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UNDIFFERENTIATED SARCOMA OF THE LIVER IN CHILDHOOD - SUCCESSFUL MANAGEMENT IN 4 PATIENTS

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Undifferentiated (embryonal) sarcoma of the liver is a rare, highly malignant, mesenchymal tumor presenting predominantly in late childhood. Four girls, aged 6 to 13 years, are reported. The first patient underwent complete resection by hemihepatectomy and received polychemotherapy for 16 months. In the second patient initially only partial resection could be accomplished. By synchronous radio- and chemotherapy the tumor decreased to a size, that it could be resected completely and was totally devitalized on biopsy specimens. Postoperative chemotherapy was finished after 8 weeks. In the third case hemihepatectomy resulted in complete removal of the tumor and chemotherapy was administered for 15 weeks post-operatively. The tumor of the fourth patient was irresectable at diagnostic biopsy. Polychemotherapy led to a significant reduction of the tumor size and resection with clear margins could be performed subsequently. Since histological devitalization amounted to about 95 %, post-operative chemotherapy was discontinued after 6 weeks. All 4 patients remain well without evidence of tumor recurrence after 48, 18, 18 and 3 months of follow-up, respectively.

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TREATMENT RESULTS OF 72 CHILDREN WITH BRAIN TUMORS

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Brain tumors are the most frequent group of solid tumors in children and comprise 20 % of all childhood malignancies. Despite substantial progress in the treatment of patients with certain forms of brain tumors the overall prognosis of patients with malignant brain tumors is still worse than that of children with malignant tumors outside the central nervous system. Therefore a combined effort of neurosurgeons, neuroradiologists, radiotherapists and pediatric oncologists is necessary to improve the long-term survival and quality of life of these patients. From January 1987 to December 1991 72 patients with brain tumors were diagnosed, 67 had primary manifestations and 5 had relapsed tumors. The diagnoses were medulloblastoma (n=10), brain stem glioma (n=8), cerebellar astrocytoma (n=9), ependymoma (n=7), astrocytoma (n=12), optic pathway glioma (n=5), craniopharyngeoma (n=3), and others (n=18). Treatment consisted of surgery (n=21), observation (n=4), surgery and radiotherapy (n=7), radiotherapy (n=1), chemotherapy and radiotherapy (n=3) and surgery or biopsy plus chemotherapy and radiotherapy (n=36). To date 19 patients died, 4 peri-operatively and 15 of tumor progression, 2 patients are lost of follow-up and 51 patients are alive with a median follow-up period of 116 weeks.

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MICROANGIOPATHIC HEMOLYTIC ANEMIA AS THE ONLY SIGN OF METASTASIS OF A BREAST CARCINOMA.

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The term "microangiopathic hemolytic anemia" (MAHA), first described by Brain et al 1962, is characterized by the intravascular fragmentation of erythrocytes due to vessel wall changes or fibrin deposits in the microcirculation.

In Nov. 1989 a 54 year old woman had a lumpectomy of the left breast and axillary lymph node-dissection (undiff.ductal carcinoma PT2N1MO, estrogen receptor expression was weakly pos., progestogen receptor was neg.) Postoperatively she underwent locoregional radiotherapy and adjuvant Tamoxifen (2o mg daily) was started. In Oct. 1991 the patient was admitted with signs and symptoms of anemia. The peripheral blood count and smear showed: Hb 9,o g/dl, MCV 1o4 fl, Hematocrit 26%, reticulocytes 8%, fragmentocytes 6% with marked polychromasia and occasional microspherocytes. The haptoglobin level in the serum was trace positive, serial serum LDH and bilirubin levels were normal. There was no concomitant disseminated intravascular coagulation. Coombs tests were negative. A diagnosis of MAHA was made. Thorough invasive and noninvasive investigations (including a bilateral iliac crest biopsy) dislosed no tumor despite a serum CEA level of 46o ng/ml. Eventually a blind liver biopsy (with all liver function tests being normal) showed a disseminated purely intravascular invasion of carcinoma cells.

4 cycles of CMF chemotherapy only brought about a minor response .We then changed to high dose monotherapy of Epirubicine 11o mg/m2 every 4 weeks. As of this writing 2 cycles produced a complete disappearence of the MAHA with no more fragmentocytes being discernible on the blood smear. Further follow up will be presented.

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IMPACT OF THE ANABOLISM OF 5-FLUOROURACIL ON CYTOTOXICITY IN COLORECTAL TUMOR CELLS *IN VITRO*

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The resistance of tumor cells to fluoropyrimidines is a complex phenomenon which has been only partially elucidated. The cytotoxic effect of 5-fluorouracil (5-FU) is mediated by several anabolic steps and a subsequent incorporation into nucleic acids. Additionally, cells are depleted of thymidine by inhibition of the enzyme thymidilate synthetase, which is efficiently blocked by 5-fluorodeoxyuridine-monophosphate in combination with tetrahydrofolate. We investigated the impact of the activating steps of 5-FU on cytotoxicity in four colorectal tumor cell lines in vitro (CCL 227, CCL 228, HT 29 and CaCo2) and the contribution of the biochemical modulation by reduced folate to the cytotoxic action.
The results revealed the differential sensitivity of the single tumor cell lines: every cell line expressed an individual resistance pattern to 5-FU and its anabolites as well as to the modulating agents [R,S]-leucovorin, [S]-leucovorin and [R,S]-5-methyltetrahydrofolate. The anabolites of 5-FU can be effectively modulated in CCL 227, but not in CaCo2. The most effective fluoropyrimidine as single agent is not necessairily the most cytotoxic one in combination with tetrahydrofolates. In all cell lines, at least one anabolic step is limiting the cytotoxic action of 5-FU. In CCL 227 this is e.g. the conversion of 5-FU to 5-fluorouridine and to 5-fluorodeoxyuridinemonophosphate via 5-fluorodeoxyuridine. HT 29 on the other hand is sensitive to all anabolites of 5-FU, but can be hardly modulated by folate indicating limitations in the folate activating steps rather than in 5-FU activation. Further studies will focus on the correlation of enzymatic activities and cytotoxicity *in vitro*. The confirmation of this results *in vivo* could contribute to the individualization of the chemotherapy with anti-metabolites in colorectal cancer.

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PHASE I/II STUDY OF EPIRUBICIN, FLUOROURACIL AND LEUCOVORIN IN ADVANCED ADENOCARCINOMA OF THE STOMACH: FINAL RESULTS

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To determine the maximum tolerated dose of epirubicin for use in combination with 5-fluorouracil (FU) and low dose leucovorin (LV), we conducted a phase I/II trial in 37 patients(pts) with advanced gastric carcinoma. The doses of FU (425 mg/m2) and LV (20 mg/m2) both given intravenously on days 1 to 5 were held constant, while the dose of epirubicin was escalated in cohorts of patients beginning at 50 mg/m2 on day 1. Cycles were repeated every 4 weeks. Significant gastrointestinal symptoms and myelosuppression were infrequently observed at the initial dose level. At a dose of 60 mg/m2 epirubicin on day 1, however, 5 of 8 pts had significant mucosal toxic effects during the first cycle of therapy. In addition, two pts treated at this dose level had grade IV granulocytopenia with insufficient recovery to permit a second course by day 28, and one pt each had severe diarrhea and nausea/vomiting. Among 37 pts with assessable disease, there were 3 complete and 11 partial responses (response rate 38%). Leucovorin modulation of FU can be safely incorporated into combination chemotherapy with epirubicin and provided a relatively active regimen for treatment of disseminated gastric cancer.

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PHASE I AND PHASE II STUDIES OF INTERFERON-ALPHA (IFN) AND 5-FLUOROURACIL (FU)/LEUCOVORIN (LV) IN COLORECTAL CANCER

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12 patients (pts) with advanced colorectal cancer entered a phase I trial for IFN sq added to standart 500 mg/m2 LV (2-hour infusion iv)+600 mg/m2 FU (midpoint-injection) once weekly for 6 weeks q 8 weeks. 3 pts were enrolled at each dose level of IFN (1 MU, 3MU, 5MU and 10 MU 3x/week). WHO-grade III/IV toxicity (diarrhea, leucopenia) occured only at 10 MU, while toxicity was insignificant at doses of 1 MU and 3 MU, and was acceptable at 5 MU. Therefore 3 x 5 MU/wk of IFN were chosen to be added to the standard FU/LV regimen in a phase II trial. 30 pts (colon:n=14, rectum:n=15; male:n=14, female:n=16; age:med.58.5 yrs [42-73]; prior treatment:n=7; med. observ. time:7.5 mo [3-21+]) were on treatment for a total of 490 wks (med.22.4 [16-112+]). Who-grade III toxicity for diarrhea (2.4%), stomatitis (2%), thrombopenia (2%), hand-foot-syndrom (2%) and nausea/vomitus (1.6%) occured during 10% of the cycles. No grade IV toxicity was seen and 9/30 pts had no side effects at all. Response evaluation showed no CR, 9 PR (30%), 4 SD (13%) and 17 PD (57%), resulting in a response rate of 13/30 (43%). Kaplan-Meier plots of survival (S) for pts with PR vs. SD/PD favored PR pts statistically significant (med. S for PR: 14.9 mo, SD/PD: 5.53 mo; Mantel-Cox [MC]: p=0.0004, Breslow [B]: p<0.004). Separate analysis for rectal cancer pts (PR: n=7, SD/PD: n=9) showed also a statistically significant benefit in S for pts with PR (med. S for PR: 13.09 mo, SD/PD: 5.89 mo; MC: p<=0.01, B: p<0.03). The phase I study shows that 3 x 5 MU/wk sq is the maximal tolerable dosis for IFN when added to FU/LV as described and that this regimen can be administered feasibly on an outpatient basis. The phase II trials proves FU/LV/IFN to be active in colorectal cancer with a remission rate of 30%, a response rate of 43% and an acceptable toxicity profile. A significant benefit in S for all pts responding to this FU/LV/IFN regimen and in particular for rectal cancer pts with no prior treatment could be revealed in this study. Thus, further phase II studies are necessary for colon cancer pts and phase III studies are warranted for pts with advanced rectal cancer.

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NON-GENITAL TUMORS METASTATIC TO THE OVARY

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We review our experience with 82 patients with non-genital cancers metastatic to the ovary. All patients were referred for evaluation of an ovarian mass. The patients had primary carcinoma of the breast (n=28), colon (n=23), stomach (n=22), pancreas (n=7), or gallbladder (n=2). The overall actuarial 5-year survival rate was 10%. 5-year survival was significantly higher in patients with metastatic colon cancer (23%) than in patients with metastatic cancer of the breast, stomach, gallbladder or pancreas, all of whom died within 58 months ($p < 0.05$). Patients with unilateral metastatic ovarian involvement had significantly better 5-year survival than those with bilateral involvement (28% vs. 5%; $p= 0.003$). 5-year survival was significantly higher in patients with disease limited to the pelvis than in those with abdominal spread (22% vs. 6%; $p< 0.04$). The 5-year survival of patients with residual disease < 2 cm or > 2 cm in diameter was 18% vs. 4%, respectively ($p= 0.002$). This pattern applied mainly to differences in patients with primary cancer of the breast or colon ($p < 0.008$). These data suggest that an aggressive surgical effort seems to be indicated in colon cancer metastatic to the ovary, as some of these patients may survive 5 years.

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MAXIMALE ANDROGEN BLOCKADE PLUS METHOTREXATE FOR TREATMENT OF METASTATIC PROSTATIC CANCER: A RANDOMISED STUDY

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In this randomised prospective study we investigated whether treatment results of maximal androgen blockade (MAB) in patients with metastatic prostatic cancer can be further improved by additional Methotrexate therapy (MTX). A total number of 61 patients (stage T1 or T2) have been included and 31 were randomised to arm A receiving MAB, i.e. orchiectomy + flutamide (3x250 mg/d). In group B 30 patients were treated with MAB + 50 mg/m² MTX (once weekly for 4 months). 53 patients are evaluable for response criteria.

Patients in group B (MAB+MTX) showed a higher response rate (CR+PR), i.e. 42,2 %, in comparison to group A, where remission rate was 29,6 %. Median duration of response was 18,3 months in group B and 17,2 months in group A. Pain relief as judged by pain scale, was more frequently observed in patients receiving no MTX treatment. Hematologic toxicity was present only in patients receiving additional chemotherapy. Six patients (23,1 %) had leukopenia - 5 grade 2, 1 patient grade 4-; 4 patients (15 %) had thrombopenia (grade 1-2) and 2 patients anemia. (grade 1). Incidence of non-hematological toxicity was similar in both treatment arms. In conclusion, patients with metastatic prostatic cancer treated with MAB and additional MTX therapy showed and increased response and improved pain palliation in comparison to those receiving MAB alone. So far, no influence on overall survival has been documented.

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SIMPLE ISH METHOD TO DETECT THE EBV IN THE HUMAN GENOME

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Epstein-Barr virus (EBV) has been strongly linked to different malignancies, including Burkitt's lymphoma, Hodgkin's and non-Hodgkin's lymphoma and nasopharyngeal carcinoma. EBV is an ubiquitous virus, primary infection with, or reactivation of the virus, however, can result in severe disease in immunocompromised individuals. Children with X-linked lymphoproliferative disease (XLP), are especially susceptible to EBV-associated fatal lymphoproliferation and malignant diseases. In order to answer the question if the EBV genome integrates always into the human genome of different types of EBV immortalized cell lines, and if there exist preferential integration sites, we performed non-isotopic *in situ* hybridization *(ISH)* experiments using the *Bam*HI-W fragment on 14 lymphoblastoid-cell lines (LCLs) and one Burkitt lymphoma cell line. The cell lines used for these studies were derived from the lymphoid tissue from a patient with XLP, one patient with infectious mononucleosis and in vitro transformed B cells. The assignement of the *ISH* signals to specific chromosomes and chromosomal bands, respectively, was enabled by a sequential demonstration of the chromosome bands after the *ISH* procedure. The EBV genome was stably integrated into the host genome of all analysed cell lines. We observed a *non-random* integration pattern of the viral genome(s) along the chromosomes which was similar in the different cell lines.

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EX VIVO STUDIES OF ONCOGENES AND P53 TRANSCRIPT LEVELS IN CML AND THEIR RELATION TO IFN-α

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Chronic myelogenous leukemia (CML) represents a unique disease model for studying molecular events which are associated with response to IFN-α therapy and which may enable prediction of sensitvity.
The constitutive and cytokine-modulated expression of certain growth regulatory genes was assessed by Northern blot analyses. Bone marrow and peripheral blood mononuclear cells of 26 Ph^{1+} chronic phase CML patients were examined for the hybrid bcr/abl, c-myc, c-fos and p53 mRNA levels both before and after a single exposure to as also after three months of IFN-α therapy. The relative abundance of the different transcripts was correlated with prognostic disease parameters and response patterns.
Results indicated that (i) constitutive mRNA levels of the hybrid bcr/abl, c-myc and p53 are positively correlated with each other but failed to relate to disease parameters (WBCC, spleen size and proportion of immature cells), (ii) constitutive c-fos transcript levels are significantly higher in patients subsequently responding to IFN-α therapy ($p<0.01$) and are positively correlated with the proportion of lymphocytes ($r=0.673$, $p<0.001$) and negatively with the proportion of immature cells ($r=-0.568$, $p<0.01$) contained in the pbmc preparations tested, (iii) acute and chronic in vivo exposure to IFN-α is accompanied by upregulation of c-fos and down-regulation of c-myc mRNA levels in responder patients, and (iv) unresponsiveness is not caused by impaired targetting or subsequent signalling because the IFN-α induced expression of 2'-5'OAS is as high in non-responder as in responder patients.

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EARLY DETECTION OF RELAPSE BY QUANTITATIVE POLYMERASE-CHAIN REACTION IN PATIENTS WITH CHRONIC MYELOGENOUS LEUKEMIA AFTER BONE MARROW TRANSPLANTATION

T. Lion[1], T. Henn[1], A. Gaiger[2]

In patients with chronic myelogenous leukemia (CML), allogeneic bone marrow transplantation (BMT) is regarded as the only therapeutic approach with curative potential. However, disease recurrence after BMT remains a major clinical problem. Early identification of patients who will eventually relapse is therefore an important challenge in the follow-up of patients after BMT. The detection of residual leukemic cells carrying the bcr/abl rearrangement by highly sensitive techniques such as *qualitative* polymerase chain reaction (PCR) was shown to be of limited value in predicting disease progression. We have adapted the PCR for *quantitative* assessment of bcr/abl rearranged cells and applied the new technique to the monitoring of residual disease in CML patients after BMT. This approach was designed to provide information on the proliferative activity of the residual leukemic cells. Twenty six CML patients were monitored by qualitative and/or quantitative PCR during a follow-up period of up to 7 years after BMT. In the majority of these cases, enzymatic amplification of the bcr/abl rearrangement by PCR turned negative within 4 months posttransplant. In four cases, increasing numbers of leukemic cells were detected by *quantitative* PCR thus indicating the presence of a proliferating clone. So far, three of these patients experienced a relapse during the posttransplant course. The *quantitative* PCR technique may therefore play a prognostic role in the monitoring of residual disease by facilitating early detection of incipient relapse.

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REVERSAL OF MULTI-DRUG RESISTANCE

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Overexpression of the MDR1 gene that codes for a P-glycoprotein is one of the mechanisms involved in resistance to anticancer drugs. This protein exports alkaloids, anthracyclins and podophyllotoxins from cells. It has been shown that verapamil inhibits the efflux of anticancer drugs, but its side effects have prevented treatment of human cancers. The purpose of our investigations was to look for useful alternatives to verapamil. We found that 0.1 µM of B8509-035 (Dexniguldipine-HCl) the R-enantiomer of niguldipine, reverses resistance to adriamycin in multidrug resistant KB-8-5 cells to the sensitivity of the parental KB-3-1 cells. The fluorescent dye rhodamine 123 is transported by the P-glycoprotein. 0.1 µM B8509-035 increases the intracellular accumulation of rhodamine 123 to a greater extent than 1 µM verapamil, as determined by measurement of the fluorescence with a flow cytometer. B8509-035 exhibits antiproliferative effects in some tumors. It is an inhibitor of protein kinase C. Another new anticancer drug and inhibitor of protein kinase C, the thioether phospholipid derivative ilmofosine (BM 41 440) also reverses multidrug resistance at therapeutic doses by blocking the drug efflux as shown by the intracellular accumulation of rhodamine 123. Our results illustrate that there are coumpounds which are very potent in reversing multidrug resistance *in vitro*. Clinical trials with B8509-035 are initiated.

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X-CHROMOSOME METHYLATION PATTERNS - A NEW STRATEGY TO DETERMINE CLONALITY IN HAEMATOLOGIC DISEASES

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Activation or inactivation of the X-chromosome is a random event and is associated with methylation of certain DNA regions. Analysis of the methylation patterns surrounding the X-chromosome locus DXS 255, which contains a hypervariable DNA region, provides a novel method for identification of clonality of haematologic diseases.
Using probe M27ß and the restriction enzyme PstI, 76% - 80% females are heterozygous at the locus DXS 255 (paternal and maternal X-chromosome can be distinguished). Methylation of the two chromosomes at the DXS 255 locus can be studied by parallel digestion of a PstI predigested DNA with the restriction enzymes MspI and its isoschizomer HpaII (MspI cleaves methylated and unmethylated DNA sequences, HpaII can cleave only unmethylated sites.
In an investigation of 60 heterozygous females, all 49 healthy females followed the normal X-chromosome inactivation pattern. Paternal and maternal X-chromosomes were present in active and inactive form at about the same level, which indicates a random inactivation and represents a polyclonal cell population. Of 11 heterozygous females with malignant diseases all showed a disproportional level of inactivation of one X-chromosome, thus indicating a monoclonal or oligoclonal cell population.
In conclusion, analysis of the methylation pattern at locus DXS 255 allows identification of clonal cell growth.

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PARENTAL ORIGIN OF CHROMOSOMES 9 AND 22 INVOLVED IN THE PHILADELPHIA-TRANSLOCATION

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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 praedisposition syndromes and sporadic tumors characterized by loss of genetic material. We were interested whether such imprinting effects might also play a role in the genesis of acquired chromosome abnormalities characterizing specific haematological neoplasms. We have therefore studied the track of inheritance of the translocated chromosomes 9 and 22 in 15 cases of Philadelphia-(Ph) chromosome-positive leukaemia utilizing unique specific chromosome band polymorphisms. We show that the translocated chromosome 9 was of paternal origin in all eleven informative cases. In contrast, the translocated chromosomes 22 were derived exclusively from the maternal copy in eleven cases with reliable polymorphisms. There was no evidence suggesting any involvement of the maternally inerited chromosome 9 or of the paternally inherited chromosome 22 in these cases. Our data therefore provide the first evidence that imprinting phenomena may play an important role in acquired tumor-specific chromosome rearrangements. Moreover, the confined participation of the paternal and maternal genetic components suggests that the bcr and abl genes which are juxtaposed by the reciprocal translocation t(9;22) may be oppositely imprinted.

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Monitoring of minimal residual disease and early detection of relapse in CML patients following non T-cell depleted bmt using the PCR.

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We have performed a two step PCR to detect bcr-abl specific m-RNA in 350 peripheral blood and/or bone marrow samples of 27 CML patients (median 10 samples, range 2 to 48 samples of each patient) following non t-cell-depleted allogeneic (n= 25) and syngeneic (n= 2) bone marrow transplantation (bmt). In 12 of our patients (44%) bcr-abl specific m-RNA could be detected following bmt. In the 2 patients after syngeneic bmt bcr-abl positive cells were detected frequently (UPN 73: observation time 25 months, 6 of 6 samples bcr-abl positive, UPN 131, observation time 23 months, 6 of 18 samples were bcr-abl positive). Both patients are in hematological remission 56 and 26 months after bmt. In 13 patients in whom serial samples were studied after some time following bmt (first sample between 6 and 78 months after bmt) bcr-abl positive cells were detected at least once in 5 patients. In 5 of 12 patients, where samples were analysed before and serially after bmt bcr-abl positivity was found in at least one sample. Bcr-abl positivity was rare in patients, who were in hematological remission for at least 2 years (2/13). Hematological relapse occured in 4 patients, all became bcr-abl positive before relapse (UPN 99: 7 of 14 samples analysed, UPN 120: 5 positive of 7 samples, UPN 123: 6 positive of 7 samples and UPN 133: 7 positive of 14 samples). Bcr-abl positivity in consecutive samples indicated a high risk of relapse (4/4), whereas the risk of relapse was low in patients, who had positive and negative results in consecutive samples (0/6). 5 (33%) of 16 patients with GVHD, but 7 (77%) of 9 patients without GVHD had bcr-abl positive cells at least once. This data indicate:
1) Transient bcr-abl positivity is usually not followed by hematological relapse, while patients, who are positive in serial samples have a higher risk of relapse
2) Syngeneic patients remained bcr-abl positive but did not relapse so far.

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GENOTYPING PATIENTS WITH GLANZMANN'S THROMBASTHENIA FOR HPA-1 TO EVALUATE THEIR PLATELET IMMUNO-MAKE-UP.

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Glanzmann's thrombasthenia (GTA) is a well defined inherited disorder of platelet function. It is caused by a deficiency or abnormality of the membran glycoprotein GP IIb/IIIa complex with bleeding due to defective platelet plug formation. Because of their bleeding disorder patients with GTA may require repeated tranfusions and are therefore at risk for alloimmunization. Of interest is alloimmunization against platelet antigens, in particular the HPA-1 system, which is expressed on GPIIIa. The formation of antibodies would render these patients susceptible to post-transfusion purpura. Because of the diminished amount of platelet GPIIIa, patients with GTA often cannot be typed for HPA-1 serologically, however. These platelets are regarded to be HPA-10. We therefore investigated the possibility of genotyping patients with GTA for their HPA-1 alloantigens.

DNA was extracted from 7 patients with GTA and controls of known HPA-1a/b type. A 23-base and a 24-base oligonucleotide primer pair flanking a region of the gene for GPIIIa containing the allelic polymorphism were used for PCR amplification. The resulting PCR products were digested with *NciI* restriction enzyme and analyzed on a 10% polyacryl-amide gel. In addition, platelet binding of anti-HPA-1 antibodies was tested by platelet immunofluorescence test (PAIFT) and the monoclonal antibody-specific immobilization of platelet antigen test (MAIPA), using various anti-CD41 and anti-CD61 MoAbs.

Genotypically, all 7 patients with GTA were found to be homozygous HPA-1a. Genotyping confirmed the serological results in controls. Platelets from patients with GTA did not react with anti-HPA-1a antisera when using the PAIFT; by MAIPA HPA-1a expression was revealed in 6 patients; results varied however, depending on the applied MoAb. No reactivity was found in the seventh patient. We show that patients with GTA can be genotyped for their HPA-1 alloantigen even when serological tests fail.

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LYMPHOPROLIFERATIVE SYNDROMES AFTER LUNG TRANSPLANTATION

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The incidence of posttransplant lymphoproliferative disease (PTLD) after lung transplantation is about 7%. It is the third leading cause of mortality outside the perioperative period. Although the immunosuppression regimen did not differ from our transplant group at large, PTLD developed in three cases since September 91. The patients varied in presentation and in the course of disease and showed nonuniform response to reduction of immunosuppression. Hence, our patients with PTLD represent a synopsis of diagnostic possibilities, therapeutic approaches and clincal outcomes. 10 months after single lung-transplantation (LTX) patient "No1" showed nodular infiltrates in the transplanted lung. On cytogenetic analysis the presence of clonal aberrations among a majority of diploid mitoses pointed to an early stage of transformation. Lymphoproliferation resolved completely after three months of reduced immunosuppression. Patient "No2" was seen with massive pleural effusions, hilar masses and a diffuse involvement of both lungs soon after a blood type mismatched double LTX. She was diagnosed as monoclonal PTLD. No tumor response could be obtained with radiation therapy. Vascular lesions and high mitotic activity of the tumor, seen in biopsy specimens, revealed transformation to highly malignant NHL. She was sucessfully treated with CHOP in addition to a monoclonal antibody specific for CD24. Jaundice and subsequent liver failure were primary symtoms of PTLD in the third patient "No3" occuring 4 months after single LTX. Reduction of immunosuppression produced no effect on the tumor but caused organ rejection. She had a rapid course and died ten days after diagnosis of monoclonal PTLD. Clonality of PTLD was determined by morphology, immunoperoxidase staining, cytogenetic evaluation and immunglobulin rearrangement analysis of biopsy specimens. In conclusion, our series showed that monoclonal tumors run an agressive and rapid course and did not respond to reduction of immunosuppression. Clonality seems to determine malignancy of PTLD and has presumably prognostic value.

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IFOSFAMIDE, MITOXANTRONE AND ETOPOSIDE AS SALVAGE THERAPY IN NON HODGKIN LYMPHOMAS

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Although several therapeutic approaches for conventional salvage therapie in non Hodgkin lymphoma have been made, successful treatment is still lacking. The combination of etoposide, ifosfamide and mitoxantrone showed a high effectiveness in relapsed and refractory NHL.
During 1986 and 1992, 56 patients (36 males, 20 females) with a median age of 66 years (range 18-89 years) were treated with a combination of etoposide (100 mg total dosage), ifosfamide (1g total dosage) and mitoxantrone ($3mg/m^2$) given on three consecutive days. Mesna was given as uroprotector.
Stages according to the Ann Arbor classification were I/7, II/3, III/6 and IV/40 patients.
33 patients suffered from high grade, 23 from intermediate grade non Hodgkin lymphoma.
Toxicity according to the WHO recommendation was as follows: Anemia grade I was observed in 10 patients. Leukopenia grade I/2 patients, grade II/1 patient and grade IV/4 patients. Thrombocytopenia was not observed.
Overall response was 32% (9 CR and 9 PR). High grade NHL showed a better response rate (18/33 patients) compared to the intermediate grade NHL's (7/23 patients). Bulky disease was a significant adverse prognostic factor, response was observed in 3/15 cases. It seems noteworthy that 15/31 cases refractory to previous treatment responded.
The combination of ifosfamide, mitoxantrone and etoposide is active in pretreated refractory and relapsed patients with non Hodgkin's lymphoma and has tolerable toxicity.

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TREATMENT OF HIGH-GRADE NON-HODGKIN'S LYMPHOMA: COMPARISON OF THREE HIGH-DOSE CHEMOTHERAPY PROTOCOLS (M-BACOD; CODBLAM IV; PROMACE-CYTABOM)

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A total of 53 pts. with high-grade NHL at various stages (stage I: 4%; stage II: 28%; stage III: 25%; stage IV: 43%) were treated with three different high-dose chemotherapy regimens (M-BACOD: 18 pts.; CODBLAM IV: 14 pts.; PROMACE-CYTABOM: 21 pts.). Overall 72% reached a complete remission (CR), with a continuous complete remission of 64%. The overall survival was 69% at 12 months and 41% at 48 months. A comparison between the three therapeutic regimens revealed comparable remission rates with CODBLAM IV being the most effective induction treatment (CR: M-BACOD: 72%; CODBLAM IV: 78%; PROMACE-CYTABOM 66%). The highest, though non-significant, continuous complete remission rate (CCR) at 12 months was found in the PROMACE-CYTABOM group (CCR: M-BACOD: 62%; CODBLAM IV: 59%; PROMACE-CYTABOM: 71%). Despite the fact that this was not a randomized study, our data indicate that all three protocols are highly effective as first line treatment of high-grade NHL. However, the long term survival of these patients is still below 50 % after 4 years and needs to be improved by a more aggressive post induction management such as bone marrow transplation.

