed. The presentation will conclude with a comparison of mechanisms of chemical carcinogenesis in solid tissues with leukemogenesis and some suggestions regarding promising areas for new research in this field.

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Therapy control of platelet transfusion using an "Ex vivo-bleeding test"

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Thrombocytopenia is the most common cause of bleeding tendency, and, if due to impaired platelet production, is best treated by platelet transfusions. For patients with acute leukemia prophylactic platelet transfusions should be considered if platelet count is below 20 000/µl. This will be underlined by a retrospective analysis at our clinic of 231 patients suffering from acute myelocytic leukemia (AML FAB M1-7) and showing an early death rate of 18% by bleeding complications. To estimate effectiveness of platelet transfusions not only stopping of bleeding symptoms and corrected count increment (CCI) should be taken into account but also whether the patient has fever, sepsis, hepato-splenomegaly or has taken special drugs [e.g. ASS]. Measuring in vivo bleeding time is of little use for low reproduction and stressing for patients. In 1985 KRATZER (Haemostasis 15: 357-362) described a new and sensitive method for the evaluation of platelet function. After modifying this method it is now possible to test platelet function even with platelet counts below 50 000/µl.

Prior tests show 1. no influence on bleeding time by plasmatic coagulation disorders (including coumarin and full dose heparin therapy), 2. great sensitivity for detecting patients with von Willebrand syndrome, 3. bleeding time is inverse correlated to hematocrit [15-55%] with constant platelet count [200 000/µl] [r=0.909; n=10], 4. keeping hematocrit constant [40%] bleeding time is linear (after log-transformation) to platelet count [10-200 000/µl] [r=0,9; n=10].

In a prospective study we investigated 61 platelet transfusions (ABO-/HLA-A-, -B- compatible/adapted) in 32 patients suffering from leukemias or solid tumours. Platelet concentrates were performed routinely using cell separators for plateletapheresis. White cell depletion was done in all platelet concentrates using standard filter systems (PL 10A®, Diamed; PL 100® und PL50®, PALL). White cell depletion varies between 98.5 and 99.6% and platelet loss between 7.5 and 16.0%. Despite white cell depletion 18% of our patients showed non-hemolytic transfusion-reactions (flush, fever, urticaria). Bleeding time was markedly improved in 83% of all platelet transfusions [measering 1h after transfusion] and 89% of them also had an improved increment [CCI>7 000]. In patients without improved increment [CCI<7 000] 71% also had impaired bleeding time. Prior incubation of samples from patients and platelet concentrates (1 h at 37°C; hematocrit 40% and 50 000/µl platelets) shows improved bleeding time in 91%; after transfusion of these concentrates 74% also shows an improved increment [CCI>7 000].

In conclusion the described ex vivo bleeding time may be helpful in controlling platelet transfusion therapy.

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NF-JUN, A NOVEL INDUCIBLE TRANSCRIPTION FACTOR ASSOCIATED WITH CELL-CYCLE REGULATION AND PROLIFERATIVE RESPONSE

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We have recently identified a pallindromic sequence within the c-jun promoter responsible for transcriptional activation of the c-jun gene in acute myelogenous leukemia (AML) cells (Embo J: 11, 1479, 1992). We here show that NF-jun activation and thus expression of c-jun is enhanced upon TNF-mediated growth-stimulation of IL-3 treated AML-blasts. Deletion of the NF-jun recognition sequence within the c-jun promoter abolished TNF-mediated reporter gene activation indicating that NF-jun binding activity is required for TNF-mediated growth-stimulation. Moreover, elimination of c-jun/AP-1 by treatment of AML- blasts with an antisense oligonucleotide to the translation initiation site of the c-jun gene was associated with relieve of TNF-mediated proliferation of IL-3 treated AML-blasts. In addition, we demonstrate that NF-jun binding activity is regulated in a cell-cycle dependent fashion in these cells. NF-jun binding activity peaked at G_0/G_1 transition and showed minimal binding activity in M-phase of the cell-cycle. Taken together, our findings indicate that activation of NF-jun is associated with cell-cycle regulation and proliferative response. Experiments are under way to study the mechanisms of cell-cycle dependent regulation of NF-jun binding such as association with other proteins known to be involved in cell-cycle control (e.g. RB-protein or cyclins).

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DETECTION OF TRANSLOCATION (14;18) WITH MULTICOLOUR FLUORESCENCE IN SITU HYBRIDISATION (FISH) TECHNIQUE

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FISH is a new technique for the detection and characterisation of genetic aberrations as changes in the number of copies of whole chromosomes, deletions, amplifications, structural aberrations and translocations. The t(14;18) (bcl-2 translocation) can be detected in about 80% of follicular lymphomas and induces the production of bcl-2 protein, which prevents apoptosis.

Whole chromosome painting probes were used to detect the chromosomes 14 and 18 on metaphase spreads and interphase nuclei of the cell line Karpas 422**. Chromosome 18 probe was labeled with biotin and stained with fluorescein, chromosome 14 probe with digoxigenin and stained with rhodamin. The location of the "stained" chromosomes were determined using light microscopy.

The location of the marked chromosomes and the location of the changed chromosome- parts allowed the detection of the t(14;18) in metaphase spreads. In interphase nuclei was to much background for a certain result. The detection of t(14;18) with whole chromosome probes is possible in metaphase spreads, but not reliable in interphase nuclei. The results should be better with specific probes. This is under investigation.

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Fluorescence in situ hybridization: techniques and applications

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During the recent years fluorescence in situ hybridization (FISH) has found widespread applications in clinical and tumor cytogenetics. The latest developments in FISH include the multicolor FISH and comparative genomic in situ hybridization (CGH).

CGH provides a new technique to search genomes for genetic imbalances. Labeled genomic test DNA, prepared from clinical or tumor specimens, is mixed (1:1) with differently labeled genomic control DNA. The mixed probe is used for chromosomal in situ suppression (CISS-) hybridization to normal metaphase spreads. Hybridized test and control DNA sequences are detected via different fluorochromes, e.g. FITC and TRITC. The ratios of FITC/TRITC fluorescence intensities for each chromosome or chromosome segment reflect its relative copy number in the test genome as compared to the control genome. We have applied CGH on a variety of genomic DNAs from different tumors. Amplified DNA segments contained in double minute chromosomes or homogeneously stained regions of tumor marker chromosomes could be mapped to their normal chromosome counterparts and new amplification sites were discovered. We have extended this technique to the analysis of archival, formalin fixed, paraffin embedded tumor specimens. Prior to the hybridization, the DNA from paraffin embedded sections from tumor tissues was amplified via PCR using a degenerated oligonucleotide as a primer (DOP-PCR) and labeled via nick-translation. This allows for a comprehensive genotype/phenotype comparison of archival tumor materials.

Multiple color FISH with selected DNA-probes can be applied to confirm candidate chromosome regions suspicious for a gain or loss of genetic material in metaphase spreads and/or interphase nuclei obtained from tumor specimens. Using multiple color FISH with pools of Alu-PCR amplified products from human YAC clones we have constructed colored chromosome staining patterns, termed chromosomal bar codes (CBCs), on human chromosomes. Analytical CBCs adapted to particular needs of cytogenetic investigations and automated image analysis can be constructed. In conclusion, we expect that conventional chromosome banding, CGH and chromosomal bar codes will provide a new integrated approach in clinical and tumor cytogenetics.

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COMPARATIVE ANALYSIS OF IMMUNOPHENOTYPIC FEATURES BETWEEN CHILDHOOD AND ADULT ACUTE MYELOID LEUKEMIA (AML)

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The remarkable difference in therapy outcome in pediatric vs. adult AML probably not only reflects better tolerability of intensive chemotherapy in younger patients but may be also a consequence of intrinsic differences in disease biology. To better define these differences we compared the immunophenotype of blast cells in childhood and adult patients with *de novo* AML at primary diagnosis. The immunophenotype of leukemic blasts from 230 pediatric patients of the AML-BFM 1987 study and 300 adult patients of the AMLCG 1981, 1986 and 1991 studies was prospectively analysed before initiation of chemotherapy with a panel of monoclonal antibodies recognizing myeloid- (CD13/33/w65/15/14), lymphoid- (CD2/4/7/10/19) and progenitor-cell-associated (CD34/10/HLA-DR/TdT) antigens using a standard indirect immunofluorescence technique. At least one of the pan-myeloid antigens (CD13/33/w65) was expressed in more than 98% of all patients, and expression of all three antigens was found in 48% and 57% of childhood and adult AML respectively. We did not observe any significant difference in the expression of the myeloid or progenitor antigens between pediatric and adult cases, the only exception being that CD13 showed a lower expression in childhood AML (64% vs. 80%). More recently, coexpression of surface antigens associated with lymphoid differentiation has been reported in AML with considerably varying incidence. In our series, coexpression of T-cell-associated antigens was detected in about 48% of the pediatric and 42% of the adult population. The proportion of CD2/4/7 positivity was 10%, 34%, 13% (children) and 8%, 29%, 14% (adults) respectively. CD10- and CD19-expression was rarely found (<2%) in either pediatric or adult patients.

In conclusion, our data confirm the value of the pan-myeloid antigens CD13, CD33, CDw65 for the immunologic diagnosis of both childhood and adult AML, but did not reveal a remarkable difference in antigen expression between these subgroups.

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CHARACTERIZATION OF HUMAN MYOCARDIAL MAST CELLS AND THEIR REDISTRIBUTION IN AURICULAR THROMBOSIS

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Mast cells (MC) are multifunctional effector cells of the immune system and involved in the regulation of microvascular and inflammatory events. We have isolated and characterized a novel type of human MC present in myocardial tissue, and compared this MC type with MC obtained from uterus, skin and lung. Myocardial MC were isolated from patients (pts) suffering from cardiomyopathy (n=16). In all pts tested, MC were found in the auricular appendix exclusively. Myocardial MC expressed the IgE R, the receptor for SCF (c-kit R), the p24 antigen (CD9), the Pgp-1 homing receptor CD44 and the ICAM-1 antigen (CD54). mAbs to CD2, CD3, CD11a, b, c, CD14, CD15, CD16, CD17, CD19 or CD35 did not recognize cardial MC. Heart MC also contained tryptase, a mast cell- specific enzyme, histamine as well as berberine sulfate- binding proteoglycans. Activation (cross linking) of either IgE R or c-kit R by specific agonists (IgE, rhSCF) as well as activation by Ca-ionophore lead to secretion of proinflammatory mediators from cardial mast cells. SCF also enhanced the IgE dependent releasability of the cells. Substance P, a skin MC agonist (10^{-4}-10^{-6}M), compound 48/80 (0.01-1000 ug/ml) and the basophil agonist FMLP (10^{-2}-10^{-7}M) were ineffective over the dose range tested. Histologic examination of auricular appendices (autopsy sections, Giemsa staining) revealed the presence of MC in the epicard, myocard as well as in the endocard. In the presence of an auricular thrombus (n=7) endocardial MC increased in number compared to control (auricular thrombosis: 4.3±0.3 versus control (n=7): 2.2±0.6 MC/mm^2). Moreover, a redistribution of MC close to the subendothelial space of the auricular appendix in auricular thrombosis was observed (auricular thrombosis: 1.9±0.5 versus control: <0.01 MC/mm^2, $p<0.002$). Together, these data suggest, that the endomyocard of the auricular appendix contains substantial amounts of mast cells. These MC exhibit immunophenotypic and functional properties similar to those found in human lung and uterus, and increase and redistribute in number in auricular thrombosis.

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INTERACTIONS OF TRANSFORMING GROWTH FACTOR-β AND LIGANDS OF THE STEROID/RETINOID RECEPTOR SUPERFAMILY

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The three mammalian isoforms of transforming growth factor-β (TGF-β) are each homodimeric peptides with each monomer consisting of 112 amino acids. The regulatory response elements in the promoters of the genes of each of the three isoforms differ markedly, resulting in differential regulation of the expression of the three TGF-βs. TGF-βs are the prototypical multifunctional growth factors. The nature of their action on a particular target cell is critically dependent on many parameters including cell type, its state of differentiation, the growth conditions, as well as the presence of other growth factors. Recently, there has been intense interest in the interface between ligands of the steroid/retinoid receptor superfamily and the three TGF-βs. It is now well established that estrogens, estrogen analogs, androgens, glucocorticoids, retinoic acid, and vitamin D3 all regulate the synthesis or secretion of the various isoforms of TGF-β. In many cases, the effects are specific for a particular isoform. Thus, treatment of vitamin A-deficient animals with all-trans-retinoic acid will induce the selective expression of TGF-β2 in many target epithelia. In addition, the above ligands frequently induce the secretion of active, rather than latent TGF-β. Although some effects of steroids and retinoids on the TGF-β system may be mediated at the transcriptional level, these effects are often post-transcriptional.

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TNF α expression in adverse drug reactions was not associated with elevated sICAM-1 and sELAM-1 plasma levels

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Intravenous amphotericin B (Am B) adminstration causes severe adverse effects, such as fever, chills and hypotension in a significant proportion of patients. Evidence is accumulating that production of acute phase cytokines like TNF α may be responsible for drug-induced febrile reactions.
Since TNF α is known to strongly upregulate adhesion molecules on cell surfaces, we serially analyzed plasma levels of circulating ICAM-1 (intercellular adhesion molecule 1) and ELAM-1 (endothelial adhesion molecule 1) in 6 patients with acute leukemia who received standardized amphotericin B application for systemic fungal infection in bone marrow aplasia.
All patients developed fever within 360 min after start of Am B infusion. In 5 patients a more than 2 fold increase in TNF α (range: 18,5 - 925 pg/ml) and IL6 (range: 154 - 1399 pg/ml) could be detected. Levels of sICAM-1 and sELAM-1 varied substantially prior to drug application (range: 120 - 761 ng/ml and 9 - 35 ng/ml, respectively). In 3 patients a minor but insignificant increase of sICAM-1 levels occurred. Plasma levels of sELAM-1 were unchanged up to 360 minutes of amphotericin B treatment.
We conclude that although TNF α strongly induces adhesion molecule expression on cell surfaces in vitro, elevated TNF α in amphotericin B-induced adverse drug reaction was not associated with increased sICAM-1 and sELAM-1 plasma levels.

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Pneumonia during bone marrow aplasia in acute leukemia was associated with increase of sICAM-1 but not sELAM-1

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The interaction between endothelium and leukocytes regulated by cytokine-inducible adhesion molecules ELAM-1 (endothelial adhesion molecule-1) and ICAM-1 (intercellular adhesion molecule-1). Whereas cytokine-induced ICAM-1 expression occurs on a variety of cell surfaces, ELAM-1 expression is restricted to endothelial cells only. Although these molecules are initially integrated into the plasma membrane, various amounts will be released into the circulation by unknown mechanisms.
Aggressive treatment of leukemia is commonly accompanied by febrile episodes and only some will indicate development of serious infections. We analyzed plasma levels of sELAM-1 and sICAM-1 during treatment of acute leukemia (n=14, 6 patients with overt leukemia, 8 in complete remission) to evaluate whether patterns of circulating adhesion molecules may differentiate severe infections from non-infectious febrile episodes. From 12 observed febrile periods only 3 were found in patients with complete remission. sICAM-1 increased considerably (2 fold and more) in 6 fever phases, 5 of which were directly related to pneumonia. In one case sICAM-1 increase occurred in fever classified as FUO in underlying HIV-infection, but this patient did also develop pneumonia lateron. In contrast, sELAM-1 increase could not be detected in any of the febrile periods. sELAM-1 levels were closely related to leukocyte counts and minimal values were constantly found in profound bone marrow aplasia.
We conclude that although ICAM-1 and ELAM-1 work in concert to regulate inflammatory reactions, severe inflammation such as pneumonia during bone marrow aplasia was associated with increase of circulating ICAM-1 but not ELAM-1 molecules. One possible explanation could be that shedding of ELAM-1 from endothelial surfaces may depend on the presence of leukocytes.

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COMBINED TREATMENT MODALITIES (CTM) OF ESOPHAGEAL (E) CARCINOMA (C).

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Due to insufficient local control in the majority of EC pts and due to distant recurrences, the dismal prognosis of EC has not essentially changed during the past two decades despite extended surgical procedures and improved radiation techniques. The 2-year-survival rates in stage IIb/III are still less than 20%. Therefore, clinical efforts in the management of EC focus on CTM including chemotherapy (CTx). Up to now, results of CTM in POTENTIALLY RESECTABLE EC have not shown that preop. CTx or CTx/RTx (radiotherapy) is superior to surgery alone with respect to resectability, local tumor control and overall survival. However, CTx or CTx/RTx responders who subsequently underwent resection had a markedly improved long term survival indicating that the inclusion of CTx in the treatment of EC may improve the prognosis. The benefit of CTx was also shown by Herskovic et al., who compared RTx (64 Gy) versus CTx (cisplatin/FU) plus simultaneous RTx (50 Gy) in EC pts (mostly stage IIA). The CTx/RTx arm resulted in a reduction of local and distant failures and a significantly improved survival. In LOCALLY ADVANCED DISEASE (LAD) preop. CTx alone failed to improve overall survival, but again patients with response to chemotherapy had an improved prognosis after R0-resection as compared to nonresponders with resection. Of note are first promising reports with intensive preop. CTx/RTx programs in LAD resulting in high local tumor control and promising survival times of patients with tumors unlikely to undergo curative resection prior to CTx/RTx.
To date, there is sufficient evidence that preop. treatment of EC may improve prognosis at least of subgroups of pts with EC. However, this has to be confirmed in well designed (proper staging including endoscopic ultrasound, etc.) randomized trials. There is also evidence that combined preop. CTx/RTx is superior to preop. CTx alone with respect to local tumor control and induction of pathologically complete remissions (20% versus 5%) and that CTx reduces the risk of distant failures.

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MULTIMODAL TREATMENT OF LOCALLY ADVANCED ESOPHAGEAL CANCER (EC): INTERIM ANALYSIS OF A PHASE II TRIAL

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Since March1991, 52 pts with locally advanced EC were entered into a phase II study with an intensive preop. chemotherapy (CTx) and simultaneous radio-chemotherapy (CTx/RTx) followed by transthoracic esophagectomy.
TREATMENT PLAN: Folinic acid 300mg/m^2, 10 min inf; etoposide (E) 100mg/m^2, 50 min inf; 5-FU 500mg/m^2 10 min inf; cisplatin (P) 30mg/m^2 1h inf, d 1-3, q d 22. On d 22 of the last CTx cycle start of irradiation (40 Gy, 2Gy/d) plus P 50mg/m^2 d2, 8 and E 100mg/m^2 d 4, 5, 6. Operation 4 weeks after end of CTx/RTx. CHARACTERISTICS of 40 pts currently off treatment: m/f 37/3; age 57(42-69); PS 1(0-1); T2 (obstructive tumors > 5cm length) 10, T3 Nx-N1 27, T4 Nx-N1 3; SCC 34, adenoca. 6. RESULTS AFTER CTx: cCR 2(5%), PR 17(42%), MR/NC 14(35%), P 6(15%), 1 tox death. TOXICITY(WHO): leukopenia 3° 52%, 4° 16%; infection 3°/4° 12%; thrombopenia 3° 36%, 4° 8%; non-hematologic toxicities were moderate only. RESULTS AFTER CTx/RTx: further response improvement in approx. 30% of the patients. Leukopenia 3°/4° 90%, thrombopenia 3°/4° 43%, 1 toxic death. RESULTS AFTER RESECTION (N=29): R0-resection (pCR) 10 (34%), R0-resection/NED 16(55%), overall R0-resection rate 90%, R1/R2-resection 3, 3 postoperative deaths (10%). RESULTS OF ALL (40) pts: cCR 3 (8%), pCR/NED 26 (65%) cCR/pCR/NED 29 (73%), 2 pre- and 3 postop. deaths. Median observation time 11 months. Calculated 2-years-survival rate is 67% for all pts and 75% for pts surviving disease-free after resection. CONCLUSIONS: This intensive multimodal treatment program is feasible and highly effective in locally advanced esophageal cancer. The overall local tumor control rate of 73% and projected 2-years-survival rate of 67% is promising. A randomized trial with this approach versus chemo-radiotherapy alone is in preparation.