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HUMAN LYMPHOBLASTOID INTERFERON, WELLFERON (WFN), IN HAIRY CELL LEUKAEMIA (HCL) - THE UK EXPERIENCE

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Pooled results from the 2 UK HCL studies in which patients received 3 MU WFN daily or thrice weekly demonstrated a major response rate of 77 %:

Response	1st Study	2nd Study	Pooled Data	
CR	8	12	20	(16 %)
PR	29	17	46	(36 %)
PRH*	5	27	32	(25 %)
MR	8	12	20	(16 %)
NR		3	3	(2 %)
IT+		7	7	(5 %)
	50	78	128	

*PR (Haematological) + Inadequate treatment (side effects)
In both studies response appeared to correlate with median cumulative dose (>450MU for patients achieving CR or PR).
30 patients from the 1st study were followed off treatment: the majority relapsed within one year. Relapse was relatively delayed in those achieving a major antileukaemic effect (<5 % bone marrow HCs).
Following maximal response 18 patients in the 2nd study went on to 3 MU weekly maintenance, and 37 observation off treatment, 7 (39%) of 18 on maintenance showed haematological relapse [5 (28%) only minimal], whilst 32 (86%) of 37 on observation relapsed [2 (5%) minimally], a highly significant difference (Chi^2 11.09, p-0.0009). (These differences held good throughout all response categories.)
75 patients' sera were screened for neutralising antibodies: all were negative. No patients developed refractory disease whilst on treatment.

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R-metHuG-CSF COMBINED WITH CHEMOTHERAPY FOR TREATMENT OF ADULT ALL - A PILOT STUDY

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14 consecutive patients with ALL (12 de novo, 2 relapsed)- median age 42 years (18 to 69), 10 c-ALL (2 bcr-abl positive), 2 pre-B ALL (1 bcr-abl positive), 1 early B-precursor ALL and one T-ALL were treated according to the chemotherapy protocol described by Hoelzer et al. In addition r-metHuG-CSF (Neupogen R) was given at a dose of 200 ug/m2 iv. in phase 1 from day 2 to day 21, and thereafter until neutrophil counts were >1000/ul on two consecutive days. In phase 2, G-CSF 200 ug/m2 sc. was started on day 2 (after CP) and continued until neutrophils recovered to more than 1000/ul on two consecutive days. These patients were compared to a historical control group treated with the same chemotherapy protocol but without G-CSF. It included only patients, who achieved CR within 4 weeks (median age 27 years, 22 c-ALL, 3 pre-B ALL, 3 early B-precusor ALL and 10 T-ALL). - 13/14 patients achieved complete hematologic remission (10 within 4 weeks and three at the end of induction treatment). One patient died because of fungal septicemia. G-CSF treated patients had a shorter duration of granulocytopenia (14.5 days vs 21.5 days) in phase 1. In phase 2 the median granulocyte count during weeks 3 and 4 was significantly higher compared to the historical control group. The number of days with fever in phase 2 was lower in the G-CSF group (1,5 days vs 0,8 days). The full dose of chemotherapy (anthracyclins, cyclophosphamide, ARA-C and purinethol) could be given in 11/13 G-CSF treated patients, while in the control group only 23 % of the patients received the protocol without dose reduction or delay. These data indicate, that, (1) G-CSF can be given along with chemotherapy in induction treatment without compromising efficacy, (2) the time of granulocytopenia in phase 1 is shortened by 7 days and the degree of granulocytopenia is amelioreated in phase 2 and (3) dose intensity (dose per time) could be increased.

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MYELODYSPLASTIC SYNDROMES: ANALYSIS OF CLINICAL AND PROGNOSTIC FEATURES IN 19 PATIENTS

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Myelodysplastic syndromes /MDS/ are clonal disorders characterized by ineffective hematopoiesis, peripheral blood cytopenias, normo- or hypercellular bone marrow and maturation abnormalities, frequently in all the three haematopoietic cell lines. 12-38% incidence of transformation to ANLL is noted. The prognosis is generally poor, the median survival is 7.5- 29 months.

A retrospective analysis of 19 consecutive patients / period: from May 1985 through May 1992, mean age: 67.1±12.2 year, sex: 11 men and 8 women/ were performed. Patients were classified according to the FAB proposals: refractory anaemia /RA/ in 10, refractory anaemia with ring sideroblasts /RA-S/ in 1, chronic myelomonocytic leukaemia /CMML/ in 1, refractory anaemia with excess of blasts /RAEB/ in 3, RAEB in transformation /RAEB-t/ in 3 cases. In 1 patient the diagnosis was ANLL following MDS. In 36% of all cases /7 pts./ leukaemic progression could be observed. From the large number of therapeutic modalities which have been tried in MDS 84% of our patients were treated with RBC transfusion, 31% with platelet transfusion, 37% with low-dose ara-C, 26% with steroid, 26% have got oral iron and vitamin supplementation. 9 patients /47%/ died during the observation time, the median survival was 14.5±18 months.

Conclusions: 1) There was an increase in the number of patients having MDS in the last years. 2) A high number of transformation to ANLL /36%/ could be observed in our material and 71% of the patients with transformation previously belonged to the RAEB and RAEB-t groups /high risk patients/. 3) The median survival of the patients was similar to the data of the literature. 4) It is necessary to introduce the latest therapeutic modalities /bone marrow transplantation, differentiation induction, human-recombinant GM-CSF/ to improve the results.

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IN VITRO CHEMOSENSITIVITY TESTING IN ACUTE LEUKAEMIA BASED ON MEASUREMENT OF INHIBITION OF NUCLEIC ACID PRECURSOR INCORPORATION (NPI)

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Multiple in vitro test systems have been studied which may indicate sensitivity or resistance to cytostatic agents in vivo. We have evaluated the applicability of the measurement of drug-induced inhibition of NPI into cellular RNA/DNA to test for chemosensitivity in acute leukaemia. The reliability of recently established criteria for the evaluation of in vitro results was determined.

69 blast cell samples from 61 patients with acute leukaemia were tested with a panel of 4 to 6 drugs prior to initiation of chemotherapy. In 40 cases the assay revealed sensitivity to at least one drug used for therapy in vivo. In this group, complete or partial remissions were achieved in 23 and 8 patients respectively (positive predictive value 89%; "early deaths" excluded). From 24 patients with resistance to all drugs 16 failed to respond to therapy (negative predictive value 76%). Five patients were classified as "possibly sensitive". Using actuarial survival analysis, patients with in vitro sensitivity showed a significantly better survival compared to patients with in vitro resistance ($p<0,005$ up to 10 month; chi-square). Mean survival time of patients with in vitro sensitivity and resistance amounted to 367±341 and 127±203 days, respectively ($p<0,003$; Kruskal-Wallis). Our data demonstrate the prognostic relevance of the NPI-inhibition assay in acute leukaemia and seem to permit the prospective use of this assay to design individual therapeutic regimens.

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SECONDARY AML WITH TRISOMY 11 IN CHILDREN

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With the improved prognosis of patients treated for solid tumors and hematologic malignancies, the clinical importance of treatment-associated secondary leukemias has increased. We therefore present two children, a 13 year old boy and a 5 year old girl, with a secondary AML and trisomy 11. The boy developed an AML-M4 60 months after diagnosis of a synoviosarcoma of the left shoulder. The girl developed AML-M2 40 months after diagnosis of an anaplastic ependymoma. The tumors were resected and treated with respective polychemotherapy and radiation regimens (CWS81 and SIOP II medulloblastoma trial) . Both children were treated with multiagent chemotherapy for AML. However, the girl died due to septicemia 4 weeks after diagnosis and the boy due to a relapse 40 months after diagnosis of AML. With approximately 50 cases reported so far, trisomy 11 remains a rare nonrandom karyotype abnormality. It has mainly been described in elderly patients with various myeloid malignancies and occurs commonly following treatment of malignant diseases and/or carcinogenic exposure. To date, our patients are the youngest in whom trisomy 11 has been reported. Within the 420 cases of childhood AML which we have successfully studied, these two patients are the only cases displaying this abnormality. In order to determine the clinical and biological significance of our observation, the acrual of additional cases with this rare abnormality is necessary.

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IL-4 INHIBITS STEM CELL FACTOR (SCF) DEPENDENT DIFFERENTIATION OF HUMAN MAST CELLS AND DOWNREGULATES EXPRESSION OF SCF-R/c-kit.

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Recent data suggest that human mast cells (MCs) express receptors (R) for interleukin-4 (IL-4) and stem cell factor (SCF-R / c-kit). In this study the effects of recombinant human (rh)SCF and rhIL-4 on growth and differentiation of human MCs was analyzed in long term suspension cultures (pb, normal donors, n=11). SCF was found to induce formation of MCs as well as tryptase (a mast cell specific enzyme) in a dose dependent way with optimal concentrations of SCF being 100 and 1000 ng/ml (tryptase values [ng/ml] on day 35: rhSCF, 100 ng/ml: 1308±679, vs control: 18±6; MCs on day 35: 12.6±6.4 vs. control: 0.0 x 10^3/ml). IL-4 per se failed to induce differentiation of MCs but was found to inhibit SCF dependent formation of human MCs in long term culture (tryptase on day 35: control: 10.6±0.1 vs. SCF: 2289.6±38.9 vs SCF+IL-4: 347.2±23.3 ng/ml). We next examined the effect of rhIL-4 on expression of SCF R (c-kit) in the human mast cell line HMC-1 by indirect immunofluorescence using anti-c-kit mAb YB5.B8 and Northern blot analyses using c-kit oligo-probe. IL-4 was found to downregulate YB5.B8 Ag (51.05±16.36 $p<0.02$) as well as expression of c-kit mRNA in HMC-1 cells. The effect of IL-4 on c-kit gene product expression was found to be dose- and time dependent and could be neutralized by anti-IL-4 mAb. Together, these data provide evidence that rhIL-4 inhibits SCF dependent formation of human MCs in vitro, probably via regulation of expression of SCF binding sites.

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CSA AND FK-506 INHIBIT SCF- / KL (kit-LIGAND) INDUCED ACTIVATION OF HUMAN TISSUE MAST CELLS

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Recent data suggest, that SCF (stem cell factor) / KL is a major regulator of human mast cells (MCs) and upregulates MCs releasability. The aims of this study were to analyze the effects of cyclosporin-A (CSA) and FK-506, two potent anti-inflammatory drugs, on SCF induced activation of human MCs. For this purpose we isolated mast cells from uterine (n=6) and lung tissue (n=4) and carried out histamine release experiments. CSA and FK-506 were found to downregulate MC histamine release induced by anti-IgE or recombinant human (rh) SCF as well as SCF dependent releasablity in a dose dependent way. Complete inhibition was observed with > 0.3 ug/ml of CSA. In lower concentrations, CSA was found to be a deactivator of unstimulated MCs, whereas rhSCF (10-100 ng/ml) was found to counteract CSA induced deactivation of MCs. CSH (cyclosporin-H), a CSA derivative with low affinity for cyclophilins (CSA R) did not alter mast cell histamine releas(ability)e induced by anti IgE or rhSCF. No effects of CSA or FK-506 on expression of SCF R (c-kit receptor) or SCF dependent formation of cAMP in human mast cells could be observed. Together our data suggest that CSA and FK-506 interfere with SCF induced activation of human mast cells presumably by interacting with cyclophilins.

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INTERCELLULAR ADHESION MOLECULE-1 (ICAM-1) IN THE PROLIFERATIVE CONTROL OF HAEMATOPOIETIC PROGENITOR CELLS

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A monoclonal antibody termed 7F7, which recognizes the intercellular adhesion molecule-1 (ICAM -1) , has been used in our experiments to modulate the influence of autologous T-lymphocytes on committed haematopoietic progenitor cells. Using a microagar culture system for human haematopoietic progenitor cells and for T-lymphocyte colony formation we demonstrate that BFU-E, CFU-Meg, CFU-GM derived colony formation was not directly affected by 7F7 antibody treatment . PHA-induced T-lymphocytic colony formation, however, was markedly suppressed by 7F7 mAb. In coculture studies between monocyte and T-lymphocyte depleted bone marrow and peripheral blood MNC and autologous T-lymphocytes preincubated with the 7F7 mAb we were able to demonstrate that ICAM-1 is involved in T-lymphocyte mediated modulation of BFU-E, CFU-Meg and CFU-GM proliferation . To further clarify the question if this effect on progenitor cell proliferation is mediated by cell adhesion blocking between T-lymphocytes and progenitor cells or by direct Ab-mediated effects on T-lymphocytes and monocytes, the 7F7 mAb was used to investigate the role of ICAM-1 in cytokine production by T-lymphocytes and monocytes. Production of TNF-alpha, IFN-gamma and IL-1 was significantly inhibited ($p<0.01$) by the incubation of mAb 7F7 with PHA activated blood MNC or isolated E rosette positive T-lymphocytes. The maximal level of inhibition was reached using saturating concentrations of 400 µl/ml of mAb 7F7 hybridoma supernatant corresponding to an inhibitory activity of 1µg of purified mAb. In contrast, GM-CSF release showed a heterogenous response over 5 experiments with an increase found in 3 experiments and a decrease in 2 experiments. Addition of increasing concentrations of supernatant or purified mAb to unstimulated MNC or T- lymphocyte cultures had no effect on cytokine release .
Our studies show that ICAM structures are involved in the T-cell mediated modulation of normal haematopoietic progenitor cells and that ICAM-1 exerts its regulatory effect via the lymphokine cascade.

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IMMUNOGLOBULINS ALTER IMMUNE CELL ACTIVATION BY SELECTIVE CYTOKINE MODULATION

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Passive immunotherapy with intravenous immunoglobulin (IVIG) has been successfully employed in primary and secondary antibody deficiency syndromes. In more recent years IVIG has been shown to also be of benefit in the treatment of various autoimmune diseases including immune thrombocytopenic purpura (ITP) and Kawasaki syndrome. In order to better elucidate the mechanisms involved in the immunomodulatory activity of IVIG, we investigated the influence of Gamimune (7S-IgG; Cutter), Gamma-Venin (5S-F(ab')$_2$; Behring) and heat-stabilized Fc fragments (Behring) on in vitro proliferative and cytotoxic immune cell response, focusing primarily on the action of IVIG on cytokine release.
Intact immunoglobulins (Gamimune, 1-10 mg/ml) reduced alloantigen-induced proliferation of peripheral blood mononuclear cells (PBMC) by more than 60% in a dose-dependent manner, whereas F(ab')$_2$ fragments and Fc fragments suppressed mixed lymphocyte reaction (MLR) only at the highest dose level tested (10 mg/ml). In a similar fashion, IVIG suppressed lectin-induced proliferation of PBMC, interferon-induced MHC antigen expression on a colon carcinoma cell line and interferon-induced macrophage activation. IVIG preparations containing the Fc fragment of the immunoglobulin molecule also inhibited the cytoloytic activity of NK cells. Immunosuppressive activity of IVIG was mediated by selective cytokine modulation. Secretion of T cell-derived cytokines such as interleukin 2 (IL-2) and granulocyt-macrophage colony stimulating factor (GM-CSF) was significantly inhibited in the presence of IVIG, whereas monocyte/macrophage-derived tumor necrosis factor a was induced. Release of IL-1, IL-6 and interferon-g during MLR was not significantly affected by IVIG.
We conclude that immunoglobulins have potent immunomodulatory properties mediated in part by selective inhibition/stimulation of endogenous cytokine production.

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EXACERBATION OF AUTOIMMUNITY DURING THERAPY WITH RECOMBINANT INTERFERON ALPHA FOR HEMATOLOGICAL DISORDERS.

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Recombinant interferon alpha (rIFN alpha) is of increasing clinical importance. In particular, it has been shown to be effective in the treatment of neoplastic hematological diseases and viral hepatitis. In the early phase of this treatment, the most prominent side effects are influenca-like symptoms, which can be attributed to an immunomodulatory effect. Although long-term tolerance of rIFN alpha is usually good, there have been some reports on the occurrence of autoimmunity associated with its long-term application. We therefore examined 65 patients (16 male, 49 female, age: 18-89 years) who had been treated for hematological disorders, for the occurrence of autoimmunity during treatment with rIFN alpha. Their diagnosis were: myeloproliferative disorders (57 pts), myelodysplastic disorders (7 pts) and hypereosinophilic syndrome (1 pt). The treatment-duration ranged from 1.5 to 48 months, the rIFN alpha dosage ranged from 6 to 25 megaU/week. Evaluation was carried out with regard to signs and symptoms of autoimmune disease or development of autoantibodies to thyroid and nuclear antigens. In 17 patients, a thyroid autoimmunity was observed, 3 of these showed signs of hypothyroidism concurrently with the thyroid autoimmunity, and one patient became hyperthyreot. Some of the patients who developed autoimmunity during IFN alpha were examined using a highly sensitive assay for thyroid antibodies (TPO and TgAb), which indicated autoimmunity at the beginning of rIFN alpha treatment. Furthermore, in two patients with psoriasis, and 1 patient with Colitis ulcerosa, rIFN alpha had to be withdrawn due to exacerbation of these diseases. These data suggest that patients with serologically dectable autoimmunity or a history of autoimmunity may develop an exacerbation of autoimmune disease during the treatment with rIFN alpha.

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NORMALIZATION OF COLONY FORMATION IN HAIRY CELL LEUKAEMIA (HCL) BY THE REMOVAL OF HAIRY CELLS, T CELLS AND THE ADDITION OF COLONY STIMULATING FACTORS

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Pancytopenia is one of the most characteristic findings in HCL. Inhibitory factors released by hairy cells might be responsible for haematopoietic failure in this disease. It has been suggested, however, that HCs alone are incapable of synthesizing potent inhibitors of myelopoiesis, and that they rather act synergistically with T lymphocytes. Therefore, we investigated the effect of the removal of HCs and/or T cells on the number of circulating progenitor cells in 6 HCL patients. The results demonstrate that the removal of either HCs (by complement mediated lysis) or T cells (by E-rosette formation) clearly improves the growth of BFU-E, CFU-GM and CFU-mix. In comparison, under the same experimental conditions, these effects could not be observed in healthy donors.
Since none of the procedures was sufficient to increase colony numbers to normal levels we determined whether or not the supplementation of the culture medium with haematopoietic growth factors (rh GM-CSF, rh IL-3) could further increase colony numbers. When the colony forming assays were performed after the removal of HCs, and upon the addition of GM-CSF/IL-3, normal colony numbers were achieved in most patients. Similar increases were observed after the depletion of T cells, and addition of growth factors.
We conclude that in HCL an inhibitory effect on haematopoiesis is exerted by HCs, but that T lymphocytes also play role in the mechanism of suppression, probably by synergizing with HCs. In addition, we postulate that a deficiency of haematopoietic growth factors contributes to the failure of the haematopoietic system. A likely candidate for an insufficient supply of growth factors is the monocytopenia usually observed in HCL.
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THE ROLE OF TNFα FOR PURGING CML PROGENITORS WITH ETHERLIPIDS IN VITRO

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The Alkyl lysophospholipid ET 18-OCH3 (ALP) is supposed to selectively inactivate leucemic cells and to activate the monocyte macraphage system. We tested the purging effect of ALP for CML by short time incubation of CML bone marrow with ALP 50ug/ml for 30 min.
ALP reduced colony growth of CML but did not affect normal progenitors. TNFa pruduction was increased in both normal and CML bone marrow after ALP treatment. as a possible mediator of ALP activity. TNFa was measured in the supernatant of 24 hour cuktures. Addition of a monaclonal TNFa antibody supressed the cytotoxic effect of ALP on CML progenitors. It had no effect on normal progenitors. ALP treated CML cells were kept in long term bone marrow culture for four weeks and assayed for clonogenicity. During the first week of culture there was additional supression of CFU-C growth but after four weeks of culture they showed about the same CFU-C recovery as the untreated control. Single colonies of ALP treated CML bone marrow were still PH positive.

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IMMUNE RESPONSE MODULATION BY PENTOXIFYLLINE IN VITRO

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Pentoxifylline (PTX) has recently been shown to modulate TNF-α production and to reduce the incidence and severity of all major complications after bone marrow transplantation, including mucositis, veno-occlusive disease, renal insufficiency, hypertension and graft-versus-host disease. In order to analyze in detail the effect of PTX on immune complications after BMT we investigated the immunomodulatory effect of PTX on immune response in vitro.
Continuous presence of PTX significantly reduced the proliferative response of peripheral blood mononuclear cells to stimulation with phytohemagglutinin and alloantigens (MLR) in a dose-dependent manner. In MLR a significant suppression (13±7%) was already achieved with 10μg/ml PTX. The concentration of 100μg/ml PTX was able to inhibit and at 1000μg/ml almost block both reactions. The inhibitory capacity of PTX in the MLR was increased by monoclonal antibodies against TNF-α (34±5% additional suppression at 100μg/ml PTX) and not reversed by the addition of rTNF-α. PTX 100μg/ml also significantly inhibited (p=0.0178) the in vitro generation of cytotoxic T-lymphocytes, when PTX was added to the culture on day 0. Profound modulatory properties can also be seen in the NK assay with a reduction of 23±3% in specific lysis at 10μg/ml and maximal reductions of 88±3% at 1000μg/ml PTX. Immunomodulatory properties of PTX were not only associated with blockage of TNF-α, but also with an impaired production of IFN-γ and neopterin. PTX treatment, however, did not affect IL-2, IFN-α, β-2 microglobulin or IL-1β production, IL-6 release was even increased.
PTX has therefore profound immunomodulatory properties in vitro, which are associated with selective inhition of cytokines release and can be enhanced by the addition of monoclonal antibodies against TNF-α but not reversed by the addition of rTNF-α.

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COEXPRESSION OF T AND B CELL ASSOCIATED ANTIGENS IN NORMAL AND MALIGNANT LYMPHOID CELLS.

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We have shown previously that CD7 and CD19 may be coexpressed on lymphoid cells in fetal bone marrow (FBM). By transformation of CD7+ and CD7+CD19+ cells from FBM with the Epstein-Barr-Virus three cell lines were established. All three cell lines are CD7+CD19+ and express the B cell differentiation antigens CD20, CD21, CD22, CD40 and are HLA-DR positive. Cell line A10 expresses also surface immunoglobulin (Ig) with kappa light chains. Cell line NC3 differs from A10 because it lacks surface Ig, but has cytoplasmic mu. The third cell line (OC3) expresses, in addition to CD7, antigens of mature thymocytes (CD2+, CD3+, T-cell-receptor (TCR) ab+). These cells also express the cortical thymocyte antigen CD1, the stem cell antigen CD34, and the early lymphoid associated CD10. All cell lines are negative for the pan-myeloid antigen CD13 and the early myeloid antigen CD33. All three cell lines have the Ig heavy chain genes rearranged. A10 and NC3 have the TCR genes in germline configuration, while OC3 has TCRb and d genes rearranged.

Thus, we have shown that i) CD7 and CD19 may be coexpressed on very immature cells carriing the stem cell associated antigen CD34, ii) CD7 is coexpressed on B cells, which are cytoplasmic or surface Ig positive, and iii) CD19 and other B cell associated antigens are coexpressed on T-cells. Results of immunophenotyping and cytogenetic studies and rearrangement patterns of TCR and Ig genes from acute leukemias coexpressing CD7 and CD19 are reported and discussed in the context of their normal counterpart.

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DOSE DEPENDENT GROWTH-INHIBITORY EFFECT OF 2-CHLORO-2'-DEOXYADENOSINE (CDA) ON MYELOID PROGENITORS IN NORMAL HUMAN LONG-TERM BONE MARROW CULTURES (LTBMCs)

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2-chloro-2'-deoxyadenosine (CdA) is a new promising substance especially developed for the treatment of lymphoid malignancies. As neutropenia and bone marrow suppression has been observed during CdA treatment, we performed Dexter-type human LTBMCs to investigate the myelosuppressive effect of CdA. 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, where we did not find an additional cell loss compared to untreated controls, numbers of CFU-GM and BFU-E were 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 (8 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 reduced progenitor cell growth might be a result of a possibly reduced secretion of IL6 or GM-CSF from the adherent layer. Therefore, concentrations of IL6 and GM-CSF were measured in the supernatant 3, 6, 12, 24, 48, 72 and 96 hours after 1/2 MC. The results show, that the levels of cytokines investigated were similar in normal and CdA-treated cultures. Therefore it can be suggested, that the dose dependent myelosuppressive effect of CdA is mediated by a direct action on progenitor cells and not by a functionally defective stromal layer.

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IMMUNE ACTIVATION AND ANAEMIA IN HIV INFECTION

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HIV and HIV-proteins have some direct effect on the haematopoietic system. However, the pathogenesis of HIV associated anaemia is still far from being understood. Recent data suggest a role of immune activation phenomena. We performed a cross-sectional study comparing blood cell counts, haemoglobin (hb) and erythropoietin levels of 63 HIV seropositive individuals with immune activation markers (IFN-γ, serum and urine neopterin, ß2-microglobulin) and with parameters of iron metabolism (serum iron, transferrin, ferritin). Compared to HIV-serogenative controls, neopterin, IFN-γ, ß2-microglobulin, as well as erythropoietin and ferritin were increased in the majority of patients, whereas transferrin was decreased. In addition, we found significant inverse correlations of hb with neopterin, IFN-γ and ß2-microglobulin levels. Significant correlations existed also between erythropoietin, the parameters of iron metabolism and hb. Finally, ferritin correlated inversely with transferrin. Thus, in patients with HIV infection low hb levels are associated with enhanced cellular immune activation and with changes of iron metabolism: lower hb is associated with lower transferrin and higher ferritin levels. The data suggest that endogenously released cytokines like IFN-γ, which inhibit erythropoiesis, may be one cause of anaemia in HIV infection.

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DOES RACEMIC FOLINIC ACID (FA) OR ITS L-STEREOISOMER (L-FA) ALTER THE PHARMACOKINETICS OF FLUOROURACIL (FU)?

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Interferon alfa significantly alters FU-PK by enhancing AUC of FU, addition of FA was shown to lower this effect (ECCO 6, 482). The question is open whether FA itself may have any influence on the kinetic behaviour of FU. Therefore in 12 patients with advanced colorectal carcinoma we tested two common dosages of FA and additionally L-FA with regard to their potential effects on FU-PK. FU schedule consisted of 370 mg/m² iv bolus daily x 5q 4 wks, in 3 consecutive monthly cycles FA was givren at 200 mg/m² (HD), 20 mg/m² (LD) or L-FA 100 mg/m² bolus respectively immediately before FU. Fu plasma levels were determined by HPLC over 60min on d_1 as baseline (B), analysis repeated on d_5 of each cycle.

Results of FU-PK parameters:

	B	HD-FA	LD-FA	L-FA
c0	27,6	23,7	25,5	25,1
AUC_{0-60}	383	399	426	387
$t_{1/2}$	9,7	11,7	11,6	10,7
Vd	23,8	27,5	25,6	26
Cl_{tot}	1,7	1,6	1,5	1,7

Conclusion: no significant change of FU-PK parameters occured at any schedule, therefore an influence of bolus FA or L-FA on FU kinetics can be excluded, suggesting an intrinsic action of Interferon alfa at this topic.

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KARYOTYPE AND PROGNOSIS IN NON-HODGKIN LYMPHOMAS

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In a prospective study we analyzed karyotypes of lymph-node biopsies from 100 patients under suspicion of malignant lymphoma. 23 cases were subsequently not confirmed pathohistologically. 12 were CLL, 9 patients suffered from Morbus Hodgkin. 42 (75%) of the remaining 56 biopsies from different non-Hodgkin lymphomas could be successfully karyotyped. The obtained data confirm the hypothesis that regardless to histologic diagnosis in non-Hodgkin lymphomas aberrations leading to loss of material from the long arm of chromosome 7 or the short arm of chromosome 17 are associated with poor prognosis. Affected patients show high tumor burden, elevated levels of serum lactate dehydrogenase (LDH 656 + 167 U/l) as well as poor response to therapy and short survival periods. It seems noteworthy that the same patients often showed additionally aberrations on chromosome 3. 14 of 24 follicular lymphomas, 2 T-cell lymphomas, 2 high grade precusor B-cell lymphomas showed clones with t(14;18)(q32;q21). Additionally we found in the two patients with high grade precusor B-cell lymphoma a specific Burkitt translocation. 6 biopsies from follicular lymphomas did not yield any mitoses. Del(18)(q21), $14q^{+}$ t(14;15)(q32;q23), inv(14)(q11q32) were observed in one case each. High karyotypic complexity was correlated with shortened survival in lymphomas bearing t(14;18).

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TREATMENT OF THROMBOTIC MICROANGIOPATHY (TMA) WITH EXCHANGE PLASMAPHERESIS, METHYLPREDNISOLONE AND VINCRISTINE AND ITS EFFECT ON VON WILLEBRAND FACTOR (vWF) MULTIMER AND SUBUNIT COMPOSITION

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TMA, a term that encompass thrombotic thrombocytopenic purpura and the hemolytic uremic syndrome, is a rare disorder of unknown cause with a high mortality rate. TMA is characterized by hemolytic anemia, thrombocytopenia, neurologic symptoms, fever and renal dysfunction. In two TMA patients vWF multimers and subunits were investigated at the time of diagnosis, following successful treatment and in one patient during relapse. A lack of the large vWF multimers was seen before treatment and during relapse whereas unusually large vWF multimers were demonstrable following successful therapy. Thus, unusually large vWF multimers may have a crucial role in the pathomechanism of TMA by inducing platelet aggregation and deposition of platelet aggregates in the microcirculation. In both TMA patients proteolysis of the vWF subunits was observed at the time of diagnosis but no longer after therapy. It is not clear whether vWF proteolysis is a primary event or occurs secondary due to endothelial cell injury.
5 patients with TMA (associated with bone marrow transplantation (BMT) in 2 and pregnancy in 1) were treated with exchange plasmapheresis 60 ml/kg/d, methylprednisolone 0.75 mg/kg i.v. 2x daily and vincristine 2 mg i.v. day 1, 1 mg days 4, 7, and 10. Treatment was successful in all patients with a normalization of all blood parameters except in 2 patients in whom persistent thrombocytopenia was due to recent BMT rather than TMA. In 1 patient 2 relapses were successfully controlled with the above regimen. We conclude that exchange plasmapheresis and administration of methylprednisolone and vincristine are effective in TMA.

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OCULAR LATE EFFECTS OF MULTIMODAL THERAPY IN ALL AND NHL IN CHILDREN

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We retrosepctively analyzed the ocular sequelae of polychemotherapy including intrathecal methotrexate, systemic corticosteroids and prophylactic cranial irradiation in children with acute lymophoblastic leukemia (n=16) and Non-Hodgkin's-lymphoma (n=2). After a median surveillance time of 4.1 years therapy induced changes of the eyes, that did not cause any symptoms were observed in 83 % of the patients: 7/18 (39 %) had a decreased tear formation, 5/17 (29 %) hat an opacity of the vitreous body and 13(18 (72 %) had an opacity of the lens. It was not possible,to determine retrospectively, which therapy caused a particular effect. Whereas corticoid therapy might have contributed to the lens opacities the reduced tear secretion is most likely radiation-induced.

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SUCCESSFUL TREATMENT OF SEVERE HYPOPROTHROMBINEMIA IN A PATIENT WITH SYSTEMIC LUPUS ERYTHEMATOSUS AND A LUPUS ANTICOAGULANT

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In this report the successful treatment of severe factor II deficiency in a seventeen year old male patient with systemic lupus erythematodes (SLE) and a lupus anticoagulant (LAC) is described.
The patient presented with fever, malaise and joint pain, and had neither a history of bleeding nor of thromboembolic events. On admission the aPTT was 142 seconds and factor II clotting activity and antigen were lower than 2%. Anticardiolipin antibodies (ACA) were considerably elevated. Other clotting factors were within the normal range. During the course of therapy with prednisolon and azathioprin an improvement of the patients clinical condition and normalization of the aPTT, an increase of factor II and a decrease of ACA (IgG and IgM) were observed.
After two years of treatment with prednisolon and cyclophosphamide the aPTT was slightly prolonged (50 seconds) whereas factor II activity and antigen and ACA had completely normalized.
This case shows an excellent response of lupus dependent coagulation abnormalities to immunosuppressive treatment.

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ALL TRANS RETINOIC ACID THERAPY - TREATMENT RESULTS IN 10 PATIENTS WITH ACUTE PROMYELOCYTIC LEUKEMIA

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Acute promyelocytic leukemia (APL), a distinct subtype of AML, is frequently associated with disseminated coagulopathy (DIC) causing a high incidence of fatal bleeding complications. APL is cytogenetically characterized by the translocation t(15;17) involving the region of the retinoic receptor α gene. In vitro all trans retinoic acid (ATRA) exhibits a differentiation-inducing and antiproliferative capacity on APL-cell-lines. Similar effects were observed when ATRA was administered to APL-patients, who achieved complete remission without any cytostatic therapy. We have treated 10 patients with APL (5 males and 5 females, median age: 36 years, range 22 - 60 years). The cytogenetic translocation t(15;17) was observed in all patients, 3 patients had additional karyotypic abnormalities. ATRA was administered as capsules (45mg/m^2 divided in two daily doses) for 90 - 106 days. 4 primarily untreated patients, 1 patient in 1st relapse after autologous transplantation and one patient with a chemotherapy-resistant relapse received ATRA without any cytostatic treatment. Two patients were treated with a combination of ATRA and "3+7" induction (60 mg/m^2 Daunorubicin d1-3, 100 mg/m^2 Ara-C d1-7) and two patients received additional chemotherapy (4 x 3g/m^2 Ara-C) due to excessive hyperleukocytosis developing during ATRA-treatment. Clinical and laboratory signs of DIC (fibrinogen level, fibrinogen degradation products) improved rapidly in all patients. Complete hematological, immunological and cytogenetic remission was obtained in all patients within 4 to 12 weeks therapy. Hyperleukocytosis developed in 3 patients and was treated in 2/3 with 4 x 3g/m^2 Ara-C. Other ATRA side effects (dryness of skin, alopecia, bone pain) were mild and improved during continuing ATRA-treatment. 7/10 patients are still in remission for 1+ to 8+ months after starting treatment. 3 patients receiving ATRA as salvage therapy after treatment failure relapsed after 2 - 7 months in complete remission. Our results indicate that ATRA therapy for APL is safe and highly effective.

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ATTENUATION OF THE HEMOSTATIC DISORDER IN ACUTE PROMYELOCYTIC LEUKEMIA BY TREATMENT WITH ALL-TRANS RETINOIC ACID.

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Acute promyelocytic leukemia (APL) is associated with disseminated intravascuar coagulation (DIC), which can be accelerated by induction of cytostatic chemotherapy and causes a markedly risk for (intracerebral) hemorrhage. Complete remissions of APL can be achieved by differentiation-inducing therapy with all-trans retinoic acid (ATRA). We studied activation markers of the coagulation system (thrombin-AT III complex (TAT), D-Dimer) in 3 patient with APL treated with ATRA. All patients had signs of DIC before induction of therapy (low plasma fibrinogen, low platelet-count) and moderate to severe bleeding tendency, activation markers were elevated (TAT >15 μg/L, D-Dimer >1500 μg/L). DIC was treated with fresh frozen plasma (3-9 U/d), no heparin was administered. APL was treated with ATRA (45 mg/m^2/d). D-Dimer levels decreased rapidly to values <300 μg/L, TAT <8 μg/L within 1 week of ATRA therapy, after 2 weeks activation markers were in the normal range (D-Dimer <100, TAT <3 μg/L). No platelet substitution was necessary during ATRA therapy, FFP substitution could be stopped after at least 4 days. Our data show, that treatment with ATRA can reduce the coagulation disorder and the risk for severe bleeding in patients with APL.

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Slow response to induction chemotherapy is an indicator of poor survival after bone marrow transplantation for acute lymphoblastic leukemia

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The prognostic value of diagnosis-remission interval on leukemia-free survival after BMT was investigated retrospectively in all 193 adult patients with acute lymphoblastic leukemia (ALL) transplanted in first remission and reported to the EBMT between 1979 and 1986. Patients achieving remission within eight weeks of diagnosis ("fast responders") had better leukemia-free survival after BMT than those with remission after eight weeks ("slow responders"): leukemia-free survival at three years was 43% vs 32% for fast and slow responders, respectively (p=0.04). The effect on leukemia-free survival was particularly severe for slow responders transplanted within three months of remission. Only 17% of the slow responders with short remission-BMT interval survived at three years. Decreased leukemia-free survival was caused by both, excess of transplant-related mortality and increased relapse incidence. In a multivariate analysis, time intervals (both, diagnosis-remission and remission-BMT) were the strongest independent prognostic factor for leukemia-free survival, probability of relapse and transplant-related mortality. We conclude that the intervals from diagnosis to remission and remission-BMT have a strong prognostic value in adult patients with ALL not only for remission duration after conventional treatment, but also for leukemia-free survival after bone marrow transplantation.

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Comparison of chemotherapy versus allogeneic bone marrow transplantation in adults with acute myelogenous leukaemia

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The present study evaluated the efficacy of chemotherapy in comparison with bone marrow transplantation for adults with acute myelogenous leukaemia. Between January 1983 and December 1991, 142 patients entered into the study. Of these patients, 31 had HLA identical sibling donor and received marrow graft in the first remission. Patients who received autologous marrow transplant were excluded from the analysis. Data were adjusted for the sex, age, FAB classification, immunological classification, WBC and time to treatment bias. Allogeneic transplants were done a median of 3.7 (1-12) months after achieving the first complete remission. The three year probability of leukaemia-free survival for the chemotherapy group was 20±11% and for the transplant cohort 58±12%. The main cause of death was relapse for the chemotherapy group and transplant related mortality for the patients who received allogeneic marrow graft. These data suggest that transplant patients may have better survival than the chemotherapy group of patients.

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ALLOGENEIC BMT IN 34 PATIENTS WITH ACUTE MYELOIC LEUKEMIA (AML)

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Between 2/82 until 5/91, 34 pts. (21 males, 13 females) with AML in first remission (n=18) and advanced disease (n=16) underwent allogenic bone marrow transplantation (BMT). 32/34 pts. were transplanted from an HLA identical sibling, 2 pts from 1 ag-mismatched sibling. Conditioning consisted of either Cyclophosphamide (120mg/kg) + TBI 10Gy single dose (n=29) or 12Gy in 8 fractions (n=3) or Vepesid 60 mg/kg + TBI 13,5Gy in 9 fractions (n=1) or Cyclophosphamide 50 mg/kg + Busulfan (7mg/kg over 4 days) + TBI 10Gy single dose (n=1). GVHD prophylaxis was performed using MTX alone (n=12), CsA plus MTX (n=20) or CsA plus methylprednisolon (n=2). 33/34 pts. were evaluable for engraftment, all of them showed complete engraftment. Currently, 15/34 pts. (44%) are alive, the median survival is 13,4 months (0,4-124 months). The probability of survival for pts. transplanted in first remission is 60%, for pts. transplanted in advanced disease 20%. The respective values for the probability of relapse are 20% for pts. transplanted in 1.CR and 70% for pts. transplanted in advanced disease. Acute GVHD grade II-IV occured in 15/33 pts. (45%). 19/34 pts. died with relapse (n=11) beeing the most frequent cause of death. 13/15 surviving pts. have a karnovsky score of 100%, the remaining 2 pts. a karnovsky score of 70% due to extensive chronic GvHD. These data show that for pts. with AML under <45 years of age allogenic BMT with on HLA identical sibling is currently the most effective treatment.

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CHRONIC MYELOGENOUS LEUKEMIA (CML) IN CHRONIC PHASE - RESULTS AFTER HIGH-DOSE BUSULFAN/ CYCLOPHOSPHAMIDE (BU/CY) AND ALLOGENEIC BONE MARROW TRANSPLANTATION

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Eight patients with CML in chronic phase (age 3-40 yrs, median 25 yrs) were transplanted with allogeneic HLA-identical bone marrow 2-51 months (median 5 months) after diagnosis following conditioning with busulfan 4 mg/kg/day over 4 days and cyclophosphamide 50 mg/kg/day over 4 days. The age of the donors was between 10 months and 38 yrs (median 22 yrs). The transplanted nucleated cell dose consisted of 0.8 - 4.9 x 10^8/kg recipient (median 4 x 10^8). Gvhd-prophylaxis was cyclosporin A and prednisone. 7 of 8 patients survive 150 - 2466 (median 560) days. 1 patient died from multiorgan-failure. The results and side effects of the BU/CY regimen do not seem to differ from classical conditioning regimens including total body irradiation.

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BONE MARROW TRANSPLANTATION AS TREATMENT FOR CHRONIC MYELOGENOUS LEUKEMIA (CML): EXPERIENCE IN 40 PATIENTS

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Between 7/83 and 4/92, 40 patients (pts) (24 males, 16 females) with Ph-chromosome positive CML were transplanted at our institution: median age was 37 years (19-57), 29 pts were in first chronic phase, 8 pts in accelerated phase and 3 pts in blast crisis at time of transplant. Conditioning consisted of Cyclophosphamide (120mg/kg) plus total body irradiation (10Gy) in 35 pts and of busulfan(16mg/kg) plus cyclophosphamide(120mg/kg) in 5 pts. Graft vs host disease (GVHD) prophylaxis was performed with methotrexate (Mtx) in 12pts, cyclosporine A (CsA) plus Mtx in 22 pts and CsA plus prednisone in 6 pts, respectively. Thirty six pts were transplanted from an HLA identical and MLC negative sibling, 1 pt from his 2 ag mismatched father, 1 pt from a 1 ag mismatched sibling and 2 pts received a syngeneic transplant. Currently, 25 of the 40 pts (62%) are alive at a median follow up of 42 months (1-105 months). The probability of survival is 65% for pts transplanted in first chronic phase and 41% for those transplanted beyond the first chronic phase; the respective values for the probability of relapse are 15% and 33%. Acute GVHD grade II-IV occured in 11/40 pts (28%); limited chronic GVHD was seen in 5/26 pts (19%) and extensiv chronic GVHD in 9/26 pts (35%) at risk. Transplant related mortality was 32% for the entire group. Twenty four of the 25 living pts (96%) have a Karnovsky score of 80-100, 1 pt has a Karnovsky score of 60. Our results confirm that a substantial proportion of pts with CML can be cured by allogeneic BMT, a therapeutic goal that currently cannot be reached by any other form of treatment.

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BONE MARROW TRANSPLANTATION IN THE TREATMENT OF HEMATOLOGICAL MALIGNANCIES AND SOLID TUMORS: THE INNSBRUCK EXPERIENCE

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Between October 1983 and June 1992, 87 bone marrow transplantations (BMT) were performed for treatment of hematological malignancies (total n=76; AML=24, CML=21, ALL=13, lymphoma=12, MDS=3, multiple myeloma=3), solid tumors (n=6; breastca=4, colorectalca=1, Ewing Sarcoma=1), severe aplastic anemia (n=4) or thalassemia (n=1). Standard risk, defined as AML in 1st remission, ALL in 1st or 2nd remission, CML chronic phase, HLA-identical sibling and 1st BMT was present in 39/87 patients (pts). Pts with hematological malignancies were conditioned with cyclophosphamide (CTX) (2x60 mg/kg/d) and total body irradiation, whereas pts with severe aplastic anemia were pretreated with CTX 50 mg/kg/d x 4. Pts with lymphoma received either TBI, VP-16 and CTX or BCNU, VP-16 and CTX as conditioning regimen. Marrow was harvested from HLA-identical sibling donors in 56 pts, from HLA-mismatched family donors in 7 pts and from 5 unrelated HLA-identical bone marrow donors. In the remaining 19 patients autologous marrow was reinfused. Cyclosporine was used as graft-versus-host disease (GvH-D) prophylaxis either alone (n=59) or in combination with methotrexate (n=9).

Overall survival probability for allogeneic BMT was 46.8%, being 74.7% for standard risk and 24.8% for high risk pts. Survival probability after autologous BMT was 31.4%. The highest survival probability was observed in pts with CML in chronic phase (78.5%), followed by 71% in pts with standard risk AML and 60% in pts with standard risk ALL. Survival probability in advanced lymphoma pts (n=12) reached 55.5%. Relapse was the most frequent cause of death (n=16) followed by CMV interstitial pneumonia (n=7). GvH-D > II was seen in 22/68 patients and cause of death in 2/22.

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PROTOADJUVANTE CHEMOTHERAPY FOR URETHELIAL CANCER: M-VAC VERSUS DOSE-ESCALATED M-VAC PLUS GM-CSF: A RANDOMISED PHASE III STUDY

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The achievement of a complete remission seems to correlate with an increased survival benefit in patients with urethelial cancer of the urinary bladder.

In the present study we investigated whether escalated M-VAC regimen (Methotrexate, Vinblastin, Adriamycin, Cis-Platinum) in combination with rh-GM-CSF results in an increased response rate in comparison to M-VAC alone. In this ongoing study so far 19 patients (12 males, 7 females) with urethelial cancer of the bladder (T2-T4) have been included. Additionally, 6 of these patients had squamous cell metaplasia. Mean age was 61,5 years (47-71,5), performace status was 0 in 17 and 1 in 2 patients on an ECOC scale. Eight patients received conventional M-VAC regimen (group A; 30 mg/m² MTX, 3 mg/m² Velbe, 30 mg/m² Adriamycin and 70 mg/m² Cisplatinum). In group B 11 patients received escalated M-VAC (30 mg/m² MTX, 4 mg/m² Velbe, 40 mg/m² Adriamycin, 100 mg/m² Cis-Platinum) and 5 µg/kg rh-GM-CSF (d 3-12). After 2 cycles patients were treated by cysterectomy, which was followed by additional 2 cycles of postoperative chemotherapy (regimen A orModern. far, 19 patients are evaluable for toxicity and 12 for response criteria. The median observation period is now 8,5 months (3-14,5 months), the median age was 61,5 (47-71,5).

Therapy	Patients	CR	PR	CR+PR
M-VAC	5	1 (20 %)	1 (20 %)	2 (40 %)
M-VAC+rh-CM-CSF	7	3 (43 %)	3 (43 %)	6 (85 %)

Patients treated with escalated M-VAC regimen in combination with GM-CSF showed an incredsed response rate, i.e. 85 %, in comparison to those treated with conventional regimen (40 %). In non of the patients with additional squamous cell metaplasia a complete remission was achieved. Side effects were comparable in both groups. Nausea and vomiting was present in 5 patients, mucositis in 3 patients, nephrotoxicity in 3 patients (1 grade 3); 1 patient had an apoplectic insult. Hematologic toxicity was present in 13 patients. Eleven patients had leukopenia (4 patients grade 1, 4 patients grade 2, 3 grade 1); 7 patients developed thrompopenia (3 patients grade 1, 2 patients grade 2, 2 patients grade 3); 2 patients had anemia grade 1, 2 patients grade 2. The median duration of leukocyte nadir was 8,3 (2-11) days in group A and 5,3 (3-8) days in group B (+GM-CSF). From these results it may be concluded that escalated M-VAC with GM-CSF supply results in increased response rates with similar incidence of side effects, but a tendency towards a shorter leukopenic phase in comparison to conventional M-VAC. Further patients' recruitment is ongoing in order to osess these initial findings.

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QUALITIY OF LIFE DURING ERYTHROPOIETIN-THERAPY IN CHRONIC TUMOR-ASSOCIATED ANEMIA

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Anemia, a common complication of malignant disease, often impairs the patients' qualitiy of life and diminishes their subjective sense of well-being to a considerable degree. We studied 24 consecutive patients (10 male, 14 female) with various neoplasias and moderate to severe anemia who were treated with 150 U/kg erythropoietin (Erypo, Cilag, Vienna), 3 x/week. Before the start of that therapy and after 8 and 12 weeks of treatment the patients responded to a questionaire. It consisted of 10 items covering physical capacity, emotional state, and social activity. Self-assessment was performed on a graded linear analogue scale ranging from very favorable (starting point 1) through neutral (point 3) to very poor (end point 5). In addition, the WHO performance status was evaluated and hemoglobin values were monitored. Response to the treatment was defined as an increase of the initial hemoglobin level by at least 20 g/l. At the start of therapy non-responders differed from responders only by a significantly higher pain score. After 8 weeks of treatment most parameters had improved in responders, but not in non-reponding patients. At that point of time we observed the highest correlation between mood and hemoglobin level ($r = -0.572$, $p < 0.001$) indicating a positive emotional reaction to both the objective improvement of the initially incapacitating anemic state and the subjective perception of that progress. After 12 weeks of therapy all items showed significant improvements in responders. Even the non-responders revealed significant gains in their subjective sense of well-being, physical capability, and social activity. In contrast to responders, however, they reported no relief of anxiety and perceived pain and nausea as unchanged. In conclusion, successful treatment of tumor-associated anemia with erythropoietin significantly improves the patients' quality of life as well as their subjective sense of well-being; to some degree psychosocial improvement seems to occur even if the patient fails to fulfill the objective response citeria.

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Bone marrow micrometastasis in colorectal cancer

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The detection of disseminated tumor cells poses a problem for conventional cytology and histology techniques. In this study bone marrow cells were purified by density centrifugation, cytospin slides were developed by use of a monoclonal antibody against epithelial cells (cytokeratin 18) and alkaline phosphatase-anti alkaline phosphatase staining.
In 19 pts. with colorectal cancer bilateral iliac crest puncture was performed prior to palliative (n=14, stage Dukes D) or adjuvant (n=5, stage Dukes A-C) immunotherapy by antiidiotypic anti= bodies. In the palliative group only 3 pts. showed sparse epithelial cells in the marrow, in the adjuvant group 4 of 5 pts. were positive, in 2 of them even in a large amount. The extent of hepatic metastasic disease and the clinical course did not correlate with the amount of epithelial cells in the bone marrow in the palliative group, in the adjuvant group the 2 pts. with a large amount of bone marrow micrometastasis showed a rapid disease progression.
Our conclusion therefore are:

1) metastatic tumor cells can be detected in the bone marrow of colorectal carcinoma pts. by this sensitive method
2) bone marrow micrometastasis do not reflect the total individual tumor load in palliative pts.
3) the presence and the number of tumor cells in the bone marrow cannot be used as a prognostic parameter in colorectal carcinoma pts.

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COMPUTED TOMOGRAPHY IN THE PREOPERATIVE EVALUATION OF SUSPECTED OVARIAN MASSES

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To evaluate the efficacy of computed tomography (CT) in 175 patients scheduled to undergo surgery for a suspected ovarian mass, we compared preoperative CT findings with the findings at surgery and histology. At surgery, 115 patients were found to have epithelial ovarian malignancies (20 stage I, 7 stage II, 50 stage III and 38 stage IV) while 60 had benign pelvic tumors. CT findings were suggestive of malignancy in 4 (7%) of 60 patients with benign tumors and suggestive of benign disease in 3 (3%) of 117 patients with malignant pelvic tumors; 30% of all scans were inconclusive. The sensitivity of CT for predicting pelvic or paraaortic lymph node involvement in 42 patients who underwent lymphadenectomy was 62% and 70%, respectively; the specificity was 100%. The results of this series suggest that the routine use of CT in the preoperative evaluation of patients does not seem justified.

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"p15E-LIKE" FACTORS IN MALIGN AND BENIGN BREAST TUMORS.

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Individuals with cancer have previously been shown to suffer from suppressed immune functions, e.g. monocyte chemotaxis. Surgical removal of the tumor often resulted in normalisation of this monocyte function, which suggested that products of the neoplasms might be responsible. A soluble factor with such an inhibitory effect on monocytes is the retroviral structural protein p15E, produced by murine leukemia viruses. Similar factors are produced by diverse human tumors. This study examines the presence of p15E like factors in sera and urine of breast cancer patients. Thirty patients (pts) with breast cancer, 29 pts. with benign breast masses and 28 healthy controls were tested blindly with the monocyte polarisation assay, using N-formyl-methionyl-leucylphenylalanine as chemoattractant. Sera of the malign tumor patients inhibited the monocyte polarisation significantly (mean inhibition 25%, SD 12.8) compared to sera of benign tumor patients (7.2%, SD 4.6) and of controls (5.1%, SD 4.2). The observed inhibition of chemotaxis could be neutralized in vitro by a monoclonal antibody to p15E. Surgical removal of the tumor resulted in a restoration of the monocyte polarisation in 18 out of 21 (86%) pts of the breast cancer group. Results testing urine samples correlated well with those of sera. These data give additional support to the concept that tumor-derived p15E-like factors are responsible for the inhibitory effect on monocyte chemotaxis in breast cancer patients, and that these factors can be found in serum as well as in urine.

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COMPUTER-ASSISTED DOCUMENTATION AND ADMINISTRATION SYSTEM FOR CANCER PATIENT CARE

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The main reason to creat a computer-assisted system for cancer patient care at the onkology department of the university hospital in Graz were the following: (1) The continously growing number of cancer patients (2) the limited number of staff and rooms and (3) the idea of gaining more time for treating each patient. At the beginning of 1989 an expert team of Joanneum Research, Institut for information systems, and the permanent staff of the oncological department started with design and development of this system. Using the relational database management system "ORACLE" and software tools such as SQL*FORMS and SQL*MENU a system was created which immediately supplies all information of the individual patient. The integration of this system in the hospital network improves the efficiency of administration and exchange of online information (e.g. radiological or histological findings) as other clinics are involved in dignosis and treatment. The "heart" of this system is the database in which all information on the patient (stock data, anamnesis, therapy, blood chemistry, etc.) is stored. An electronic appointment book has been installed. For improved information exchange with other departments and general practitioners about 100 predefind forms have been designed and can be printed out on demand. WordPerfect has been integrated for textprocessing of medical reports. The whole system is controled via menues.Using Oracle the system runs on different hardware configuratons which is important if studies will be carried out in cooperation with other departments. This system has been in use on VAX with 7 terminals and 1 integrated PC since April 1990.

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COINCIDENCE OF CROHN'S DISEASE AND LEIOMYOSARCOMA OF THE TERMINAL ILEUM: CASE REPORT

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A fifty-one-year old male was admitted to hospital for investigation of fever, progressive weight loss, stool irregularities and generalized fatigability. The patient's past medical history was non contributory. Physical examination revealed a pale, subfebrile, and almost cachectic patient. Laboratory investigations disclosed an elevated ESR, leukocytosis, thrombocytosis and a severe microcytic, hypochromic anemia. The patient underwent a complete gastrointestinal diagnostic workup. Radiologically, there was evidence of a characteristic involvement of the terminal ileum with cobblestoned appearance of the mucosa as well as a 5-6 cm stenosis proximal of the bauhini valve. Abdominal computed tomography suggested the presence of a large intraluminal tumorous lesion. Because of crescent symptoms of intestinal obstruction, laparotomy was performed with resection of the terminal ileum. Pathologic examination revealed a tumour of 6 cm in diameter within an ulcerative chronically inflammed mucosa. Histology revealed a leiomyosarcoma in a typical Crohn's disease affected terminal ileum. Although malignant small bowel tumours have been described in association with regional enteritis, and leiomyosarcomas, in fact, account for approximately 15% of all small bowel neoplasms, there are some specialities in this case. First, no concurrent incidence of leiomyosarcoma and Crohn's disease has ever been reported before. Furthermore, malignant tumours in inflammatory bowel disease usually arise after long immunosuppressive therapy and/or in a defunctioned ileum.

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TREATMENT RESULTS IN MALE BREAST CANCER.

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A retrospective review of patients suffering from male breast cancer was carried out at the Department of Clinical Oncology and the Department of Radiotherapy of the teaching hospital of the University of Graz.Twenty-four evaluable cases were analysed,the clinical features with adequate follow-up are presented..Nine patients were in Stage I,7 in Stage II,2 in Stage IIIa,4 in Stage IIIb and 3 in Stage IV. Of 23 patients who were treated with mastectomy,22 had modified radical mastectomy and postoperative irradiation to the chest wall +- peripheral lymphatic areas,one patient underwent radical mastectomy and postoperative irradiation.Another patient had an excision biopsy only,followed by irradiation.One patient received tamoxifen,another one CMF-regimen in an adjuvant setting.Local recurrence developed in 1 /23 patients (4%) treated with mastectomy and radiation therapy to the chest wall and peripheral lymphatics.Four patients (17%)developed distant metastases Five-year and 10-year overall survival (Kaplan-Meier) is 93% and 87%,respectively,when all causes of death are included.As observed in former reports,the stage of disease at initial presentation seems to be a parameter that significantly contributes to survival in male breast cancer patients.To what extend improved local control by adequate local therapy,as surgery and postoperative radiotherapy,may improve overall survival,remains to be discussed.
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MUCIN-LIKE CANCER ASSOCIATED ANTIGENS (CA 549, CA M 26, CA M 29) IN SERA OF PATIENTS WITH PRIMARY OR ADVANCED BREAST CANCER.

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In patients with breast cancer no satisfying tumor markers were found, yet. The aim of our investigation was to compare the usefulness of newly developed tumor markers with the most common combination carcinoembryonic antigen (CEA) with cancer antigen (CA) 15-3. We evaluated the concentrations of tumor associated antigens in the sera of 153 female patients with histologically proven mammary cancer. In 84 of these patients evidence or progression of disease was clinically confirmed, whereas 69 patients after mastectomy without any evidence of disease served as control group. The following tumor markers were determined: CEA, CA 15-3, the mucin markers CA 549, carcinoma-associated mucin antigen (CA M) 26, CA M 29 and the proliferation markers tissue polypeptide antigen (TPA) and tissue polypeptide specific antigen (TPS). The performance of the standard marker CA 15-3 (sensitivity 59,6% /specificity 80%) was exceeded by CA M 26 (59,5%/92,3%) and by CA M 29 (67,1%/87,3%), but not by CA 549 (50%/91,3%). Especially in patients with primary breast cancer and local recurrence CA M 26 and CA M 29 seem to be superior to CA 15-3. Slightly better but not statistically significant results were realized combining CEA with CA M 26 or CA M 29 when compared with the standard combination CEA/CA 15-3. The proliferation marker TPS seems only to be superior to TPA in patients with primary breast cancer with metastases. In ROC-diagrams both markers (TPA and TPS) show similarly results to the mucin-like markers, but reflect the clinical situation only in metastasized cancer. None of the investigated tumor markers shows a significant relationship between the serum concentration and the localization of metastasis. It should be noted that the overall results were unsatisfactory concerning the detection of small tumor masses. Therefore the clinical use of the investigated markers remains limited to the follow-up of patients after modified radical mastectomy or in monitoring the effect of therapy on patients with metastasized breast cancer.