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EPSTEIN-BARR VIRUS ASSOCIATED LYMPHOPROLIFERATIONS.

H. Stein.

In recent years, techniques, probes, and reagents became available to reliably visualise individual Epstein-Barr virus (EBV)-infected cells, to assess EBV gene expression, and to analyse the clonal composition of EBV genomes in human tissues. Application of these techniques to more than 1000 lymphoid tissue specimens revealed (1) characteristic cellular and compartimental distribution patterns of EBV-infected cells in normal lymph nodes, reflecting the interference of EBV with physiologic B cell differentiation pathways, (2) an association of EBV with various mono- and oligoclonal lymphoproliferations ranging from benign conditions to overtly malignant lymphomas, and (3) characteristic patterns of EBV gene expression among EBV-associated lymphoproliferations. In the context of the established immortalising and transforming properties of EBV, the findings support the concept of an etiologic role of EBV for cases of certain lymphomas such as Burkitt`s lymphoma, anaplastic large cell lymphoma, plasmablastic lymphoma, Hodgkin`s disease, and lymphomas arising in immunocompromised individuals. In contrast, lymphomas harbouring EBV in only proportions of the tumour cells (such as cases of peripheral T cell lymphoma and some B cell lymphoma types) argue against an etiologic role in the primary process of malignant transformation for the virus in these instances. Since in many of these cases a proportion of the EBV-infected tumour cells express the EBV oncoprotein LMP (latent membrane protein) the virus may influence, however, the proliferative properties as well as the morphological and molecular phenotype of the neoplastic cells.

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PATTERN OF CYTOKINES IN LEUCOPENIC FEVER

H.T. Steinmetz, M. Bertram, A. Riediger, M. Kochanek, S. Ritter and V. Diehl

BACKGROUND : While recombinant cytokines gain more and more significance for treatment of malignant and infectious disease, only little is known about the physiologic role of natural cytokines.
METHODS : To gain an insight in the regulatory network in leucopenic fever 20 patients with AML were documented concerning clinical and laboratory course by daily evaluation (e.g. blood-cell count, fever, microbiology, medication). All patients were treated with standard chemotherapy. Fever was treated according to the guidelines of the PEG-II trial for fever in granulocytopenia. Serum was frozen in aliquots before chemotherapy. During leucopenia serum was collected at least twice a week and frozen within 2 hours. In case of fever additional samples were frozen. TNF and IL-6 serum-levels were determined by Medgenix-ELISA, G-CSF and GM-CSF by Quantikine-ELISA (Biermann).
RESULTS are summarized in the table :

	Before ChTh without fever	WBC < 1000/mcl without fever	WBC < 1000/mcl with fever
TNF	n.r.	n.r.	irregular increase
IL-6	n.r.	n.r. *	increase
G-CSF	n.r.	increase	additional increase
GM-CSF	n.r.	irregular	irregular

Abbr.: ChTh = Chemotherapy, WBC = white blood cell count, n.r.= normal range (compared to healthy volunteers),
* minor increase (<100 pg/ml) in local infection without fever or recovery of WBC.
Detection of TNF-alpha is a rare event in leucopenia. TNF-serum level increases in severe infection or fever during recovery of blood-cell count. Il-6 normally could be detected during episodes of fever independent from blood-cell count. The course of IL-6 serum-level mostly runs parallel with G-CSF serum-level. G-CSF is upregulated by falling leucocytes/leucopenia or fever respectively. There is no clear correlation between GM-CSF levels and leucopenia, fever or clinical course.

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TNF IS A INDICATOR FOR DEATH DUE TO INFECTION IN CHEMOTHERAPY-INDUCED LEUCOPENIA

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BACKGROUND : It was the intention of the research to determine the relative risk of development of fever in chemotherapy-induced leucopenia and to detect additional factors correlated with ICU-admission or death during leucopenia.
METHODS : All patients of 4 oncologic wards (72 beds) beeing suspected to have either the diagnosis of AML, ALL, lymphoblastic NHL or relapsed M.Hodgkin were registrated. In case of confirmed diagnosis and chemotherapeutic treatment the clinical and laboratory course was documented by daily evaluation. All patients were treated with standard chemotherapy (AML : age <60 TAD9/HAM, >60 AVA 7/5; ALL and lb-NHL : BMFT-study; M.Hodgkin : Dexa-BEAM). They received selective oral antibiotic prophylaxis and antibiotic treatment according to the PEG-II intervention trial for fever in granulocytopenia. Serum was frozen in aliquots before chemotherapy. During leucopenia serum was collected at least twice the week. TNF-serum levels were determined by ELISA (Medgenix).
RESULTS : 156 patients (56 AML, 25 ALL, 39 NHL, 25 HD, 11 other) were registrated with 374 admissions during 14 month.
Table: 91 patients with 230 episodes of chemotherapy-induced leucopenia <1000 cells/mcl for more than 2 days were documented.

DIAGNOSIS	EPISODES	FEVER	DEATH
AML	121	101 (84%)	8 (7%)
ALL	67	33 (49%)	6 (9%)
NHL	16	11 (69%)	-
M.HODGKIN	26	12 (46%)	-
TOTAL (N 91)	230 (100%)	137 (60%)	14 (6%)

The mean TNF-serum level was 215 pg/ml in survivors and 88 pg/ml in non-survivors. Preliminary data show an increase in TNF-serum level in the days before death due to infection. Additional risk factors (e.g. days in leucopenia, age) will be reported.

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CORRELATION OF ANTI-X A AND D-DIMER VALUES WITH OCCURRENCE OF POSTOPERATIVE VENOUS THROMBOSIS IN PATIENTS TREATED WITH UNFRACTIONATED OR LOW MOLECULAR WEIGHT HEPARIN (LMWH)

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Little is known about D-Dimer concentrations in patients (pts) with or without postoperative deep vein thrombosis (DVT) before and under prophylactic antithrombotic treatment. In addition, conflicting results have been reported on the correlation between anti-Xa activity and occurrence of DVT after surgery. Therefore, anti-Xa-(Heptest) and D-Dimer (ELISA) levels were investigated in pts undergoing hip arthroplasty who were treated with unfractionated heparin (UH) or LMWH (CY 216) in a double-blind, randomized multicenter trial (GHAT, Arch Orthop Trauma Surg, 1992). Before surgery mean D-Dimer values of pts with postoperative DVT (n=34) assessed by phlebography were significantly ($p<0.05$) higher than those of pts without DVT (n=38). This was true also after surgery irrespective of whether UH or LMWH was applied. In the LMWH group mean postoperative anti-Xa levels were significantly higher than in the UH group. However, no significant difference was found in the anti-Xa activity in any group between pts with or without DVT. Whereas anti-Xa activity seems to be of limited value in the prediction of postoperative DVT, D-Dimer levels after and even before surgery may select pts with high risk of postoperative DVT offering a basis for individual antithrombotic treatment.

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A RELIABLE APPROACH FOR SEQUENCING CLONE-SPECIFIC CDR-III REGIONS IN B-LYMPHOMA.

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The CDR-III regions are part of the rearranged immunoglobulin heavy chain genes in B cells. In B cell-derived lymphoid malignancies, the CDR-III region of the malignant clone represents a unique marker by which very low numbers of malignant cells in the blood, bone marrow or other tissues can be detected using PCR. For the generation of clone-specific primers, the CDR-III regions of the NHL or ALL must be sequenced. Direct sequencing of respective PCR fragments amplified with universal primers frequently results in a suboptimal quality of the autoradiogram, due to co-amplification of a variable background of normal B cells, which all carry individual CDR-III regions. On the other hand the subcloning of PCR fragments usually requires an enzymatic modification of the fragment ends, which often results in a reduced efficiency of subcloning.
We chose to subclone the CDR-III PCR fragments from NHL or ALL samples, amplified by the use of universal primers, directly into a special vector (TA Cloning System, Invitrogen). This cloning system takes advantage of the activity of Taq Polymerase to add single deoxyadenosines to the 3'-ends of the PCR products. The TA vector provides the complementary deoxythymidines for subcloning. Purification of the PCR fragments was not required and an aliquot of the PCR reaction put directly into the ligation reaction enabled an efficient subcloning. Recombinant clones can be identified by the white appearance of the bacterial colonies. Restriction analysis of miniprep DNA showed that 90% of these clones carried a PCR fragment integrated into the vector. After purification of plasmid DNA with PEG, the different cloned CDR-III regions were sequenced by cycle sequencing. For this, about 20 ng of plasmid DNA was required, representing only a small fraction of the miniprep DNA. Between 6 and 10 different clones from each ligation were sequenced and all yielded high quality autoradiograms. From this sequence data, clone-specific primers were prepared and used for the detection of occult lymphoma cells in patients with high grade NHL. Sequential peripheral blood samples from patients in which G-CSF was used to mobilise peripheral stem cells are being studied. The aim of our molecular study is to see what effect G-CSF has on the level and/or persistence of minimal residual disease and to which extent, if any, peripheral stem cell harvests are contaminated with lymphoma cells. PCR data from our patients will be presented.
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THE ROLE OF ONCOGENES AND TUMOUR SUPPRESSOR GENES IN CELL SURVIVAL AND NEOPLASATIC TRANSFORMATION

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Physiological cell death, apoptosis, is responsible for removing obsolete and potentially dangerous cells of various lineages and is therefore indispensable for normal development. This death process is controlled by protein products of genes which are also important in neoplastic transformation, oncogenes and tumour suppressor genes.
bcl-2 is the first example of a new class of oncogenes, which regulates cell survival but does not influence proliferation or differentiation. Enforced *bcl*-2 expression prolongs survival of several cytokine dependent cell lines after factor deprivation and *bcl*-2 transgene expression extends survival of B and T lymphocytes *in vivo* and *in vitro* even in the presence of various cytotoxic agents.
bcl-2 appears to play an important role in clonal selection which operates on developing B and T cells to guarantee survival of lymphocytes with useful antigen-receptors and death of those with autoreactive, useless, or no receptors. *bcl*-2 transgene expression inhibits death of B but not T cells in *scid* mice which are unable to generate productive antigen receptor gene rearrangements. Furthermore *bcl*-2 transgene expression in anti-HY TCR transgenic mice inhibits death of immature thymocytes expressing a TCRα/β heterodimer which cannot bind to self MHC molecules and also delays, but does not abrogate, deletion of autoreactive T cells. These data suggest *bcl*-2 is normally upregulated during positive selection as a result of antigen receptor engagement, but on its own is insufficient to prevent deletion of autoreactive lymphocytes.
Long term analysis of Eμ-*bcl*-2 transgenic and (*bcl*-2+*myc* or *ras* or v-*abl*) double oncogene transgenic mice identified *bcl*-2 as a weak transforming oncogene on its own, which collaborates in neoplastic transformation with *ras* (weak), v-*abl* (intermediate) and *myc* (strong), identifying *bcl*-2 as an oncogene which contributes to tumorigenesis by inhibiting apoptosis.
The tumour suppressor genes *p53* and *FAS/APO-1* (*lpr*) were recently shown to be essential for some induction mechanisms leading to apoptosis. One of the big challenges in the near future is to determine the relationship between these three gene products and identify novel genes which control physiological cell death.

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p53 mutations and mdm-2 amplification in renal cell cancers

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Alterations of the p53 gene may be the most frequent mutations in various human cancers. Little is known about the genetic alterations associated with renal cell cancer. DNA from 53 primary human renal cell tumors was screened for the presence of mutations of p53, using the polymerase chain reaction and single strand conformation polymorphism analysis, followed by direct DNA sequencing. Five cases showed mobility shifts. Sequencing of these DNA's revealed 2 cases of nonsense mutations (codon 182, 192); a case of missense mutation (codons 285); and 2 cases of the same silent mutation at codon 213. All tumors with nonsense and missense p53 mutations were cases with poor prognosis, i.e. the highest pathological grade and/or advanced Robson stage. Therefore, alterations of p53 may be associated with the development of renal cell carcinoma of higher grade and/or stage. The frequency of mutations altering the p53 gene (3/53:5.8%) was low compared to the reported frequency of allelic loss of 17p (location of p53 gene) by RFLP analysis (15-30%) in renal cancers. This suggests the existence of another tumor suppressor gene in the region of p53 which when mutated, is associated with these cancers. The mdm-2 protein binds and possibly can inactivate p53 when overexpressed; it is amplified in sarcomas. Southern analysis showed that it was not amplified in renal cell cancers. In summary, the p53 gene is infrequently altered in renal cell cancer, but when mutated, it is associated with a bad prognosis. Allelotyping suggests that at least one more tumor suppressor gene is located on 17p, and it is frequently abnormal in renal cell cancers.

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High-Dose Chemotherapy and ABMT in Three Patients with Relapse of Mediastinal Large-B-Cell Lymphoma with Sclerosis

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Mediastinal large-B-cell lymphoma with sclerosis (MLCL) is a histopathologic entity characterized by clinical features like young age, prevalence of females over males, rare occurrence of superficial lymphnodes and involvement of unusual extranodal sites. Complete remissions can be achieved with conventional therapy regimes like CHOP in 60 - 89%, resulting in long term remissions in 59 - 79%. But treatment after relapse so far never resulted in long periods of survival.
Based on experiences that recurrent high grade NHL can have a benefit from high-dose chemotherapy (HDT) we treated three patients with relapsed MLCL with high dose carboplatin (1500 mg /m²), etoposide (2000 mg/m²) and ifosfamide (10 g/m²) and subsequent ABMT.
All 3 patients (26/w, 49/w, 22/m with initial stages IIb, IIa and IVa) recieved under conventional chemotherapy (CHOP/ COPBLAM) and radiation complete remissions of short duration (only 2 - 4 months). Salvage therapy led to partial remissions with disease progression in therapy free intervals. After HDT the 22-year-old man died 10 days after ABMT of therapy related toxicity and disease progression. The two other patients responded with PR, but 7 respectively 8 weeks after ABMT both had disease progression. The 28-year-old woman died under palliative chemotherapy 11 months later, the other is still alive with disease 6 months after ABMT.
High-dose chemotherapy only led to short lasting response in 2 patients. It remains to be determined if relapsed MLCL can be cured by HDT and ABMT.

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SHORT-TERM EFFECTS OF CHEMOTHERAPY ON THE PATTERN OF MONONUCLEAR CELLS AND THE SOLUBLE IL-2 RECEPTOR IN PERIPHERAL BLOOD OF PATIENTS WITH HIGH-GRADE NON-HODGKIN'S LYMPHOMA

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Using different methods (fluorescence microscopy,flow-cytometry, ELISA), we studied the short-term effects (before, after 3 and 7 days) of chemotherapy (CHOP-Bleo,CHOP,MACOD-B,COP-BLAM) on the pattern of mononuclear cells and the concentration of the soluble IL-2 Receptor (sIL-2Rc) in the peripheral blood of patients with high-grade non-Hodgkin's lymphoma (NHL).
Comparing 15 healthy subjects and untreated patients, we found that within the populations of T-cells (CD3,CD4,CD7,CD8), B-cells (CD19), monocytes (CD14), NK-cells (CD16,CD57) and activated cells (CD25,CD26,CD71,HLA-DR-classII), only CD3- and CD4-positive cells showed a slight decrease in the relative and absolute number. However, the concentration of the sIL-2 Receptor was increased significantly (patients:294+/-120U/l; control group:83+/-23U/l). All other parameters did not show any clear difference.
After 3 and 7 days of chemotherapy, we discovered a significant reduction of the portions of CD14- ,CD19- ,CD57- positive cells as well as a slight increase of CD3- and CD4-positive cells.
In this time the concentration of the sIL-2 Receptor was determined to be diminished to 70% of starting value.
Our data suggest a cellular immunodeficiency and indicate the possible use of the sIL-2 Receptor as an early marker of tumor response in the treatment of high-grade non-Hodgkin's lymphoma.

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SEQUENCE-DEPENDENT EFFECTS OF ARA-C AND ETOPOSIDE ON MYELOID CELL LINES AND BLAST PROGENITORS OF ACUTE NONLYMPHOBLASTIC LEUKEMIA (ANLL)

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The combination of ara-C and etoposide (VP-16) has shown promising results in the treatment of ANLL, although conflicting in vitro data exist as to whether a synergistic or antagonistic mode of action is to be presumed. The present study investigated the sequence-dependent interactions of cytosine arabinoside and etoposide against 6 myeloblastic cell lines, the blast progenitors (CFU-L) of 7 newly diagnosed patients with ANLL and 7 normal controls (CFU-GM). Cells were incubated with 2μM VP-16 for 30 or 90 min., or with 10 μM ara-C for 60 or 90 min.. Preincubation with 2 μM VP-16 for 30 min. was followed by 10 μM ara-C for 60 min.. Preincubation with 10 μM ara-C for 60 min. was followed by 2μM VP-16 for 30 min.. Simultaneous application consisted of both 2 μM VP-16 and 10 μM ara-C for 90 min.. The therapeutic effectiveness was determined by measurement of cell proliferation in liquid suspension culture (LSC) and by evaluation of colony formation in methylcellulose (CFU-GM and CFU-L). Monoincubation with either etoposide or ara-C had the least antiproliferative effect compared to combined or sequential application in all culture systems. By prolonging the incubation time from 30 to 60 minutes for VP-16, and from 60 to 90 minutes for ara-C it was possible to increase the degree of inhibition, though signifcantly only for ara-C ($p < 0.05$). In LSC assays monoincubation with VP-16 proved to be least inhibitory, even after increasing the time of exposure to 90 min., compared to either combination ($p < 0.05$). With KG-1, KG-1a, HL-60, K 562, as well as 4 out of 7 ANLL samples, the highest degree of inhibition was obtained by preincubation with VP-16. In DU 528, the remaining 3 ANLL patients, as well as all 7 controls simultaneous exposure was most inhibitory. Our study shows that the combination of ara-C and etoposide has greater antiproliferative effects compared to monoincubation with either drug. We did not observe any clearcut antagonistic effects.

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CORRELATION OF CYTOKINE LEVELS WITH CLINICAL PARAMETERS IN PATIENTS WITH HODGKIN'S DISEASE

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Expression of a variety of cytokines have been detected in cell lines and primary specimen from Hodgkin's Disease. It has been suggested that these molecules maybe involved in the interaction between the tumor cells and reactive bystander cells and related to clinical symptoms such as fever and night sweat. To analyse whether cytokines are present in the sera of patients with HD we determined the concentrations of the molecules by ELISA in a large panel of patients which were treated with protocols of the German Hodgkin Study Group. The concentrations of cytokines were compared to a number of clinical and serological parameters.
The concentrations of IL1α, IL1ß, IL2, IL3, IL4, GM-CSF, TNFα, TNFß and sCD23 were not elevated in sera of patients with HD. In contrast elevated concentrations of sIL2 receptors (sIL2R), IL6 , G-CSF, IL7 and IL8 were detected in lymphoma patients as compared to normal sera. There was a strong correlation between advanced stages of HD (stage III and IV) with enhanced levels of sIL2R, IL6 and IL7 and the presence of B symptoms.

	% above normal range	
	patients with HD	controls
sIL2R	77	4
IL6	72	2
G-CSF	39	5
IL7	39	3
IL8	46	5

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ACTIVATION OF PROTEIN KINASE-A IN MURINE T-HELPER 2 CELLS ALTERS T-CELL RECEPTOR INDUCED IL-4 AND IL-10 SECRETION

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The main intracellular signalling pathway activated by the T-cell receptor (TCR) is the phosphatidyl-inositol-biphosphate second messenger system. Evidence has accumulated that protein kinase-A (PK-A) activation inhibits TCR-mediated signal transduction in T-helper 1 cells. Here we analyze the effect of PK-A activation on TCR-induced interleukin synthesis in T-helper 2 (TH2) cells.

IL-4 and IL-10 are two major lymphokines produced by TH2 cells. Activation of PK-A by isobutyl-methyl-xanthine (IBMX) has different effects on the TCR-triggered synthesis of the two interleukins. While TCR-induced synthesis of IL-4 is enhanced by low concentrations of IBMX secretion of IL-10 remains unaffected. At higher concentrations of IBMX enhancement of IL-4 secretion is less marked. When TH2 cells are stimulated by a phorbolester plus calcium ionophore IL-4 secretion is enhanced almost fourfold whereas IL-10 production is unaffected by PK-A activation. This effect is observed at all concentrations of IBMX tested. The same results were obtained when analyzing IL-4 and IL-10 messenger-RNA by Northern-blotting.