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PHASE II STUDY OF NAVELBINE PLUS MITOMYCIN C AS SALVAGE THERAPY FOR METASTATIC BREAST CANCER

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Twenty-five patients (pts) with metastatic breast cancer were treated with a combination of the semisynthetic 5`Nor-vinca-alkaloid navelbine (30 mg/m2, iv on days 1 and 21) and mitomycin C (15mg/m2, iv on day 1). Treatment cycles were repeated every 6 weeks. All pts were refractory to first-line chemotherapy, and 14 pts had also failed previous hormonal treatment. Two pts were pre-, and 23 postmenopausal. The median age was 62 years (range, 42 to 75), and the median WHO performance status was 1 (range, 0 to 2). Sites of the metastases were soft tissue or lymph nodes in 16, pleuropulmonal and bone each in 9 pts., and liver in 5. Ten pts had single, and 15 pts multiple sites of metastases. After a median of 3 treatment cycles (range, 1 to 4), 6/17 evaluable patients had objective tumour response (35%; 1 CR, 5 PR). Nine pts were stable (53%), and 2 had progressive disease. Eight pts are too early. Haematologic toxicity was observed in 13 pts, including WHO grade III-IV leukopenia and/or thrombocytopenia in 4. All other treatment associated side effects (nausea/emesis in 9, alopecia in 4, stomatitis and infection in 3 pts each, and peripheral neuropathy in 2) were mild to moderate. In conclusion, navelbine plus mitomycin C combination chemotherapy was well tolerated and seems to represent an active salvage regimen for patients with refractory breast cancer.

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ABNORMAL ERYTHROCYTE DEFORMABILITY IN MALIGNANT TUMORS

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We made some haemorheological examinations in patients, suffering from malignant tumors. We used the following examinations: whole blood viscosity, yield shear stress, plasma viscosity and three tests for erythrocyte deformability: initial filerability rate /IRFR/, clogging particules /CP/, red cell transit time /RCTT/. These three parameters were examined by St. George s filtrometer.

In 3 cases the whole blood viscosity was elevated and in 9 patients the yield shear stress too. In the half of the patients the plasma viscosity was elevated, due to elevated fibrinogen concentration and abnormal protein fractions.

We found an interesting phenomenon examining the erythrocyte deformability. If we used buffer erythrocyte solution /10 %/ the deformability was decreased only in six cases. On the other hand if we used the own plasma the erythrocyte deformability was abnormal in 12 patients.

In seven cases the erythrocyte deformability was normal in buffer solution, but was abnormal in plasma solution.

We think: in malignant tumorsthe abnormal erythrocyte deformability due to abnormal plasma factors.

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A PHASE II STUDY OF D-VERAPAMIL (DVPM) PLUS DOXORUBICIN IN ADVANCED COLORECTAL CANCER

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In October 1991 we initated a study of reversal of multidrug resistance in patients (pts) with metastatic colorectal cancer (CC). 16 pts (10 males, 6 females) with advanced, bidimensionally measurable CC were entered in the study, all of whom were refractory to first-line chemotherapy with 5-FU/leucovorin combination chemotherapy. Pts median age was 56 years ((range, 46-66), and all had a WHO performance status of 0 or 1. Treatment consis ted of oral DVPM (1200-1400 mg/d x 3d) and doxorubicin (75 mg/m2) given by iv bolus on the 2nd day of DVPM.Courses were repeated every 3-4 weeks. The median number of treatment cycles is 2 (0-6). Cardiovascular side effects (that all reversed spontaneously on cessation of DVPM) comprised hypotension (syst.BP ≤90mmHg) in 9/16 pts, PQ-prolongation (>0.20s) in 5, 2nd-degree heart block in 1, junctional ryhthm in 2, and sinus bradycardia (f<50/min) in 3. One pt developed acute doxorubicin-related congestive heart failure and was discontinued after the 1st cycle. GI-toxicity was modest with nausea/vomiting WHO grade I-II in 3/16, and grade III in 1; oral mucositis grade I-II occurred in 2, and grade III in 4 pts. Myelotoxicity of grade III-IV was noted in 8/16 pts, leading to reduction of the doxorubicin dose in 5. At present, 11/16 pts are evaluable for response, and 4 are too early. Only 1 pt with a large pelvic mass had a PR, documented by CT scan; 4 pts had SD, and 6 PD. It is concluded that DVPM plus doxorubicin has only minor activity in CC when given in this dose and schedule.

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RESPONSIVENESS OF NORMAL AND TUMORIGENIC MAMMARY EPITHELIAL CELL LINES TO HEMATOPOIETIC GROWTH FACTORS

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Hematopoietic growth factors (HGF) are widely applied during post-chemotherapeutic treatment of patients with malignant disease. Yet, their effects on nonhematopoietic cell growth are poorly explored. To investigate the implication of HGFs in regulation of mammary epithelial cell growth we have studied the effect of IL-3, IL-4, IL-6 and GM-CSF on anchorage-independent growth properties of 4 cell lines constituting a novel in vitro model system for mammary epithelial cell tumorigenesis. The human breast epithelial cell line Hu-MI was established by microinjection of SV40 DNA into milk epithelial cells, the precursor cells of breast cancer. Hu-MI cells grow strictly anchorage-dependent and do not induce tumors in nude mice. In none of our experiments the tested HGFs could induce clonogenic growth of such cells. From this cell line we have selected sublines HuMI-T, HuMI-TTu1 and HuMI-TTu2, reflecting different stages of transformation. HuMI-T cells grow anchorage-independent but do not induce tumors. Their clonogenic growth is significantly inhibited by IL-3, IL-4 and IL-6. HuMI-TTu1 and HuMI-TTu2 cells are clonogenic as well as tumorigenic; yet, they show distinct stages of tumoral differentiation. Both tumorigenic lines are growth inhibited by IL-3 and IL-6. IL-4, however, inhibits clonogenic growth of HuMI-TTu1 but stimulates proliferation of HuMI-TTu2 cells. None of the tested lines was responsive to GM-CSF. Our results indicate that responsiveness of human breast epithelial cells to HGFs might be in relation with their transformational stage.

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NERVE GROWTH FACTOR (NGF) STIMULATES THE CLONAL GROWTH OF HUMAN TUMOR CELL LINES IN VITRO

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We investigated the effect of 2.5S NGF purified from mouse submaxillary glands on the clonal growth of various human malignant cell lines. Cell lines included the following: 2 myeloid leukemia cell lines, 2 lymphoma cell lines, 10 glioma cell lines, 2 neuroblastoma cell lines, a colorectal cell line, a breast carcinoma cell line, an ovary carcinoma cell line, an osteosarcoma cell line, 2 lung carcinoma cell lines, a hepatocellular carcinoma cell line. NGF was tested (0,5-500 ng/ml) in human tumor cloning assays (HTCA) in agar. The cells were continuously exposed to the growth factor for the complete HTCA period. NGF showed growth stimulation in two gliomas and in two lung carcinomas (two-fivefold). Growth stimulation was dose dependent and could be abolished by preincubation of NGF with neutralizing antibody. Single colonies stimulated by NGF had a high secondary plating efficacy arguing against an induction of differentation by the factor. In the other cell lines there was no significant growth modulation by NGF. Since NGF will be investigated in clinical trials, this observation should be further studied in vitro and in vivo.

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RESCUE WITH LYMPHOBLASTOID INTERFERON ALPHA AFTER LOSS OF RESPONSE DUE TO THE OCCURRENCE OF INTERFERON NEUTRALIZING ANTIBODIES

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The occurrence of interferon-neutralizing antibodies (NA) has been shown to be associated with loss of response. This phenomenon has been observed mainly during treatment with recombinant interferon (rIFN) alpha. - We studied the loss of response to rIFN alpha due to neutralizing antibodies in patients with myeloproliferative disorders and thrombocytosis. 2 of 31 patients (6.5%) became resistant to rec IFN alpha 2c after 3 and 8 months of treatment, respectively. This resistance could not be overcome by dose increases. One of these two patients, a 54 year old female, treated for thrombocytosis due to polycythemia vera, subsequently received rec IFN alpha 2b until she became resistant again 4 months later. A retrospective analysis of neutralizing antibodies (NA) showed moderate titers after treatment with rIFN alpha 2c against all three subtypes of rIFN alpha (levels of NA against: rIFN alpha 2c: 63 lU, rIFN alpha 2b: 36 lU and rIFN alpha 2a: 40 lU). These antibody levels rose to 90 lU against IFN alpha 2c, 51 lU and 64 lU against rIFN alpha 2b and alpha 2a, respectively, after treatment with IFN 2b. During the following trial with rIFN alpha 2a, the patient did not respond. However, the levels of NA rose to 400 lU against rIFN alpha 2c, 159 lU and 179 lU against rIFN alpha 2b and alpha 2a, respectively. At this time a cross reactivity to ly-IFN could be observed (NA: 10.3 lU). After a period of chemotherapy, the patient was treated with lyphoblastoid IFN alpha and responded, as a drop in platelet counts showed. Continued response was observed, despite a rise of the antibody levels against rIFN alpha 2c, alpha 2b and alpha 2a to 565 lU, 283 lU and 225 lU, respectively, and the cross reactivity with ly-IFN disappeared. This investigation shows that neither dose increases nor the application of various rIFN alpha subgroups can restore clinical response after loss due to NA. Ly-IFN alpha, on the contrary, has been shown to be effective even after the occurrence of NA against rIFN alpha, and may be used for rescue therapy.

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CLINICAL EXPERIENCES WITH INTERFERONE (INTRON-A) THERAPY IN HAEMATOLOGICAL PATIENT MATERIAL

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Since the beginning of the year 1991 we have had opportunity to use Intron-A in the treatment of multiple myeloma (13 cases),essential thrombocytaemia (2 cases), hairy cell leukaemia (3cases) and chronic granulocytic leukaemia (4 cases). In multiple myeloma 1 patient's disease progressed during the 5 month periode. Eight of them could have been treated for more than half a year, 3 of them treated with combined M-2 protocol+Intron-A induction therapy improved rapidly and later could have been taken on Intron-A maintenance monotherapy. One patient was treated with Intron-A between M-2 regimens only, 2 was given Intron-A induction therapy without any cytostatics and another one maintenance Intron-A treatment after completion of cytostatic induction.During the treatment of 2 patients with essential thrombocytaemia the platelet count decreased in a fortnight followed by improvement of splenomegaly as well. Three patients with hairy cell leukaemia had been treated successfully:pancytopenia improved significantly in all cases,the splenomegaly in twoo. Among 4 patients with CGL one had been treated with Intron-A induction monotherapy achieving complete remission in 4 months. In 3 cases Intron-A was given as maintenance therapy. The most frequent side effects were:increase of bone pain (1 case),mental depression (2 cases), fever (5 cases),leukopenia (2 cases) and thrombocytopenia (1 case).

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GRANULOCYTE-COLONY STIMULATING FACTOR AS AN ADJUNCT TO TREATMENT OF HAIRY CELL LEUKEMIA WITH ALPHA INTERFERON OR PENTOSTATIN

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Recombinant human Granulocyte-Colony Stimulating Factor (rhG-CSF) (5 mcg/dg/day) was administered in 3 patients with Hairy Cell Leukemia (HCL) as an adjunct to treatment with alpha Interferon (2 patients) or Pentostatin (1 patient) in order to prevent initial myelosuppression by these agents. Whereas in the patient with severe neutropenia (neutrophil count $\leq$ 100/mcl) only a partial response by G-CSF was observed (maximal value 300/mcl), 2 patients with moderate neutropenia (650 and 1130/mcl, respectively) completely responded with neutropoiesis achieving neutrophil counts of 2010 and 4200/mcl within two weeks of G-CSF treatment. In one patient initial reconstitution of granulopoiesis was followed by a subsequent drop in granulocyte counts most likely due to salmonellosis. Interruption of G-CSF therapy revealed inadequate neutrophil counts without external G-CSF in 2/3 patients, clearly suggesting a positiv effect of this hematopoietic growth factor on granulopoiesis. Our results suggest that compromised neutropoiesis in HCL can be effectively stimulated by G-CSF despite simultaneous administration of alpha Interferon or Pentostatin and suggest a potential benefit of this cytokine in reducing neutropenia associated morbidity in the early phase of treatment.

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SUCCESSFUL TREATMENT OF DRUG INDUCED AGRANULOCYTOSIS WITH GRANULOCYTE-COLONY STIMULATING FACTOR

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Recombinant human granulocyte colony stimulating factor in a dose of 300 mcg subcutaneously twice daily was administered in 5 patients with drug induced agranulocytosis (4 Thiamazol induced, 1 Metamizol induced). In 3 patients with substantial myelopoesis in bone marrow at diagnosis neutrophil counts rose to more than 500/mcl in 2 days after starting G-CSF therapy. In 1 patient in whom no bone marrow aspiration has been performed, neutrophil counts recovered on day 3 and in 1 patient showing hypocellular bone marrow at diagnosis recovery of neutropoesis (> 500/mcl) took 4 days. The fast response in our patients which was much more rapid than the spontaneous recovery reported previously in patients with drug induced agranulocytosis and the marked leucocytosis following rh-G-CSF therapy (range: 18.000-36.000/mcl) suggest a stimulatory effect of rh-G-CSF on granulopoesis and indicate a potential benefit of this cytokine for reducing neutropenia associated morbidity in drug induced agranulocytosis.

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KINETICS OF INTERLEUKIN-6 AND TUMOUR NECROSIS FACTOR SECRETION BY MURINE MACROPHAGES STIMULATED WITH MALARIA ANTIGEN PREPARATIONS

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In previous studies, elevated levels of the cytokines interleukin-6 (IL-6) and tumour necrosis factor (TNF) have been found to correlate with poor outcome of human malaria. In this work, heatstable antigen preparations of Plasmodium vinckei were used to study antigen dependent cytokine production of murine peritoneal macrophages. For determination of IL-6 and TNF activities, the cell lines B9 and Wehi 164 were used in bioassays as previously described. A significant production of IL-6 and TNF could be registered after 2 hours and 1 hour stimulation, respectively. When stimulation was performed in presence of polymyxin B, the antigen dependent production of IL-6 and TNF was reduced by 20 %. The patterns of IL-6 and TNF secretion after malarial antigen stimulation were compared to those obtained with endotoxin stimulation and differences could be observed. Maximal secretion levels for IL-6 were not reached after 6 hours stimulation whereas TNF showed its maximum secretion rate between 1.5 and 2 hours. In the presence of anti-TNF antibody, the antigen dependent IL-6 synthesis was lowered, indicating TNF dependency of IL-6 synthesis upon antigen stimulation. At concentrations higher than 0.5 mg/ml, it was found that pentoxyphilline (PTX) inhibited TNF secretion, but in contrary IL-6 secretion was clearly increased by PTX. Similar effects were observed with exogenous antigen preparations of Plasmodium falciparum on human macrophages, when the production of IL-6 and TNF were studied. These kinetic studies provide new perspectives about the importance of macrophages as a source of cytokines in malaria compared with other parasitic diseases.

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IMPROVED BIOASSAY FOR QUANTIFICATION OF TRANSFORMING GROWTH FACTOR-ß (TGF-ß) IN MALIGNANT EFFUSION.

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Bioassays are used for detection and quantification of polypeptide regulatory factors such as cytokines and tumor growth factors in biological fluids. We have been dealing with a bioassay (mink lung cell growth inhibition assay) for detection of TGF-ß-like activity in malignant effusion. Both methods used, i.e. ^{3}H-thymidine incorporation as well as MTT-test showed great variations in sensitivity over the time, when recombinant human (rh-)TGF-ß was used for establishing standard test conditions. In order to improve test results several clones with 3 distinct morphologies were developed out of the original cell line. These clones differed in sensitivity to the growth inhibitory effect of rh-TGF-ß as well as in autocrine growth factors supply and response to them, as detected by neutralisation with monoclonal antibodies (MoAb). Most constant results were obtained when C_1/C_2-clones were used as targets in growth inhibitory assay. Repeated testing with rh-TGF-ß showed good reproducibility and an ED 50 of< 20 pg/ml. With this assay we are able to detect TGF-ß-like activity in malignant effusions derived from cancer patients. Specificity controls are performed by neutralisation with anti-TGF-ß-MoAb. This assay provides a useful tool for detection and quantification of TGF-ß-like activity in clinical samples such as malignant effusions and in tumor cell culture supernatants or extracts.

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MEMBRANE MICROVISCOSITY, FATTY ACID COMPOSITION AND LIPID PEROXIDATION CAPACITY OF NEONATAL RED CELLS

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Our preliminary data indicate that the membrane microviscosity and the proportion of polyunsaturated C18 fatty acids are lower, the lipidperoxidation capacity and the proportion of arachidonic acid (20:4n-6) are higher in neonatal calf erythrocytes as compared with the values that have been found in adult cattle. Lipid peroxidation capacity (LP_C) has been estimated by comparing the malondialdehyde (MDA) content of the erythrocytes before (LP_0) and after (LP_{24}) the autoxidative test: $LP_C=LP_{24}-LP_0$. The results were expressed as nmol MDA x g^{-1}Hb. In the autoxidative test, a 10% suspension of washed erythrocytes has been incubated in isotonic NaCl solution containing 10 mM Veronal-Na-HCl buffer (pH 7.4) in air atmosphere for 24 hours at 37°C. The lipids have been extracted from the erythrocytes by isopropanol-chloroform 11:7 (v/v) without previous hemolysis. Fatty acid methyl esters were separated on 10% DESS-PS column using a Hitachi MOD 263 gas-liquid chromatograph connected to data processor. Membrane microviscosity was estimated by measuring the emission anisotropy of the fluorescent lipid probe 1,6-diphenyl-1,3,5-hexatriene (DPH) at two different temperatures (20°C and 37°C). The autoxidative test resulted in the significant enhancement of lipidperoxidation and the remarkable decrease in the proportion of polyunsaturated fatty acids in calf erythrocytes. The high sensitivity of red cell membrane and fetal hemoglobin against autoxidation could explain the faster ageing and the shorter lifespan of neonatal cells.

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STUDY OF SERUM CHOLESTEROL IN PRIMARY AND SECONDARY MYELOFIBROSIS: DOES A RELATION BETWEEN ITS LOWERING AND THE EXTENSION OF THE FIBROTIC PROCESS EXIST?

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Serum cholesterol level of 27 patients suffering from primary myelofibrosis was studied between 1980 and 1991. Their mean age was 64.2 years. Diagnosis was based on the generally accepted criteria including the histological examination of bone marrow. The mean value of the total cholesterol was 3.4 mmol/l (range 1.8-5.06 mmol/l). This cholesterol level proved to be significantly lower than that of healthy persons and of a group of patients suffering from chronic idiopathic thrombocytopenic purpura (ITP). Mean cholesterol value of 29 patients with chronic ITP proved to be 4.95 mmol/l. Similar data have been described by Marini et al in 1989. A further decrease of serum cholesterin was observed during the disease process and a relation between the degree of cholesterol lowering and the fibrotic process seems to be very probable. Similar tendency in the change of cholesterol level could be observed in chronic myeloproliferative disorders such as polycythaemia vera and chronic granulocytic leukaemia. Our data suggest that the cholesterol metabolism is influenced by the abnormal function of the megakaryocytic cell lineage manifesting in myelofibrosis.

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ANTI RH(D)-IGG THERAPY IN PATIENTS WITH HEAVILY PRETREATED ITP

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The administration of Anti Rhesus D specific IgG has been shown to be an alternative treatment possibility for immune thrombocytopenia. The therapeutic effect is mediated by blocking of the RES.
We report on the results of Anti D treatment in 6 patients with therapy refractory ITP. All patients had previously received corticosteroids, 5 patients had received interferon α, 2 patients in addition immunoglobulines, 2 immunosuppressants and/or chemotherapy, 2 patients had undergone splenectomy. The 6 patients received totally 7 cycles Anti D, doses ranging from 1200 to 6000 ug. Patients after splenectomy and/or immunosuppressive therapy with Imurek or cytostatic agents (n=3) did not respond to the administration of Anti D, in contrast to the other patients. The response was short lived in 2 patients, long term remission was observed in one patient, one patient was not evaluable because of early splenectomy after Anti D treatment. Responders showed decreases of serum hemoglobin indicating mild hemolysis. Response occured at dose levels from 1200 to 6000 ug.
We conclude (1) that only patients with cell sequestration into the spleen will respond to Anti D, (2) that previous chemotherapy blocks Anti D effects and (3) that no clear dose/effect relation has been found. Long term results indicate that heavily pretreated patients are no candidates for Anti D treatment.

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SENSITIVITY-TESTING OF CANDIDA SPECIES AGAINST CLINICAL-RELEVANT ANTIMYCOTICA

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Patients with acute leucemia are not only compromised by bacterial but also by fungal infections. Fungal colonization and subsequent infections can be promoted by the use of antibiotics, corticosteroids and immunosuppressive drugs. According to epidemiological data of Candidoses, Candida albicans and Candida tropicalis cause more than 90% of all nosocomial fungal infections. We examined the sensitivity of clinical isolates of C. albicans and C. tropicalis against amphotericin B, ketoconazole, fluconazole and 5-flucytosine (5-FC) by means of MIC determination. The used media were Sabouraud -Glucose-bouillon for amphotericin B, nitrogen base-bouillon for 5-FC and a chemically defined medium for ketoconazole and fluconazole.
The MIC 90 for Candida albicans were for amphotericin B 0.63 mg/l, for 5-FC 2.5 mg/l, for ketoconazole 0.3 mg/l and for fluconazole 0.31 mg/l. New experimental data show that sensivity-testing for 5-FC and fluconazole are necessary.

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ERYTHRON TURNOVER OF TRANSFERRIN MOLECULES (ETU) IN VARIOUS HAEMATOLOGICAL DISORDERS

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Radioisotope examinations of the erythrokinetics in patients with aplastic anaemia, primary myelodysplastic syndrome (refractory anaemia and sideroblastic anaemia), osteomyelofibrosis and haemolytic anaemia were performed. We investigated the importance of changes of several conventional erythokinetic parameters as well as the erythron turnover of transferrin molecules (ETU). The examination procedure is well utilizable for definition and distinction of osteomyelofibrosis, true polycythaemia, myelodysplasia and aplastic anaemia. It makes possible a qualitative interpretation of the actual state of erythropoiesis as a basis for differential diagnostics in clinical practice. Erythrokinetics provides a more direct evaluation of erythropoiesis than morfological methods, and differentiates conditions with dominant proliferative or maturation disturbances, which is important for appropriate management of e.g. myelodysplastic syndromes. Many hematological disturbances produce a typical picture during examination of the erythrokinetics, quite different from others. The main sphere of indiacation of the examination are conditions with cytopenia, which pathogenesis are not revealed by sipmler laboratory procedures.

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COMPLICATIONS OF IMPLANTABLE CATHETERSYSTEMS FOR SYSTEMIC AND REGIONAL CHEMOTHERAPY

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Implantable catheter devices are increasingly used for systemic and regional chemotherapy. As we know about possible complications beside of well known advantages, incidence and sort of problems in these systems will be studied.
From january until december 1991 117 patients received implantable catheter devices and where included in this investigation. Pharmacia (Port a Cath) and PFM (Jet Port) systems were used and placed via central venous access, as well as intraarterially (A.hepatica propria, Truncus coeliacus, A. mammaria int., intraaortic).

99 out of 117 (84,6%) received an intravenous system, 18 patients (15,3%) an intraarterial. Catheterassociated problems were observed in a total of 23 patients (19,6%), 16 (69,5%) of them had i.v. and 7 (30,5%) had i.a. catheters.
In the i.v.section mechanical problems (75%) predominated septical complications (25%), whereas all i.a. catheter associated problems turned out to be caused mechanically. 11 in a total of 23 complications had to be treated by surgical intervention, deaths associated with catheter/port complications had not to be observed.

In summary it can be stated that totally implantable intraarterial catheter systems provide application of highly efficient regional chemotherapy, whereas devices with central venous access facilitate systemic therapy and supportive care.
Results of our investigation show the possibility of severe complications in several cases, which can be reduced to acceptable dimensions by interdisciplinary cooperation, use of adequate catheter systems, and furthermore carefull handling by experienced and skillful staff.

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CD45-RA ANALYSIS OF $CD34^+$ HEMATOPOIETIC CELLS TO DISCRIMINATE BETWEEN EARLY AND LATE PROGENITORS

G. Fritsch, D. Printz, P. Buchinger, G. Mann, A. Zoubek, H. Gadner

Mononuclear cells (MNC) isolated by density centrifugation of cord blood and normal bone marrow samples, and of peripheral blood (PB) from patients treated with GM-CSF, were double stained with anti CD34 MAb (8G12) versus CD45, CD45-RB, CD45-RO and CD45-RA, respectively, and analyzed by flow cytometry. In all specimen, $CD34^+$ MNC expressed CD45 at a low to very low level, while the expression of CD45-RB was similar or slightly higher. In contrast, CD45-RO and CD45-RA can subdivide the $CD34^+$ population into fractions negative, dim ($^+$) and normal positive ($^{++}$) for these subgroups. In bone marrow, the majority of the $CD34^+$ MNC is RA^{++} and RO^-, but there are also $34^+/RA^+$ and $34^+/RA^-$ cell fractions. In PB, most cells are $34^+/RA^-$ with varying proportions of $34^+/RA^+$ and $34^+/RA^{++}$ and a variable expression of RO. In cord blood, the hematopoietic progenitors are usually $34^+/RA^-$ and $34^+/RO^-$. Culture of sorted MNC in semisolid medium revealed that clusters and dispersed (late) CFU-GM originated from $34^+/RA^{++}$ cells, while the $34^+/RA^-$ MNC formed compact and multiple centre, white and red colonies derived from early progenitors. Addition of 20 ng SCF (Amgen) per ml of medium containing $34^+/RA^-$ sorted cord blood MNC led to a change of many BFU-E to CFU-mix which was not, to this extent, seen in blood and bone marrow. We conclude that early hematopoietic cells are $CD34^+/CD45\text{-}RA^-/CD45\text{-}RO^-$ and that differentiation leads to CD45-RA expression detectable by flow cytometry.

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COMPARATIVE STUDY TO ENRICH FOR LEUKAPHERESIS-DERIVED CFU PRIOR TO FREEZING

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Leukapheresis-derived cell concentrates (buffy) collected for autologous transplantation are comprised of 95% to 99% erythrocytes most of which will lyze during the freezing/thawing procedure. We studied the recovery of CFU after purification of such cell concentrates via density centrifugation and examined 21 leukapheresis products obtained from 5 patients during recovery from myelosuppressive treatment. Using cell counting, clonogenic assay and flow cytometry, the results were compared with those obtained from the low density MNC preparations of peripheral blood (PB) samples drawn prior to each apheresis. Both the MNC and CFU were enriched by 40 to 300 fold per ml of buffy compared to 1 ml of PB, and very similar distributions of CFU-mix, CFU-GM, and BFU-E were found after culture of PB MNC, unseparated buffy MNC and low density buffy MNC. Most erythrocytes were pelleted into the high density cell fraction during density centrifugation. Compared to the original buffy cell suspension (=100%), the recovery of total cells (= volume) in the interface cell fraction ranged only between 1%-10%, whereas 49%-68% of the MNC and 68%-92% of the CFU were recovered. Only 0.1-1% of the CFU were grown from the high density cell fractions. In conclusion, we recommend density separation of cells collected by leukapheresis since most of the CFU are recovered whereas the volume of the transplant to be frozen (and thus the amount of DMSO to be infused during transplantation) is reduced to 10% or below.

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PROTECTIVE EFFECT OF DEOXYCYTIDINE ON 2-CDA TREATED HUMAN BONE MARROW CULTURES

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The lymphocytotoxic effect of chlorodeoxyadenosine (CdA), a purine analogue resistant to adenosine deaminase, requires the phosphorylation by the enzyme deoxycytidine kinase. This effect on lymphocytes can be antagonized by coadministration of deoxycytidine (dCyt), a competitive substrate of deoxycytidin kinase.
Recent in vitro studies[1] have shown, that not only growth of lymphocytes but also colony formation by myeloid progenitors derived from normal human bone marrow is dose dependently inhibited by CdA.
The aim of this study was to examine the effect of various doses of dCyt (10^{-6} to 10^{-3}M) on CdA mediated growth inhibition of myeloid progenitor cells in vitro. Our results show that coadministration of dCyt (>10^{-4}M) to CdA containing cultures (160nM) protected colony formation by human bone marrow derived progenitor cells (CFU-E, BFU-E, CFU-GM) in the methylcellulose system. However, the protective effect of dCyt was markedly different on the various subclasses of progenitor cells depending on their maturation stage. Thus, coadministration of 10^{-4}M dCyt completely reversed the growth inhibiting effect of CdA on CFU-E colony formation, whereas the colony formation of the immature progenitors BFU-e and CFU-GM was only restored to 50% of control cultures. If the concentration of dCyt was increased to 10^{-3}M, the protective effect for BFU-E and CFU-GM in the presence of a maximally growth inhibitory dose of CdA (160nM) reached almost 80% of control cultures.
The fact that CdA mediated growth inhibition of CFU-E could be completely restored, but that of BFU-E and CFU-GM only incompletely despite a higher concentration of dCyt (10^{-3}M), led us to the suggestion, that beside phosphorylation by dCyt kinase additional mechanisms may be operative for the toxicity of CdA in the more immature progenitors (BFU-E and CFU-GM).