Presented data indicate that PK-A activation may lead to enhancement of TCR-induced interleukin synthesis in TH2 cells. Based on our results we propose different activation pathways for IL-4 and IL-10 in TCR-stimulated TH2 cells which can be distinguished by their susceptibility to PK-A activation.

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INTERFERON-ALPHA-2C AND LOW DOSE ARA-C FOR THE TREATMENT OF PATIENTS WITH PH-POSITIVE CML: PRELIMINARY RESULTS OF THE AUSTRIAN MULTICENTER PHASE-II STUDY

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In a phase-II trial 50 patients with newly diagnosed Ph-positive chronic myelogenous leukemia (CML) were treated with interferon-(IFN)-a-2C (Berofor®) at daily doses of 3.5 MU subcutaneously and low dose cytosine arabinoside (LD AraC, Alexan®) added for ten days every months at a dose of 10 mg/m2 subcutaneously following an initial reduction of WBC to less than 20 G/L with hydroxyurea (HU,Litalir®). In case of a leukocyte nadir above 10 G/L, the AraC dose was increased to 20 mg/m2 for ten days per month.

Within a median observation period of nine (range, one to 23) months 45 patients finished the HU phase and 41 patients received a median of four (range, one to 18) IFN and LD AraC cycles. Side effects, largely WHO grades one and two, observed during the IFN and LD AraC treament consisted of fever (29%), muscle and bone pain (32%) , nausea (27%), leukopenia (46%), thrombocytopenia (31%), cutaneous reactions (17%), gastrointestinal toxicity (15%), neurotoxicity (17%) , infections (7%), anemia (10%) and hair loss (15%).

Among the 41 patients, who received at least one cycle of IFN and LD AraC, complete hematological remission (CHR) was achieved in 18 patients (44%) and partial hematological remission (PHR) in eight patients (20%). Cytogenetic analysis was performed every six months. In 32 patients evaluable at present 13 cytogenetic responses (41%) including five major cytogenetic responses were observed.

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MODULATION OF MULTIDRUG RESISTANCE (MDR1) BY DEXNIGULDIPINE-HCL IN COMBINATION WITH VAD OR VECD IN PATIENTS WITH REFRACTORY MYELOMA

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Resistance in multiple myeloma is frequently associated with a 170 kD transmembrane glycoprotein, which is encoded by the MDR1 gene. Circumvention of multidrug resistance (MDR) in myeloma patients was recently shown for the drugs verapamil and cyclosporin A. In the present phase II trial we applied dexniguldipine-HCl, a dihydropyridine derivate, to myeloma patients with either (1) progressive disease (PD) after at least two courses of VAD (vincristine, doxorubicin, dexamethason) or VECD (vincristine, epirubicin, cyclophosphamide, dexamethason) or (2) stable disease (SD) after at least two courses of VAD/VECD and no improvement of tumor parameters following two additional courses of VAD/VECD. The study protocol consisted of identical VAD or VECD courses combined with dexniguldipine-HCl at a dose of 2500 mg p.o. daily for eight consecutive days starting three days before each VAD/VECD cycle. Basic tumor parameters were examined after each treatment course, bone marrow analysis was performed after three courses.

At present eleven patients with a median age of 56 (range, 44 to 71) years were included in the trial. Myeloma paraprotein classes were IgG (n=9), IgA (n=1) and BJ (n=1). Pretreatment consisted of local radiotherapy in five patients and systemic chemotherapy in all patients. Seven patients received two or more different schedules. A median of 4 (range, 2-15) VAD or VECD courses were applied before study entry. At that time seven patients had PD and four patients SD. Until now 19 courses with dexniguldipine-HCl (VADM or VECDM) were applied with a median of 2 (range, 1-5) courses. Nine patients are evaluable for response, one patient has just been entered and one died due to fungal septicaemia during the first cycle. The following responses were seen: partial response (n=1), SD (n=7) and PD (n=1). In 5 of 7 patients with SD a decrease of paraprotein ranging from 21% to 54% of pretreatment values was observed. Beside some infectious episodes, toxicity was moderate and did not exceed that of previous cycles without dexniguldipine-HCl.

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AEROSOLIZED NATURAL INTERLEUKIN 2 FOR TREATMENT OF ADVANCED MALIGNANCY: RESULTS OF A PHASE I TRIAL.

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To investigate the toxicity and clinical efficacy of aerosolized nIL-2 (Biotest) 15 patients presenting with advanced malignancy were entered into a phase I trial. 13 patients suffered from metastasizing renal cell carcinoma, 2 patients from advanced bronchial carcinoma. At start the patients received either 50000, 150000 or 300000 U nIL2 applied as a single dose. If no adverse events were observed, treatment was continued with the same dose 5 times daily for six weeks. In addition to standard investigations, a detailed evaluation of the respiratory function was performed once weekly and soluble interleukin 2 receptor serum levels and numbers and/or phenotype of lymphocytes in the bronchoalveolar lavage fluid were studied.

Treatment with aerosolized nIL-2 was well tolerated. Most prominent toxicity appeared to be resistant cough in all patients treated with 5x300000 U/d. No febrile reactions or other constitutional side effects were observed. A dose-dependent increase of the numbers of T lymphocytes, macrophages and eosinophile granulocytes could be demonstrated in BAL fluid. In addition, the treatment resulted in an increased expression of adhesion molecules on lymphocytes. 1 patient suffering from renal cell carcinoma achieved a partial remission after 6 weeks of treatment with 5x50000 U/d. We conclude that treatment with aerosolized nIL-2 is biologically active and well tolerated and should be further tested in clinical phase II trials.

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CML - THE PROGNOSTIC IMPACT OF HISTOLOGICAL VARIABLES IN A MULTIVARIATE REGRESSION ANALYSIS

J. Thiele, H.M. Kvasnicka, B.R. Titius, H. Stein, V. Diehl and R. Fischer

An immunohistochemical and morphometric study was performed on bone marrow trephine biopsies in 130 patients with Ph^{1+}-CML to evaluate the prognostic significance of clinical as well as histological disease features at time of diagnosis. To identify all cell elements of the megakaryopoiesis we used the monoclonal antibody CD61 (Y2/51), for erythro- and normoblasts Ret40f and for the demonstration of macrophages PG-M1. Density of argyrophilic fibres was determined per fat cell-free marrow area. Based on a multivariate analysis-derived risk model, the reproducibility of the prognostic score described by Sokal and coworkers was tested. Additionally we calculated the disease-specific loss in life expectancy. Our prognostic model (Cox model) consisted of the variables: age, spleen size, peripheral erythro-normoblasts, pseudo-Gaucher cells, and fibre density. To assess the validity of this new CML score, a receiver operating curve (ROC) of sensitivity and specificity was constructed. The improved prognostic efficiency of this newly developed risk model in predicting death within three years after diagnosis of CML was shown in comparison with generally accepted staging systems. Immunohistochemistry revealed that not the total number of macrophages, but only the subfraction of pseudo-Gaucher cells exerted a significant impact on survival. It was feasible to calculate the number of atypical micromegakaryocytes and pro- and megakaryoblasts. This abnormal and immature cell population revealed a significant correlation with fibre density and prognosis.

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TESTING IN VIVO SUPPRESSION OF HUMAN T CELLS WITH ANTIBODIES IN THE hu-T-cell-SCID CHIMERA MODEL

S. Thierfelder, U. Zengerle and G. Hoffmann-Fezer

Following ip injection of human lymphocytes a minority of severe combined immune deficient mice (SCID) show proliferation of human T, B and histiocytic cells on the peritoneum, subsequent blood T cell chimerism and lethal human graft-versus-host disease (Hoffmann-Fezer et al, Eur.J.Immunol.92, Blood 93). In the present study we show that injections of single or synergistic monoclonal antibodies against human T cell antigens (anti-CD3,4,5,7) suppress normal or leukemic T cells.
We therefore standardized a preclinical screening model where a 100% SCID mouse mortality (within 3 weeks) from human T cell leukemia/lymphoma (Jurkat cell line) was prevented or delayed according to antibody and therapeutic scedule of antibody treatment.
The screening model indicates the cell-depleting effect of unconjugated immunosuppressive and anti-leukemic human T cell antibodies and should be useful for testing combination therapy with other drugs.

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CENTRAL SLEEP APNOEA SYNDROME AND MULTIPLE MYELOMA

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Vigilance or mental disorders in patients with multiple myeloma suggest the possibility of hyperviscosity because of hyperproteinemia associated with myeloma. We report on a 73-year old male patient with 1989 diagnosed k-light-chain myeloma (IIIA) and impressive vigilance disorder caused by central sleep-apnoea syndrome.
Till 6/92 the myeloma has been treated with accumulative 20 courses of orally Melphalan / Prednisone and osteolytic lesions in the skull base (12/89: 49 Gy), vertebral column (12/89: 30 Gy) and left femur (4/90: 40 Gy) were irradiated. In 10/92 the patient was admitted to hospital because of progressive daytime sleepiness. Total serum protein and electrophoretic serum protein distribution showed normal values. The skull-MRT presented small inconspicious symmetric lesions in the occipital white medulla. Description of several apnoeas during sleep by the patients wife and impressive vigilance reduction even during the physicians visitations were conspicious for sleep-apnoea-syndrome. Basic diagnostic measurements (EKG: heart rate; pulsoximetry: oxygen saturation; laryngeal microphone: snoring noises) revealed 67 oxygen-desaturations averagely per hour and indicated severe sleep-apnoea syndrome. Consecutive polysomnography confirmed the central form of this syndrome. Under treatment with nasal BIPAP-ventilation nocturnal apnoeas were reduced to 6 per hour and daytime sleepiness disappeared. During hospitalisation in 3/93 because of gastroduodenitis n-BIPAP was discontinued. As a consequence daytime sleepiness and vigilance reduction returned. After consequent continuation of n-BIPAP these symptoms resolved again.
Beneath daytime sleepiness and reduced vigilance the description of nocturnal breathing stops were main clues to sleep-apnoea syndrome and led to the elusive above mentioned examinations, especially pulsoximetry.

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LONG TERM LOSS OF CD4/CD45RA+ SUPPRESSOR-INDUCER T-CELLS IN HODGKIN'S DISEASE AFTER LYMPHOID RADIATION THERAPY

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Lymphocyte subpopulations from 62 patients with Hodgkin's disease from our institution[1] were analysed by flow cytometry. Data were considered for patients who received lymphoid irradiation solely or in combination with polychemotherapy and compared with healthy control persons and patients who received only chemotherapy. While chemotherapy altered the lymphocyte subpopulations transiently, radiation therapy caused far-reaching and persisting changes. The well-known loss of CD4+ T-cells with decreased CD4/CD8 ratio occured in our series too and persisted up to 17 years. A frequent finding was the distinct loss of CD4+/CD45RA+ suppressor-inducer fraction of T-cells, known as "naive" T-cells, too. CD4+/CD29+ helper-inducer cells ("memory" T-cells) remained the predominant fraction of CD4+ T-cells for years after radiation therapy. Concomitantly increased the percentage of CD57 or CD56-defined NK-cells as well as in some cases CD19 or CD20 positive (CD5-negative) B-cells . Further analyses will show the clinical relevance of these findings.

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REGULATION OF ADHESION MOLECULES AND ACTIVATION MARKERS BY CYTOKINES IN B-CLL CELLS IN VITRO

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In normal and malignant B cells many functions such as homing to microenvironment, cell-cell interactions, extravascular migration etc. are regulated by different types of adhesion molecules. In B cell malignancies early development of systemic disease appears to correlate with the expression of certain adhesion structures. The regulation of these structures by cytokines which might be produced in an autocrine or paracrine fashion is yet poorly understood. We investigated the effect of several cytokines (IL-1, IL-2, IL-4, IL-6, IL-10, TNF-a) on the expression of adhesion molecules (CD11a, CD44, CD49d, CD54, Lecam) and B cell activation associated markers (CD21, CD23, CD25) in highly purified B CLL cells. Furthermore, the influence of these cytokines on expression of bcl-2 in CLL cells was examined. TNF-a significantly induced expression of CD54 and CD49d, IL-4 upregulated these structures to a lesser extent. The other cytokines tested had no influence on adhesion molecule expression. CD25 was upregulated by IL-4 and IL-10. Furthermore, IL-10 significantly induced expression of CD21. bcl-2 expression was induced by IL-4 whereas TNF-a caused downregulation of constitutive expression of bcl-2 in B-CLL cells. The influence of combinations of these factors, either added concomitantly or in a consecutive fashion, on bcl-2 and induction of apoptotic cell death was examined. In further studies the functional consequences of adhesion molecule expression on binding to matrix proteins and stromal cells will be examined.

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ANERGY IN T-LYMPHOCYTES: IMPLICATIONS ON TUMOR GROWTH

G. Trenn

Work of different laboratories has shown a suppressed immune response in tumor-bearing organisms. Various mechanisms have been identified which may explain this phenomenon. In these models tumor cells passively escape from the immune surveillance since they do not present specific or sufficient cell surface antigens to activate immunocompetent T-lymphocytes. Evidence, however, is accumulating that the tumor cells may play an active role in the inhibition of host specific immune functions by inducing an anergic state in T-lymphocytes. The characterization of the state of cellular anergy as well as the identification of the biochemical events leading to this state are a prerequisite for the development of new strategies of immunotherapy.

Jenkins et al. have described a transient state of cellular anergy in T-lymphocytes which is characterized by an inability of T-helper cells to synthesize interleukins upon T-cell receptor stimulation while cellular responsiveness to exogenous interleukin-2 remains unaffected. This state of cellular anergy is due to an "incomplete" stimulation of the T-cell-receptor which does not induce cellular activation. Here we present evidence that a state of cellular unresponsiveness can also be induced in cytolytic T-lymphocytes which temporarily lose their ability to lyse antigen-specific target cells and to synthesize γ-interferon upon T-cell receptor stimulation. Analysis of intracellular signalling events reveals a block in the proximal part of the T-cell-receptor triggered activation cascade prior to the activation of protein-kinase C.

Strategies to either prevent the induction of cellular unresponsiveness or to bypass the block in signal transduction in unresponsive cells are discussed.

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EFFECT OF CICLOSPORIN A ON THE ANTI-T-CELL RECEPTOR INDUCED SECRETION OF IL-10 AND IL-13 IN A MURINE T-HELPER2 CELL LINE

G. Trenn, Ch. Teschendorf, J. Sykora, A. Gessner and G. Brittinger*

T-helper 2 cells can be distinguished from T-helper 1 cells by their unique pattern of lymphokines secreted after T-cell receptor (TCR) stimulation. Here we analyze the effect of ciclosporin A (CSA) on the TCR-induced synthesis of a set of lymphokines in a T-helper 2 cell line. Using a novel assay system to detect secretion products in the supernatant of stimulated cells we present evidence that interleukin-4 (IL-4) synthesis is completely blocked by low concentrations of CSA. While IL-6 secretion is not affected at all by CSA the interleukins IL-10 and IL-13 show an "intermediate" susceptibility to the inhibitory effect of CSA. At a CSA-concentration of 100 ng/ml IL-10 secretion in response to TCR-stimulation is reduced by app. 50%. Higher concentrations of CSA do not result in further reduction of IL-10 secretion. Similar results were obtained for IL-13, a recently described lymphokine with yet unknown functions.

Results imply that TCR-stimulation of T-helper 2 cells activates various intracellular signalling pathways which can be distinguished by a different susceptibility to the inhibitory effect of CSA. We conclude that CSA does not generally suppress interleukin synthesis - a mechanism very well characterized for the IL-2 secretion- but rather that this drug interferes with the immunoregulatory network by changing the pattern of secreted lymphokines in response to an antigenic stimulus. Possible implications of these findings on the immunosuppressive effect of CSA are discussed.

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DIAGNOSIS OF PANCREATIC ADENOCARCINOMA BY THE DETECTION OF RAS GENE MUTATIONS IN DUODENAL SECRETIONS

L.Trümper, A. Gause, W. Jung, G. Jacobs, J. Roth, M. Pfreundschuh, H. Daus

Adenocarcinoma of the pancreas is a frequent human cancer with a high mortality rate. Since it is frequently diagnosed at an advanced stage, sensitive, reliable and non-invasive tests to distinguish between malignant and inflammatory lesions of the pancreas would greatly enhance the diagnostic reliability of imaging procedures currently available. We have developed a molecular test based on the polymerase chain reaction (PCR) that allows the detection of oncogene mutations highly specific for pancreatic adenocarcinoma. Mutations of the ras oncogene are found in more than 90% of pancreatic adenocarcinomas, usually at the first two positions of codon 12. PCR amplification and sequencing of PCR products from a pancreatic carcinoma cell line and from paraffin-embedded carcinoma tissue showed the presence of mutations at these positions. To facilitate screening for these mutations, a non-isotopic test to detect mutations was developed based on a 2-hour minigel separation of heat and formamide-denatured PCR products (non-isotopic SSCP). We show that mutations at a single position are reliably detected with this screening test, even when "wild-type" and "mutated" alleles are mixed in different ratios. Carcinoma specific ras mutations were detected in pancreatic secretions obtained by routine endoscopic procedures from patients with carcinoma, but not from patients with inflammatory conditions. To our knowledge, this is the first molecular test for the detection of pancreatic adenocarcinoma. Detection of RAS mutations in duodenal secretions will greatly enhance our ability to diagnose pancreatic carcinoma by non-invasive procedures.

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P 53 MUTATIONS ARE PRESENT IN SINGLE REED STERNBERG CELLS OF HODGKIN'S DISEASE: IMPLICATIONS FOR PATHOGENESIS AND ORIGIN OF HODGKIN'S DISEASE

L. H. Trümper, D. Gray, H. Griesser, R. Gascoyne, M. Pfreundschuh and T. W. Mak

Structural alterations of the p53 gene resulting in increased stability of the mutated p53 protein and subsequent inability to function as a "guardian of the genome" (D. Lane) have been shown to be important events in a number of human tumours. Since Hodgkin and Reed Sternberg (H&RS) cells of Hodkgin's disease, but not the bystander lymphocytes of Hodgkin's nodes, can be stained with p53 antibodies (Gupta et al, B. J. Hemat.), p53 mutations may also play a role in the pathogenesis of Hodgkin's disease. We employed a single-cell based reverse-transcriptase polymerase chain reaction (RT-PCR) assay to detect p53 mutations in single H&RS cells. p53 mRNA was detected in most H&RS cells from three cases of nodular-sclerosing Hodgkin's disease, but not the small "bystander" lymphocytes. H&RS cells had been isolated from lymph node suspensions with a micromanipulator after identification by morphological criteria and αCD15 immunofluorescence. After two rounds of amplification for exon 5 to 9 specific sequences, PCR products were cloned and sequenced. Direct PCR sequencing of single cell products gave inconsistent results. At least 5 independently isolated clones from each of 7 H&RS cells were sequenced twice each, and a single mutation at codon 246 (Met to Val) was consistently present in all clones from 5/7 H&RS cells. Therefore, p53 mutations may play a role in the pathogenesis of Hodgkin's disease, and the presence in H&RS cells of a common mutation may point towards clonality of these cells.

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ADVANCES IN THE TREATMENT OF CHRONIC MYELOGENOUS LEUKEMIA BY INTERFEERON.