[1] Petzer A., Bilgeri R., Zilian U., Geisen F., Haun M., Konwalinka G.: Inhibitory effect of 2-CdA on granulocytic, erythroid and T-lymphocytic colony growth. Blood 78,1992:pp 2583-2587

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THE INFLUENCE OF METEOROLOGICAL PHENOMENA ON HAEMOSTASIS

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Many haemophiliacs develop bleeding without any known trauma. Comparing continuously these spontaneous bleedings and meteorological phenomena, the obvious connection was noticed between the bleedings and the beginning of anticyclonic activity.

The most suitable explanation concerns the field of biometeorology. It explores the influence of the weather on the live world. About twenty four hours before a storm (cyclonic activity) there is an increase in positive air ion concentration, liberating serotonin from platelets and neurons. It has a potent central effects (change in mood and behaviour), facilitates allergical reactions, make worse pain in previously damaged tissues, provokes the rise of stress induced hormones and leads to bronchoconstriction (asthma) and vasoconstriction (hypertension).

On the contrary, with the beginning of the anticyclonic activity, the rise in negative air ion concentration leads to hyperactivity of monoamine oxidase and serotonin degradation, thus proning to unexplained and sudden bleedings.

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CHARACTERISTICS OF HEMOSTASIS IN DIFFERENT STAGES OF CHRONIC LYMPHOCYTIC LEUKEMIA (CLL)

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Hemostasis alterations have been investigated in some hematological malignancies but not in CLL. We examined how hemostasis is affected in CLL and the connection between the progression of the disease and the hemostasis disturbances. In 80 CLL cases belonging to stages of Rai 0-IV, prothrombin activity (PT), activated partial thromboplastin time (APTT), thrombin time (TT), fibrinogen level, antithrombin III (AT III) activity, ethanol gelation test (EGT), FDP, platelet count, ADP, epinephrine and collagen induced platelet aggregation were examined. We also measured the ATP release from the platelets in 25 patients. We found that there were various hemostasis alterations in CLL already in early stages. We observed a gradual decrease of platelet aggregation and ATP release from the platelets in stages 0-III, when the platelet count was within the normal range. EGT became positive and AT III activity decreased in more advanced stages (II-IV), PT decreased only in stage III-IV. Fibrinogen, TT and APTT did not change with the progression considerably. Decreased platelet functions occured more frequently in patients with high WBC count. With the improvement of the disease hemostasis alterations improved as well. With respect to the causes of blood clotting abnormalities, the bone marrow in lower stages, the spleen and liver involvement, the activation and consumption of some coagulation factors in higher stages can be taken into consideration. Hemostasis examinations may help the staging and indicate the progression and also the regression of CLL.

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Restrictive cardiomyopathy in AL-Amyloid and LCDD

Restrictive cardiomyopathy is observed in cases of endomyocardial fibrosis, endocarditis fibroplastica Löffler, Boeck's disease and metabolic disorders. As paraproteinamic haemoblastosis can coincide with amyloidosis especially in the final phases echocardiographic and bioptic myocardial damages are not surprising accordingly. Lately, casuistics on cardial Participation in the so-called LCDD of various etiologies have multiplied. Thus, we consider the diagnosis by echocardiography of the early phase of paraproteinosis coinciding with LCDD to be of great importance. The casuistics of a first diagnosis of a lambda-chain Bence-Jones plasmozytoma reached by the findings of a restrictive cardiomyopathy is presented in the following. In November 1990, we admitted a 67 year old female patient for the clarification of cardial insufficiency symptoms which had been existing for month. Echocardiography revealed a restrictive cardiomyopathy; etiology showed an l-chain plasmozytoma of the lambda type with extended infiltration of the bonemarrow; radiologically no foci in the bones; results of peripheral blood-count and retention parameters stage I; protein analysis showed a distinct nephrotic syndrome on the basis of high excretion of light chains. Apart from L.C., in rectal biopsy histologically and immunohistologically we found minimal deposits of amyloids in submucosa. Up to present under therapy of melphalane (Alkeran^{R}), prednisone and colchicine satisfactory general condition; no haematological progression of the plasmozytoma; beginning decrease of clinical and echocardiographical cardial symptoms as well as a slight improvement of the nephrotic syndrome. The following consequences resulting from this casuistics: In the diff. diagnosis of restrictive cardiomyopathy early forms of paraproteinamic haemoblastosis with LCDD should be taken into consideration. A cardial indication for plasmozytoma therapy, particularly that of a light chain plasmozytoma must be propounded. These circumstances necessitate myocardial biopsy in order to clarify the question of LCDD or amyloid. Echocardiographic results concerning restrictive cardiomyopathy which until now have been related to amyloidosis must be verified with regard to LCDD.

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INFLUENCE OF LYMPHOMA CELL PROLIFERATIVE ACTIVITY ON THE EFFICACY OF CHEMOTHERAPY

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Flow cytometric analysis of DNA is accepted as the technique by which to assess the percent of cells in the various phases of cell cycle. Proliferative activity determined by the fraction of cells in S-phase is a good indicator of tumour growth rate. We investigated the cell suspensions of diploid lymph nodes of 14 patients (pts) with non-Hodgkin's lymphomas (NHL). According to the Working Formulation, 4 were included in low grade malignancy, 3 in the intermediate grade and 7 in high grade malignancy. Overall median percent of cells in S-phase was 19% for a total group without significant differences among the various histological subtypes. Based on values of S-phase and regardless of the histological types, pts were divided into two groups. In the first group were 8 pts who presented with s phase ≥15%. A second group, 6 pts presented with S-phase <15%. All the pts received combined chemotherapy. In the first group, complete and partial remissions were achieved in 5 pts, and there were 4 in the second group. Two pts of the first group died and one of the second group. Remission was longer in the first group (3 vs 7 months), but there were no significant differences in the duration of survival.

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Is combined modality treatment superior to radiotherapy alone for CS IA-IIB Hodgkin's disease

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From January 1979 to December 1988 34, patients (15 males and 19 females) were pathologically staged as IA - IIB Hodgkin's disease and treated with radiotherapy (RT) alone (22 pts) or RT plus six cycles of MOPP (12 pts). Indications for combined modality treatment were: bulky mediastinal mass; ES >50; age over 40 years. Median age for whole group was 29 years (range, 14-49 years). Overall CR was obtained in 30 of 34 pts (88%). Relapse was documented in 8/30 (26.6%) and five of eight in the irradiated area, with a follow-up ranging from 18 to 126 months. Five year probability of survival was 90% for the first and 75% for the second group (p=ns) and disease free survival 63% and 54% respectively (p=ns).
We conclude that additional chemotherapy does not improve the results of radiotherapy for patients with IA-IIB stage of Hodgkin's disease.

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Systemic chemotherapy of cutaneous lymphomas

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Skin lesions may be primary or secondary manifestations of various types of lymphomas, and the most usual form being mycosis fungoides (MF). We present 32 patients (pts) who showed advanced cutaneous lesions. They were treated with systemic chemotherapy. Only 4 pts were pre-treated with PUVA.The histological diagnosis of mycosis fungoides was established in 22 patients (69%), while 10 cases were diagnosed as having lymphomas of other types (non-MF).Clinical staging was established according to the extensity of skin changes and involvement of lymph nodes and visceral organs.Eleven patients were in stages I and II, and 21 were in stage IV. All patients were treated with a combined chemotherapy regimen (COP, MOPP, CHOP). Complete remission was achieved in 14 pts, partial response in the same number of patients and no effect or progression of disease was seen in 4 pts. Duration of remission was relatively short (median 11 months, range 1 to 144 months) and twelve patients have relapsed (38%). The median survival for the whole group was 22,5 months (range 3-160 mo) with no statistical difference between MF and non-MF groups. The younger patients with longer premycotic period and uninvolved lymph nodes and viscera whose remission lasted as longer as one year have more favourable prognosis. We conclude that systemic chemotherapy is effective in the treatment of patients with cutaneous lymphomas.

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SOLUBLE TNF-RECEPTORS IN CHRONIC LYMPHATIC LEUKAEMIA (CLL) AND HAIRY CELL LEUKAEMIA (HCL)

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The release of soluble cytokine receptors has been suggested to represent a mechanism of cytokine regulation. Recently, a soluble form of tumor necrosis factor receptor (sTNF-R) has been discovered. This TNF binding protein is probably involved in the modulation of TNF-activity in many diseases. To investigate its possible diagnostic and prognostic value in lymphoproliferative disorders we studied sTNF-R in CLL and HCL.
Levels of the 60 kDa sTNF-R were determined by an ELISA technique (GR Adolf et al, J. Immunol. Meth., 1991) in the serum of 22 patients with CLL and 6 patients with HCL, and compared with healthy donors (HDs) (n=26). The following results were obtained: B-CLL 5,03 $\pm$ 2,18 ng sTNF-R/ml, HCL 4,74 $\pm$ 1,56 ng/ml, HDs 2,24 $\pm$ 0,54 ng/ml. The levels in B-CLL and HCL were significantly higher than in HDs ($p < 0,01$).
The concentration of sTNF-R in serum correlated well with activity and stage of disease. Interestingly, the only patient with T-CLL, studied so far, showed normal levels of sTNF-R with no change during the progression of the disease.
The findings indicate that sTNF-R is a disease parameter in B-cell leukaemias. It has to be determined, however, whether an increase of sTNF-R represents a physiological breakdown of surface receptors or whether sTNF-R is released to bind and thereby to neutralize TNF in these disorders.

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ELDESINE PLUS BLEOMYCINE AS SALVAGE TREATMENT IN NON HODGKIN LYMPHOMAS.

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Though great achievement in the treatment of NHL has been made, salvage therapy is still necessary for patients having a refractory or relapsed course of disease. Most published schedules have considerable toxcity and poor long term outcome. So we deviced a regimen of comparable low toxicity, consisting of bleomycine (15 mg) and eldesine (5 mg) combined with irradiation (16 pts.) or cytostatics non cross resistant to firstline therapy (asparaginase and methotrexat, 56 pts.). Most patients were pretreated with regimens including anthracyclines.
Bleomycine and eldesine were given as a 2 - 24 hours continuous infusion. Influence of the different infusion durations will be discussed. Prophylactic antipyretics were given in all patients.
The medical records of 100 patients, 55 male and 45 female with a median age of 59 years (range 21-88) treated at our department between 1.1.1985 and 1.5.1992 were analysed retrospectively.
Ann Arbor stage was I/5, II/8, III/17 and IV/70 patients. 6 patients suffered from low grade, 48 from intermediate grade and 46 from high grade non Hodgkin lymphomas.
Overall response was 38% (13% CR, 25% PR). There was no difference in the response between the histological subtypes.
Toxicity measured according to the WHO-recommendation was as follows: Leukopenia grade I/5 patients, grade II/8 patients and grade IV/7 patients.
Thrombocytopenia grade I/2 patients, grade II/4 patients and grade III/1 patient. Anemia grade I was seen in one patient, grade II/3 patients, grade III/1 patient and grade IV/1 patient. In contrast to published data short time infusion of bleomycine/eldesine was superior and therefore this treatment can be easily applied in ambulatory patients.

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COMBINATION CHEMOTHERAPY OF ADVANCED HODGKIN'S DISEASE WITH COPP VERSUS ABVD

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The poster reports the result of a randomized comparative trial of COPP vs. ABVD at the National Cancer Institute, Department of Hematology /Budapest/.
Between January 1985 and March 1988 45 patients with advanced Hodgkin's disease received COPP /22 patients/ or ABVD /23 patients/. 9 and 16 patients entered complete remission treated by COPP and ABVD, respectively. Toxic manifestations after COPP were myelosupression and neurotoxicity whereas alopecia and gastrointestinal symptoms occured most freguently after ABVD. We concluded that primary ABVD treatment is a successful alternative of COPP in the management of advanced Hodgkin's disease.

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AGE AS PROGNOSTIC FACTOR IN HIGH-GRADE NON-HODGKIN'S LYMPHOMA

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In a multicentre study the data of 131 consecutive patients were analyzed retrospectively to get information about the prognostic importance of age in patients with high-grade non-Hodgkin's lymphoma. The median age at presentation was 57.3±16.3 years. Sixty five percent of the patients belonged to stage I and II, 35% to stage III and IV. Patients were treated according to different protocols (CVP, CHOP-Bleo, Pro-MACE-COPP) without any reduction of dose in elderly. Remission was achieved in 90 cases (81 complete and 9 partial remission). The mean follow-up time 44 months, the median survival 19.8 months. At 7 years 24% of the patient is alive. To see the effect of age on response rate and survival patients were divided into three groups: 1. patients younger than 60 years of age: 2. patients between 60-70 years: 3. patients older than 70 years. Overall response rate was slightly better in patients above 60 than in younger ones, 74% and 63% respectively. However, the survival did not differ significantly. Multivariate analysis revealed that the most important predictive factors for survival are remission and stage of disease. Only above 70 years get the age some prognostic relevance. Our results indicate that advancing age is not an absolutely poor prognostic factor and, that high-grade non-Hodgkin's lymphomas could be treated effectively in the elderly.

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EXPERIENCES REGARDING THE TREATMENT OF T-CELL LYMPHOMAS

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Among the NHL, T-cell malignant lymphomas are of lesser proportions, 10-20 % in the Western hemisphere. They require special attention due to their poorer prognosis and variable therapeutic response. During the past five years, we cared for 22 T-cell malignant lymphomas (17 males, 5 females). The average age of the patients was 42,6 years (20-71 years). At present six patients are living, 16 have died. Histologically, there were high grade non-classified: 9, T-lymphoblastic lymphoma: 6, Lennert lymphoma: 4, T-angioimmunoblastic:1, T-CLL: 1, T-zona lymphoma: 1. The most commonly employed treatment was CHOP and ProMACE. Average survival was 25,0 months (3-75 months). The expected outcome of the treatment was made worse by the fact that 3/4 of the patients were already in stage III and IV at the time of the diagnosis (stage II-6, stage III-10, stage IV-6). 55 % of the patients exhibited B symptoms. Out of 14 post-mortem examinations, 6 patients showed severe infections as contributing to their death; we lost one patient in CR as a consequence of infection, which demonstrates the importance of good supportive therapy. Despite the treatment less than half of the patients could be brought into complete remission and 6 patients were non-responders, demonstrating the difficulties encountered in the treatment of this group of patients.

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SERUM LEVELS OF TRYPTOPHAN AND NEOPTERIN IN MULTIPLE MYELOMA
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In serum of patients suffering from multiple myeloma were determined the concentrations of tryptophan and neopterin. Tryptophan levels were significantly reduced and neopterin levels significantly elevated both in groups of patients in progression and in remission when they compared to controls. The degree of difference was the most expressed in the advanced stage of disease. The serum protein level was not influenced decisively to the statistically results.
According to their results beside neopterin level the cheeper and seeming more sensitive serum tryptophan determination may be suitable too for the investigation of the cellular immune reactions, and so among others for the determination of the clinical status in the multiple myeloma.

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EARLY CLINICAL SYMPTOMS OF MALIGNANT TRANSFORMATION IN OUR PATIENTS WITH ANGIOIMMUNOBLASTIC LYMPHADENOPATHY
A.Bányai, K.Dankó and Gy.Szegedi

Frizzera et al. first described Angioimmunoblastic Lymphadenopathy (AILD) as a systemic disease characterised by generalised lymphadenopathy, hepatosplenomegaly, fever, weight loss and frequently appearance of skin rashes. The lymph node histology shows diffuse obliteration of architecture by heterogeneous proliferation of immunoblasts and small blood vesel endothelium. Investigators found that most of patients had remission and recovered but about 35% of cases transformed to malignant lymphomas.
Because the malignant progression couldn't be recognised early by histology we worked up retrospective the histories of our 35 patients with AILD. Our aims were to find early signs of worse prognosis. We found that the 13 patients with developed malignant lymphomas were significantly younger, their lymphadenopathies started as localised and some laboratory dates showed early specific abnormalities as signs of transformation to malignant tumors.

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INCIDENCE OF LYMPHOMAS AND OTHER CANCERS IN HIV-INFECTED AND HIV-UNINFECTED PATIENTS WITH HEMOPHILIA
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Objective: To determine the types and rates of cancers in excess in the presence of human immunodeficiency virus 1 (HIV-1) infection.
Design: Cohort analytic study of HIV-positive and HIV-negative hemophiliacs followed up to 12 years.
Patients: 1927 patients with hemophilia, of whom 1141 were HIV-1 positive.
Results: The incidence of non-Hodgkin's lymphoma (NHL) was 2±0.6% 10 years after seroconversion. The annual incidence of NHL rose from 0.04% to 2% per year over the 12-year period following seroconversion. The greatest absolute risk of lymphoma was in the oldest age group, the relative increase was 38-fold in subjects < 40 years and 12-fold in older patients compared with the normal population. The incidence of Kaposi's sarcoma (KS) was increased 200-fold. The incidence of cancers other than NHL and KS was not increased in HIV-positive hemophiliacs. HIV-negative subjects had no significant increase in cancer incidence.
Conclusion: HIV-infection has restricted effects on cancer incidence. Improvements in therapy of HIV-infection prolong survival and may lead to further increases in HIV-associated lymphoma.

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VAD-REGIMEN (VINCRISTINE-ADRIAMYCIN-DEXAMETHASONE) TO ACHIEVE COMPLETE REMISSION IN PATIENTS WITH MULTIPLE MYELOMA
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In the past therapy for cure for multiple myeloma has only been possible with continued high-dose chemotherapy, radiotherapy and allogeneic bone marrow transplantation. Intensified chemotherapy using high-dose melphalan and/or adriamycin can lead to partial remission in up to 74% of patients.Nine patients with multiple myeloma stages II and III received chemotherapy according to the VAD-regimen. In three patients (one pretreated with standard therapy and two receiving only the VAD-regimen) a complete remission was achieved. The M-gradient was abolished in these cases and the plasma cell count in bone marrow biopsies was less than 5% of total cells. In six patients a partial remission was obtained. Only mild side effects (nausea, vomiting, myelosuppression - WHO - grade 2) were observed but did not necessitate a change in the drug administration regimen. Longterm observations are necessary to study the duration of remission and the effect on quality of life and survival time.

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BONE MARROW TRANSPLANTATION IN PATIENTS WITH SEVERE APLASTIC ANEMIA: EXPERIENCE IN 36 PATIENTS.

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We report on 34 allogeneic and 2 syngeneic bone marrow transplantations between 1981 and 1991. 12 of the patients were female, 24 male, the mean age was 16,5 years (2,6 yrs - 37,2 yrs.). All allogeneic recipients were transplanted from an HLA identical, MLC negative family donor. The mean marrow cell dose they received was 3,65 x 10^8 (0,77 - 8,8) mononuclear cells/kg body weight. All patients were multiply pretransfused. Conditioning consisted of 50mg Cyclophosphamide/kg body weight, given on four consecutive days in all patients. Rejection prophylaxis was irradiation with 300 - 400 rad for 8 patients (total body- n=7, total nodal irradiation n=1), or transfusion of non irradiated donor buffy coat cells (n=24).
At present 30 of 36 (83%) patients are alive in complete remission. The medium surveillance is 54 months (8,8 - 125,5 months) after bmt. The probability of survival for the whole group is 81%. 9 Patients developed acute GVH-disease grade II - IV, 14 Patients chronic GVH-D (lim. n=7, ext. n=7).
6 of 36 patients died (17%), two related to chronic GVH-d, one because of bone marrow rejection, three died of septicemia.
Our results confirm that allogeneic bmt is the treatment of choice in young patients with severe aplastic anemia, who have a suitable family donor.

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Chemotherapy versus allogeneic bone marrow transplantation in adults with acute lymphoblastic leukaemia

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The efficacy of chemotherapy and bone marrow transplantation for adults with acute lymphoblastic leukaemia was analyzed. Between January 1983 and December 1991 100 patients entered into the study. Twenty eight patients with HLA identical sibling donor received marrow graft in the first remission. Patients who received autologous marrow transplant were excluded from the analysis. Data were adjusted for the sex, age, FAB classification, immunological classification, WBC and time to treatment bias. Allogeneic transplants were done a median of 4.0 (1-12) months after achieving the first complete remission. The three year probability of leukaemia-free survival for the chemotherapy group was 28±8% and for the transplant cohort 52±12%. The main cause of death was leukaemia recurrence for the chemotherapy group. Transplant related mortality was the main cause of death in a group of patients who received allogeneic marrow graft. These data suggest that transplant patients may have more favourable prognosis than patients on chemotherapy.

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Combination of cyclosporine and methotrexate for GVHD prophylaxis: Long term follow-up

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From May 1985 to June 1989, 76 patients with leukaemia were randomized to receive either cyclosporine (CSP) alone (N=39) or CSP combined with methotrexate (MTX) (N=37) as a GVHD prophylaxis. Patients received a total body irradiation and cyclophosphamide followed by HLA identical sibling marrow infusion. The incidence of moderate to severe acute GVHD was higher in the CSP group compared with the CSP+MTX group (20 (51%) versus 9 (25%) (P=0.02). There was no significant difference among the two groups regarding chronic 'GVHD. Median follow-up for the CSP+MTX group was 64 (range 78-34) and for the control group 59 (range 76-36) months. Survival was significantly better for the CSP+MTX group (54±10%) than for the control group (38±12%). Leukaemia-free survival was higher for the CSP+MTX group (50±12%) compared to the CSP group (30±15%).

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REGULATION OF SOLUBLE CD23 SERUM LEVELS IN B-CLL

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CD23 generally known as the low-affinity receptor for IgE (FceRII) is expressed on the surface of B-lymphocytes at the intermediate stage of differentiation and on B-CLL cells. Its soluble form, sCD23 (IgE-BF) has potent BCGF-activity. Recently, it has been shown, that sCD23 is significantly elevated in sera of B-CLL patients. The clinical implications of this finding, however, are still unclear.
To get further information on the role of sCD23 and the in vivo mechanism of its regulation, we repeatedly investigated 40 cases of B-CLL with RAI stages O-IV. Additionally to the measurement of sCD23 serum levels, we examined the expression of CD23 on MNCs in 17 cases. The results indicate that in all patients with B-CLL sCD23 was highly elevated (median 4544, range 284-20200) as compared to normal individuals (median 113, range 27-1504) and other lymphoproliferative disorders (HCL, T-CLL, HL, low grade/high grade (lg/hg) NHL, ALL, MM). Only within the group of lg NHL of B-cell phenotype similar serum levels to B-CLL were measured. Serum concentrations of sCD23 correlated with disease activity as evaluated by RAI stage, lymphocyte doubling time and distinction between active and indolent forms of B-CLL, but not with absolute lymphocyte counts. The response induced by chemotherapy was reflected by a decrease of sCD23 serum levels.
The FACS analysis revealed that 75% of MNC in B-CLL were CD23 positive. The CD23 antigen was located independently of stage on the malignant CD19/CD5 positive population. While in lymphomas the CD23 antigen was restricted to the CD19/CD5 positive population, it was found only on CD19 positive cells in healthy donors. Since sCD23 serum levels were only weakly correlated to the absolute numbers of CD23+ MNC in peripheral blood and neither related to the density of CD23 molecules on the cell surface of MNC nor to the product of these two parameters, sCD23seems to reflect the tumor mass rather than the number of leukaemic cells.
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BLEEDING COMPLICATIONS IN ACUTE PROMYELOCYTIC LEUKAEMIA

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Haemorrhagic tendency is a frequent manifestation of acute leukaemia, in particular APL. Procoagulant substances released from leukaemic blast cells may induce DIC by activating conventional coagulation pathways or primary fibrinolysis.
Between 1981 and 1991 we diagnosed 42 adult patients with acute myelocytic leukaemia.
We treated 6 acute promyelocytic leukaemias, using different chemotherapeutic regimens.
Three of the six patients /50 %/ hat major bleeding at diagnosis. We found hypofibrinogenaemia, elevated fibrinogen /fibrin degradation product level, prolonged PTT, TT and clotting times.
Our main strategy in the management of the DIC complicating acute leukaemia is to eradicate the underlying cause.
Induction chemotherapy was administered immediately after diagnosis. These patients received platelet transfusions to correct thrombocytopenia, fresh frozen plasma to replace clotting factors and cryoprecipitate to correct hypofibrinogenemia.
Complet remission was obtained at 4 of these 6 patients. In two cases we achieved partial remission. The mean duration of survial is 24.5. months.

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FAMILIAL LEUKAEMIA/LYMPHOMA IN SZABOLCS-SZATMÁR-BEREG COUNTY

J. Jakó, L. Szerafin, I. Póth, P. Nagy, D. Smanykó and T. Babicz

Twelve cases of familial malignant haematologic diseases were found by authors. Demonstrating the clinical pictures and the development of eleven pairs (Hodgkin's disease - non-Hodgkin's lymphoma, Hodgkin's disease - chronic lymphocytic leukaemia, non-Hodgkin's lymphoma - acute lymphoblastic leukaemia, hairy cell leukaemia - acute lymphoblastic leukaemia, chronic lymphocytic leukaemia - acute myelogenous leukaemia, non-Hodgkin's lymphoma - chronic lymphocytic leukaemia, non-Hodgkin's lymphoma - non-Hodgkin's lymphoma, chronic myelogenous leukaemia - non-Hodgkin's lymphoma and three times chronic lymphocytic leukaemia - chronic lymphocytic leukaemia) and one triad (non-Hodgkin's lymphoma - acute myelogenous leukaemia - acute myelogenous leukaemia) of cases authors want to give data about occurrences of familial leukaemia/lymphoma in their county.

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UNCOMMON FORMS OF CHRONIC LEUKAEMIAS

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Detailed study of uncommon forms of chronic leukaemias including hairy cell leukaemia (ten cases), T-cell chronic lymphocytic leukaemia (two-cases), B-cell prolymphocytic leukaemia (two-cases), adult T-cell leukaemia-lymphoma (one case), Ph chromosome negative chronic granulocytic leukaemia (three cases), and eosinophil leukaemia (one case) are presented. Morphological, cytochemical, conventional immunological markers and monoclonal antibodies were used for the diagnosis of lymphoproliferative disorders. Splenectomy was the treatment of choice for hairy cell leukaemia, some refractory cases were treated with interferon. Combined chemotherapy produced variable responses in the other lymphoproliferative processes, but the single adult T-cell leukaemia-lymphoma showed rapid progression. Patient with eosinophil leukaemia proved to be Ph chromosome negative confirmed by molecular genetic analysis. In this case a long haematological improvement was achieved by interferon therapy.

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CHEMOTHERAPY FOR POLYCYTHAEMIA VERA

Gy. Ujj, B. Telek, A. Kiss, and K. Rak

129 patients with polycythaemia were treated between 1974 and 1992. The male-female ratio was 1.63. 31 patients were lost during the follow up. Phlebotomy alone was used in 12 cases and at the rest of the patients chemotherapy and phlebotomy were applied together. 59 patients were treated only with dibromomannitol (DBM), most of them several times, while in 27 cases P^{32} and other cytostatic drugs - including DBM in 19 cases as well - were used. Among patients followed more than three years, four secondary acute leukaemia were observed (one out of 38 DBM treated and three out of 19 patients treated with DBM plus other cytostatic regiment). In the above mentioned groups one and five myelofibrosis occurred, respectively. A female preponderance was observed among myelofibrotic patients.

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AN EVALUATION OF THE EFFECTIVENESS OF PROTOCOLS YU-84 AND YU-87 IN THE TREATMENT OF ACUTE LYMPHATIC LEUKEMIA IN CHILDREN

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159 children with acute lymphatic leukemia (ALL) were treated with 2 different protocols at the Centre for the Treatment of Leukemia in Children, Department of Hematology and Oncology, Pediatric Clinic, Šalata University Hospital in Zagreb, from 1st January, 1984 to 31st December, 1989. The results of treatment were analysed in order to determine which of the 2 protocol applied was the most effective. 82 children were treated with protocol YU-84 from 1st January, 1984 till 31st May, 1987. 77 children were treated with protocol YU-87 from 1st June, 1987 till 31st December, 1989. The first complete remission was achieved in 64 (78%) of the children treated with protocol YU-84; and in 73 (94.8%) of the children treated with protocol YU-87; the differences are highly significant statistically ($p<0.01$). The first relapse occured in 25 (39%) of the patients treated with protocol YU-84 and in 18 (24.6%) of those treated with protocol YU-87; although differences exist they are not statistically significant ($p = 0.06$). Survival probability of 48 months was 50% for the patients treated with protocol YU-87 and 73.5% for the patients treated with protocol YU-87; the differences are statistically highly significant ($p<0.01$).