S. Tura for the Italian Cooperative Study Group on chronic myeloid leukemia (ICSG - CML)

Alpha interferon has proved effective in the treatment of a number of hematologic malignancies. In CML early reports showed that this treatment was able to induce a cytogenetic conversion in 30-50% of the patients. Recent data coming from the M.D. Anderson Hospital, (Houston) and from multicentre studies in several European countries show that karyotypic conversion is more frequent in low risk patients and is associated with survival prolongation. The I.C.S.G. - CML set up, in 1986, a prospective study randomizing r-IFN alpha 2A vs hydroxyurea (HU) in CML patients at diagnosis.
Out of 322 pts, 218 were assigned to the IFN arm and 104 to the HU arm (2:1 random ratio). The main results of this study are: patients in the IFN arm show better karyotypic conversion, longer time to progression to accelerated phase and longer survival, with respect to those treated with conventional chemotherapy. Survival is closely related to the degree of karyotypic response: as a matter of fact, survival is 90% at 60 mo. in patients who achieve either a complete or a major karyotypic response at least once. Strongest predictors of karyotypic response are: hematologic response to IFN alone within the first 8 mo. of treatment, low relative risk, normal platelet count and very low percentage of blast cells in the peripheral blood.

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CELLULAR IMMUNOTHERAPY FOLLOWING ALLOGENEIC BONE MARROW TRANSPLANTATION (BMT) IN A MURINE LEUKEMIA MODEL.

L. Uharek, B. Glass, T. Gaska, M. Zeis, W. Gassmann, H. Loeffler, W. Mueller-Ruchholtz.

Graft-derived lymphocytes are considered to exert an import part of the antileukemic effect of allogeneic BMT. We investigated (1) whether the transfer of donor-derived spleen cells at time of BMT could provide additional antileukemic activity and (2) wether the incubation of the spleen cells with IL-2 enhances their graft-versus-leukemia (GVL) activity. *Methods:* Balb/c mice were injected with $5x10^5$ A20 (B cell leukemia) cells 2 days prior to lethal (7.5 Gy) total body irradiation (TBI) and transplantation of either syngeneic Balb/c or allogeneic MHC-matched ($H\text{-}2^d$) DBA bone marrow cells ($2x10^7$ cells). In this experimental system chronic but no lethal acute GVHD occurs. In different experimental groups donor-derived spleen cells with or without IL-2 preincubation (24 hrs) were added. Leukemia-free survival (LFS) was monitored until day 120 post BMT. *Results:* Following syngeneic transplantation LFS and median survival time (MST) were 11% and 39 days, respectively. Allogeneic MHC-identical transplantation did not improve these results and resulted in a LFS of 10% and a MST of 39 days. The addition of spleen cells to the allogeneic BM graft reduced the relapse rate significantly and a LFS of 32 % and a MST of 62 days was achieved. Activation of spleen cells by incubation with IL-2 resulted in a LFS of 63%. *Conclusions:* (1) The experimental model presented allow the investigation of cellular immunotherapy following bone marrow transplantation. (2) In this BMT model, characterized by only marginal GVL activity and without acute GVHR, an improved antileukemic effect could be achieved by the addition of allogeneic MHC-matched spleen cells. Experiments to identify the antileukemic cell population are in progress.

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MOLECULAR BIOLOGY OF CHROMOSOMAL ABERRATIONS IN T-CELL-NEOPLASIAS

M. Uppenkamp

Clonal chromosomal abnormalities are a feature of many hematopoietic neoplasms. They are carried throughout the malignant cells indicating that they occurred prior to clonal expansion. This implies that they may be critical to pathogenesis of the neoplasm. Furthermore the majority of the translocations are nonrandom, so that similar translocations are observed among clinicopathological entities.
By analogy to B-cell tumors, molecular genetic analyses of chromosomal translocations in T-cell proliferations have demonstrated rearrangements of T-cell receptor (TCR) genes as a consequence of chromosomal breakage. Patients with T-cell acute lymphoblastic leukemia, chronic lymphocytic leukemia of T-cell type, adult T-cell leukemia, and prolymphocytic leukemia show a predominance in aberrations, such as translocations and inversions, of the TCR-α/δ locus on chromosome 14q11. However, all four TCR genes may be affected by chromosomal abnormalities. The disruption and the joining of the TCR genes into new context may lead to deregulation of proto-oncogenes and formation of hybrid genes, which are important in the development of the T-cell type involved. Several mechanisms will be discussed that may mediate the process of joining between chromosomes in T-cell neoplasias. Molecular characterization of translocations is of utmost interest that will provide further insight into the pathogenesis of T-cell tumors.

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RESTRICTION FRAGMENT ANALYSIS OF IMMUNGLOBULIN- AND T-CELL RECEPTOR GENES: DIAGNOSTIC VALUE IN ACUTE LEUKEMIAS

M. Uppenkamp, K. Scheepers, I. Gana Dresen, S. Petrasch, P. Meusers, E. König and G. Brittinger

We investigated retrospectively the immunglobulin and T-cell receptor gene rearrangements in 28 patients with acute leucemias, to determine the value and applicability of this molecular diagnostic method in this heterogeneous group of patients. The major breakpoint region (M-bcr) of the bcr-abl rearrangement was also analysed in 24 patients. Three out of 15 patients with acute myeloblastic leukemia exhibited receptor gene rearrangements. One of these patients was diagnosed of having a leukemia of mixed lineage with a myeloid and a lymphoid clone. One patient showed T-cell receptor gene rearrangements, but did not express a T-cell phenotype. The third patient was found to have a restriction fragment length polymorphism for EcoRI of the λ-chain gene.

Acute lymphoblastic leukemias of B-cell type frequently harbored inappropriate T-cell receptor gene rearrangements (lineage infidelity). Four patients who were initially diagnosed of having an acute undifferentiated leukemia could be assigned to the myeloid or lymphoid lineage, retrospectively.

We conclude that restriction fragment analysis of the immunglobulin and T-cell receptor genes should not be performed routinely in the diagnosis of acute leukemia. This molecular analysis, however, is an extremely valuable tool for determining the lineage of leukemias that cannot be classified by conventional phenotyping.

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DNA INDEX (DI) AND ITS PROGNOSTIC VALUE IN NON-HODGKIN'S LYMPHOMAS OF LOW MALIGNANCY.

I. Urasiński, A. Krygier-Stojałowska, T. Urasiński

The prognostic value of DNA Index (DI) in acute lymphoblastic leukemia (ALL) and non-Hodgkin's lymphomas(NHL) of high malignancy is well known; reports on correlation between DI and survival in patients with NHL of low malignancy are still confusing. The study comprised 107 pts. diagnosed as having NHL of low malignancy. They were divided into 4 subcategories: immunocytic lymphoma 45 pts, cb-cc lymphoma 8 pts, cc lymphoma 10 pts and B-CLL 44 pts. Pretreatment DNA cytometry in Feulgen stained lymphoma cells was performed using microscopic image analyser "Morphoquant"; peripheral blood lymphocytes served as a standard for DNA diploidia. The follow-up time was within the range of 24-126 mo. (median 63 mo.). Statistical analysis was performed with the use of logrank test.

There were 70 hyperdiploidic and 37 diploidic pts. A tendency towards better prognosis could be seen for pts. with B-CLL. There was no statistical difference in probability of survival (p-S) between diploidic and hyperdiploidic pts. neither for the whole group studied nor in 4 histopathological subcategories.

We conclude that DI has no prognostic significance in NHL of low malignancy.

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TAXOL IN COMBINATION WITH CISPLATIN, ETOPOSIDE AND 5-FLUOROURACIL IN GASTRIC CANCER CELL LINES.

U. Vanhoefer, A. Harstrick, H. Wilke, M. Stahl, N. Schleucher, C. Schmiedel and S. Seeber.

Taxol is a new antimicrotubule agent isolated from the Western yew tree Taxus brevifolia with high activity in ovarian carcinoma, breast cancer and melanoma. In ovarian cancer phase II studies of taxol in combination with cisplatin are ongoing.

We studied the cytotoxicity of taxol alone and the interaction of taxol with cisplatin, etoposide and 5-Fluorouracil (5-FU) in two human gastric cancer cell lines (HM2 and HM51).

METHODS: Cytotoxicity of the individual drugs and the drug combinations were measured with the sulforhodamine B (SRB)-assay . Exponential cell growth was shown for both cell lines during the 96 h incubation period ($5x10^3$ cells/microculture well). A continuous drug exposure (72 h) was used; cytotoxicity was measured after 96 h. The concentration to inhibit cell growth by 50% (IC 50) was obtained from semilogarhytmic dose-response curves. The interactions of taxol with cisplatin, etoposide and 5-FU were assessed using the isobologram methodology (50% isobolograms) and classified as "synergistic", "additive" or "antagonistic". All experiments were done in triplicate.

RESULTS: Taxol was highly active in HM2 and HM51 with a IC 50 of 0.2 and 0.1 uM respectively. Significant synergism was observed for the combinations of taxol /etoposide and taxol /5-FU in HM2. Interestingly the highest degree of syergism was seen if low doses of taxol were combined with high concentrations of either etoposide or 5-FU. However both combinations were less than additive in HM51. The combination of taxol / cisplatin was antagonistic in both cell lines.

CONCLUSIONS: These data may provide a basis for the rational development of combination therapy with taxol in gastric cancer. The combination of taxol with either etoposide or 5-FU appears promising since synergism was demonstrated in one cell line, whereas the combination of taxol and cisplatin was clearly antagonistic in both cell lines.

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GLUTATHIONE METABOLISM IN TWO HUMAN GASTRIC CANCER CELL LINES WITH DIFFERENT SENSITIVITY TO DNA DAMAGING AGENTS.

U. Vanhoefer, H. Wilke, M. Stahl, A. Harstrick, H. Walles, M.E. Scheulen, C. Schmiedel, and S. Seeber .

Resistance to chemotherapy which might in part be mediated by alterations in the glutathione (GSH) pathway is a major problem in the treatment of gastric cancer. We investigated the activity of GSH-dependent enzymes, the distribution of alpha-, mu-, and pi-subunits of glutathione-S-transferases (GST) and the cellular content of GSH in two human gastric cancer cell lines (HM2 and HM51) with different degrees of resistance to DNA damaging agents. METHODS: Activity of GST, glutathione peroxidase (GPX), -reductase (GRD) and cellular content of GSH were measured spectrophotometrically in the 150.000 g supernatant (cytosolic fraction). Enzyme activities were expressed per mg soluble protein (EP). Isoenzymes of GST were determined by SDS-PAGE (12.5%) and immunoblotting (Western blot) using specific monoclonal antibodies against alpha-, mu- and pi-subunits.

RESULTS: The cell lines HM2 and HM51 showed significant differences in their chemosensitivity to cisplatin, doxorubicin and cyclophosphamide. HM2 was approximately 6-8 fold more resistant to cisplatin and doxorubicin but 8 fold more sensitive to cyclophosphamide than HM51.

Enzym activities :	GST	GPX	GRD	GSH concentration
HM51 (mU/mg EP)	*530	*40.8	30.0	24.5 (nmol/mg EP)
HM 2 (mU/mg EP)	371	26.6	38.5	19.5 (nmol/mg EP)

HM51 expressed mu- and pi-classes of GST, while HM2 showed only the pi-class. *(p value < 0.05)

CONCLUSIONS: There was a significantly increased activity of GST and GPX in HM51, which might be associated with the resistance to cyclophosphamide. The relative resistance to cisplatin and doxorubicin in HM2 does not seem to be related to alterations in the glutathione metabolism.

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DECREASED PROGENITOR CELL GROWTH IN BONE MARROW SAMPLES INFILTRATED BY NON-HODGKIN's LYMPHOMA CELLS IS APPARENTLY NOT DEPENDENT ON TUMOR NECROSIS FACTOR-ALPHA.

K. Vehmeyer, T. Liersch, B. Wörmann, and W. Hiddemann

Our investigation was based on data previously reported describing impaired growth capacity of hemopoietic progenitor cells in patients suffering from CLL. In this study we were interested in exploring the growth behaviour of bone marrow (BM) progenitor cells from patients with different types of B cell non-Hodgkin's lymphoma (NHL), with or without microscopic BM infiltration. In the study 15 lymphocytic, 18 lymphoplasmocytic/cytoid, 9 centrocytic, 8 centroblastic/centrocytic, 10 low-grade unclassified, 26 centroblastic, 2 B lymphoblastic, and 3 immunoblastic NHL were included. The functional capacity of BM progenitor cells (GM-CFU-c) was analysed according to the method of Metcalf. In 35 cases, BM involvement (test group) was detected both by cytology and histology and a significant inhibition of the growth capacity of progenitor cells was detected as compared with controls of patients without hematological malignancies (n = 49) (median colony growth per 10^5 cells: test group / control group: 0 / 14 using G-CSF; 6 / 85 with IL-3 + GM-CSF + G-CSF. In 56 cases, BM infiltration was not ascertained by histology and cytology. In this group, the progenitor cells demonstrated normal growth behaviour (median colony growth per 10^5 cells: 10 with G-CSF; 87 with IL3 + GM-CSF + G-CSF).
According to previous reports, we questioned the possible role of TNF-α in the suppression of GM-CFU-c. In 10 cases of NHL with BM infiltration, anti-TNF-α scarcely affected the colony growth. In 30 cases of NHL without BM involvement, however, a significant enhancement of GM-CFU-c was detected by anti-TNF-α (4.8 times increase in median). It is noteworthy that only in cultures supplemented by G-CSF, both alone and in combination with IL-3 and GM-CSF, was the colony growth considerably increased. Similarly, in the control probes (n = 34) anti-TNF-α enhanced the number of GM-CFU-c (3 times increase in median).
In conclusion, our data show that TNF-α does not play an important role in the suppression of GM-CFU-c in BM probes infiltrated by B NHL cells. The molecular mechanism of GM-CFU inhibition and the prognostic value of stem cell assay in the lymphoma staging needs further examinations.

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VIABILITY AND GROWTH CAPACITY OF CELLS IN FRESH-FROZEN PLASMA: IMPLICATIONS FOR GRAFT-VERSUS-HOST DISEASE

K. Vehmeyer, and J. U. Wieding

Transfusion-associated graft-versus-host disease (TA-GvHD) is a rare complication of blood transfusion. In a few cases TA-GvHD has been associated even with the transfusion of fresh-frozen plasma (FFP). This was unexpected since the white cell contamination of plasma is very low and the majority of these cells do not normally survive the freezing and thawing process. For better understanding the risk involved in TA-GvHD with conventional FFP we performed experiments on the growth capacity of lymphocytes and hemopoietic progenitor cells before and after freezing. FFP was prepared from blood donations with leukocyte counts of less than 10000/μl. Plasma separation was performed with a triple-bag system using the top-and-bottom technique. The proliferation capacity of lymphocytes was determined using phytohemagglutinine (PHA) as mitogen. The growth capacity of hemopoietic progenitor cells was investigated in a stem cell assay in the presence of stem cell factor, interleukin-3, granulocyte/macrophage colony stimulating factor, granulocyte colony stimulating factor and erythropoietin. The colonies were counted after 14 days of incubation. Before freezing the plasma bags contained (on average) 260 ml and 125 leukocytes per μl (= 32×10^6). In all non-frozen plasma samples the lymphocytes were able to proliferate and hemopoietic progenitor cells showed colony forming capacity. Plasma bags contained on average 5680 colony forming units (CFU) per 260 ml. After freezing and thawing, roughly 30% of the mononuclear cells remained intact as detected by acridine orange staining. The lymphocytes, however, lost their PHA-inducible proliferative capacity (n = 7). In contrast, a few hemopoietic progenitor cells still exhibited colony forming capacity. In 3 out of 9 preparations, the plasma bags contained 32, 55 and 70 CFU respectively, calculated per 260 ml plasma. This investigation demonstrates the residual growth capacity of white blood cells remaining in plasma. Despite freezing and thawing, normal FFP preparations contain substantial amounts of viable cells, some of which still have potential growth capacity. In conclusion, even if the cell contamination of plasma is extremely low, TA-GvHD cannot be excluded since the critical amount of leukocytes inducing a TA-GvHD is not definitively known. This especially concerns children suffering from congenital immunodeficiency syndroms. Therefore, effective measures are warranted in plasma preparation in order to achieve a further reduction of white blood cell contamination or a reduction of their growth capacity e.g. by irradiation.

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LONG TERM THIRD REMISSION IN ADULT PATIENTS WITH ACUTE MYELOID LEUKEMIA RELAPSING AFTER AUTOLOGOUS BONE MARROW TRANSPLANTATION

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We report about two female patients aged 30 (Pat. A) and 25 years (Pat. B) with de novo acute myeloid leukemias (FAB M2 and M4) without cytogenetic abnormalities. Both had been treated with standard regimens for initial remission induction and early consolidation and relapsed after autologous bone marrow transplantation (ABMT).
Pat. A was given cyclic maintenance therapy for 5 months and was autografted another 5 months later after conditioning with busulfan and cyclophosphamide. First relapse occurred 7 months post ABMT. Reinduction of complete remission was achieved with one course of TAD-9 (6-thioguanin, cytarabine, daunorubicin) without subsequent maintenance or consolidation therapy. A second relapse was documented 21 months post ABMT. Sequentially administered high dose cytarabine plus mitoxantrone (S-HAM) followed by granulocyte-macrophage colony-stimulating factor (GM-CSF) induced a third complete remission continuing for a follow-up period of 63 months by now without any further antileukemic treatment.
Pat. B received cyclic maintenance therapy in first remission for up to 3 years and relapsed 6 months after treatment termination. Second complete remission could be induced by one course of S-HAM. 5 months later, ABMT was conducted after conditioning with busulfan and cyclophosphamide. A second relapse was documented 6 months after ABMT. Treatment with S-HAM followed by GM-CSF resulted in a third complete remission lasting for 27 months by now under cyclic maintenance therapy with subcutaneous cytarabine in combination with oral idarubicin.
Dominant toxicity of intensive relapse therapy included hemocytopenia grade IV with blood cell support for 26 weeks, infections grade IV with pulmonary involvement, mucositis grade IV in Pat. A and hemocytopenia grade IV with blood cell support for 10 weeks, infections grade IV with presumed fungal pneumonia and marked dermatosis in Pat. B. Hospitalization was required for 9 weeks and 5 weeks, respectively. Irreversible or secondary/late toxicity has not been observed.
It can be concluded that intensive reinduction chemotherapy with S-HAM followed by GM-CSF may be a highly effective treatment modality in selected patients with AML relapsing after ABMT.

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CONSTITUENTS OF AUTOCRINE IL-6 LOOPS IN MYELOMA LINES

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Stimulation of autonomous cell growth, induced by auto- or paracrine IL-6 dependent mechanisms, is a controversial hypothesis concerning neoplastic plasma cell growth. We examined 7 myeloma cell lines of varying stages of B cell differentiation (U-266, RPMI 8226, LP-1, OPM-2, IM-9, L-363, HS-Sultan) in regard to expression and constitution of IL-6 receptor (IL-6r), its binding capacity for the ligand, the production and secretion of IL-6 and the effect of IL-6 on proliferative activity. Immunoblotting with different anti-IL-6r mab's revealed ubiquitarity of the gp 80 molecule in all cell lines, although diverse reactivity and intensity of staining was striking. Comparison of results from Western blotting with *in situ* analysis by immunocytochemistry revealed: (1) the presence of rather low numbers of gp 80-molecules on all cell lines, (2) extreme heterogeneity in receptor expression within subsets of the neoplastic clone (3) different IL-6r density within the various cell lines and (4) different reactivity of methods and antisera used. Immunostaining of gp 130, the signal transducer molecule of the IL-6r revealed positive results in only two cell lines (LP-1; RPMI 8226), whereas immunoblotting consistantly gave negative results. Binding capacity of Il-6r was tested using immunofluorescent ligands. Intact binding could only be demonstrated in 34.6% and 7.4% of the LP-1 and the L-363 cell lines respectively supporting the immunocytochemical findings of only very low numbers of IL-6r per cell.
Highest levels of IL-6 were found in the supernatant of the LP-1 plasma cell leukemia line which displays the slowest *in vitro* growth. This may reflect (1) that the maximal *in vivo* renewal capacity of this cell line is driven by an IL-6 induced effect of other growth factors, (2) functional receptor deficiency or (3) malfunction in signal transduction. When U-266 cells were starved in medium with 1% to 3% serum for 48hs, up to 4 fold increase in ^{3}H-thymidine uptake could be induced by refeeding with 10% serum but not with IL-6 within the same time period. We are currently refining this system and testing the influence of IL-6 on second messengers and proto-oncogenes in order to more clearly define the biologic effects of IL-6 in plasma cells.