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EXTRAMEDULLARY RELAPSE WITHOUT BONE MARROW INVOLVEMENT IN ACUTE MYELOGENOUS LEUKEMIA (AML). REPORT OF TWO CASES AND REVIEW OF THE LITERATURE

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Granulocytic sarcoma (myelosarcoma) are localized tumors containing myeloblasts without evidence of leukemia in blood and marrow. Myelosarcoma is the first clinical manifestation of AML in about 0.5% of cases. Typical sites include the respiratory and urogenital tract, orbita, lymphoid organs, small bowel and soft tissues. Most of these patients develop typical AML with bone marrow involvement after a median time of 13.7 months but some patients who received chemotherapy were apparently cured. - We studied the incidence of myelosarcoma in patients with AML who achieved complete remission after chemotherapy. Over a period from 1979 to 1992, 100 patients with relapse were studied. In 2 (2%), the relapse occured as myelosarcoma. - Case 1 is a 68 years old male with AML (FAB M2). He achieved complete remission after 3 courses of DAT and received two cycles of consolidation treatment. He was in unmaintained complete remission for 9 years. In 1992, he was admitted because of obstructive renal failure. A CT scan showed a tumor of the prostate which infiltrated the seminal vesicle and extended to the orifices of both ureters. A biopsy of the prostate showed an infiltration with myeloblasts. The peripheral blood counts were normal and the bone marrow contained < 5% blasts. After treatment with mitoxantrone and ARA-C the tumor regressed, no infiltration with myeloblasts could be demonstrated in the prostate tissue which was obtained by electroresection. - Case 2 was a 20 years old male with AML (FAB M2). He achieved complete remission after 1 cycle of "3+7" in 1/88. After consolidation he underwent allogeneic bone marrow transplantation. 9 months later (3/89) he developed myelosarcoma of the stomach and the bladder. No involvement of the bone marrow could be demonstrated. However, 1 month later he developed full-blown AML with bone marrow involvement and died in 5/89. - In the literature (MED-LINE research between 1969 and 1991) we found only 2 published similar cases. Our data suggest that this pattern of relapse may be more common.

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PCR-DETECTION OF t(15;17) POSITIVE CELLS IN PROMYELOCYTIC LEUKEMIA

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The t(15;17) which juxtaposes the PML gene with the gene encoding the retinoic acid receptor alpha (RARalpha) is the chromosomal hallmark of acute promyelocytic leukemia (M3). The translocation creates PML-RARalpha fusion RNAs which can be specifically detected by a polymerase chain reaction (PCR) using 5'primers from PML and 3'primers from RAR (deThe et al., Cell 66: 675-684, 1991). We have used this technique to analyze samples of three patients with M3 and t(15;17) before, during and after treatment with all-trans retinoic acid (ATRA) and conventional chemotherapy. PCR was positive at diagnosis in two of the three patients. One of the two PCR-positive patients had a short clinical remission, but relapsed early and died of a cerebral hemorrhage due to DIC after 6 months. Residual t(15;17) cells could be detected in blood and bone marrow samples of this patient at any time. The other patient is still in remission after 6 months. Interestingly, t(15;17) cells disappeared from the peripheral blood of this patient after chemotherapy, at least at a detection level of approximately $1/10^4$ cells. Our data indicate that (1) t(15;17) PCR can be used for the diagnosis of M3 in many, but not all patients, probably due to a certain heterogeneity of breakpoint location; (2) it is useful for the detection of minimal residual disease, especially since there may be a group of patients whose blood samples become negative after treatment.

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INVESTIGATION OF THE MULTIDRUG-RESISTANCE PROTEIN IN THE LEUKOCYTES OF HAEMATOLOGICAL PATIENTS AND IN AN IN VITRO EXPRESSION SYSTEM

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Multidrug resistance is a major problem in the treatment of leukaemic patients. One of the major causes of both the primary and secondary multidrug resistance is the presence of the MDR1 gene product, the P-glycoprotein, actively extruding several kinds of drugs, in the plasma membrane of the leukaemic cells. In order to estimate the amount of this protein in the blast cells present in peripheral blood, we have developed a quantitative immunoblot technique in which the electrophoresed and electroblotted cellular proteins of ficoll-separated leukocytes are detected by various monoclonal and polyclonal anti-MDR1 antibodies, generated against specific peptide regions of the MDR1. The amount of the MDR1 mRNA measured in the leukocytes by Northern blotting is compared with the amount of the MDR1 protein on the immunoblots and with the clinical, cytochemistry and FACS cell typing data of the respective patients. Initial studies were performed on the cells of 37 patients with diagnoses of AML, ALL, CGL and CLL, and the relationship between the level of MDR1 and the response to chemotherapy is currently analyzed. The *in vitro* expression system, in which *Spodoptera frugiperda* (Sf9) cells are infected with the MDR1 recombinant baculovirus and produce a large amount of the MDR1 protein, can be utilized as a quantitative control for the protein in the immunoblot method. The *in vitro* expressed protein is also used to generate further polyclonal antibodies against the full length protein, since such antibodies are expected to recognize the MDR1 protein with a greater sensitivity and specificity.

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MOLECULAR CHARACTERIZATION OF THE bcl-2 MAJOR BREAKPOINT REGION IN LYMPHOMA: NUCLEASE-SENSITIVITY AND PROTEIN-BINDING CAPACITY

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The *bcl-2* major breakpoint region (mbr) is a chromosomal hotspot involved in the translocation t(14;18) which is the most frequent translocation in human lymphoma and occurs at the pre-B cell stage of development. 70% of follicular lymphomas break within this remarkably well focused region which comprises only 150bp. We hypothesized that the mbr must have certain structural features or contain DNA elements which make it a preferred target for illegitimate recombination. We first tested the region for its nuclease sensitivity. Incubation of nuclei from a B-cell line with S1 nuclease produces a band on Southern blots which maps to the mbr, indicating the vulnerability of the chromatin in this region. Using supercoiled plasmids containing the *bcl-2* 3'end as a substrate we also detected an endogenous nuclease activity in extracts from early B-cells which is able to cleave the mbr. Moreover, a 68bp DNA fragment from the 5'end of the mbr specifically binds to at least one protein present in these extracts. These findings suggest that the *bcl-2* major breakpoint region is a preferred target of endogenous enzymatic cleavage in early B-cells and that protein-DNA interactions may facilitate the translocation process by alteration of the chromatin structure.

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PCR-MEDIATED ANALYSIS OF THE FMS ONCOGENE IN MDS PATIENTS - CORRELATION WITH CYTOGENETICS

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Point mutations at position 301 and 969 in the c-fms oncogene have been shown to enhance its transforming activity in vitro. These point mutations have been demonstrated to occur in patients with acute myeloid leukemia (AML) or myelodysplastic syndrome (MDS). In this study we compare cytogenetic data of patients with MDS (RA,RAEB or CMML) or AML secondary to MDS with the results of the molecular biological analysis.
20 bp long fragments of the c-fms oncogene containing codon 301 were specifically amplified from DNA isolated from patients with MDS using the polymerase chain reaction. Dot blots were hybridized with endlabelled wild type and mutant oligonucleotides.
DNA from patients with a 5q deletion hybridized only with the wild type oligo, indicating the absence of a mutation in this fms region. Quantitative PCR using the co-amplification of part of the beta globine gene as reference confirmed observations of fms hemizygosity in 5q- patients already found by quantitative Southern blotting.
DNA from 2 of 4 patients with translocations involving chromosome 5q showed cohybridization with both wild type and mutant allele. This might indicate the possible involvement of the fms oncogene in this translocation.
Sequencing of the mutation positive PCR products in order to confirm the nature of the point mutation is in progress.

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CYTOCHALASINS FOR DETECTION OF MULTIDRUG RESISTANCE MODULATORS: A QUANTITATIVE FUNCTIONAL BIOASSAY

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Multidrug resistance (MDR) is characterised as an intrinsic or acquired resistance of cells to cytotoxic activity of a group of structurally and functionally diverse chemotherapeutic agents. Recently, the clinical importance of the MDR phenomenon and its reversion has been stressed by several preclinical and clinical studies.
We present evidence that Cytochalasin B and D (CCB, CCD) are substrates for the MDR efflux pump and act as specific and sensitive indicators for MDR phenotype and its modulation.
MDR^+ (KBC1) and MDR^- (KB3-1) human cancer cell lines were exposed to ^{3}H-CCB or unlabeled CCD: Accumulation of ^{3}H-CCB was significantly higher in sensitive KB3-1 than in resistant KBC1 cells. This corresponds to the antiproliferative effects of CCs (^{3}H-thymidine uptake, colorimetric assay) in these cells lines. Furthermore, as a direct measure of the significantly higher intracellular CC concentrations in the sensitive parental cells, a significantly higher number of bi(multi-)nucleated cells - cytokinesis block due to the CC's microfilament activity - has been demonstrated at significantly lower CC concentrations as compared to their resistant counterparts. This MDR dependence of the CC's inhibiting effect on cytokinesis was then used to study agents with MDR reversing activity. Treatment of MDR^+ KBC1 cells with Verapamil, Quinidine, or Staurosporine during exposure to CCs resulted in similar patterns of both CC retention and cell division block as detected in the sensitive parental line. Thus CCs offer a suitable tool for detection and quantification of MDR phenotype and potential reversing agents.

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A 45kDa PROTEIN BINDS TO A RECOMBINATORIAL SIGNAL AT THE t(14;18) CHROMOSOMAL BREAKPOINTS IN LYMPHOMA

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The t(14;18) which juxtaposes the *bcl-2* oncogene with the immunoglobulin heavy chain (IgH) locus represents a model system for chromosomal recombination in neoplasia. While the break on chr.14 occurs during the attempted DH to JH rearrangement and is probably mediated by *V(D)J*-recombinase, the actual mechanism of translocation is still unclear. Using gel retardation assays as well as Southwestern blotting we have identified a 45kDa protein (bp45) which binds to a homopurine-homopyrimidine stretch in both major (mbr) as well as minor breakpoint regions (mcr) of *bcl-2*. The sequence contains a human analogue of the recombinatorial element CHI in E.coli. Homologous sequences from the immunoglobulin DH and JH regions cross-compete for the binding of bp45 indicating that this protein specifically binds to the breakpoints on both chromosomes. The protein is highly expressed in early B-cells at a time when the t(14;18) translocation takes place. The tissue distribution of the protein as well as the localization of the binding sites suggest that bp45 is involved in the translocation and that site-specific homologous recombination is part of the process. Since the DNA binding motif is also found at breakpoints of other translocations bp45 may possibly play a more general role in illegitimate recombination.

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DETECTION OF ACTIVITY OF P-GLYCOPROTEIN IN B-CELL CHRONIC LYMPHOCYTIC LEUKEMIA USING RHODAMINE 123

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Depending on the methods used there exist contradicting data concerning the incidence and clinical relevance of multidrug resistance (MDR) in B-cell chronic lymphocytic leukemia (B-CLL). Using two different methods we studied 22 consecutive patients suffering from B-CLL for the expression of MDR. In a first step we applied a functional, flow cytometric assay to peripheral blood samples of all B-CLL cases which accurately detects the retention/efflux of the dye rhodamine 123 (Rh123). For dual fluorescence analysis the leukemic cells were stained with a phycoerythrin-conjugated, B-cell directed MoAb (Leu12/CD19, Becton Dickinson). Thereafter fluorescence gates were placed around the PE-labeled cells and Rh123 efflux was selectively assessed in these cells in the presence or absence of MDR inhibitors (verapamil or B859-35, Byk Gulden, Konstanz). 16 (73%) B-CLL cases showed a significant Rh123 efflux which was completely abolished in the presence of MDR inhibitors. The percentage of Rh123 effluxing cells ranged from 13 to 70% (median 37%). 6 (27%) cases showed no efflux. In a second step we analyzed the expression of MDR1 mRNA using polymerase chain reaction (PCR) in 16 samples to confirm the results of the functional Rh123 assay. To quantitate the PCR-products we separated the MDR1- and ß-mikroglobulin-products (internal control) by polyacrylamide gel electrophoresis. The gel was stained, photographed and the bands were quantitated by a computer assisted imaging system. The MDR1 mRNA levels (standardized to that of multidrug-resistant KB-8-5 cells) correlated well with the results of the Rh123 efflux assay. MDR expression, obtained by both methods, was neither correlated to Rai stage nor to prior treatment or disease progression.
We conclude that the flow cytometric measurement of cellular Rh123 retention/efflux is an efficient and sensitive tool to assess the functional activity of P-gp in tumor samples.

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DETECTION AND CLINICAL RELEVANCE OF GENETIC ABNORMALITIES IN PEDIATRIC ALL: A COMPARISON BETWEEN CYTOGENETIC AND PCR ANALYSES

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Certain subtypes of ALL with a t(1;19)(q23;p13) or a t(9;22)(q34;q11) have been associated with poor prognosis. To test whether the employment of PCR improves the detection rate of these clinically relevant genetic anomalies we have developed a multiprimer-PCR protocol which facilitates the detection of each of the four chimeric E2A/PBX1 and BCR/ABL mRNAs in a single reaction. This protocol was used for the evaluation of bone-marrow or blood samples from 251 children with ALL in whom cytogenetic analyses had been performed. Twenty one patients carrying the E2A/PBX1 rearrangement and three with the BCR/ABL transcripts were detected by PCR. Twelve of these cases had escaped the detection by conventional cytogenetic analysis. In two of twelve patients with a typical t(1;19)(q23;p13), no E2A/PBX1 transcripts were identified by PCR, thus suggesting the presence of different molecular rearrangements. Residual leukemic cells were detected by PCR in five of eight patients who were followed during complete clinical remission. We conclude that the routine use of PCR may have an important impact on both clinical diagnosis and monitoring of minimal residual disease in patients with B-cell precursor leukemia who carry the E2A/PBX1 or BCR/ABL fusion genes.

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INVOLVEMENT OF INTERLEUKIN-6 (IL-6) IN THE BIOLOGY OF HUMAN MALIGNANT MELANOMA

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We have previously demonstrated that human malignant melanoma cell lines (MML) produce immune and growth regulatory peptides such as IL-1,IL-3 and basic fibroblast growth factor (bFGF).
In the present study we extended our recent finding that MML produce and secrete IL-6. Seven of 10 MML - culture supernatants contained IL-6 (22pg-800pg/ml) as detected by RIA. IL-6 production and release could be increased by incubation of MML with PMA, IL-1ß and TNF-α -but not with Interferon-α (IFN-α), IFN-gamma and bFGF -in 4 of 7 lines tested.IL-6 was also shown to be present in these MML by immunoperoxydase staining with MoAb. Furthermore we found by FACS analysis that MML express the binding capacity for fluoresceine-conjugated IL-6 suggesting the expression of IL-6 receptors on these cells. Preincubation of MML with IFN-gamma and/or TNF-α resulted in an upregulation of the binding site for IL-6. The latter was also substantiated by competition studies with unlabeled IL-6.
Additionally we have evidence that recombinant IL-6 acts under certain conditions as a negative growth signal for MML in vitro.
Our results suggest, as human MML produce and respond to IL-6, that this cytokine has a role in melanoma biology.
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Poor prognosis of malignant mastocytosis associated with changes of surface antigens: A case report

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A 60 year old man was admitted to the medical academy hospital, Dresden, because of a hepato-splenomegaly, weight loss and weakness that had presented 4 weeks before. Physical examination showed that the liver was 4 cm at the MCL and spleen 2 cm and abdominal lymphadeopathy confirmed by sonography. The ESR was 90 mm/hour. Hemoglobin was 6,4 mmol/l; leukocyte count 7.9 * 10 g/l. Bone marrow cytology with cytochemistry and histology including elektronmicroscopy confirmed the presence of malignant mastocytosis. By monoclonal antibodies CD2 and CD4 antigens (FACS and APAAP) could be found on the mast cell surface. A diagnosis of malignant mastocytosis with T-cell antigens was accepted and he received 2 cycles of combination chemotherapy with CHOP-Bleo. A laparoscopy with liver biopsy was done and the histology showed an increase of mast cell infiltrations in the liver parenchyma. The autopsy confirmed the diagnosis of a malignant mastocytosis. Conclusion: Rapid deterioration, organic infiltration, atypical mast cells and changes of surface antigens give valuable information for diagnosis and prognosis.
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DEFECTIVE FUNCTION OF PERIPHERAL BLOOD MONOCYTES IN PATIENTS WITH NON-HODGKIN'S LYMPHOMAS.

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Bactericidal activity and the production of oxygen radicals by isolated monocytes were investigated in 37 patients was impaired in late stage ($p<0.01$) and was normal in patients in early stages and in remission.
Both INT and chemiluminiscence were significantly lowered and delayed ($p<0.001$ in INT and $p<0.01$ in cl), with respect to normal control.
10 mg/ml zymosan corrected the INT to normal.
Indomethacin at 1 µg/ml corrected the monocyte deficiency increasing the cl response to near normal.
CONCLUSION:

1-Bactericidal activity decreased in late stage of NHL and were normal in early stages and in remission.
2- A partial restoration of monocyte function after in vitro incubated with PGE2 inhibitors.
3- The technique of chemiluminiscence is the best method for purpose of routine measurement of oxygen metabolites in clinical practice.

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AGRANULOCYTOSIS INDUCED BY ANTITHYROID THERAPY - EFFECTS OF G-CSF TREATMENT : A CASE REPORT

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Severe neutropenia occurs as a rare reaction to a variety of drugs and is the principal undesirable side effect of anti-thyroid therapy with Methimazole. Here, we report on a 26-year-old female patient with leucopenia and agranulocytosis induced by Methimazole. Because of Basedow's disease diagnosed 2 years ago, she was treated with Methimazole for 18 months. With a daily dose of at least 15 mg an eumetabolic state was obtained. A severe tonsillitis and gastroenteritis with high fever and an initial leukocyte count of 800/ul with total absence of neutrophils as well as a typical bone marrow (exhibiting a complete arrest of myelopoesis at the stage of promyelocytes with a normal megakaryo- and erythro-poesis) lead to the diagnosis of an antithyroid therapy induced agranulocytosis. The Methimazole therapy was immediately stopped. After one week of antibiotic treatment, the lymphocyte counts ranged from 1.700 to 2.900/ul, neutrophils still absent. Therefore, a treatment with G-CSF (granulocyte colony-stimulating factor) in a dose of 480ug/d s.c was started and well tolerated without any side effects. 24 hrs after the first application the leukocyte count rapidly rose up to 7.700/ul consisting of 57% granulocytes with a maximum of 47.100/ul (87% granulocytes) after the 3rd injection. As the patient showed a good recovery from her infections, G-CSF therapy was stopped after the 3rd day of application. In the following 7 days the leukocyte count gradually fell to normal numbers, stabilizing at about 6.500/ul (61% granulocytes). In parallel, the platelet counts rose from initially 228.000/ul to 743.000/ul after the 3rd day of G-CSF treatment and gradually declined to normal numbers within the following week. Up to now, G-CSF has not been described as a major stimulating factor in megakaryopoesis. Thus, the concomittant increase of platelets might be explained by the rapid marrow recovery after depression caused by the severe infection and/or by possible toxic side effects of the antithyroid treatment.We conclude that G-CSF is a potent agent for shortening the period of drug induced neutropenia complicated with severe infections thus leading to an earlier recovery of the patient.

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DIFFERENTIAL REGULATION OF INFLAMMATORY CYTOKINES IN PBMNC AND MYELOID CELL LINES BY TYPE I INTERFERONS

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Cytoreductive effects of interferon (IFN) alpha in myeloproliferative diseases are well established. In subpopulations of patients with Ph1+ CML treatment with IFN alpha results even in a reduction or disappearance of the malignant cell clone which might protect patients from progression to the lethal blast crisis. However, the mechanism of action of IFN in CML remains to be elucidated. Beside antiproliferative activity against hemopoietic progenitor cells regulation of the cytokine network can be responsible for clinical efficacy and certain side effects of IFN alpha. We therefore studied regulation of gene transcription of several cytokines with proinflammatory or antiproliferative activity by type I interferons after *in vivo* application and in *in vitro* models: Peripheral blood mononuclear cells (PBMNC) from patients with Ph1+ CML in chronic phase were seperated 4-6 hours after application of IFN alpha or beta. For in vitro examination PBMNC from CML patients or normal volunteers were incubated with various doses of IFN alpha or beta with or without hemopoietic growth factors. Furthermore, the monocytic cell line THP-1 was studied for monokine regulation by IFNs. RNA separated from such cell samples was examined for transcription of TNF α, IL-1β, IL-6 and IL-8. After application of IFN to CML patients in vivo a reduction of mRNA transcripts for IL-8 was observed, whereas message for TNF-α was induced. In vitro experiments in the MNC from CML patients or normal controls revealed upregulation of TNF and IL-6 and downregulation of IL-8 and IL-1-β suggesting that shifts of the mononuclear cell population under treatment with IFN was not responsible for the observation in vivo. In THP-1 cells a comparable regulation of monokine transcription was seen as in MNC populations thus providing evidence that the differential regulation of cytokine cascades by IFNs may occur in the same cells rather than in different monocyte subpopulations. In view of recently reported pathophysiologic implications of various cytokines in progression of CML understanding of the regulation of cytokine production by type I IFNs increasingly gains importance and represents the focus of further detailed studies.

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INVESTIGATION OF THE FIBRINOLYTIC SYSTEM - A TWO YEAR FOLLOW-UP STUDY IN PATIENTS WITH DEEP VEIN THROMBOSIS

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Impairment of the fibrinolytic system is generally accepted to contribute to the development of deep vein thrombosis (DVT). However, limited information is available concerning the time-dependent evolution of hypofibrinolysis.
187 patients (110 females, 77 males, aged 15 - 63 years) who had suffered from DVT were investigated 2 years after the thrombotic occurrence. Laboratory investigation included the assessment of the total fibrinolytic capacity (fibrin difference assay, euglobulin clot lysis time, fibrin plate method) and measurement of tissue-type plasminogen activator (t-PA) and plasminogen activator inhibitor (PAI-1).
Hypofibrinolysis was found in 27 % of all patients. Patients who had suffered from recurrent DVT demonstrated higher PAI-1 activities compared to patients with one thrombotic event ($p < 0,001$).
54 patients were reinvestigated two years later. 17 patients who had been classified in the first investigation as Non- or Poor-Responder demonstrated permanent hypofibrinolysis. In contrast, from 30 patients who had been classified in the first investigation as Responders only 11 patients showed normal fibrinolytic capacity.
In conclusion, a dynamic follow-up of the fibrinolytic capacity is recommended in order to obtain detailed information about the evolution and significance of hypofibrinolysis.

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RESULTS OF AUTOLOGOUS BONE MARROW TRANSPLANTATION (ABMT) IN 20 ALL PATIENTS AFTER A 5-YEAR FOLLOW-UP. Annaloro C., Mozzana R., Butti C., Della Volpe A., Soligo D., Uderzo M., Lambertenghi-Deliliers G.

The role of ABMT in prolonging EFS in ALL is still a matter of debate. From 1985 to 1991, 20 ALL patients (17 adults and 3 children, 14 male and 6 female, median age 18 years, range 10-39) underwent autografting. In 7 adult patients, at high risk of relapse according to conventional prognostic factors, ABMT was performed in 1st CR as late intensification, 9 to 16 months (median 10) after achievement of CR; previous chemotherapy included aggressive regimens. In 6 adult patients, ABMT was performed soon after liquoral remission (median 1 month) following isolated central nervous system (SNC) relapse. The remaining 7 patients, including the three children, received ABMT in CR after one or more hematological relapses. In all but 3 cases, bone marrow (BM)was harvested in the same phase in which ABMT was performed. In all patients conditioning regimen included HD Ara-C (3 g/m^2 every 12 hours over 2 consecutive days), HD cyclophosphamide (60 mg/m^2/day over 2 consecutive days) and TBI, 1000 cGy fractionated over 3 consecutive days. No transplant related deaths were recorded; recovery of peripheral blood values occurred in all cases. As at March 31, 1992, the median follow-up from ABMT was 54 months (range 6-66). Four patients transplanted in 1st CR were in continuous (C) CR and 3 had relapsed; the median EFS had not been reached and the 5-year EFS chance was 57%. Four patients transplanted after meningeal relapse were in CCR and 2 had relapsed (1 CNS and 1 BM); the median EFS had not been reached and the 5-year EFS chance was 53%. One patient transplanted after hematological relapse was in CCR and 6 had relapsed; the median EFS was 6 months.The present experience emphasizes the role of ABMT in prolonging EFS after isolated meningeal relapse; the results of our small series are far better than expected from conventional rescue chemotherapy. The antileukemic activity of previous chemotherapy on BM disease, as well as of the conditioning regimen on extramedullary disease, may have contributed to the above figures. Although regarding high-risk adult ALL cases, the results of the 1st CR series do not allow any reliable conclusion on ABMT in this disease-phase. The discouraging EFS in patients transplanted in CR after hematological relapse is in agreement with other reports in the literature and is mainly attributable to the difficulties in managing poor quality CR with a heavy residual disease-burden Substantial improvements in grafting techniques are therefore required to hypothesize a less fortuitous role for ABMT in these patients.

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EXPLORATORY ANALYSIS OF PROGNOSTIC FACTORS IN LONG-TERM CLINICAL TRIALS WITH CENSORED OUTCOME. AN EXAMPLE : CHRONIC MYELOGENOUS LEUKEMIA

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Clinical trials are rarely designed with the main aim of determination of prognostic factors. Such analysis is usually exploratory in nature and may be helpful in hypothesis generation and design of further trials. The aim of this paper is to investigate two methods for the identification of prognostic factors with censored data- the Cox proportional hazards regression model and the Classification and Regression Tree (CART) technique. The Cox-model is used to assess the impact of independent risk factors on the outcome variable, while CART is concerned with extracting prognostically homogeneous subgroups of patients. This method separates the population into two groups in each of a series of steps using each potential prognostic variable and the "best" split is selected based on a "goodness of split" statistic. The terminal nodes of the tree indicate the prognostic subgroups which can then be ranked. The CML-study is a multicentre three-arm randomized clinical trial (Busulfan, Hydroxyurea, IFN-α), in which a total of 624 patients from 60 centres in Germany and Switzerland were randomized. 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 analysis). Since missing values lead to exclusion of patients from the analysis for both techniques, completeness of data also played a role in the initial variable selection. Of 427 Ph-positive patients, 367 patients had complete data for all 17 variables and were included in the analysis. Of seven prognostically significant variables, five were common to both methods - age, Karnofsky-Index, blasts, erythroblasts in peripheral blood and organomegaly-related symptoms. The results complement each other in the sense that a maximum of information is gained about the study population.

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PULMONARY METASTASECTOMY AS SECONDARY TREATMENT FOR TESTICULAR TUMORS

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Patients presenting with synchronous and metachronous metastases of nonseminomatous testicular tumors are candidates for pulmonary metastasectomy after definitive primary treatment, which includes semicastration, eventually retroperitoneal lymphadenectomy, and definitely chemotherapy. 87 such patients aged from 14 to 79 years (m : 27 yrs) were channelled to pulmonary metastasectomy from 1972 - 1989, this number representing 15.8% of the pulmonary metastasectomy performed during the same period. Of the 96 operations performed, 9 were redo operations.Both median sternotomy and posterolateral standard thoracotomy and later transverse thoracotomy were standard surgical approaches used to remove the single and multiple metastases randomly distributed in both lungs. Wedge and atypical segment resections (75%),lobectomy (13.5%),segmentectomy (7.3%), pneumonectomy (4.2%) and mediastinal lymphadenectomy (100%) were performed.Of the 96 patients, metastasectomy was radical in 67 and non-radical in 20. The 30-day mortality was 2.5%. The life table shows a survival rate of 79%, 64% and 58% at 1 year, 3 resp.5 years. A comparison of the 5 year survival rate following pulmonary metastasectomy for other malignancies - breast cancer: 33%, hypernephroma: 38%, colorectal carcinoma: 19%, and osteosarcoma: 22% - shows good prognosis for testicular tumors. Of all the prognostic factors examined, radical operation seems to be the most significant factor influencing the prognosis.

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HEPATOLIENAL CANDIDIASIS IN PATIENTS WITH ACUTE LEUKEMIA - EXPERIENCE IN DÜSSELDORF

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A distinct syndrome called hepatosplenic or chronic visceral candidiasis is increasingly described in patients with acute leukemia. According to previous reports this infection is characterized by persistent fever unresponsive to antibiotics, abdominal symptoms, often with elevated serum alkaline phosphatase levels, and typical ultrasonographic and CT findings. Standard therapy is amphotericin B and flucytosine, but some authors recently described superior results with long-term high-dose fluconazole treatment. We report our experience in 5 patients with that syndrome during the last two years.

All patients suffered from acute leukemia (AML = 4, ALL = 1) and had fever during chemotherapy-induced bone marrow aplasia. Ultrasonography and CT scanning demonstrated typical lesions in liver and/or spleen. Three patients had significant increases in the candida titers, two patients had elevation of alkaline phosphatase levels.

Three patients received triazoles for treatment (1 patient itraconazole 500mg/d for 9 days, two patients 400 mg fluconazole/d for 13 d and 29 days, resp.). Two of them defervesced within two days, one had a prolonged critical clinical course with defervescence after two months of treatment, but death due to sudden cardiac death occurred eight weeks later.

Two patients received intravenous amphotericin B and flucytosine. One had a good response with defervescence within three days, the other had a prolonged febrile course for three weeks before defervescence.

The radiologic abnormalities disappeared completely in two patients within three months, while in the other three radiologic abnormalities persisted for more than 9 months, but without clinical signs of ongoing infection.

We conclude that hepatosplenic candidiasis is a syndrome which covers a spectrum of disease activity ranging from asymptomatic radiologic abnormalities slowly regressing after achieving complete remission without long-lasting antifungal therapy to a life-threatening disease despite specific treatment. Therapy should be individual for each patient and adjusted to the clinical course of disease. In our experience defervescence is the critical factor which may allow cessation of further antifungal treatment after recovery of the neutrophil count.

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COMBINED IN VIVO/EX VIVO T-CELL DEPLETION AS GVHD PROPHYLAXIS IN PATIENTS WITH ACUTE LEUKAEMIA IN FIRST REMISSION: IS SURVIVAL IMPROVED?