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Enhancement of point mutation detection in the p53 gene by single strand conformation polymorphism (SSCP) using MDE-gel matrix as compared to conventional polyacrylamide gels

D.Vogel, I.Heide, C.Rochlitz, D.Huhn, A.Neubauer

The rapid and sensitive detection of point mutations grows increasingly important for understanding the molecular mechnisms of human diseases. With regard to malignant cells, activation of proto-oncogenes and inactivation of tumor-suppressor genes mainly occur due to single point mutations. One of the most frequently mutated genes in human cancers is the p53 gene. SSCP has become the most important technique to screen a given DNA sequence for point mutations. The technique is based on altered migration speeds through solid supports of single stranded DNA fragments carrying mutations. We previously characterized a number of liver metastases regarding p53 gene mutations. Eight cases with known mutations and one wild-type sequence were selected and SSCP was carried out in a blinded fashion. SSCP was performed using conventional protocols; only electrophoresis at room temperature with 10% gylcerol was carried out (Genomics 5:874). On the other hand, SSCPs were done using a novel gel matrix called MDE. Of eight known mutations, conventional SSCP done at room temperature detected only one mutation. In contrast, MDE-SSCP revealed mutations in the correct eight cases. These data show that MDE-SSCP seems to be superior compared to conventional SSCP performed at room temperature with 10% glycerol.

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Mutations in exons 11 - 23 in the retinoblastoma (RB) susceptibility gene seem to be rare in acute myeloid leukemia (AML): Analysis by MDE-SSCP.

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It is now widely accepted that malignant transformation is not only the result of one genetic aberration, but of a cumulative genetic damage leading to multiple molecular abnormalities in protooncogenes and tumor suppressor genes. AML is a heterogeneous disease with the most frequent genetic aberration being an activation of the *ras* protooncogenes, which occur in approximately 20% of the cases. It has been reported that, in AML, in up to 40% of the cases no RB-protein can be detected in the tumor cells, suggesting that inactivation of this important tumor suppressor gene product may contribute to leukemic transformation/progression (Oncogene 6:1343). Further, inactivation of the RB-gene in "knockout" mice leads to abnormalities within the hematopoietic system. We thus studied the mechanisms by which RB is inactivated in AML. In a first screen, 13 adult AML cases, and in addition 5 erythroleukemias, were studied for point mutations in the RB coding region (codons 375 - 800), since, in this area, the "hot spots" seem to be localized. Using MDE-SSCP, no clear-cut point mutation was detected. Single cases were sequenced, and, again, no mutations were found. These data show that point mutations of the RB gene within codons 375-800 do not seem to be frequent in AML.

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Today's concept of cancer registration - requirements and consequences

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Former concept of cancer registration

Goal: Collection of a standardized minimized data set regarding cancer patients to describe incidence and course of the different cancer types in the FRG.

Requirements: A high ratio of completeness according to incidence and data fields. Actuality of the data was of minor interest. Supporting clinical tasks was a kind of welcome by-product.

Consequences: The EDP-solution was easy because of the simple data structures and centralized data collection. Little effort was necessary according to data coordination. The goal was not achieved mainly because of the low acceptance rate by the clinical departments that didn't see much benefit from this data collection.

Today's concept of cancer registration

Goal: Provide the clinical departments with EDP-tools in order to support clinical tasks such as therapy management, writing doctor's letters, providing electronic patient records. Data collection is merely a by-product of these activities.

Requirements: Besides the completeness of incidence and of data a high degree of actuality is required. The data has to be collected where it comes into existence and has to be available there.

Consequences: The actuality and high reliability of the data can only be achieved with a doctor's help. Distributed data collection demands a lot of data coordination work. Data safety and data security are another big tasks. Acceptance by the clinical departments can only be gained if the effort for data collection is minimized and the application programs are useful for solving the clinical tasks.

Conclusion: Cancer registration has to be integrated into the clinic information system in order to:

- avoid redundancy in data collection.
- support clinical tasks for all patients (not only cancer).
- enable the connection between administration data and department data.
- share infrastructure of EDP-department of the clinic.
- minimize efforts for system maintenance.

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PROPAGATION OF HUMAN PRELEUKEMIAS IN SCID MICE

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Preleukemia results from alterations in the pluripotent stem cell pool and evolves from the clonal expansion of a single stem cell. Existing in vitro culture technology does not permit the routine propagation of most human preleukemias. Abnormalities commonly seen in cultures of marrow from patients with preleukemia include either decreased or absent clonal growth, abortive cluster formation, and defective maturation of cells within the colonies. Previous work has shown the usefulness of mice with severe combined immunodeficiency (SCID) for the growth of normal human bone marrow as well as for ALL, AML and CML in blast crisis. To date, no in vivo model exists for human preleukemias. We show here that bone marrow samples from patients with preleukemia also grow in SCID mice that receive human growth factors. Up to $5x10^7$ cells were injected intravenously into sublethaly irradiated (400 cGy) SCID mice, followed by intraperitonaly injections of 7µg PIXY-321 (IL-3/GM-CSF fusion protein) per mouse every other day. After 12-19 weeks 5 out of 6 human MDS-samples (RAEB, CMML) and 1 human Essential Thrombocythemia sample were growing in the mice, as proven by FACS-analysis using an anti-human-CD45-moAB. The percentage of positive staining cells ranged up to 10% in the peripheral blood, 60% in the bone marrow, and 50% in the spleens of the mice. In addition studies are under way to identify the known karyotypic abnormalities of the pathologic human clones in the mouse bone marrows and spleens. The findings demonstrate that SCID mice provide a reproducible system for the propagation of human preleukemias. This model will allow closer studies of the pathobiology of preleukemias as well as in vivo expansion of preleukemic cells for therapy studies.

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POINT MUTATIONS IN THE GM-CSF RECEPTORα CODING SEQUENCE IN PATIENTS WITH ACUTE MYELOID LEUKAEMIA.

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Mutations of signal transducing molecules have been described in haematological malignancies. They could confer a growth advantage and contribute to the pathogenesis of the disease. Knowledge of such mutants may also further our understanding of the normal signal transduction. We have therefore sought mutations in the GM-CSF receptor α chain (GM-CSFRα) in patients with acute myeloid leukaemia (AML). The α chain binds GM-CSF and also modulates signalling events which are mediated by the β chain. To detect mutations we have used Single Strand Conformation Polymorphism (SSCP) analysis on radioactive reverse transcription (RT) PCR products. 6 PCR fragments of ca. 300 bp were amplified to span the entire length of the α chain coding sequence. The products were run on non-denaturing polyacrylamide gels under 2 separate conditions (10% glycerol, 20°C/no glycerol, 4°C). In SSCP analyses mutations are indicated by band shifts. In 4 cases out of 32 AML blast samples a major band shift (ca. 50% of the total) was detected. Sequencing revealed 4 different point mutations. 2 of them are conservative. 2 substitute amino acids: one mutation (Ala 17->Gly) lies in the signal peptide and therefore does not affect the mature protein. The other mutation (Arg 164->Gln) is not likely to influence the receptor structure either. We therefore believe that these mutations represent germline polymorphisms. In a further 2 patients a minor band shift (10% or less) was detected suggesting a minor subclone with an α chain mutation. These minor bands are not suitable for direct sequencing and subcloning is in progress. Our results indicate that there are frequent polymorphisms/mutations of the GM-CSFR α chain.

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FUNCTIONAL ANALYSIS OF THE FLK2 RECEPTOR TYROSINE KINASE IN TRANSGENIC MICE

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Signal transducton initiated by the interactions of growth factors with their specific receptors is an important mechanism of regulating normal cell growth and differentiation. Flk2 is a recently identified receptor tyrosine kinase (RTK) predominantly expressed in primitive hematopoietic cells. In order to elucidate the role of Flk2 in early hematopoiesis, we developed several constructs containing the cDNA of wild type or mutated murine Flk2 driven by different tissue specific promoters. Furthermore, chimeric receptors consisting of the extracellular ligand-binding domain of human colony-stimulating factor 1 (CSF-1) and the transmembrane and tyrosine kinase domains of murine Flk2 were cloned to facilitate the stimulation of Flk2 despite the unavalibility of the natural ligand. In vitro analysis has shown biological activity of the chimeric receptor by stimulation with human CSF-1. The constructs were used to generate transgenic mice. Germline transmission was identified by PCR and Southern blot analysis. Hematopoietic tissues of animals carrying the chimeric transgene were subjected in vitro to human CSF-1 and effects on hematopoietic cell development and differentiation were analyzed.

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CHARACTERIZATION OF THE CAUSATIVE GENETIC DEFECTS IN NINE PATIENTS WITH HEMOPHILIA B

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Factor IX (FIX) is a vitamin K-dependent plasma protein essential for normal hemostasis. Lack of functional FIX results in the hereditary bleeding disorder hemophilia B. We describe the molecular basis of hemophilia B in nine patients, who were investigated at our department. Characterization of the mutations was performed by amplification of all eight exons and exon-intron junctions by PCR and subsequent genomic sequencing of the products. We identified the following causative mutations: FIX Vienna 1: a deletion of nucleotides (nt) 20530-20532 in exon VI leading to the loss of the codon for Gly-184. FIX Vienna 2: a deletion of nt 6343-6362 in exon II resulting in a premature stop-codon at nt 6378-6380. FIX Vienna 3: a point-mutation at nt 17704 (C>G) in exon V resulting in the substitution of Gln-97 by Glu. FIX Vienna 4: a point-mutation at nt 17761 (C>T) in exon V leading to a stop-codon at Arg-116. FIX Vienna 5: a point-mutation at nt 10415 (C>G) in exon IV resulting in the substitution of Pro-55 by Ala. FIX Vienna 6: a point-mutation at nt 6583 (C>T) in exon II altering Thr-38 to Ile. FIX Vienna 7 a point-mutation (G>C) at nt 31276 in exon VIII leading to the substitution of Trp-385 by Cys. FIX Vienna 8: a deletion of nt 6700 in exon III resulting in a premature stop-codon at nt 10422-10424. FIX Vienna 3 was found in two obviously unrelated patients. FIX Vienna 1 and 8 are both novel hemophilia Bm-variants. FIX Vienna 4 and 5 have been previously described, whereas FIX Vienna 2,3,6 and 7 are novel FIX-mutants.

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CYSTOSARCOMA PHYLLOIDES AND OVARIAN THECOMA: TWO RARE GYNECOLOGIC TUMORS WITH AN IDENTICAL CYTO-GENETIC ANOMALY : TRISOMY 12

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Ovarian thecoma (OT) and Cystosarcoma phylloides (CP) are both rare gynecologic tumors that have to the best of our knowledge not been studied cytogenetically before. We would thus like to present two patients with OT and CP respectively and trisomy 12 and discuss the possible role of this autosome in cancer pathogenesis.

Case I: A 42-year old woman who was seen for continous vaginal bleeding. CT-scans of the abdomen revealed a solid, well defined mass of 6.5x6.5cm located between rectum and uterus. On histology, the diagnosis of ovarian thecoma was established. Chromosome complement was: 47,XX,+12.

Case II: A 44-year old woman who had noticed a lump in her left breast. The tumor, measuring 6x4cm was excised and proved to be cystosarcoma phylloides. Cytogenetically, the cells showed 47,XX,+12.

Aberrations of #12 have occasionally been reported before in gynecologic tumors such as papillary serous adenocarcinomas of the ovary, ovarian dysgerminoma or a malignant mixed Müllerian tumor. Moreover, #12 is involved in a variety of rearrangements, structural as well as numerical, in a large number of different benign and malignant tumors like lipomas/liposarcomas, leiomyomas and testicular tumors. This may be an indication that the role of genes on #12 lies in the promotion of proliferative processes in neoplastic tissue rather than in the initiation of specific tumors.

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REVERSIBLE HEMOLYSIS AND CHRONIC RENAL FAILURE FOLLOWING AUTOLOGOUS BONE MARROW TRANSPLANTATION (ABMT) - A CASE OF HEMOLYTIC UREMIC SYNDROME ?

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Nonhematological toxicity of conditioning regimens for bone marrow transplantation may involve a variety of organ systems including microvasculature. Here we report on a 45-year old female patient with a HUS-like complication after ABMT. She was diagnosed to have Ph-pos c-All in 5/92 and initially treated according to the high risk stratum of the German multicenter ALL/AUL (04/89) trial, including consolidation with HD-AraC/ Mitoxantrone. In 12/92 she was admitted for ABMT in CR1. The conditoning regimen was hyperfractionated total body irradiation 14,4 Gy and cyclophosphamide 200 mg/kg, and she was autografted with immunomagnetic-bead-purged marrow. On day 0 (Day of ABMT) creatinine clearance was reduced by 50 %, with normal values for serum creatinine and urea. After 3 weeks she developed fluid retention and an increase in serum creatinine and urea, associated with hematuria and proteinuria (7,6 g/day). Furthermore, signs of microangiopathic hemolysis, such as elevated LDH, decreased serum haptoglobin, increased red blood cell transfusion requirement, elevated conjugated bilirubin and temporary appearance of fragmentocytes in peripheral blood smears were recognized. Coombs test was negative and von Willebrand factor multimer (vWF-M) pattern appeared normal. Renal biopsy was not performed because of refractory thrombocytopenia. We considered hemolytic uremic syndrome (HUS) as possible cause and started repeated plasmapheresis (PPh) for 10 days. Renal function irreversibly deteriorated requiring chronic hemodialysis. Hemolysis however completely reversed and the patient was discharged 3 weeks later. This is the first case out of 42 autotransplants at our institution with posttransplant renal failure. Some recent reports described the incidence of hemolysis and renal impairment with variable outcome in BMT patients, however the difficulties of differential diagnosis and differential therapy in this special group of patients remains unsolved. Since renal function and microangiopathic hemolysis developed differently in our aggressively pretreated patient, and since vWF-M were normal, we suggest that her HUS-like syndrome may be distinct from classical HUS in non-transplanted patients, and may be related to preexisting renal damage.
We cannot conclude on the therapeutic impact of Pph in such a case, but since the potential benefit outweights the therapy-dependant risk, we propose the immediate onset of Pph if a HUS-like syndrome is suspected.

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MOLECULAR ANALYSIS AND in vitro EXPRESSION OF A SEVERE CRM-NEGATIVE FACTOR X VARIANT, FX'VIENNA'.

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Factor X (FX) is a vitamin K-dependent plasma protein which plays a central role in blood coagulation. Cross reacting material negative (CRM(-)) FX deficiency is characterized by a lack of detectable FX antigen in the plasma; in most such cases the mechanism accounting for the absence of detectable antigen is not known. Here we describe the molecular basis of a CRM(-) FX deficiency in a patient with a severe bleeding diathesis. The propositus is a 4 year old boy who sufferd a cerebral hemorrhage within his first year of live. His PT(48 sec) and APTT (52 sec) are very prolonged. The FX activity level is < 1% and the FX antigen level is <5%. The PT and the APTT of his parents are normal. Their FX activity (mother :42%;father:61%) as well as their FX antigen (mother:50%;father:65%) is reduced. Enzymatic amplification of all eight exons of the propositus revealed a single missense mutation (G-A) in exon VI resulting in a change from Glu(GGG)+201 to Gly(GAG). Both parents are heterozygous for this mutation. To elucidate the mechanism which leads to the lack of FX antigen in the propositus we compared the processing of FXVIENNA and wild type FX in a transient expression system. Wild type and mutant FX cDNA's were expressed in the human embryonic kidney cell line 293. The nascent protein was puls labelled with 35S-Met, immunoprecipitated using a polyclonal FX antibody and analyzed on SDS-PAGE. Results showed that FXVIENNA is produced at roughly the same amount as normal FX. It is secreted into the cell supernatant in its two chain form and is not degraded within the first 12 hours after synthesis. We then expressed the two constructs in the presence of 10% and 30% of plasma to investigate the influence of plasma proteases on the stability of the expressed proteins. Only 52% of mutant FX was detectable 36 hours after expression in the presence of plasma when compared to the plasma-free expression system. The wild type FX construct showed no alteration in FX levels when expressed in the presence of plasma in the media. Our data suggest that FXVIENNA is stable intracellularly and appears to be secreted normally. Less FX is present in the mutant construct, however, when the expression is carried out in the presence of plasma in the media and compared to the wild type construct. Our data therefore suggest, that instability of the mutant protein is reponsible for the lack of detectable FX antigen in the propositus.

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COMBINED IMMUNOPHENOTYPING AND INTERPHASE CYTOGENETICS

Klaus Weber-Matthiesen, B. Schlegelberger, W. Grote

Proof of clonal proliferation is possible by various methods, some being the Southern blot analysis, cytogenetics and more recently also interphase cytogenetics enabling detection of numerical chromosome aberrations in interphase cells. Each of these techniques is in the position to reveal clonal proliferation owing to specific genetic characteristics, such as gene rearrangements and chromosomal changes. However, all have in common that they do not render information concerning the nature of the detected clones, for example their cell lineage. It is sometimes impossible to define, whether a cytogenetically aberrant clone really corresponds with the cell population identified as tumor cells by the pathologist. Questionable in respect of this may be also the interpretation of data obtained by cytogenetic analysis of Hodgkin's disease. Whilst the chromosome analysis of Hodgkin's disease mostly reveals complexly aberrant karyotypes - mostly within the tri- or tetraploid range -, discrete chromosomal changes affecting only a single chromosome occur in approximately 10% of cases. Are these really Hodgkin's cells? Or do they represent chromosomally aberrant and clonally proliferating "bystander cells", the function of which is receiving more and more attention? We hope to answer these and other open questions using a new technique that was developed by us to combine fluorescence immunophenotyping and interphase cytogenetics. We refer to it as "Fluorescence-immunophenotyping and Interphase Cytogenetics as a Tool for Investigation of Neoplasms (FICTION)"

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WEEKLY THERAPY WITH FOLINIC ACID (FA)/HIGH-DOSE 5-FLUOROURACIL (5-FU) 24-HOUR INFUSION AS SALVAGE THERAPY IN PRETREATED PATIENTS WITH METASTATIC COLORECTAL CARCINOMA: A MULTICENTER PHASE-II STUDY OF THE ARBEITSGEMEINSCHAFT FÜR INTERNISTISCHE ONKOLOGIE (AIO)

H.J. Weh, U. Klaassen, H. Wilke, J. Dierlamm, R. Siegmund, H.J. Illiger, A. Schalhorn, E.D. Kreuser, U. Hilgenfeld, B. Steinke, O. Burkhard, A.Zoller, W. Weber, R. Subert, J. Pfitzner, R. Kribel, and D.K. Hossfeld

Using a weekly 24-hour infusion of high-dose 5-FU (2600 mg/m^2) with folinic acid (500 mg/m^2), Ardalan et al (JCO 9, 1991, 625 - 630) recently reported the remarkable remission rate of 30 % in 10 patients pretreated with conventional 5-FU/FA regimens.
Stimulated by this report, between 1/92 and 12/92 the AIO has conducted a multicenter study in pretreated patients with metastatic colorectal carcinoma using the same regimen as proposed by Ardalan et al with the exception that FA was given as 1-hour infusion prior to 5-FU. All patients had to have measurable and documented progressive disease. After 6 infusions (one course) response to therapy was evaluated. Only in cases with PR or SD with improvement of the patient's clinical condition therapy was continued, in all others cases it was stopped. So far 57 patients are evaluable for response and 48 for toxicity.
Clinical data of the patients: mean age 57 yrs (range 31 - 74), sex: 36 male, 21 female pts. Prior treatment: 5-FU/FA regimens (n = 42), 5-FU/IFN (n = 13), several types of chemotherapy: 16 pts, preceding metastasectomy: 10 pts. Primary response to treatment: 13 PR, 20 SD, 18 PD, 6 unknown. Metastatic sites most frequently involved: liver (n = 47), lung (n = 17), Karnofsky performance status: 80 - 100 % (n = 40), 60 - 70 % (n = 17).
Results: Therapy resulted in 5 PR (9 %), 32 SD (56 %), 19 PD (33 %) and one toxic death. Response rates were strongly influenced by response to primary treatment: 26/32 (81 %) pts with a prior PR or SD again achieved PR or SD, but only 8/18 (44 %) of those with prior PD. Median duration of SD/PR was 3 months, median survival for all pts 8 months, for those with SD/PR 12 months, and for those with PD 4 months.
Toxicity: One toxic death was observed. In the other pts toxicity was moderate. 51 toxicities grade 2 or 3 were seen with diarrhoea (n = 11), nausea (n = 11) and mucositis (n = 11) being most often encountered.
Conclusions: Although the remission rate was low, SD could be achieved in most pts for a median of 3 months. Median survival of 8 months is relatively good for pretreated patients./// Department of Oncology and Hematology, University Clin. Eppendorf, Martinistr.52, W-2000 Hamburg 20, Germany

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THROMBOHEMORRHAGIC COMPLICATIONS IN CHRONIC MYELOPROLIFERATIVE DISORDERS

A. Wehmeier

Bleeding, arterial and venous thrombosis are the main cause of morbidity in chronic myeloproliferative disorders (MPD) and may result in disabling functional defects (e.g. pulmonary embolism, stroke). About 30% of patients die from thrombohemorrhagic complications. However, risk factors for such complications are not well defined, and the optimal treatment for prophylaxis of bleeding and thrombosis in MPD is unknown.
The nature and frequency of complications are influenced by MPD subgroup and previous bleeding and thrombotic events. Other factors such as age, hematocrit, and platelet count may be of limited predictive value. Although platelet dysfunction probably plays a significant role in abnormal hemostasis in MPD, platelet parameters tested so far (mainly aggregation, morphology, secretion, arachidonic acid metabolism) lack a reproducible correlation to bleeding or thrombotic episodes. The development of thrombohemorrhagic complications on the basis of a labile hemostatic balance in MPD seems to be a multifactorial process that cannot be assessed by a single variable.
Control of abnormal cell proliferation seems to be the most effective way to reduce bleeding and thrombosis in MPD. Treatment with alkylating agents or radiophosphorus is increasingly replaced by alpha interferon as effective cytoreductive therapy. Regular phlebotomy may be necessary to lower the hematocrit. Antiplatelet drugs are valuable to ameliorate or even abolish microcirculatory disturbances but may increase the risk of bleeding complications.

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ALTERED EXPRESSION OF THE RETINOBLASTOMA GENE PRODUCT IN HUMAN HIGH GRADE NON-HODGKIN'S LYMPHOMAS

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The retinoblastoma gene (RB) is a growth suppressor gene on the human chromosome 13q14. It encodes a 105 kDa phosphoprotein (p105) with DNA-binding capacity. P105 is thought to be involved in cell cycle control. Inactivation of RB is responsible for the development of retinoblastomas and occurs frequently in osteosarcomas and small cell lung cancer. In this study we looked at the RB-structure and expression in cell lines and primary lymphoma samples from patients with high grade non-Hodgkin's lymphoma (NHL). 45 primary high grade NHL, the B-lymphoblastoid cell line IM-9 and the NHL cell line WSU-NHL were studied for RB structure by Southern blotting and for RB-expression by Northern blotting, Western blotting and immunocytochemistry. In all experiments freshly cryopreserved material was used. Southern and Northern experiments were performed with the 0.9kb and 3.8kb RB-cDNA probe. For the detection of p105 two different anti-p105-monoclonal antibodies were used in immunocytochemistry and Western blotting experiments. No RB mRNA and no p105 could be found in IM-9 cells. 26 high grade NHL samples (58%) showed no p105 expression. In the subgroup of centroblastic lymphomas 16 out of 21 and in Burkitt's lymphomas 5 out of 8 showed no p105-expression.
CONCLUSIONS: P105 expression is absent in 58% of high grade NHL, particularly in centroblastic and Burkitt's lymphomas, suggesting that inactivation of RB may play a crucial role in the pathogenesis of high grade NHL.
Key words: RB-expression - high grade NHL

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OLIGOCLONAL EXPRESSION OF T CELL RECEPTOR B CHAIN VARIABLE REGION GENES IN T LYMPHOCYTES INFILTRATING HUMAN SOLID TUMORS.

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At the site of disease with specific T cell infiltrates the total T cell population can be expected to contain subpopulations of cells proliferating in response to antigens. Therefore, the hypothesis has been that in solid tumors infiltrated by T cells the T cell receptor V region repertoire may show an oligo or monoclonal pattern. We tested this hypothesis in freshly isolated tumor infiltrating lymphocytes (TIL) obtained from malignant melanomas (MM), hepatocellular carcinomas (HCC) and a lung metastasis from renal cell cancer (RCC) responding to treatment with in vitro tumor sensitized lymphocytes (IVS) and IL-2 utilizing a quantitative polymerase chain reaction technique as described previously (Weidmann et al. Cancer Res 52 (1992) 5913). In 5 patients with HCC and 8 patients with MM restricted TCR Vß repertoires were detected when the analysis was compared to that of PBL from 8 healthy individuals. Sequencing analysis of 2 predominantly expressed Vß regions in TIL from 2 patients with HCC reveiled sequence homology in the majority of the clones sequenced indicating a clonal proliferation of T cells possibly in response to a tumor associated antigen or i.e. to a viral antigen. However, the distribution patterns of Vß regions in TIL within both patient populations were diverse, suggesting that different antigens or restriction elements were responsible for T cell proliferation. In a responding lung metastasis from RCC treated with IVS and IL-2 and strongly infiltrated by lymphocytes, gene expression of Vß 13.1 was 28%, twice as much as in the non responding renal tumor indicating that Vß 13.1 expressing cells may have been responsible for tumor regression in the lung nodule. Altogether the data may suggest restricted TCR Vß repertoires in TIL from human solid tumors possibly in response to tumor associated antigens.

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INTRODUCTION OF A MODEL TO STUDY THE IN VITRO T CELL RESPONSE TO AUTOLOGOUS CYTOKINE GENE ENGINEERED TUMOR CELLS.

E. Weidmann, J. Brieger, K. Fenchel, D. Hoelzer, L Bergmann, P.S. Mitrou.

T cells with specific cytotoxicity against autologous tumor have been described in a variety of tumors. Preliminary clinical protocols introducing the administration of in vitro activated autologous T cells for treatment of melanoma have been promising. However, such therapies are extremely time and cost consuming and thus and for physiological reasons the in vivo activation of specific cytolytic effectors would be preferable. We are currently setting up an in vitro study preceding the clinical use of cytokine gene engineered tumor cells for tumor vaccination. The aim of the study is to determine whether tumor cells transfected with IL-2 and/or IFN-gamma genes are capable of inducing a specific autologous T cell response. Our working hypothesis is that IL-2 serves as an unspecific stimulus at the site of T cell recognition of the target in addition to T cell receptor mediated activation and that IFN-gamma enhences MHC expression and thus antigen presentation by the tumor cells.
IL-2 and IFN-gamma genes were obtained in M13/MP18 vectors and for easy expansion subcloned in bluescript vectors. For subsequent selection of cells transfected with both genes, IL-2 was cloned into an expression vector including the neomycin resistence gene and IFN-gamma in a vector with the hygromycin resistence gene. Tumors and PBL were obtained from patients with squamous cell cancer of the head and neck. PBL were cryopreserved for later use and tumor cells are growing in culture. After transfection of the cells with both genes by lipofection, cocultures with PBL will be performed and studied for cytokine release, for specific cytotoxicity of outgrowing lymphocytes and for their T cell receptor V region gene expression repertoire.

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STRATEGIES FOR THE TARGETED INHIBITION OF TUMOR CELL GROWTH TRANSMITTED THROUGH THE ERBB-2 AND EGF RECEPTORS
W.Wels, R. Beerli, L-M. Harwerth & N.E. Hynes

The erbB-2 and EGF receptors are overexpressed in many human tumors due to gene amplification. High levels of erbB-2 are found in adenocarcinomas arising at sites including breast, ovaries, lung and stomach. EGFR overexpression is found in gliomas and epidermoid and squamous carcinomas. The tumor enriched expression and extracellular accessibility make these receptor proteins appropriate targets for tumor cell directed therapy. We have taken an approach based upon the isolation of specific monoclonal antibodies (mAb) which bind to the extracellular domain of the receptor proteins. The variable domains of mAbs binding the erbB-2 and EGF receptors have been cloned by reverse transcription of hybridoma cell RNA and specific cDNA amplification using PCR techniques. Fusion genes coding for single chain antibody molecules (scFv) were made by joining the light and heavy chain variable domains with a synthetic nucleotide linker encoding a 15 amino acid peptide. These scFv encoding genes: scFv(225) and scFv(FRP5) specific for, respectively, the EGF and the erbB-2 receptors have been used in 2 types of experiments both aimed at the selective inhibition of tumor cell growth. 1) A retroviral vector encoding the scFv(225) gene has been used to infect cells which express the human EGFR. Intracellular expression of scFv(225) affects both the EGFR activation and EGF dependent growth of cells. 2) Recombinant immunotoxin genes were constructed by the addition of sequences encoding a modified *Pseudomonas* exotoxin A (ETA) to the scFv encoding DNA. The bacterially expressed recombinant immunotoxins bind specifically and with high affinity to the appropriate receptor and display both in vitro and in vivo cytotoxic effects selective for tumor cells expressing high levels of the erbB-2 and EGF receptors.

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Somatostatin-Receptor(In-111-octreotide)- versus Transferrin-Receptor(Ga-67)-Scintigraphy in Patients with Non-Hodgkin's-Lymphoma

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Somatostatin receptors have been demonstrated on peripheral B- and T-lymphocytes and in a high density on surgically removed lymphoma specimens of high grade non-Hodgkin-lymphoma (NHL). Somatostatin receptor scintigraphy with the somatostatin analog octreotide could demonstrate lymphoma infiltration at various localisations in patients with NHL. Ga(67)-scanning is a well established ancillary staging procedure for NHL espacially usefull in determinating vitality of residual lymphoma after chemo- and/or radiotherapy. Gallium (Ga) resembles iron with respect to transferrin binding and transferrin receptor uptake. This study was designed to evalute the use of the new method of somatostatin receptor scintigraphy for staging of NHL and to compare it with the established transferrin receptor scintigraphy. For staging or re-staging, 17 consecutive patients with NHL (14 high grade, 3 low grade) were investigated within 7 days with 185MBq Ga-67-citrate and 37MBq In-111-octreotide. Planar scans were acquired 24 and 72 hours after Ga-67-injection and after 4 and 24 hours in In-111-scintigraphy. Impulse rate of lymphoma localisation was determined with region-of-interest(ROI)-technique in comparison to a control-ROI. Results were compared with the results of the other staging procedures (ultra-sound, CT- and MR-scan). Thirteen patients had active disease, four were in complete clinical remission by standard evaluation. Ga-67-scans and In-111-octreotide-scans had comparable specificity, but Ga-67-scans showed significantly more intensive receptor uptake (mean target-background ratio t/b = 1,37/1) than In-111-octreotide (t/b = 1,31/1) in patients with active disease, revealing a higher sensitivity for the Ga-67-scan. Only in one patient (1 of 2 immunoblastic lymphomas) In-111-octreotide scintigraphy was superior to Ga-67-scanning. Investigations of the potential diagnostic use of somatostatin analogs in malignant lymphoma are going on in order to define a specific indication for the faster but more expensive In-111-octreotide-scintigraphy.

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HIGH -DOSE CHEMOTHERAPY COMBINED WITH WHOLE-BODY-HYPERTHERMIA IN ADVANCED DISSEMINATED MALIGNANCIES: A PHASE I TRIAL
G. Wiedemann, E, Knop, S. Eleftheriadis, and T. Wagner

In extensive animal studies on tumor xenografts growing in nude mice we have shown, that the therapeutic efficacy of a given dose of selected alkylating agents increases steeply with raising tumor temperature. Surprisingly, under these conditions the systemic toxity did not nearly increase to the extent to which the therapeutic effect rose. Therefore we started a phase I trial in cancer patients with advanced disseminated malignancy of unfavourable histology (all patients were heavily pretreated with chemotherapy and radiotherapy) to study the systemic toxicity, side effects, pharmacokinetics and therapeutic efficacy of a combined treatment with ifosfamide (IFO), actinomycin D (AD) and whole-body-hyperthermia (WBH; 41.8°C for 1hr).The drug doses were stepwise increased from 5 g/m^2 to 7 g/m^2 (IFO) from 0.8 mg/m^2 to 1.4 mg/m^2 (AD). A total of 19 treatments was given to 8 patients. No toxic death occurred. 1 patient suffered from severe nephrotoxicity. The myelotoxicity remained unexpectedly low. No symtoms of brain or liver toxicity have been observed.
In 8 patients we observed 3 PR and 1 NC. 1 patient had tumor progression. The treatment dependent antitumor effect was not evaluable in 2 patients because the study design was changed. In 1 treated patient the antitumor effect will be studied later.

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STIMULATION OF Vγ9δ2 T LYMPHOCYTES BY CD5 EXPRESSING B LYMPHOMA CELLS
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γδ T cells represent a minority of T cells in human peripheral blood. Although there have been reports of reactivity against (myco)bacterial antigens and heat shock proteins, the biological role of γδ T cells is not well understood. Corresponding to γδ T cells in the T cell compartment, CD5+ B cells represent a small subset of B lymphocytes, which is thought to be involved in the maintenance of natural immunity and autoimmunity.
We present data, which indicate that after in vitro culture of PBL for 8 days, depending on the presence of CD5+ B lymphoma cells as bystander cells and distinct bacteria, the percentage of γδ T cells dramatically enlarged (70% vs. 4%). In addition the absolute count of γδ T cells increased ($3x10^5$ vs $2x10^3$), implicating an extensive proliferation of γδ T cells. FACS analysis revealed, that all the γδ T cells displayed the Vγ9δ2 phenotype, which is their most common phenotype in peripheral blood.
Our in vitro system might give new insights in the interaction of B lymphoma cells with the immune system, the initiation of autoimmunity by bacteria and the antigens recognized by γδ T cells.

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RATIONALE OF MULTIMODAL TREATMENT (MT)

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The major goals of MT (periop. chemotherapy (CTx) ± irradiation (RTx) ± growth factors or other cytokines, CTx/RTx alone) are to improve local tumor control and/or to reduce the risk of distant failures. The preclinical rationale of MT is based on mathematical models and in vitro and in vivo experiments investigating aspects of tumor cell kinetics, growth regulation mechanisms, the role of microenvirement and tumor surrounding tissues, cell repopulation after cytoreductive therapy, primary and acquired drug resistance, timing of perioperative treatment, etc. The informations obtained from a wide variety of these tumor models serve as a sound foundation for clinical trials of early CTx. However, the striking successes achieved in these animal tumor systems have not been readily duplicated in man, although promising clinical results were achieved with pre-/postoperative CTx ± RTx or CTx/RTx alone in various tumor entities (tumors of the aerodigestive tract, breast cancer, osteosarcoma, etc.). At that point in time, the frequently discussed preclinical and clinical pros and cons of the various kinds of MT (pre- or postop. treatment, CTx/RTx alone) are well known, but, for most tumor entities there is still no clear recommendation which kind of MT should be used or at least investigated in a given clinical situation. The basis for such a decision depends on the expected outcome of local therapy (usually surgery (Sx) and the patterns of relapse thereafter.

HIGH LOCAL TUMOR CONTROL RATE WITH NON-MUTILATING Sx: Low risk of distant failure -> *no perioperative treatment*, high risk of distant failure -> *pre- or postop. CTx.*

HIGH LOCAL TUMOR CONTROL RATE WITH MUTILATING Sx: Low risk of distant failure -> *preop. CTx ± RTx*, high risk of distant failure -> *preop. CTx ± RTx ± postop. CTx.*

RESECTABLE BUT LOW LOCAL TUMOR CONTROL RATE WITH Sx: Low risk of distant failure -> *postop. RTx or preop. CTx ± RTx or CTx/RTx alone*, high risk of distant failure -> *preop. CTx ± RTx or CTx/RTx alone.*

IRRESECTABLE OR DISEASE UNLIKELY TO UNDERGO R0-RESECTION: -> *preop. CTx ± RTx or CTx/RTx alone.*

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METHOTREXATE PHARMACOKINETICS AND PROGNOSIS IN OSTEOSARCOMA

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The influence of methotrexate (MTX) pharmakokinetic parameters on the efficacy of high dose MTX in osteosarcoma was analysed. MTX plasma peak values from 202 patients in 1743 treatment courses and more detailed pharmacokinetic data on 153 patients in 1072 treatment courses from the cooperative osteosarcoma studies COSS-80, COSS-82 and COSS-86 were investigated. A mean threshold peak level of $\geq$ 1.000 umol/l for the repeated MTX courses of individual patients was found significantly correlating to prognosis in study COSS-80 (17 % versus 63 % actuarial 10 year MFS, $p<$ 0.00003). The MTX peak level was found closely correlated to AUC. AUC however was a less powerful determinator of prognosis, than the mean threshold MTX peak value. In patients receiving DDP as one of the additional drugs to MTX, the peak values and AUC as well were significantly increased (1.4 versus 1.27 umol/l, 6698 versus 5796 h·umol/l, $p<$ 0.001) and only a few patients (6 %) did not achieve mean threshold MTX peak values. In addition, following restriction of the hydration fluid after the MTX infusion from 4.5 to 3.0 l/m^2/24 h, the early MTX half life time and the AUC but not the MTX peak value, were found significantly increased (3.4 versus 3.06 h, 6777 versus 5975 h·umol/l, $p<$ 0.001) . These changes may have shifted downwards the threshold MTX peak level, explaining the lack of influence of the 1.000 umol/l threshold peak level in studies COSS-82 and COSS-86. The small number of patients with mean peak levels below 1.000 umol/l may have embarassed the discrimination of the assumed lower threshold MTX peak level in these studies.
Conclusion: MTX pharmakokinetics significantly influence the efficacy of MTX in osteosarcoma. The hydration volume thereby seems to play a crucial role.

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FC$_\gamma$RIII-AUTOANTIBODIES IN TWO PATIENTS WITH NEUTROPENIA AND NK CELL-DEFICIENCY

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Neutropenia was diagnosed in a patient with ITP and recurrent sinusitis and oral candidosis, and in a second patient with myasthenia gravis and postoperative wound healing disturbances. Positive direct and indirect immunofluorescence tests revealed autoantibodies against granulocytes in the patients' serum. In addition, a deficiency of CD16(Fc$_\gamma$RIII)+ NK cells was shown using flow cytometry. In order to determine the specificity of the autoantibodies an ELISA was established, in which soluble CD16 from supernatants of PMA-activated granulocytes or NK cells was coated to the plates via the CD16-antibody 3G8. This test revealed CD16-autoantibodies in serum of both patients. Furthermore, rate of phagocytosis of E.coli by granulocytes was partially inhibited by preincubation with the patients' serum.
In conclusion, autoantibodies against Fc$_\gamma$RIII may cause an immunodeficiency syndrome with neutropenia, NK cell-deficiency and inhibition of the rate of phagocytosis of granulocytes.

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RECOGNITION OF HUMAN RENAL CARCINOMA CELL LINES BY AUTOLOGOUS CYTOLYTIC T LYMPHOCYTES (CTL) : ATTEMPTS TO DEFINE CTL TARGET ANTIGENS.

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Clinical studies demonstrated a therapeutic effect of lymphokines like interferon-gamma on renal cell carcinoma (RCC). Such observations suggested that RCCs are potentially immunogenic. This assumption prompted the search for RCC-associated tumor antigens that can be recognized by autologous CTL generated in vitro from tumor-bearing patients.

Renal carcinoma cell lines as well as corresponding normal kidney cells were established in tissue culture from patients. Mixed lymphocyte tumor cell cultures (MLTC) performed with tumor cells and autologous peripheral blood lymphocytes led to the isolation of tumor reactive CTL. In the renal carcinoma model MZ1257, HLA-A2 was identified as the restriction element for recognition of MZ1257-RC cells by autologous CTL. Moreover, two out of four HLA-A2-positive renal carcinoma cell lines, including MZ1257-RC, were recognized by allogeneic tumor reactive CTL clones derived in the human melanoma model AV.