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Transplant related mortality and relapse after bmt has a negative influence on the outcome of patients transplanted for acute leukaemia in first remission. Transplant related mortality includes graft-versus-host disease, infections and graft failure. To prevent gvhd and associated infections without increased graft rejection, a protocol of combined in vivo/ex vivo T-cell depletion (Campath IgG 20 mg i.v. for 5 days and Campath IgM T-cell depleted graft with no further immunosuppression was initiated. Up to now 15 adult patients (median age 39 years, 21-51) were transplanted. No rejection and no acute or chronic gvhd occured. 2 patients relapsed after bmt and died. 1 further patient died due to interstitial pneumonitis. 12 patients are alive in CR. With a median follow up of 9 Mo (1-21) probability of survival is 72%, disease free survival is 77% and transplant related mortality is 7%. We compared these results to 3 historical control groups with different regimens of gvhd prophylaxis. 1. MTX group (n = 15): With a median follow up of 127 (108-140) months after bmt probability of survival is 40%, dfs is 40% and transplant related mortality is 60% (mainly gvhd + infection). 2. Campath group (only ex vivo T-cell depletion, n = 25): With a median follow up of 79 (55-95) months probability of survival is 52%, dfs is 43% and transplant related mortality is 36% (mainly rejection and infection). 3. MTX/CSA group (n = 30): With a median follow up of 40 mo (4-59) after bmt probability of survival is 72%, dfs 67% and transplant related mortality is 17%.

In comparison to the historical control groups combined T-cell depletion effectively prevents acute and chronic gvhd without increase of graft rejection and transplant related mortality is reduced. Survival and dfs is at least comparable to the MTX/CSA group and longer follow up is needed for definite analysis.

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HIGH-DOSE CHEMOTHERAPY AND GM- OR G-CSF FOR MOBILIZATION OF PERIPHERAL BLOOD STEM CELLS IN PATIENTS WITH NON-SEMINOMATOUS GERM CELL TUMORS

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Numerous chemotherapy regimens provide adequate levels of circulating stem cells in the peripheral blood (PBSC) for collection via the cytapheresis technique. We evaluated six patients with non-seminomatous germ cell tumors (NSGCT) treated with a minimum of 4 chemotherapy cycles of CDDP, VP16 and Ifosfamid (0.15, 1.0 and 8.0 g/m^2 total dose per cycle for 5 days; PEI) followed by GM- or G-CSF (5 µg/kg BW/day for an average of 10 days). The average duration of the neutropenia (< 0.5 x 10^9/l) after chemotherapy was 5.5 days (range 1-19 days). With increasing number of treatment courses a tendency to prolonged and deeper aplasia was observed. The flow cytometrical investigation (dual-color staining) of the circulating CD34+ cell recovery yielded the highest values after the second chemotherapy course (1-2.5% of the total WBC). The peak values of CD34+ cells were reached simultaneously with the monocyte peak and after the WBC's had recovered above 1.0 x 10^9/l. G-CSF contributed to higher cell counts as compared to GM-CSF (18.3 ± 3.2; n = 15 vs 6.7 ± 1.3 x 10^9 WBC/l; n = 10; mean ± SD and up to > 1000 x 10^9 platelets/l). Due to the small number of patients evaluated the clinical relevance of this phenomenon remains unclear. A mean of 5.8 stem cell aphereses per patient (range 2-10) after PEI-chemotherapy cycles was performed. Initially a minimum of 2.5 x 10^8 MNC's, 8.0 x 10^4 CFU-GM's was collected. Currently 2.0 x 10^6 CD34+ cells per kg BW were determined as the lowest limit of hematopoietic progenitor cells to be collected.

Thus it can be concluded, that in patients with metastatic NSGCT the escalated PEI regimen followed by hematopoietic growth factors provides appropriate counts of circulating stem cells. The collection of PBSC for autografting should be performed during the early chemotherapy courses, thereby reducing the number of necessary apheresis procedures. The retransfusion of PBSC combined with GM- or G-CSF may enable to further escalate the PEI regimen without increasing treatment morbidity and to achieve a higher dose intensity, which may result in a higher cure rate for patients with far advanced germ cell tumors.

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MYELODYSPLASTIC SYNDROMES:
RESPONSE TO AGGRESSIVE CHEMOTHERAPY AND PROGNOSTIC FACTORS FOR TREATMENT OUTCOME

C. Aul, V. Runde, A. Heyll, and W. Schneider

50 patients with advanced MDS presenting with a Karnofsky score of at least 60% were treated with conventional antileukemic therapy. The median age of patients was 52 years (range, 16-72). MDS subtypes at the start of treatment were RAEB in 2 and RAEB-T in 17 cases. 31 patients were treated after transformation of MDS to AML. For remission induction the following protocols were used: TAD-9 n=40, double induction TAD-9/HAM n=2, double induction TAD-9/TAD-9 n=2, HAM n=1, Ara-C/idarubicin n=5. In this series, 28 patients (56%) entered CR, and 7 patients obtained a PR, defined according to the CALGB criteria. Early death occurred in 5 cases, and 10 patients (20%) had refractory disease. No unusual toxicities of chemotherapy were noted. The median duration of bone marrow aplasia (leukocytes < 1x10^9/l and/or platelet count < 20x10^9/l) for patients achieving CR after TAD was 19 days (range, 6-39). Despite maintenance treatment, the median duration of CR was relatively short (11 months). The factors most strongly associated with successful remission induction were: 1. a comparatively low blast count (< 30%) at the start of therapy (CR rates: 82% vs. 31%) 2. presence of Auer rods in granulocyte precursors (61% vs. 38%). We conclude that intensive chemotherapy can be successfully administered to patients with MDS. Treatment is particularly successful in younger patients with Auer rod-positive blasts and should be commenced before patients transform to overt AML.

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SERUM DEOXYTHYMIDINE KINASE IN MYELODYSPLASTIC SYNDROMES

C. Aul, V. Runde, U. Germing, N. Gattermann

Deoxythymidine kinase (TK) which catalyses the phosphorylation of deoxythymidine to deoxythymidine monophosphate is an important salvage pathway enzyme in the synthesis of DNA. Previous studies have shown that measurements of the serum TK (sTK) activity provide prognostic information in patients with Hodgkin's disease, Non-Hodgkin's lymphomas, multiple myeloma and acute leukemia. The aim of the present study was to examine the value of sTK in the myelodysplastic syndromes (MDS). Using a radioenzyme assay, sTK was determined in 114 patients classified as having RA (n = 17), RARS (n = 15), RAEB (n = 35), RAEB/T (n = 22) and CMML (n = 25). Compared to 69 healthy probands (mean ± SDM: 3.1 ± 1.2 U/µl), 87 (76%) MDS patients presented with pathological sTK levels at the time of diagnosis. Excessively elevated enzyme activities (> 100 U/µl) were measured in 16 patients, all belonging to advanced subtypes of MDS (RAEB, RAEB/T and CMML). sTK levels were not found to be correlated with known risk factors in MDS, such as peripheral leukocyte counts (r = 0.29), hemoglobin concentration (r = 0.12), platelet counts (r = 0.12) and percentage of medullary blast cells (r = 0.17). As shown by life table analysis, the sTK activity had a statistically significant influence on the survival probability of MDS patients. The median survival of patients with sTK levels < 10 U/µl was 40 months, as compared to 11 months in patients with sTK levels > 10 U/µl (Breslow: $p < 0.005$, Mantel-Cox: $p = 0.001$). Raised sTK levels were not correlated with an increased risk of AML transformation ($p = 0.44$).
These data suggest that the serum TK activity may be used as an independent prognostic parameter for assessing the survival of MDS patients.

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SEQUENTIAL TREATMENT WITH RECOMBINANT HUMAN GRANULOCYTE-MACROPHAGE COLONY-STIMULATING FACTOR (GM-CSF) AND HUMAN ERYTHROPOIETIN (EPO) IN PATIENTS WITH MYELODYSPLASTIC SYNDROMES

C. Aul, V. Runde, A. Ebert*, L.E. Feinendegen*, and W. Schneider

The efficacy of sequentially applied GM-CSF and EPO was examined in 7 patients with myelodysplastic syndromes (MDS). GM-CSF was administered subcutaneously at a dosage of 150 µg/m^2/d for 10 days. EPO was given subcutaneously at a dosage of 100 U/kg/d from day 11 to day 38 or longer if response occurred. FAB-subtypes were: RA n=3, RARS n=2, RAEB n=2. All patients were transfusion-dependent (2-6 units of packed red blood cells per month) and had markedly increased serum EPO levels (range, 159-3618 mU/ml). Prior to therapy and 4 to 6 weeks after initiation of treatment, ferrokinetic studies including red cell iron utilization, plasma iron clearance rate, red cell life span studies and whole body iron distribution analysis were performed in each patient. At present, one patient is not yet evaluable. Another patient died on day 39 of treatment due to septic complications. Of the remaining 5 patients two patients became transfusion-independent after 2 months of treatment with EPO. In 3 cases no increase in hemoglobin concentrations was found. With respect to the responders' delayed improvement in hemoglobin values, we cannot exclude that the beneficial effect resulted from the administration of EPO alone. Side effects, only observed during ap–pli–cation of GM-CSF, included fever in all cases, bone pain in 3 cases and pleural effusion and arrhythmia in one patient. Additional patients will be required to evaluate the efficacy of this treatment protocol.

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HOW FREQUENT ARE MYELODYSPLASTIC SYNDROMES?

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Although most haematologists perceive a rising prevalence and incidence of MDS, reliable epidemiological data on these disorders are largely lacking. The bone marrow register of the University of Düsseldorf allowed us to assess among other epidemiological features the incidence of MDS, which was compared to that of AML. Among a total of 18,416 different patients registered between 1975 and 1990, 584 cases of MDS (3.2%) and 506 cases of AML (2.8%) were identified. Over the study period, the percentage of newly diagnosed MDS rose from 1.3% to 4.5%, while there was no upward trend for AML. Among all patients undergoing bone marrow biopsy, the proportion of those over 60 years of age increased from 41.9% in 1975 to 54.1% in 1990. We found a strong correlation between the proportion of elderly patients and the relative frequency of MDS diagnoses. 31 patients (5.3%) were classified as secondary MDS because of previous treatment with cytotoxic chemotherapy and/or irradiation for a variety of malignancies. 12 patients were identified in whom occupational exposure to organic solvents could not be ruled out.
For calculating age-specific incidence rates, the analysis was confined to the town district of Düsseldorf (575,000 inhabitants), because exact demographical data were available for this population. In the last quinquennium of the study period (1986 - 1990), myelodysplastic syndromes were more frequent than AML in the age group 50 to 70 years (4.9 vs. 1.8/100,000/year). In patients over 70, the incidence of MDS was more than three times that of AML (22.8 vs. 6.7/100,000). In this age group, men had a higher incidence of MDS (33.9/100,000) than women (18/100,000). Crude annual incidence (all age groups) was also higher for MDS (4.1/100,000) than for AML (2.1/100,000) in recent years.
We conclude that MDS are relatively common haematological neoplasias. The rising incidence in recent years is probably not due to changes in etiological factors, but may reflect increased awareness on the part of physicians and extended use of diagnostic procedures in elderly patients.

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RESULTS OF A PILOT PHASE II STUDY WITH IFN ALPHA 2 B AND LOW DOSE ARA-C FOR TREATMENT OF CHRONIC PHASE CML

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Treatment of chronic phase CML results in cytogenetic remissions in 20 % of the patients. The fact that in this group of patients an excellent long term survival has been demonstrated suggests that induction of cytogenetic remissions might protect patients from progression into blastic transformation. Recently increased cytogenetic response rates has been reported after combined therapy with rIFN alpha and low dose ara C.
We report on the results of the pilot phase of a multicenter trial with rIFN alpha 2b and low dose ara C in 34 patients with chronic phase CML. Treatment consisted of 5 MU rIFN alpha 2b/d s.c. and 20 mg Ara C for 14 days every fourth week. Combined therapy of rIFN and low dose Ara C was tolerated and the toxicity profile moderate. Major side effects were flu like syndrome, thrombopenia (1 > WHO 2) and mucositis (1 > WHO 2). Preliminary clinical results indicate a hematologic response rate of 60 %. Reduction of the Ph1 positive clone of more than 10 % was observed in 50 % of the patients (median observation time 3 months). The confirmation of this increased cytogenetic response rate and the question whether these patients are also protected from progression to the blast crisis will be the objective of a consecutive clinical trial.

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IL-2, IFN-γ AND IL-3 DIFFERENTLY AFFECT CIRCULATING PRECURSORS OF CTL (CTLp) IN MAN

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Synergism of specific antigen and cytokines for clonal expansion and differentiation of CTLp has generally been accepted. However, the primary effect of cytokines on CTLp without challenge by specific antigen remains unclear.

We investigated, wether systemic treatment with cytokines affects the clone size of circulating alloreactive CTLp in man. We used limiting dilution analysis to determine CTLp frequencies in patients with non-hematologic diseases before and after three days of subcutaneous application of either IL-3 (2.5, 5.0 or 10.0 μg/kg/d), GM-CSF (5μg/kg/d), IFNγ (400μg/d), IFNα (5x10⁶ IU/d) or IL-2 (4.8x10⁶ IU/m² BD). Simultaneously clone sizes of circulating autoreactive CTLp were determined by split well analysis.

Preliminary data suggest only minor influences of low dose IL-3 on CTLp frequencies. Intermediate dose IL-3 reduced numbers of circulating CTLp. Further escalation of dose interestingly overcame this effect and lead to significant expansion of these cells. Significant expansion was also observed under systemic treatment with IL-2. In contrast IFNγ markedly diminished the clone size of circulating alloreactive CTLp. GM-CSF and IFNα did not exhibit measurable effects. Furthermore we could not find autoreactive CTLp at any time tested.

In conclusion systemic application of IL-2, IFNγ or IL-3 differently affects circulating precursors of CTL in man. This may have impact on the responder status to specific antigens in vivo.

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EMPIRIC THERAPY FOR FEBRILE PATIENTS WITH SEVERE GRANULOCYTOPENIA: RESULTS OF TWO DIFFERENT ANTIMICROBIAL REGIMENS

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The clinical efficacy of two empiric antimicrobial treatment regimens for infection in neutropenic patients with acute leukemia during induction and intensification therapy was evaluated. The first program of therapy, applied in 38 patients, consisted of three sets of antibiotics (gentamycin + carbenicillin or azlocillin, amikacin + cefradine or cefuroxime, netilmycin + cefotaxime or ceftazidime) used one after another in case of persistent fever in spite of treatment for 72 hours. In addition, flucytosine was applied after 72 hours of antibiotic therapy. It was replaced by amphotericin B after next 72 hours in case of ineffective treatment. In the second program, used in 22 patients, amikacin with piperacillin or azlocillin were applied. Amphotericin B was given after next 48 hours if no response was observed. Vancomycin was added after 72 hours of persistent fever. Ureidopenicillin and amikacin were then replaced by cefoperazon or ceftazidim with netilmycin, and in case of lack of improvement imipenem was given after next 48 hours. The response rates in patients during induction and intensification therapy for the first regimen was 68% and 96%, and for the second 75% and 95%, respectively.

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LOCALIZATION AND CLASSIFICATION OF MALIGNANT TUMORS BY IMMUNOSCINTIGRAPHY, RECEPTOR SCANNING, AND POSITRON-EMISSION-TOMOGRAPHIE (PET)

R. Bares and U. Buell

Current procedures for in-vivo tumor-detection are aiming at morphological tissue alterations caused by malignant disease. More specific immunological or metabolic changes were up to now accessible to experimental in-vitro tests only. Recently, three nuclear medicine imaging techniques have been developed with the potential to provide the missing data. Immunoscintigraphy uses radiolabelled monoclonal antibodies against tumor associated antigens (e.g. CEA, CA 12-5). After i.v. injection antibodies bind at antigen positive tumor sites and can be localized by gamma camera imaging. Relevant findings could be obtained in local recurrencies of colorectal (differentiation between tumor and scar) and ovarian carcinomas (peritoneal spread). Successful tumor detection can also be achieved by receptor scanning after administration of radiolabelled ligands such as diestradiol (breast cancer) or somatostatin analogs (GEP-tumors, malignant lymphomas). PET using radiolabelled substrates of carbohydrate, amino, or nucleinic acid metabolism yields quantitative information on metabolic and/or proliferative activity of tumors. Clinical studies in patients with pancreatic cancer or malignant lymphomas indicate that improved tumor detection and treatment monitoring can be achieved by measuring uptake of 18-F labelled deoxyglucose. Thus, immunoscintigraphy, receptor scanning, and PET complement conventional diagnostic procedures in oncology and may help to optimize treatment by specific tumor data (receptor/antigen status, metabolic activity).

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ACCUMULATION OF GENETIC LESIONS DURING THE GENERATION OF MYELOID NEOPLASIAS

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Leukemogenesis has long been thought to be a multistep process. The advent of molecular biologic technology now has provided the tools to characterize several of the genetic alterations that cause disorders in the control of cell proliferation, differentation, and intercellular communication, and in turn lead to malignant transformation. Two sorts of genomic lesions have emerged as the salient components of this process: the activation of oncogenes coupled with the inactivation of tumor-suppressor genes. Relatively few of these genetic defects are exclusively associated with a specific leukemia subtype, such as the PML-RARα recombination underlying t(15;17) in AML-M3. Chimeric oncogenes as e.g. BCR-ABL or DEK-CAN corresponding to t(9;22) and t(6;9) are observed in different myeloid and lymphoid neoplasias. A third category of tumorgene alteration is not only present in a broad spectrum of hematopoietic malignancies as well as solid tumors, but is also detectable in premalignant states (e.g. myelodysplastic syndromes) and even in clinically normal individuals. Cases in point are RAS gene and P53 mutations. These data, complemented by cytogenetic and X-chromosome inactivation studies, suggest that the accumulation of genomic lesions constitutes the critical determinant for the manifestation of myeloid neoplasias. Respective molecular genetic parameters bear major clinical significance for the subclassification and monitoring of leukemia patients.

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DIFFERENTIATION OF BLAST CELLS IN MDS AND ACUTE MYELOID LEUKEMIA BY AUTOMATED IMAGE ANALYSIS.

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Cytologically undifferentiated bone marrow blast cells from 12 patients with MDS (RA,RARS) who consequently developed a secondary acute myeloid leukemia (AML) were analysed by high resolution image analysis including texture and color features of cell nucleus and cytoplasm. All features were calculated in a decision tree algorithmn to determine the subtypes of blast cells. No significant differences in blast cell morphology between MDS blasts and subsequent AML blasts were found. In contrast we found significant differences between blasts from secondary AML and primary (de novo) AML. The results suggest, that clonal evolution of blast cells in MDS might be a prerequisite for the progression into acute myeloid leukemia. The clear distinction of blast morphology between primary AML and secondary AML points to different entities of leukemia. Morphology of blast cells determined by image analysis might be a useful tool to distinguish blast subtypes especially in myeloid malignancies.

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INTERPHASE CYTOGENETICS BY FLUORESCENT IN-SITU-HYBRIDIZATION (FISH) FOR MONOSOMY 7 ASSOCIATED MYELOID DISORDERS.

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FISH with chromosome specific probes is a promising tool for detection of numerical chromosome aberrations in interphase nuclei and terminally differentiated cells. By bypassing the need for metaphase preparations it may avoid the bias resulting from the analysis of merely proliferating cells and provide useful clinical information in hematological disorders. We studied peripheral blood and bone marrow nuclei of 15 previously karyotyped cases of monosomy 7 associated myeloid disorders [3 childhood myeloproliferative disorders, 2 myelodysplastic syndromes, 10 acute myeloid leukemias (AML)] using a chromosome 7 specific centromeric probe (7Z1/2, ONCOR). Validation of optimized hybridization conditions on 32 disomic controls produced two signals in 96,8% ± 2,1% of nuclei. Monosomy 7 was readily confirmed in all study cases (13% to 95% of nuclei) during active disease. In two remission samples of AML, 3% and 4% of nuclei respectively had one signal only, therefore ranging below the established detection threshold for monosomy 7. In 3 cases a minority (23% to 32%) of nuclei were found to be monosomic by FISH, while virtually all metaphases were so by standard cytogenetics. In two of them, the discrepancies between both methods can be explained by a more active proliferation of the malignant clone. Metaphases of the third contained a small acrocentric marker, which by FISH could be identified as pericentromeric region of chromosome 7. Interphase nuclei here were indistinguishable from normal cells.
Conclusions: FISH yealds rapid and important complementary results to classical karyotyping. However, in this setting, it is not suitable for the detection of minimal residual disease. Combining FISH with membrane immunophenotyping should considerably strengthen the power of the method - studies using this strategy are currently in progress.

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PLASMA CELL LEUKEMIA AND BURKITT'S LYMPHOMA IN A CARDIAC TRANSPLANT RECIPIENT

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Patients receiving immunosupressing therapy after organ transplantation are at risk to develop unusual types of lymphoproliferative disease. We present a case of cardiac transplant recipient with two different B-cell neoplasms.
In 1988 at the age of 54 he received a cardiac transplant for ischemic heart disease followed by immunosuppressive therapy with azathioprin, prednison and cyclosporin. In 1989 a fracture of the thoracic spines 11 and 12 was noted. In 1990 an increase of the light chains type kappa was found. In 7/91 his condition deteriorated with back pain, nausea, hematemesis and impairment of renal function. Clinical examination revealed no lymphadenopathy, no hepatosplenomegaly. The main laboratory findings were: LDH 7920 U/l; creatinine 3.9 mg/dl; BUN 134 mg/dl; hemoglobin 8.6 g/dl; WBC $14x10^9$/l with 29% immature plasmacells, platelets $40x10^9$/l. Immunphenotyping of peripheral blood mononuclear cells showed that 50% of them expressed cytoplasmatic kappa light chains and CD 38. The bone marrow differential revealed 57% of the nucleated cells to be plasmablasts and plasmacells. Chromosome analysis of peripheral mononuclear cells demonstrated a clone with trisomy 13 and a translocation 8;14. On the day after his admission he required laparatomy for a massive upper gastrointestinal bleeding. A tumor in the bulbus duodeni was resected, histologic examination showed a Burkitt's lymphoma. Chemotherapy with vincristin, adriamycin and steroids was started but his condition deteriorated and he died. Post mortem examination showed infiltration of the lungs, the liver, the kidneys and the bone marrow by plasmacells.
This case is unusual as two different B-cell neoplasms developed in a immunosuppressed patient. Another unusual finding is the diagnosis of a Burkitt's lymphoma which is infrequently found in transplant patients.

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COAGULATION INHIBITORS IN PULMONARY CANCER PATIENTS

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Multiple mechanisms are involved in the pathophysiology of hypercoagulability in cancer due to balance between coagulatory and anticoagulatory system. 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 and profibrinolytic active PC but is now able to influence local activation of complement system on phospholipid bilayers. Therefore PS functions as a link between coagulation and complement system. Pulmonary cancer patients are known to have an increased risk of thromboembolic complications.In the present study we have investigated the role of PC, PS and C4bBP in patients suffering from small-cell (SCC, n=30) and non-small-cell (NSCC, n=50) pulmonary cancer.
Methods: Protein C concentration: ELISA protein C and Protein S concentration: EID protein S from Boehringer Mannheim,FRG. Free PS was determined after precipitation of C4bBP bound PS with C4bBP-ASSERA^R-PLATE test from Diagnostika Stago, FRA. All results are expressed in percent of increase or decrease ± SE as compared to healthy controls (n=52).
Results: **SCC-pat.**: increase of PStotal 44.23% ± 5.96%; p<0.001), of PSbound 62.5% ± 4.62%; p<0.001, of C4bBP 46.6% ± 7.91%, decrease of PSfree 12.6% ± 1.02%; p<0.001, PC 9% ± 5.02% n.s change. **NSCC-pat.**: increase of PStotal 48.2% ± 4.97%; p<0.001, of PSbound 57.5% ± 4.92; p<0.001, of C4bBP 84.5% ±6.83%; p<0.001, of PC 7.45% ± 2.87%; p<0.02, decrease of PSfree 6% ± 2.29% p<0.01.
Conclusion: Decreased PSfree-levels, due to increased binding to C4bBP is one reason for the elevated risk of thromboembolic complication in patients suffering from pulmonary cancer.

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USE OF A YAC CLONE CONTAINING THE BCR GENE FOR THE DETECTION OF THE PHILADELPHIA CHROMOSOME IN HEMATOLOGICAL MALIGNANCIES

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The Philadelphia (Ph1)-chromosome is the result of a reciprocal translocation between chromosomes #9 and #22 (t(9;22)(q34;q11)). It is present in 95% of patients with CML and is an important marker of this disease. In adult ALL, the Ph1-chromosome is the most frequent chromosomal abnormality and associated with poor prognosis. For the rapid detection of the t(9;22) fluorescence in situ hybridization (FISH) could become an invaluable tool. So far, approaches using chromosome specific DNA libraries and flanking cosmid probes have been used. We report preliminary data on experiments using the yeast artificial chromosome (YAC) clone D107F9 (kindly provided by Dr. H. Riethman, Philadelphia and Dr. T. Cremer, Heidelberg) for the detection of the Ph1-chromosome in a patient with Ph1-positive ALL with the breakpoint in the major breakpoint cluster region of the BCR gene. The insert of this clone is 215 kb in size and contains genomic sequences of the BCR gene (Lengauer et al., Cancer Res., in press). In situ hybridization resulted in specific signals on chromosomal band 22q11 on the normal homolog, on the telomeric part of the Ph1 chromosome and on the 9q+ chromosome. In this patient, the signals in the cell nuclei were of limited quality and not sufficient for interphase diagnostics. Further studies including two color hybridization with additional cosmid probes mapping to the c-abl protooncogene and more patient material are in progress. These analyses will prove, if the YAC clone D107F9 also spans across the minor breakpoint cluster region and thus is a suitable tool for the general detection of the bcr-abl fusion in acute leukemia.

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TRISOMY 8 IN MYELOID LEUKEMIAS: SENSITIVITY OF DETECTION ON PREVIOUSLY STAINED BLOOD SMEARS

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With the development of fluorescence in situ hybridization (FISH), "interphase cytogenetics" has become possible. So far, only scarce data on the application of this technique to previously stained blood smears have been reported and a systematic assessment of the hybridization quality on peripheral blood smears has not been performed. In this study we used FISH with an alphoid probe (D8Z1) for the detection of trisomy 8 on previously stained peripheral blood smears. The results were compared with hybridization experiments performed on slides with chromosome spreads prepared for G-banding analysis (methanol/acetic acid fixed slides = MAF-slides). In parallel, each patient was examined by conventional cytogenetic techniques. So far, 5 healthy volunteers (controls) and 15 pts. have been examined, 9 with AML, 4 with CML-blast crisis and 2 with RAEB. In the controls, similar results were obtained for blood smears and MAF-slides (2 signals: 92.8%+/-3.1% vs. 92.2%+/-4.2%, 3 signals: 2.1%+/-0.9% vs. 1.5%+/-0.6%). 11 pts. did not exhibit a trisomy 8 on classical cytogenetic analysis. Their FISH results did not show any significant differences on peripheral blood smears and MAF-slides. Again, the frequency of nuclei exhibiting three signals was low (1.9%+/-0.6% vs. 2.2%+/-0.8%) and two signals were seen in 92.2%+/-4.2% (blood smears) versus 89.1%+/-6.3% (MAF-slides) of the nuclei. Two pts. had a trisomy 8 in all metaphases. Using FISH three signals were detected in the majority of cells in these cases (82.6% and 67.1% on blood smears, 79.1% and 52.3% on MAF-slides). In the remaining two pts. a subclone with three (pt.14) and four (pt.15) chromosomes #8 was present. Again, these abnormalities were detected equally well on blood smears and MAF-slides. We conclude that using a chromosome #8 specific alphoid probe FISH can be performed on blood smears with the same sensitivity and specificity that is achieved by using MAF-slides. As morphological features of the cells remain preserved, a correlation of phenotype and karyotype in blood smears is possible.

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A NEW CENTRAL VENOUS ACCESS MINI-PORT IMPLANTED ON THE PROXIMAL FOREARM OR ON THE CHEST WALL

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A new mini-port system (Pharmacia Deltec, Freiburg, FRG) was used for central venous access in 37 pts. Fifteen pts. (with solid tumors x8, lymphomas x4, acute leukemias x3) had the port positioned on their proximal forearm, 22 pts. (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 peripheral access (P.A.S.) port and 87 days (range 0-480) for the chest wall port with cumulative observation periods of 3778 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 pts. with the P.A.S. port and 18 of 22 pts. with the chest wall-positioned mini-port. There were 6 pts. with peripheral vein thrombosis, 3 pts. with reversible port occlusion, 3 pts. with port infections for the P.A.S. port and 1 pt. with port infection, 1 pt. with dislocation of the catheter tip, 1 pt. with unsuccessful implantation, 2 pts. with paravasations, 1 pt. with port occlusion for the chest wall position. However, loss of function and/or explantation were the consequences for only 5 mini-ports (P.A.S. port x2, chest wall port x3). A new electromagnetic catheter tracking system (Cath-Finder, Pharmacia) was used in 8 pts. for implantation of P.A.S. port. When compared with x-ray detection the system predicted the catheter tip accurately in 7 pts. and changed surgical procedure by predicting wrong positioning in 3 pts. In conclusion, the new mini-port can be used like the older and larger systems with less cosmetic damage. Chest wall positioning of the port was accompanied with less frequent complications and thus, acceptance for the central chest wall position was better than for the position on the proximal forearm by 22 pts., 9 physicians, and 8 nurses interviewed. At the moment we are testing positioning of the mini-port on the distal upper arm. Klinikum Steglitz, Freie Universität, Hindenburgdamm 30, 1000 Berlin 45, FRG

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INTENSIVE CHEMOTHERAPY WITH VINCRISTINE, HIGH-DOSE ADRIBLASTIN, CYCLOPHOSPHAMIDE, PREDNISONE AND ETOPOSIDE (VACPE) IN HIGH MALIGNANT NON-HODGKIN LYMPHOMAS - AN ONGOING PHASE II TRIAL

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The prognosis of high-grade non-Hodgkin lymphomas (NHLs) depends on the induction of an early initial complete remission (CR). Several trials therfore focus on dose itensification of the induction therapy by addition of hematopoetic growth factors for reduction of hematotoxicity.