By transfection and subsequent gene cloning steps, we plan to identify the genes coding for antigens on MZ1257-RC cells that are recognized by autologous or allogeneic CTL, respectively. We did not succeed in cloning the genes after transfection of genomic DNA prepared from tumor cells. However, recently several genes coding for HLA-A2-restricted antigens expressed on AV melanoma cells were cloned using the following approach: COS-cells were co-transfected with the cDNA prepared from AV melanoma cells and with the HLA-A2 gene. Afterwords, antigen-positive transfectants were detected with the help of CTLs in TNF assays.

In general, this strategy requires the availability of a cloned gene coding for the appropriate antigen-presenting HLA molecule, a cDNA library prepared from the tumor cell line and CTL suitable for TNF-detection assays. These prerequisites are now fulfilled for the renal carcinoma model MZ1257.

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CYTOTOXICITY OF ANTINEOPLATIC DRUGS AT DIFFERENT TEMPERATURES DETERMINED ON TWO DIFFERENT HUMAN TUMOR CELL LINES: FEASIBILITY OF THE MTT-ASSAY

W. Wößmann, A. Siemens, G. Wiedemann, T. Wagner

In vitro cytotoxicity of hyperthermia alone or combined with cisplatinum (CDDP), mitoxantrone (Mito) or mafosfamide (Mafo) was investigated in the human breast cancer cell line Mx1 and the lung cancer cell line Lx1 with the tetrazolium based colorimetric assay (MTT). Experimental conditions simulated in vivo models and clinical trials: temperatures ranged from 32°C to 43°C, for 1 hour each, combined with a 4 dose drug range for each cytostatic agent which enclosed 50% cell survival (ID_{50}) at 37°C.

Hyperthermia alone up to 42°C (1 hr) had no influence on cell survival; 43°C (1 hr), however, reduced the survival fraction to 67% (Mx1) and 82% (Lx1), respectively.

The cytotoxicity of CDDP, Mito and Mafo showed clear dose dependencies at all temperatures. Compared to Lx1 the Mx1 cell line was significantly more sensitive to these drugs ($p<0.001$) with ID_{50} values being half as high as for Lx1. The thermal enhancement of drug cytotoxicity was both drug and dose dependent. The Mafo effect was furthermore influenced by the type of cell line.

CDDP-cytotoxicity was not affected by raising temperatures from 32°C to normothermia, but hyperthermia at 42°C reduced ID_{50} values for both cell lines by 50%. In both cell lines for Mito existed a linear correlation between temperature and cell survival. For Mx1 cells the ID_{50} for Mafo was reduced by the factor 2.2 for each additional 5°C. The cytotoxicity above normothermia was even more pronounced. The Mafo effect on Lx1 cells was not increased to a markable degree by hyperthermia (42°C), but was significantly lower at hypothermia.

These results suggest that the MTT-assay already used for chemosensitivity screening can even be helpful for fast screening of drugs used for thermochemotherapy in different tumors before testing promissing drugs in in vivo models.

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IS HODGKIN'S DISEASE AN INFECTIOUS DISEASE?

J.Wolf and V.Diehl

That Hodgkin's disease (HD) is a true malignant disorder of the lymphatic system, has been strengthened by the impressive cure rates of anticancer treatment like radiation and polychemotherapy. This success, however, is still counterfaced by a major lack of understanding of the pathogenetic events leading to HD. No convincing model exists neither to define the cell of origin of HD nor to explain the interaction between the putative malignant Hodgkin/Reed-Sternberg (HD/RS) cells and the surrounding bystander cells.

Rather, several lines of evidence question the concept of Hodgkin's disease starting as a true malignant disorder: (I) The epidemiological pattern of HD strongly resembles that of an infectious disease. (II) In early stages HD exerts pronounced clinical and biological features of an atypic immuneresponse. (III) Despite extensive investigations the HD/RS cells could not unequivocally be defined as the definite malignant cell population in HD. These cells are not specific for HD. Also, fundamental attributes of malignant cells, aneuploidy and clonal origin, cannot consistently be demonstrated in HD/RS cells. Vice versa, in many cases, where clonality or chromosomal aberrations were identified in HD tissue, their derivation from HD/RS cells could not unequivocally be demonstrated. In experiments performed in our laboratory, for instance, EBV-positive B cells harbouring numerical and structural chromosomal aberrations grew out from lymphatic tissue affected by HD after transplantation into SCID mice.

In summary, Hodgkin's disease in early stages might be understood as the unsuccessful attempt of the organism to eliminate a cell expressing a yet undefined target antigen. This antigen might have been introduced into the cell by viral infection (e.g.the EBV latent membrane protein LMP1), but could also be encoded by a cellular gene. The cell expressing the putative target antigen might be a precursor cell of any hematopoetic lineage. Growth promotion of this cells could be mediated by viral transformation and/or cytokine stimulation from the reactive environment. In the course of the disease, unability of an altered immunesystem to eliminate this target antigen expressing cell coincides with a stepwise transformation, probably triggered by an inherent genetic instability, thus leading to outgrowth of a fully transformed malignant cell clone in late stages of the disease.

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CHEMOTHERAPY OF MULTIPLE MYELOMA: A RETROSPECTIVE STUDY

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Although chemotherapy of multiple myeloma became more aggressive over the last three decades, median survival time of patients could not be prolonged significantly. Cytostatic regimens enlarged, but toxicity increased equally. The Alexanian protocol melphalan / prednisone seems to be one of the most effective therapeutic regimens in treatment of multiple myeloma. As a retrospective study we examined median survival time of 140 patients (pts.) (66 male, 74 female) stage II and III (Durie & Salmon classification) initially treated with several regimens. Melphalan was administered either orally (8 mg/m^2 day 1-4, MPpo) or intravenously (15 mg/m^2 day 1, MPiv) combined with prednisone 60 mg/m^2 day 1-4 (40 vs. 50 pts.). Initial therapy was VCMP in 15 pts., VAD in 7 pts., other regimens were applied in 28 pts.. There were no differences between both major groups concerning age, sex, stage of disease or renal dysfunction. 62% of the pts. suffered from IgG myeloma, 25% from IgA myeloma, 7% from Bence Jones myeloma, 2% from non-secretory myeloma, 1% from IgD or IgM myeloma, 3% of the pts. produced biclonal paraprotein. Median survival time of all patients was 54 months, ranging from 56 months in pts. initially treated with MPiv to 41 months in pts. treated with MPpo, 26 months in the VCMP treated group, respectively. Survival time was significantly shorter in patients who did not respond to induction therapy. Complete remission was seen only in 2 pts. treated with MPiv. Partial remission was more frequent in the orally treated melphalan group.

As survival time seems to be prolonged and toxicity is moderate, we prefer MPiv treatment as initial therapy of multiple myeloma.

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MULTIMODALITY TREATMENT IN SMALL CELL LUNG CANCER (SCLC)

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Overall treatment results in SCLC have not been improved significantly during the past decade. A median survival of 12 to 18 months and a 5-year survival rate of 5-10 % is seen in patients without distant metastases. Approaches to improve this stagnant situation include the use of multimodality treatment strategies. A large series of clinical trials have been performed comparing chemotherapy to chemotherapy plus radiotherapy. Although the results of these trials are conflicting, a recently published meta-analysis showed a significant advantage for the patients receiving radiotherapy. Wether concurrent chemo-radiotherapy or early administration of radiotherapy is superior to a sequential treatment strategy or late administration of radiotherapy, has not been clarified until now. Several phase II studies with concurrent chemo-radiotherapy described high 2-year survival rates, but further randomized trials and longer follow-up periods are necessary to confirm these data. A further approach to improve the prognosis for limited stage patients represents surgery. Possible treatment schedules are surgery followed by adjuvant chemotherapy or neoadjuvant chemotherapy followed by surgery. In non randomized trials 3-year survival rates of 30-50 % have been described for patients with N_0 or N_1 disease. However, the only randomized trial currently available comparing chemotherapy to chemotherapy followed by surgery did not demonstrate any difference in survival. In conclusion, further clinical trials are necessary to define the optimal treatment schedule and subgroups of patients who may profit from multimodality treatment strategies.

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INHIBITION OF HEMATOPOIETIC DIFFERENTIATION IN EMBRYONIC STEM CELLS BY ANTISENSE VAV TRANSCRIPTS

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The vav protooncogene is expressed exclusively in hematopoietic cells. Its expression is not restricted to a specific lineage or stage of differentiation.The 95kD protein contains an array of characteristic motifs indicative of a transcriptional activator and/or a signal transducing molecule.Vav interacts with activated membrane receptors and with Tyrosine phosphorylated cytoplasmic proteins. Nothing is known about the impact of Vav on hematopoietic stem cell growth and development. We therefore studied vav expression during the hematopoietic differentiation of embryonic stem cells (ES-cells, CCE-line) in vitro. Colonies developing from single ES-cells in methylcellulose and in the presence of Stem Cell Factor, Erythropoietin and IL-1 develop hematopoiesis in a well defined temporal order that recapitulates the in vivo ontogenesis. The onset of embryonic erythropoiesis as monitored by the expression of the embryonic β-globin chain (βh1) occurs on day 6, the expression of the myeloid marker CD11b on day12. By Northern Blot Analysis vav is first detected on day 10. However, low levels of vav as detected by Reverse Transcriptase (RT-) PCR analysis are detected already in undifferentiated ES cells and an upregulation is observed as early as day 2.To study the significance of vav for the early steps of hematopoietic differentiation, we derived ES cell lines expressing anti-vav mRNA transcripts. Stable transfection was done with an expression vector containing a 1.9 kb vav cDNA-fragment in antisense direction transcribed from the phosphoglycerate-kinase (pgk) promoter. 4 cell lines were obtained that expressed vav antisense transcript levels similar to or stronger than the hematopoietic cell line MEL. Maintained under regular culture conditions in the presence of leukemia inhibitory factor (LIF) the antisense expressing ES-cell lines show growth kinetics similar to the parental cell line CCE. The plating efficiency of the vav antisense expressing clones did not differ significantly from the neo control clones. Though the number of developing colonies was normal, the percentage of colonies that turned visibly red as an indicator for erythropoiesis was significantly reduced from 73 -85% in the neo control lines down to 2 to 22% in the vav antisense transfected cell lines. Cytological examination of the colonies confirmed the absence of erythro- and myelopoietic cells. Northern Blot analysis showed that GATA-1, PU.1 and CD11b are only weakly expressed or totally absent in the colonies derived from the antisense transfected ES-cells We conclude that the disruption of the hematopoietic program by non functional vav appears to affect very early stages of hematopopiesis even before the activation of transcriptional factors involved in erythro-myelopoiesis. These results present the first evidence that vav has a critical role in the development of hematopoietic cells from primitive cells.

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DETECTION OF MINIMAL METASTATIC TUMOR CELLS IN PERIPHERAL BLOOD AND BONE MARROW BY RT/PCR OF CK 18 AND PTHRP

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Gross metastatic spread marks the turning point to bad prognosis in the course of solid neoplasms. The significance of small numbers of disseminated tumor cells in the bone marrow or circulation, which has been shown to be a significant prognostic factor in breast cancer, still has to be defined in other entities. Conventional detection of small numbers of tumor cells is based on histological or immunhistological methods. Single metastatic tumor cells can be distinguished from surrounding tissue by their tissue-specific markers of differentiation. The expression of cytokeratin 18 (CK 18), the most divergent of the class I keratins, is strictly connected to epithelial differentiation. Parathormon Related Protein (PTHrP) plays a crucial role in fetal calcium homeostasis. PTHrP is not expressed in most adult tissues, but reactivated during malignant transformation of some tumors, especially in bone marrow metastases of breast carcinoma. A method for the detection of CK 18 and PTHrP transcripts in total RNA based on primer-specific reverse transcription and amplification by polymerase chain reaction (RT/PCR) was developed. RT/PCR of ß-actin served as control for RNA integrity. Specifity was checked by nested PCR with internal primers. Mononuclear cells of the bone marrow (BM) from donors free of neoplastic disease were negative for CK 18 (0/10) and PTHrP (0/5). The sensitivity of the method was tested in mixing studies of tumor cells from cell culture with mononuclear cells of the peripheral blood (breast carcinoma MCF-7 for CK 18, kidney carcinoma 786.0 for PTHrP). By RT/PCR of CK 18 one tumor cell in 10^3 mononuclear cells, by RT/PCR of PTHrP one tumor cell in 10^2 mononuclear cells of the peripheral blood was detectable.
RT/PCR of CK 18 and PTHrP may be a useful tool for the detection of minimal metastatic tumor cells. Its application to bone marrow specimens in bronchial and breast carcinoma may help to elucidate the prognostic significance of minimal metastatic disease in these settings.

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Human Thrombocytes contain the type I IFN-induced MxA-Protein

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The MxA-protein is an intracellular protein specifically induced by type I-Interferons (α,β,ω). Upon stimulation with IFN-α this protein is dose-dependently expressed in granulocytes, lymphocytes and monocytes both in vitro and in vivo.
To investigate whether thrombocytes also contain MxA-protein, thrombocytes obtained via thrombocyte enriched plasma were lysed and assayed for the presence of MxA-P. $25*10^6$ thrombocytes had no measurable MxA-P, but contained 0.2U MxA-P within 48 hours after the first rIFN-α2b-injection. At day 5 and 10 further increases of MxA in the thrombocytes (0.5 - 0.8U) were measured. Immunochemical staining of the thrombocytes with monoclonal antibody directed against MxA-P revealed also Mx-positivity of thrombocytes after IFN-injections. Furthermore, staining of bone marrow cells showed that also megakaryocytes under IFN-therapy contain high amounts of this MxA-protein. Apparently, in the presence of IFN-α megacaryocytes are capable of synthetizing MxA-P, which subsequently appears in the circulating thrombocytes. Presently, functional studies are underway to determine the efficacy of Mx-positive thrombocytes.

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Therapy-induced human rIFN-α2a antibodies from patients with hairy cell leukemia recognize conformational epitopes of rIFN-α2a

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Of 59 patients with hairy cell leukemia 15 developed measuable rIFN-α2a antibodies in their serum under a continuous rIFN-α2a-therapy. 9 of these 15 patients with the highest rIFN-antibody titer experienced a relapse of their disease despite continuous IFN-α2a-therapy. rIFN-α-antibodies from 5 such patients were highly purified by sequential protein G and rIFN-α-affinity chromatography. They neutralized IFN-α2a and IFN-αk, but not IFN-α_1,α_4 and α_8. To study the epitopes on rIFN-α2 recognized by these antibodies rIFN-α2 fragments were produced by digesting rIFN-α2 with Staphylococcus aureus V8 protease. Three main digestion IFN-fragments were obtained. When run on SDS-PAGE none of these fragments were recognized by the human therapy-induced IFN-antibodies. However, when the same IFN-fragments were separated in an electrophoresis under native conditions, all digestion products were identified by the human and rabbit antibodies. Similar results were obtained employing trypic fragments.These results strongly suggest that hightitered human rIFN-α2a antibodies are directed against conformational, but not sequential epitopes on the rIFN-α2a molecule. Therefore, conformationlly dysformed rIFN-molecules are likely to induce an immune response and subsequently a clinical resistance in patients treated with such a cytokine preparation.

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THERAPY OF LYMPHANGIOLEIOMYOMATOSIS WITH GOSERELIN AND RADIATION: A CASE REPORT

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Lymphangioleiomyomatosis (LAM) is a rare tumor of the lymphatic sytem which predominantly occurs in women of childbearing age. Assuming hormonal triggering mostly unsatisfactory treatments with progesterone, tamoxifen and oophorectomy have been tried.
A 17-year old Turkish woman, who acquired a retroperitoneal and mediastinal LAM immediately after her first delivery, was treated with the LHRH-agonist goserelin (Zoladex). Although an effective hormonal castration with suppression of LH and FSH was achieved, after 5 months of treatment there was neither regression of the abdominal and mediastinal tumor nor clinical benefit. Therefore percutaneous radiation of the abdominal mass including the ovaries with 30,2 Gy was given. After radiation there was a slow but steady improvement and the patient was discharged. A control examination 12 months later that included CT-scanning revealed 50% tumor reduction of the abdominal and mediastinal tumor mass. 5 years after onset of LAM the patient is still free of symptoms and CT-scanning shows continuous partial remission.
In this case of LAM long-term suppression of the ovarian function by radiation is the fundamental point of treatment. A short suppression over a period of 5 months by goserelin was of no success.

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PRIMARY SYMPTOMS IN LOW-GRADE NON-HODGKIN-LYMPHOMA AND PLASMA CELL DYSCRASIA: A RETROSPECTIVE ANALYSIS OF 444 PATIENTS

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444 out-patients with low-grade lymphoma were seen in Düsseldorf over a period of 15 years. In a retrospective analysis the primary symptom, which made the patient contact the doctor was evaluated. About half of the patients (47%) was free of any symptoms and diagnosis was made by chance. Symptomatic patients most frequently complained about fatigue and exhaustion (13%), followed by bone pain (12%) and lymph node enlargement (10%). B-Symptoms were primary symptom only in 6% of all low-grade lymphomas. Other symptoms such as gastrointestinal discomfort (5%), skin symptoms (4%), eye symptoms (2%), dyspnoe (1%) and neural disturbances (1%) were rarely presented. The highest percentage of asymptomatic patients was found in chronic lymphatic leukemia (55%), the lowest in centrocytic-centroblastic (cc-cb) lymphoma (4.7%). Bone pain was exclusively seen in multiple myeloma (35%). Another specific symptom were changes of the skin in T-cell lymphoma (50%). Symptoms of the eye were almost specific for patients with macroglobulinemia Waldenström and were observed in 10% of these cases. Gastrointestinal discomfort was a frequent primary symptom in cc-cb lymphoma (16%) and was rarely seen in other low-grade lymphoma. Lymph node enlargement was equally observed in chronic lymphatic leukemia, cc-cb lymphoma and T-cell Lymphoma.
Conclusion: in nearly one half of all cases diagnosis of low-grade lymphoma is established in an asymptomatic patient. Fatigue and exhaustion are the most frequent primary symptoms in the other half. Rather specific primary symptoms are: 1. bone pain in multiple myeloma, 2. skin lesions in T-cell lymphoma, 3. gastrointestinal disturbances in cc-cb lymphoma and 4. ocular symptoms in macroglobulinemia Waldenström.

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LYMPHOPLASMACYTIC LYMPHOMA WITH MONOCLONAL SERUM IGG IMMUNOGLOBULIN: REVIEW OF 6 CASES

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Lymphoplasmacytic lymphoma is a type of low-grade Non-Hodgkin-Lymphoma often combined with monoclonal serum IgM immunoglobulin (M.Waldenström). Association with monoclonal IgG immunoglobulin is rare.
Over a period of 14 years 62 lymphoplasmacytic lymphoma were diagnosed: 27 showed no monoclonal serum immunoglobulin, 29 had monoclonal IgM and 6 monoclonal IgG immunoglobulin. There were 4 women and 2 men between 55 and 79 years who had monoclonal serum IgG immunoglobulin. In 3 cases serum IgG concentration at diagnosis was elevated, in the other 3 IgG was within normal range. The maximal serum concentration of IgG measured at diagnosis was 6020 mg/dl. 3 Patients were asymptomatic, one had peripheral neuropathy, one ocular symptoms due to a retrobulbar lymphoma and one complained about abdominal pain due to a splenic lymphoma. 2 patients showed lymphocytosis (31.000/µl and 27.000/µl). Diagnosis was established by bone marrow biopsy in 5 cases, in the case of the retrobulbar lymphoma no bone marrow infiltration could be found and diagnosis was made by uvulectomy. This patient was treated with retrobulbar radiation, another one received splenectomy of the splenic lymphoma. Chemotherapy was not required in these 2 cases. Of the other 4 patients 2 remained asymptomatic for 42 and 14 months. 2 patients were treated with Chlorambucil and Cortison as first line therapy because of peripheral neuropathy and progressive anemia resulting from bone marrow infiltration. Although both patients initially responded to chemotherapy, treatment had to be changed after 4 and 11 months to azathioprin and CVP. Follow up of all 6 cases ranged from 4 to 47 months (medium 26 months).

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CARDIAC HIGH-GRADE NON-HODGKIN-LYMPHOMA CAUSING SEVERE HEART FAILURE: A CASE REPORT

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Cardiac manifestation of high-grade Non-Hodgkin-Lymphoma (NHL) in the final course of the disease is found in 20%. Cardiac manifestation with heart failure as initial symptom is rarely seen and associated with poor prognosis. In many cases ante-mortem diagnosis is not achieved.
We wish to report a case of a 68-year-old patient, who complained about arrhythmias over a period of 4 months and acquired severe progressive heart failure NYHA IV with dyspnoe, peripheral oedema and pleural effusions. CT-scanning showed tumor of the mediastinum and pericardium with compression of both atria and superior and inferior vena cava syndrome. Bone marrow biopsy, pleural fluid cytology, gastroscopy and bronchoscopy gave no hints at the primary tumor. Diagnosis was made by thoracotomy, which showed high-grade NHL of polymorphic subtype of the pericardium and mediastinum. Final tumor staging was IIAE . As the tumor mass was huge, debulking was not possible. After thoracotomy chemotherapy (CHOP) was given and heart failure disappeared within one week. We applied 5 courses of CHOP and reached complete remission. 2 courses of IMVP-16 and radiation with 35 Gy were performed for consolidation. 1 year after diagnosis the patient still is in complete remission.

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THE EFFECT OF METHYLENE BLUE VIRUS INACTIVATION ON FRESH FROZEN SINGLE DONOR PLASMAPHERESIS PLASMA

T. Zeiler, H. Riess, G. Wittmann, R. Zimmermann, N. Schwella, H.G. Heuft, R. Eckstein[1] and D. Huhn

To investigate the effect of virus inactivation of fresh frozen plasma on coagulation capability we prepared 22 aliquots (250 ml each) of 11 ACD plasmapheresis plasmas. One group (VIP) was submitted to virus inactivation by addition of methylene blue to a final concentration of 1μmol/l and exposure to visible light (B.Lambrecht, DRK Blutspendedienst, Springe, Niedersachsen). The other group (FFP) was stored as usual. PT and PTT, fibrinogen, factors II, V, VII and VIII, AT3, C1 inhibitor, protein C, protein S and plasminogen were tested in VIP, FFP (after thawing) and the native plasma. The values of the native plasma were set as 100 %.
With the exception of a factor VIII recovery of 41% (versus 61% in FFP) VIP meets the requirements for fresh frozen plasma (i.e. 70% activity of the initial bulkware). We could find a statistical significant decrease ($p<0.003$) in recovery of factors II, V and VIII in VIP compared to FFP (paired samples t-test). Fibrinogen, factor VII, AT3, protein C, protein S, C1 inhibitor and plasminogen were less affected. The global tests PT (80% vs. 93%) and PTT (39.7 sec. vs. 34.4 sec.) revealed a statistical significant ($p<0.001$) decrease in coagulation capability of VIP (paired samples t-test).
We conclude that VIP is sufficient regarding the requirements for fresh frozen plasma but the transfusing physician should be aware of the reduced coagulation capability of VIP that might result in altered dosage.

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PATHOLOGICAL ACTIVATION OF INTESTINAL MUCOSAL LYMPHOCYTES: PREREQUISITE FOR THE DEVELOPMENT OF INTESTINAL LYMPHOMAS?

M. Zeitz

Primary gastrointestinal lymphomas occur in several clinical settings: Gastric B-cell lymphoma is associated with H. pylori induced gastritis and there is preliminary evidence that growth of the malignant B-cell clone is dependant on T-cell activation by H. pylori antigens. Another form of intestinal B-cell lymphoma is immunoproliferative small intestinal disease (IPSID) which is accompanied by intestinal infections and in its early stages is responsive to broad spectrum antibiotics.
By far most cases of small intestinal T-cell lymphomas occur in association with celiac sprue or other forms of enteropathy. Based on there phenotype and on there reactivity with mAB HML-1 which specifically labels mucosal lymphocyte antigen these tumors seem to develop from intraepithelial lymphocytes (IEL) and are designated as enteropathy-associated T cell lymphomas (EATL). In untreated celiac sprue there is a higher rate of mitotic figures in IEL and it has been shown that a strict gluten-free diet can prevent the development of EATL. Therefore chronic stimulation by gluten might lead to the development of EATL. In recent studies we showed that the presence of activation markers on EATL usually is accompanied by villous atrophy. However there are IEL-derived T-cell lymphomas not associated with villous atrophy, interestingly these tumor cells lack activation markers. Using the intestinal epithelial cell line HT 29 we showed that activated T-cells produce factors reducing viability and proliferation of epithelial cells and increasing MHC class II expression. These findings rise the question whether factors released by activated tumor cells of EATL induce small intestinal transformation seen in celiac sprue.
In conclusion, most primary gastrointestinal B- and T-cell lymphomas are associated with chronic inflammatory diseases of the mucosa and are partially responsive to stimulation by specific antigens. Therefore chronic activation of the mucosal immune system might lead to malignant transformation of mucosal lymphocytes.

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IMMUNEPHENOTYPIC ANALYSIS OF PERIPHERAL BLOOD STEM CELL SAMPLES FRACTIONATED BY COUNTERFLOW CENTRIFUGAL ELUTRIATION (CCE)

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In allogeneic bone marrow transplantation depletion of T-cells has been performed to decrease the incidence of graft-versus-host-disease (GvHD), but has been found to be associated with a higher relapse rate. Peripheral blood stem cells (PBSC) are widely used for autologous transplantations. A major hinderance for the use of peripheral blood stem cells (PBSC) in allogeneic transplantation is the high rate of contamination with lymphocytes, resulting in a high risk for GvHD. We studied the separation of PBSC (n=10) by CCE to deplete lymphocytes and to enrich hematopoietic progenitor cells. Three different cell fractions were obtained and characterized by flow cytometry, colony forming capacity and cell size. The viability was not reduced by the procedure. Colony forming assays were performed on methylcellulose as day-14 BFU-E and CFU-GM. The ratio of BFU-E to CFU-GM was prior to CCE 1.78 (±1.14), in fraction 28 ml/min 6.93 (±6.04) and in the rotor off fraction 1.23 (±0.76).

Fraction	BFU-E	CFU-GM	CD 34+	CD 3+	CD 19+	CD 56+
24ml/min	0,01±0,03	0,00	0,15±0,04	78,79±3,2	8,68±8,16	4,77±2,45
28ml/min	19,22±9,1	3,81±5,80	0,32±0,08	79,08±5,1	1,93±2,09	7,21±4,72
Rotor off	80,76±9,1	77,86±36,40	4,51±1,97	3,34±0,73	1,27±0,88	1,31±0,6

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GENERATION OF EARLY HEMATOPOIETIC PRECURSORS IN LONG TERM BONE MARROW CULTURES (LTBMC) AFTER MARROW PURGING WITH ET-18-OCH_3

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Alkyl-lysophospholipid derivatives (ALP) are currently being tested as candidates for bone marrow (BM) purging prior to autologous BM transplantation in different malignancies (1). Preclinical studies revealed preferential membrane-toxicity of some ALP towards neoplastic tissues with relatively sparing normal cells (2). We evaluated the toxicity of the ALP ET-18-OCH_3 (1-0-octadecyl-2-0-methyl-*rac*-glycero-3-phosphocholine; ET) towards early hematopoietic precursors by testing progenitor regeneration of non-purged and ET-purged BM from autologous LTBMC in 3 different patients with malignant hematologic diseases in complete remission (AML, NHL, Hodgkin's disease). Marrow was obtained during harvest operation and used for initiation of LTBMC (30 - 40 flasks/trial, 2 x 10^7 cells/flask in supplemented Mc Coy's media, 33°C, 5% CO_2) and purging experiments. Purging was performed with 75 µg and 125 µg ET/ml/2 x 10^7 cells (4 hrs, 37°C, 5% CO_2). Adherent LTBMC feeder layers (3-4 weeks) were irradiated with 875 rad for complete elimination of hematopoietic progenitors and recharged with cryopreserved purged and non-purged BM cells (1 x 10^7 cells/flask). In weekly intervals, adherent layer (AL) and supernatant (SN) LTBMC cells were completely removed and evaluated for progenitor generation in short term colony forming unit (CFU)-progenitor assays. We have seen sufficient CFU-generation from ET-purged and non-purged marrow cells for up to 8 weeks of LTBMC (>40 CFU/flask), however, with a decline of CFU-counts over time. Total CFU-counts from LTBMC with purged BM were slightly but not significantly reduced when compared with non-purged control. CFU-development in the AL approximated or exceeded those present in the SN from LTBMC with purged and non-purged BM. Although high-dose purging with 125 µg ET/ml partly inhibited initial CFU-proliferation (weeks 0-2) in 1 patient, nearly equal CFU-counts were seen after 4 and 8 weeks of LTBMC compared with non-purged control. In conclusion, ET-purging did not add significant toxicity to cryopreservation for early hematopoietic precursors as measured by LTBMC.
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1. Vogler W. R. et al. (1992) Blood 6:1423 2. Okamoto S. et al. (1987) Blood 69:1381.
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IDENTIFICATION OF ANTIBODIES TOWARD PUBLIC CLASS I HLA DETERMINANTS IN HIGHLY SENSITIZED PATIENTS WITH HEMATOLOGIC DISEASES

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The supply of platelets in thrombocytopenic patients with malignant diseases is often complicated by developing antibodies toward HLA determinants. Conventional techniques to identify the specifity of HLA antibodies or to provide a sufficient number of cross-match negative platelet concentrates in highly sensitized patients are time consuming and not universally available.
We applied a modified program to the 2x2 table analysis of lymphocytotoxicity test results for identification of HLA antibodies (Transplantation 50; 427). This program incorporates a list of public antigens shared by cross reactive class I HLA antigens. These public antigens belong to well known cross reacting groups (CREGs). In a series of 4485 sera we retrospectively identified 504 sera with >5% panel reactivity (PRA) from 140 patients with hematologic disease or germ cell tumors for further evaluation. The analysis yielded significant information on antibody specificity for 321 (64%) of these sera and for 109 (78%) patients, respectively. We detected antibodies to public HLA determinants in 163 and to private determinants in 158 sera. The search for antibodies to public antigens was most efficient in 260 sera with 30 to 90% PRA showing CREG specificity in 49%. For selected patients data are presented showing that the election of compatible donors with regard to the antibody specifity of the patient is more efficient than searching for HLA identical donors. Our method offers an attractive approach to improve the transfusion practice in platelet refractory patients.

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MDR1 GENE EXPRESSION IN ACUTE MYELOID LEUKEMIA: AN UPDATE

S. Zöchbauer, A. Gsur, P. A. Kyrle, K. Lechner, and R. Pirker.

The expression of the MDR1 gene was determined in the leukemic cells in patients with acute myeloid leukemia (AML) at diagnosis and correlated with clinical outcome. In 79 patients, MDR1 RNA expression was assessed by slot blot analysis by means of a radiolabelled MDR1 cDNA (probe 5A). MDR1 RNA expression of the leukemic cells was negative in 37% and positive in 63% of the patients. The complete remission (CR) rate of induction chemotherapy was 76% for patients without MDR1 RNA expression as compared to 54% for patients with MDR1 RNA expression (p=0.05). The median duration of overall survival was 19 months for patients without MDR1 RNA expression but 8 months for patients with MDR1 RNA expression (P=0.02). In 52 patients, P-glycoprotein expression was determined by immunocytochemistry by means of monoclonal antibody C219. In patients (N=25) with 0-5% staining cells, the CR rate was 74% as compared to 40% in patients (N=27) with >5% staining cells (P=0.01). The median duration of OS was 15 months in the former group of patients and 6 months in the latter group (P=0.06). The data indicate that the MDR1 gene is frequently expressed in AML and that its expression is associated with a poor prognosis.

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SOLUBLE INTERLEUKIN-2 RECEPTORS ABROGATE IL-2 INDUCED CELLULAR ACTIVATION IN MICE AND MEN

U. Zorn, I. Dallmann, J. Grosse, H. Kirchner, H. Poliwoda and J. Atzpodien

Soluble interleukin-2 receptors (sIL-2R) exert a potential role in immunoregulation. We investigated the ex vivo effects of sIL-2R on several interleukin-2 (IL-2)-dependent activation events. Proliferation of the IL-2-dependent mouse cell line CTLL-2 and isolated human PBMC stimulated with recombinant IL-2 (rIL-2) was suppressed by sIL-2R added to the culture medium in a dose-dependent way. Preincubation of sIL-2R with rIL-2 did not enhance this suppression. Cytotoxicity of rIL-2-stimulated human PBMC against the human cell lines K562 and Daudi was correlated inversely to the concentration of sIL-2R in the culture medium during rIL-2 stimulation. sIL-2R concentrations higher than 4.0 pM produced a significant decrease in cytotoxicity ($p<0.01$). Light microscopy of IL-2-stimulated PBMC revealed no signs of cellular activation when high dosages of sIL-2R had been added. The effect of different sIL-2R concentrations added to cultured human PBMC on secondary IL-2 and sIL-2R production was tested by ELISA. Initial supply with high sIL-2R dosages yielded weak increase and subsequent slow reduction of IL-2 levels. In contrast, strong secondary IL-2 production followed by rapid clearance was observed when low sIL-2R concentrations had been added. Endogenous shedding of sIL-2R in response to rIL-2 was abrogated by the initial exogenous addition of high amounts of sIL-2R whereas low exogenous addition of sIL-2R was followed by a continuing endogenous production of sIL-2R after five days of culture. Our studies may lead to a better understanding of IL-2-related immunoregulation in the preclinical and clinical settings.

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ALL-TRANS RETINOIC ACID (tRA) AND LOW DOSE HOMOHARRINGTONINE (HHT) IN THE TREATMENT OF ACUTE PROMYELOCYTIC LEUKEMIA (APL)

P. Zou[1], S.J. Song[1], P. Meusers[2] and G. Brittinger[2]

From October 1989 until May 1993 18 out of 22 patients with newly diagnosed APL were intended to treat with two courses (day 1-14 and 29-42) of tRA (80 mg/day) and low dose (1 mg/day) HHT, a cephalotaxine alcaloid. One patient received aclacinomycin (5 mg/day) instead of HHT. Postremission treatment included 2-3 cycles of daunorubicine (DNR) (40 mg/day 1-3) and cytarabine (A) (200 mg/day 1-7).
Typical coagulation abnormalities could be demonstrated in 17 patients. Four patients died of bleeding complications before treatment initiation and 2 patients after the first course of tRA + HHT.
Evaluation of treatment results was performed in 15 patients completing two courses of tRA + HHT:
Complete remission was achieved in 9 patients (60 %) and partial remission in 3 patients, who were lost to follow-up for socioeconomic reasons. In 2 of 3 non responding patients complete remission could be induced after 2 to 3 cycles of DNR+A. Median survival probability in patients with complete remission was 6 (6+ to 38+) months, with 3 patients relapsing after 6 and 36 months.
Complete remission in APL was obtained by tRA without bone marrow hypoplasia by cell differentiation, thus diminishing typical bleeding complications and avoiding the incidence of hyperleukocytosis syndrome by treatment with low-dose HHT. Postremission consolidation is mandatory.

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Correlation of Growth-Stimulation by rhGM-CSF for AML Progenitors and Priming Effects on Ara-C-Toxicity

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Hemopoietic growth factors like rhGM-CSF have the potential to prime responsive cells for cytotoxic drugs. We have investigated priming effects on AML progenitors and normal progenitors during a clinical trial. In vitro and in vivo results showed an enhancement of ara-C cytotoxicity for both, leukemic and normal progenitors.

Bone marrow cells of 20 patients with newly diagnosed AML were separated on Ficoll-Hypaque gradients, pretreated with 100U/ml rhGM-CSF or rhIL-3 or control medium, treated with 0-100μM ara-C, washed and seeded into a CFU-L assay based on methylcellulose with IMDM and rhGM-CSF. CR marrows were evaluated likewise with a CFU-GEMM assay.

Ara-C toxicity was quantified from dose response-curves of CFU-L or CFU-GM colony numbers using the median effect principle to obtain LD50s. LD50s of non-primed controls were compared to LD50s altered by priming with rhGMCSF for 24h or 48h or with rhIL-3 for 48h; their ratio (primed/non-primed) is abbreviated as priming index, PI. Growth stimulation during pretreatment with GM-CSF or IL-3 was measured by the colony numbers obtained without ara-C and is called stimulation index, SI, here. The following table shows some of these data for CFU-L and in vitro exposure, only.

pretreatment:	GM-CSF 24h	GM-CSF 48h	IL-3 48h
increased ara-C tox.	5 of 9	12 of 15	6 of 10
median SI	0.723	0.957	0.713
median PI	0.957	0.288	0.614
correlation SI-PI	0.750	0.361	0.733
significance p <	0.025	0.05	0.025

We conclude that growth stimulation during pretreatment with GM-CSF and enhancement of ara-C toxicity are not independant variables. Yet, although growth stimulation is a predictor for PI, it does not account for all variation in PI as indicated by mediocre correlation coefficients. Especially pre-expsoure to 48h GM-CSF seems to activate other mechanisms responsible for priming. Candidates for such mechanisms might be pharmakokinetic changes during priming.

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Abstracts received after deadline

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MOLECULAR BIOLOGY OF SECONDARY HIGH GRADE LYMPHOMAS.

M. Kneba

Since the original description of an immunoblastic sarkoma in a patient with preexisting CLL in 1928 by Richter, the malignant transformation (MT) of malignant lymphoma from a low- to a high grade tumor is well described occurring in up to 80% of patients during their clinical course. MT represents an abrupt transition in tumor biology and is associated with a change to high grade (large cell) morphology, an increase in lymphoma cell proliferation rate and a more aggressive clinical course. However, the critical molecular events that drive the transformation process are poorly understood in most cases. Aquired spontaneous or therapy-induced sequential genetic lesions of the primary lymphoma or development of a true secondary tumor originating from a clonally unrelated, newly transformed B-cell, have been considered as a cause of MT. A significant number of non Hodgkin's lymphomas (NHL) are characterized by non randomly occurring chromosomal aberrations involving protooncogenes (bcl-2, bcl-1/cyclin D1, c-myc) The frequency and type of these genetic alterations varies in the different clinicopathologic cathegories of the Kiel classification. Detailed investigation of these events in transformed lymphomas has shown that MT represents the malignant progression by secondary genetic lesions of the primary lymphoma rather than the emergence of a truly separate neoplasm in the majority of cases. The most aggressive secondary high grade lymphomas arise after the aquisition of a t(8;14) translocation deregulating the extremely potent oncogene c-myc in a primary t(14;18)- positive cb-cc lymphoma. Other genetic lesions leading to MT include EBV and the tumor suppressor gene p53.

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CYTOKINE GENE TRANSFER IN CANCER THERAPY

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Potential strategies for gene therapy of cancer include corrections of the genetic defects by homologous recombination, antisense approaches, protection of non-neoplastic cells by transduction of resistance genes into normal cells and transfer of prodrug activating enzymes into tumor cells. We are pursuing strategies to augment host immunity to cancer by introducing cytokine genes into tumor cells or into antigen presenting cells. In a paracrine model, we are investigating the potential therapeutic effects of cytokines secreted by other cell types in close proximity to tumor cells.
Prerequisites for an efficient cellular immune response against tumors are the preferential expression of specific antigenic determinants - such as idiotypes on cells in lymphoma and possibly Hodgkins disease - and the activation of effector cells capable of recognizing and eliminating these neoplastic cells. In the CMS-5 murine fibrosarcoma model, we have shown that local secretion of IL-2 by IL-2 transduced tumor cells can activate and expand specific cytotoxic effector cells leading to tumor rejection and immunological memory. This effect can be further augmented by the co-secretion of IFN-γ after transduction with a retroviral vector containing both the IL-2 and IFN-γ genes. In contrast, transduction with GM-CSF, which in other models has been shown to be a potent inducer of immunity, appears to lead to enhaced growth of transduced tumor cells. These data demonstrate that host immunity to cancer can be augmented by cytokine gene transfer. However, therapeutic effects are highly dependent on the strategies and model systems chosen.

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