We conducted a phase II trial in patient with high-grade NHLs except lymphoblastic lymphomas using high-dose chemotherapy ± GM-CSF applicated in a short intervall. In detail, patients receive vincristine 2mg d1, adriamycin 25mg/m^2 d1-3, cyclophosphamide 800 mg/m^2 d1, prednisone 60 mg/m^2 p.o. d1-7 and etoposide 120mg/m^2 d1-3. in patients >60 yrs adriamycin is only applicated on d1+2 and etoposide reduced to 100mg/m^2 on d1-3. This cycle will be repeated every 3 weeks up to 5 cycles in stages I-III and 6 cycles in stage IV. Additionally, radiotherapy will be applicated for consolidation.

Up to now, 40 patients (pts.) with newly diagnosed high-grade NHLs are included into the study. 9/40 pts. are too early for follow-up and 3/40 pts. had early deaths. 28/40 pts. (11m, 15f) are evaulable for response. Most histological subtypes are centroblastic (12/28), pleomorphic T-cell lymphoma (3/28), immunoblastic (3/28), anaplastic Ki1+ (2/28) and 6 others. 4 pts. had stage I, 7 stage II, 6 stage III and 9 stage IV. In 21/28 (81%) a CR could be achieved. The median relapse free survival is not yet reached. The probality for CCR is 63%. The toxicity was moderate and may be reduced by GM-CSF. VACPE is a highly effective schedule in high-grade NHLs.

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INTERLEUKIN-2 BOLUS INFUSION AS LATE CONSOLIDATION THERAPY IN 2nd REMISSION OF AML

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Despite intensification of chemotherapy in acute myelocytic leukemia (AML), the remission duration remains unsatisfactory due to persistance of minimal residual disease (MRD). Here, the induction of graft versus leucemia (GvL) like reactions with interleukin-2 (IL-2) is an interesting new therapeutic approach. Therefore we conducted a multicenter phase II trial with IL-2 late consolidation in patients (pts) with 1st relapse of de novo AML.
The patients receive two cycles of IDAraC (2x600mg/m^2 d1-4) and VP-16 (100mg/m^2 d1-7) as relapse therapy and a third cycle as early consolidation. After a rest of 4 weeks, IL-2 is administered in a dosage of 9x10^6 IU/m^2 rIL-2 (EuroCetus, Frankfurt, FRG) as 1h infusion on day 1-5 and 8-12. After a rest of 4 weeks, this cycle will be repeated up to 4 cycles totally. Patients undergoing ABMT BMT receive IL-2 staring latest 6 weeks after ABMT, too.
Up to now, 31 patients were included into the study. 18/28 (64%) evaluable patients achieved CR after chemotherapy. 9 pts received IL-2 late consolidation, 3 after ABMT. 3 pts relapsed before IL-2 therapy, 2 pts refused further therapy and 4 pts are too early for evaluation. The median remission duration of pts, who received IL-2, is 14 months. 3 pts relapsed after 5,11 and 13 months. 6 pts have an ongoing remission (3+, 9+, 10+, 16+, 19+, 27+). The toxicity was moderate, so that the schedule could be applicated in most cases in an outpatient manner . Only two patients both after ABMT developed severe infections (sepsis, pneumonia) after IL-2. The follow-up of immunological parameters revealed a significant lymphocytosis with an increase of CD56$^+$ cells, induction of endogeneous TNF-alpha, IFN-gamma, IL-6 and adhesion molecules. 3/8 patients meanwhile exceeded 1st remission duration indicating a benefit of Il-2 for relapse free survival in AML.

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TOXICITY OF HIGH-DOSE CARBOPLATIN , ETOPOSIDE AND IFOSFAMIDE IN PATIENTS WITH LYMPHOMA AND GERM CELL TUMORS

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High dose chemotherapy (**HDT**) with autologous stem cell rescue (**ASCR**) is increasingly used for the treatment of solid tumors and hematologic malignancies. Beside dose, schedule and drug combination in a HDT regimen, pretreatment is one major factor that influences the incidence of non-hematological side effects.

Between 8/89 and 3/92 we treated 12 pts with germ cell tumors (**GCT**), 7 pts with non-Hodgkin's lymphoma (**NHL**) and 3 pts with Hodgkin's disease (**HD**) with identical doses of carboplatin 1500 mg/m^2, etoposide 1200 to 1600 mg/m^2 and ifosfamide 10 g/m^2 followed by ASCR.

Of the pts with GCT who all had intensive cisplatin pretreatment 8/12 (67%) had a rise in serum creatinine > 1,2 mg/dl and 1/12 (8%) required temporary hemodialysis. Mucositis ≥ WHO III^o occured in 11/12 (92%), diarrhoea ≥ WHO III^o in 4/12 (33%), hepatic toxicity ≥ WHO III^o in 2/12 (17%) and peripheral nervous toxicity ≥ WHO I^o in 4/12 (33%) pts.

Pts with NHL/HD who had all received only non-cisplatin pretreatment and irradiation, tolerated the HDT better. Only 1 pt (10%) had a rise in serum creatinine > 1,2 mg/dl and no pt required hemodialysis. Mucositis ≥ WHO III^o occured in 6/10 (60%) and diarrhoea ≥ WHO III^o in 1/10 (10%). No hepatic toxicity ≥ WHO III^o or peripheral nervous toxicity ≥ WHO I^o was observed.

CONCLUSION: Carboplatin, etoposide and ifosfamide can be used in a HDT combination. Manageable, but substantial side effects occur. Pretreatment with cisplatin results in a significantly higher incidence of renal toxicity. HDT should not be routinely used before trials have shown it's superiority over standard-dose treatment.

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AEROSOL APPLICATION OF AMPHOTERICIN B (AMB) AS PREVENTION OF INVASIVE PULMONARY ASPERGILLOSIS.

J.Beyer, C.Weyer, G.Barzen, G.Risse, K.Miksits, G.Farshidfar, D.Huhn and W.Siegert.

Prolonged periods of neutropenia after intensive chemotherapy or bone marrow transplantation (bmt) are the major risk factor for invasive pulmonary aspergillosis (IPA).

Between 8/89 until 2/92 we used aersol amb in 40 pts undergoing autologous bmt to obtain pharmacological data and to investigate it's clinical use. Amb was dissolved in sterile water at 10 mg / 5 ml and nebulized using a "lifetec jetair δ 10^R" air compressor with a "respirgard II^R" nebulizer at a flow rate of 8 ml/min. Pts started inhalations 6 days prior to bmt and continued twice daily for 20 min until leucocytes >1.0/nl were reached. In 15 pts amb serum levels were regularly monitored. In 4 healthy volunteers the organ distribution of radiolabeled amb was studied. **Results:** Radiolabeled amb showed a homogeneous pulmonary distribution. Of the total activity a median of 2.6% (range 2.4-3.3%) were found in the lungs immediately after inhalation and 1.4% (range 1.1-1.6%) at 14h post inhalation. Serial amb serum levels in pts pre- and 1h post inhalation were < 0.1 μg/ml in 150/168 (89%) samples. Between 0.1 μg/ml and 0.2 μg/ml of amb was measured in 18/168 (11%) samples. Treatment was well tolerated with minimal toxicity (nausea and bad taste). Inhalations had to be discontinued in 10 pts with WHO IV^o stomatitis or for other reasons. In one pt in whom aerosol amb had to be stopped on day -3 after only five inhalations, IPA was documented after his death on day +21 despite i.v. amb treatment. There were no other cases of IPA. **Conclusions:** lack of toxicity, absence of relevant serum levels and good pulmonary distribution warrant prospective evaluation of aerosol amb as prophylaxis of IPA in neutropenic pts.

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PROTECTIVE EFFECT OF DEOXYCYTIDINE ON 2-CDA TREATED HUMAN BONE MARROW CULTURES

R. Bilgeri, A. L. Petzer, U. Zilian, F. H. Geisen, M. Haun and G. Konwalinka

The lymphocytotoxic effect of chlorodeoxyadenosine (CdA), a purine analogue resistant to adenosine deaminase, requires the phosphorylation by the enzyme deoxycytidine kinase. This effect on lymphocytes can be antagonized by coadministration of deoxycytidine (dCyt), a competitive substrate of deoxycytidin kinase.

Recent in vitro studies[1] have shown, that not only growth of lymphocytes but also colony formation by myeloid progenitors derived from normal human bone marrow is dose dependently inhibited by CdA.

The aim of this study was to examine the effect of various doses of dCyt (10^{-6} to 10^{-3}M) on CdA mediated growth inhibition of myeloid progenitor cells in vitro. Our results show that coadministration of dCyt (>10^{-4}M) to CdA containing cultures (160nM) protected colony formation by human bone marrow derived progenitor cells (CFU-E, BFU-E, CFU-GM) in the methylcellulose system. However, the protective effect of dCyt was markedly different on the various subclasses of progenitor cells depending on their maturation stage. Thus, coadministration of 10^{-4}M dCyt completely reversed the growth inhibiting effect of CdA on CFU-E colony formation, whereas the colony formation of the immature progenitors BFU-e and CFU-GM was only restored to 50% of control cultures. If the concentration of dCyt was increased to 10^{-3}M, the protective effect for BFU-E and CFU-GM in the presence of a maximally growth inhibitory dose of CdA (160nM) reached almost 80% of control cultures.

The fact that CdA mediated growth inhibition of CFU-E could be completely restored, but that of BFU-E and CFU-GM only incompletely despite a higher concentration of dCyt (10^{-3}M), led us to the suggestion, that beside phosphorylation by dCyt kinase additional mechanisms may be operative for the toxicity of CdA in the more immature progenitors (BFU-E and CFU-GM).

[1] Petzer A., Bilgeri R., Zilian U., Geisen F., Haun M., Konwalinka G.: Inhibitory effect of 2-CdA on granulocytic, erythroid and T-lymphocytic colony growth. Blood 78,1992:pp 2583-2587

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rhIL-1-beta PHASE I TRIAL IN PATIENTS WITH ADVANCED MALIGNANCIES.

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To study safety, tolerance and biological effects of IL-1 administered subcutaneously, patients with advanced malignancies are assigned to receive rhIL-1ß at dose levels of 10, 30, 60, and 100 ng/kg (6 patients per dose level). IL-1 (E.coli; Syntex/Behringwerke) is given once daily for 7 days, followed by 21 days of monitoring. Up to now, 6 patients entered dose level I (10 ng/kg) and 4 patients entered dose level II (30 ng/kg). Patients who responded to IL-1 by tumor regression or stable disease received additional cycles of IL-1 at the same dose (dose level I, n = 16 cycles; dose level II, n = 5 cycles). One patient had WHO grade III fever (30ng/kg), all other displayed grade II fever. Two patients had grade II hypotension without need for therapy. Other side effects (all grade I) included chills, local erythema at the site of injection and herpes labialis. One patient with malignant melanoma had a partial remission (dose 10 ng/kg), and 5/10 patients had stable disease (2 malignant melanoma; 2 renal cell carcinoma; 1 parotic carcinoma). An acute phase response was induced in all patients. IL-6 reached maximum serum levels 4-6 hours after IL-1 administration (up to 200 pg/ml following 10 ng/kg IL-1). Sustained thrombocytosis - starting at day 5-7 for up to 4 weeks - was observed in 18/21 treatment cycles. Considering the induction of high-levels of IL-6 by IL-1ß, the increase in platelets might be mediated by IL-6. Peripheral blood progenitor cells were not significantly increased by IL-1 when compared to pretreatment levels. This study indicates that IL-1ß can be given safely to patients and stimulates a sustained rise in platelet counts.

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INVESTIGATION OF THE METABOLISM OF MITOXANTRONE

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The investigation of the metabolism of the anticancer drug mitoxantrone received an additional impetus since evidence was found for a new metabolic pathway in man, pig and rat which based on the oxidation of the phenylenediamine moiety of mitoxantrone. A naphthoquinoxaline derivative of mitoxantrone which was detectable in the urine of patients, rat and pig was the unequivocal proof of this biotransformation.

This metabolite is the product of an intramolecular cyclization reaction occuring either in a quinone-diimine intermediate or alternatively, in a radical cation of mitoxantrone. Both intermediates could be formed in vitro via enzymatic or non-enzymatic oxidation of mitoxantrone and are potentially capable to act as alkylating agents as proved by their reaction with glutathione after enzymatic activation of mitoxantrone with activated horseraddish peroxidase.

Evidence for the alkylating property of oxidized mitoxantrone has been achieved after incubation of primary cultures of rat hepatocytes. By action of the hepatocytes mitoxantrone is transformed into the corresponding mono-L-cysteine- and the monoglutathione conjugates. The biotransformation products have been identified by high-performance liquid chromatography and spectral comparison with authentic reference compounds prepared by chemical synthesis. Structural proof of the reference compounds has been obtained by application of nuclear magnetic resonance spectroscopy and electrospray mass spectrometry.

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INTERVENTIONAL ANTIMICROBIAL THERAPY IN FEBRILE NEUTROPENIC PATIENTS WITH CEFOTAXIME/PIPERACILLIN VERSUS IMIPENEM/CILASTATIN AND EFFICACY OF EARLY TREATMENT WITH ITRACONAZOLE

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

A prospective, randomized trial was designed for investigating Cefotaxime/Piperacillin (Cef/Pip) vs Imipenem/Cilastatin (Imi/Cil) as empirical therapy and Itraconazole as early antifungal treatment in febrile neutropenic patients. Totally, 139 patients were included. 43/70 patients receiving Cef/Pip (61%) and 40/69 patients treated with Imi/Cil (58%) responded. Episodes with fever of unknown origin (FUO) had the best response with 82% (23/28 pat.) under Cef/Pip, 79% (19/24 pat.) under Imi/Cil. Non-responders got additional therapy with Gentamicine and were randomly assigned to treatment with or without Intraconazole. The overall response of FUO was 100%.

In 30 patients, bacteremia was diagnosed without any localization of infectious focus. 7/12 pat. (58%) responded with Pip/Cef, 11/18 pat. (61%) with Imi/Cil. By adjusting treatment to antibiogram the response was increased to 90% (27/30).

In 25 episodes a pneumonia developed after start of fever. A complete recovery occured in 11% (1/9 pat.) unter Cef/Pip, in 12,5% (2/16 pat.) under Imi/Cil. By further therapy according to antibiogram or with Amphotericin B/5-Flucytosine the response was elevated to 76% (19/25 pat.).

28 patients with initially diagnosed pneumonia were randomized to treatment with Imipenem/Cilastatin or with Imipenem/Cilastatin/Itraconazole. The response was 50% (9/18 pat.), and 70% (7/10 pat.) respectively. By supplement of the antibiotic combination 93% of patients responded.

In conclusion there was no difference in efficacy between Cefotaxime/Piperacillin and Imipenem/Cilastatin in the treatment of FUO and secondarly documented infections. The preliminary results of initially diagnosed pneumonias suggest a benefit of the combination with Itraconazole. It is necessary, however, to await results in further infection cases.

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BISPECIFIC ANTI-CD3xANTI-CD19 AND ANTI-CD28 ANTIBODIES MEDIATE T-CELL ACTIVATION IN VITRO AND DELAY OF LYMPHOMA GROWTH IN XENOTRANSPLANTED SCID-MICE.

H.Bohlen, T.Hopff, U.Kapp, J.Wolf, A.Engert, V. Diehl and H.Tesch*

It has been shown that bispecific antibodies which recognize epitopes on the CD3-complex and tumor-antigens can force pre-activated T-cells to react against tumor cells. For the activation of resting T-cells, however, a second activation signal is required. In this study this signal was given by crosslinking of the CD28 molecule on T-cells.

Monocyte depleted peripheral blood leukocytes from patients with CLL and cell lymphomas (leukemic phase) were cocultured with anti-CD3xanti-CD19 bispecific antibodies together with anti-CD28 antibodies. FACS analysis after 8 days of culture showed that the total number of tumoral B-cells decreased by 40-90%. Simultaneously the IL2-receptor (CD25) and the CD38 activation marker on T-cells were upregulated. Further analysis of the T-cell subsets revealed that CD4 positive T-cells were markedly overrepresented in the stimulated cultures (CD4/CD8 ratio-range in 6 patients: 6-15 fold). PBLs stimulated with anti-CD3xanti-CD19 bispecific antibodies and anti-CD28 were assayed after 5 days for specific cytolysis against autologous B-lymphoma cells. 25% specific cytotoxic activity against autologous B-lymphoma cells could be detected when anti-CD3xanti-CD19 bispecific antibodies were added, whereas the parental antibodies showed only background activity.

To demonstrate the efficacy of this protocol *in vivo* SCID-mice were subcutaneously transplanted with human B cell lymphoma tissue. After visible tumor growth the mice were treated with autologous purified T-cells($1x10^7$/mouse) and subsequently injected with anti-CD3xanti-CD19(25µg/mouse each) plus anti-CD28 (group I), anti-CD3xanti-CD19 alone (group II) or parental antibodies (group III).

A significant delay in tumor growth of 50% on day 30 could only be detected in 3/5 mice from group I which had been treated with bispecific antibodies and anti-CD28 T-cell activating antibodies.

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TREATMENT OPTIONS IN PATIENTS WITH "LOW RISK" NON-SEMINOMATOUS GERM CELL TUMOURS (NSGCT)

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With the use of combination chemotherapy (CTX) 80 - 85 % of patients (pts) with testicular germ cell tumours can expect to be cured from malignant disease. For the subgroup of pts with "low risk" NSGCT, cure rates up to 95 % can be achieved and current improvements in therapy for this group of patients aim at the reduction of toxicity without compromising long term survival. However, different definitions of "low risk", taking into account metastatic sites only (Indiana University), additional tumour markers (EORTC) or LDH-levels (MSKCC) complicate the comparison of treatment results. Between 10 - 20 % of pts will be assigned to another "risk group" when analysed according to the different definitions of "low risk" trials.
In "low risk" pts 3 cycles (cy) of Platin (P), Etoposide (E) and Bleomycin (B) have been demonstrated to be as effective as 4 cy of the same regimen and are regarded as "standard" (92 % cont. CR/NED; Indiana University). The omission of B in 3 cy of PEB resulted in significantly worse results (67 % vs 86 % cont CR/NED; Indiana University), while 4 cy of PE were equivalent to 4 cy of PEB (88 % vs 92 % cont. CR/NED; EORTC). The substitution of cisplatinum (P) by carboplatin (C) in combination with etoposide gave inferior results in a randomized trial comparing 4 cy of CE- to 4 cy of PE-chemotherapy (78 % vs 87 % cont. CR/NED; MSKCC). Promising results were obtained in a phase-II-study of 4 cy of CEB treatment (90 % cont. CR/NED; MRC). This regimen of 4 cy CEB is currently compared to 3 cy of "standard" PEB (Indiana-regimen) in a randomized phase III study of the German Testicular Cancer Study Group. Outside of clinical studies 3 cy of the PEB-regimen should be regarded as standard treatment for pts with "low risk" NSGCT.

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LATE CARDIAC TOXICITY IN LONGTIME SURVIVORS OF HODGKIN'S DISEASE (HD)

C. Bokemeyer, A. Schrader, J. van Rhee, H. Köhne-Wömpner, H.-J. Schmoll, H. Poliwoda

83 consecutive patients (pts) out of 219 pts treated for HD between 1965 and 1988 at Hannover University Medical School, who had been in continuous CR for more than 24 months (mo) were evaluated for late cardiotoxic effects of therapy. The pts received an electrocardiogram (ECG) and a measurement of left ventricular ejection fraction (EF) and an echocardiogram in selected cases.
Pts characteristics: Median age: 37 years (18-74); median follow-up: 96 mo (27-243). Risk factors for cardiac disease: diabetes 3 pts, hypercholesterinemia 4 pts, hypertension 13 pts. 48 of 83 (58%) pts were non-smokers (NS), 19/83 (23%) smokers (S) and 16/83 (19%) gave up smoking more than 5 years ago (FS). Treatment consisted of radiotherapy (RTX) in 27% of pts, chemotherapy (CTX) in 15% and combined CTX and RTX in 58% of pts; 63/69 pts (91%) with RTX had received mediastinal RTX.
Results: 18/83 (22%) pts had a pathological EF (7 pts) (ejected left ventricular blood-volume lower than 50% or decreased regional wall-motility) or borderline (11 pts) EF (EF between 50 and 55%). Echocardiography performed in 4 cases showed no specific changes attributable to prior therapy. 13/83 (16%) pts showed ECG-changes only (left ventricular repolarization disturbances (7 pts), AV-block (4 pts), incomplete right bundle branch block (4 pts), left ventricular hypertrophy (2 pts), extrasystoles (2 pts), left anterior hemiblock (2 pts), myocardial infarction (1 pat), LGL-syndrome (1 pat). In 6/13 (46%) pts more than one ECG-alteration occurred. Additionally 9/81 (11%) pts showed abnormalities in both EF and ECG.
31 of 81 (38 %) pts complained about subjective cardiac signs.
Conclusion: Significant or borderline reductions of cardiac EF occurred in 27/81 (33 %) pts after treatment of HD. They were not associated with potential risk factors for cardiac disease, but appeared to be more common in pts receiving combined RTX and CTX, particularly after the use of anthracycline containing regimens (ABVD). Prospective studies are needed to determine the incidence and to define the potential of "cardioprotective substances" for the prevention of late cardiac toxicity in pts with curable malignant tumors.

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METASTATIC LEYDIG-CELL TUMOURS OF THE TESTIS: REPORT OF THREE CASES

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Leydig-cell tumours (LCT) constitute 1.6 - 3.6 % of all testicular neoplasias. Only 10 % of these are malignant with the potential for metastases (METS), which predominantly involve regional lymph nodes (LN), liver, lung or bones. Fifty percent of LCT have altered estrogene (ES) and/or androgene (AN) serum levels.
From 1990-92 3 pts with metastatic LCT were treated at Hannover University Medical School. **Pts age** at diagnosis: 54, 62 and 65 years. **Hormone parameters:** normal in 1 pt, decrease of AN and elevation of gonadotropins in 1 pt and increase of AN and ES in 1 pt. **Metastatic sites, treatment and outcome:** Pat 1: Retroperitoneal LN METS at first diagnosis (FD); tumour free after surgical resection for 6 months (MO). Pat 2: METS in retroperitoneal LN, liver and lung 7 years after FD; chemotherapy (CTX) (first line: platin(P)/etoposide(E); second line: epirubicin), followed by incomplete resection and subsequent hormonal therapy (1.: tamoxifen; 2.: lonidamine); PD and death 8 years after FD (1.5 years after detection of METS). Pat 3: Retroperitoneal LN METS 6 MO after FD; incomplete resection, chemotherapy (P/E/Bleomycin(B)); minor response and alive with disease for 17 MO after FD.
The three cases reported confirm the large variability of the time interval between FD and development of METS in pts with LCT. Although one pat achieved a minor remission after treatment with platinum-based combination CTX, no effective CTX is established for metastatic LCT and responses to standard regimens used for testicular germ cell tumours (PEB) have only rarely been reported. For cases with completely resectable metastases surgery offers the most effective treatment option.

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EVALUATION OF LATE EFFECTS ON GONADAL FUNCTION AFTER TREATMENT OF HODGKIN'S DISEASE (HD)

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83 consecutive patients (pts) (43 women, 40 men) (median age 37 years (18-74)) of 219 pts treated for HD at Hannover University Medical School between 1965 and 1988, who were in CR/NED for more than two years, were evaluated regarding sex hormone status, fertility and influence on relationship. Treatment consisted of chemotherapy (CTX) in 16 % of pts, radiotherapy (RTX) in 26 % and combined CTX and RTX in 58 % of pts.
Results: Of 26 eligible women (17/43 excluded for: age > 47 years 4 pts; current contraceptive medication 12 pts;, ovarectomy 1 pat) 13 (50%) showed signs of ovarian insufficiency (OI): Elevated follicle stimulating hormone (FSH) and luteinizing hormone (LH) and decreased estradiol (E_2). 10/26 (38 %) had persistent, 1 (4 %) transient amenorrhea for 9 months. Age at the end of therapy appeared to be important with OI occurring in 1/8 women (13%) younger than 25 years compared to 11/18 (61 %) women older than 25 years.
26/40 men (65 %) showed elevated FSH-levels as an indicator of testicular tubular damage (median 13.4 mU/ml (1.5 - 32.3)). Median values of LH and androgenes were within the normal range (8/40 (20 %) with decreased androgene levels). In 12 men undergoing sperm analysis 25 - 180 mo after therapy for HD, 7/12 (58 %) were infertile (CTX only 14 %, combined CTX + RTX 86 %). 2/12 were classified as subfertile. In 6/7 (86 %) men infertility was associated with elevated FSH levels.
3/40 (8 %) men fathered healthy children, 3/43 (7 %) women became pregnant, but lost their children due to spontaneous (1) or voluntary (2) abortion. 14/83 (17 %) pts reported a negative influence on sexual function and relationship after treatment for HD.
Conclusion: OI associated with amenorrhea after treatment for HD represents a common problem in about half of the pts. It appears to be significantly more common in women older than 25 years at the end of treatment. In 65 % of men treated for HD elevated FSH was found, indicating testicular tubular demage and possibly infertility. Sperm banking should be routinely offered to male pts with the wish to procreate children. Furthermore, pts should be counselled concerning possible distress on relationship and sexual activity caused by treatment of HD.

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STUDIES ON THE FORMATION OF ACTIVE AND INACTIVE METABOLITES OF IFOSFAMIDE BY DIFFERENT ORGAN SYSTEMS.

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The precondition for the antineoplastic effect of ifosfamide (ifo) is the oxidation of the oxazaphosphorine ring system. The 'ring oxidation' leads to the formation of alkylating mustard by several steps. 'Side chain oxidation' produces the cytostatically inactive metabolites 2- and 3-dechloroethyl-ifosfamide. This metabolic pattern gains growing interest in the discussion of ifosfamide-related nephro- and neurotoxicity.
The metabolic capacity of rat liver, kidney, brain and lung, therefore, was investigated.
Ifosfamide (ifo) and the metabolites 4-hydroxy-ifosfamide (4-OH-ifo)/aldoifosfamid, Ifosfamide-mustard, Carboxyifosfamide, 4-Ketoifosfamide, 2-deschloroethyl-ifosfamid (2-d-ifo) and 3-deschlorethyl-ifosfamide (3-d-ifo) were determined in microsomal preparations by HPLC. In rat liver microsomes formation of Carboxyifosfamide and 4-keto-ifosfamide could not be demonstrated. The maximum velocity of the formation of 3-d-ifo exceeded the 2-d-formation fourtimes. In supernatant all metabolites were formed. Predominating metabolite of the 'ring oxidation' was carboxyifosfamide.
Preparations from rat and rabbit lung tissue were incubated and the formation of all metabolites with the exception of 4-ketoifosfamide could be observed. Neither brain from the rat nor kidney from rat and rabbit demonstrated metabolic activity.

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EVALUATION OF REPRODUCTIONABILITY OF PATIENTS WITH HEMOBLASTOSIS

A. Borstel, A. Franke, G. Müller, R. Uhle, W. Weise

Results of the study about the reproductionability of patients with hemablastosis are evaluated.
1079 patients wich the diagnosis of hemoblastosis were treated with a chemotherapy in the Department of Hematology/Oncology of the clinics of Internal Medicine of the Medical Academy of Magdeburg. Within this number were 147 female and 191 male patients in the age of reproductionability. 16 deliveries and 7 abortions of the females occured and 5 of the male patients got 6 children.
The natural course of lymphogranulomatosis is not affected by pregnancy. In contrast the course of an untreated high malignant NHL can be affected in a prognostically unfavourable way. There is no diaplacental affection of the child by the lymphoma.
The manifestation of an acute leucaemia in early pregnancy in general leads to abortion, a manifestation in late pregnancy mostly results in early delivery.
Application of antineoplastic chemotherapy in the first third of pregnancy results in a remarkable risk of teratogenicity and abortion. That's why it is suggested to perform an abortion after regression of diseases or remission.
During polychemotherapy in late pregnancy there isn't an increased risk of malformed off spring, low birth weight and premature are possible.

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IDENTIFICATION AND EXPRESSION CLONING OF A NOVEL TRANSCRIPTION FACTOR, NF-JUN, REGULATING THE c-JUN GENE IN MYELOID LEUKEMIA CELLS

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We have identified a novel transcription factor termed NF-jun which regulates transcriptional activation of the c-jun gene. Expression of c-jun is associated with cellular proliferation. NF-jun can be isolated from nuclear proteins of rapidly proliferating cells such as various human myeloid leukemia cell lines and primary AML sample, but is not detectable in nuclear extracts of peripheral blood monocytes or growth- arrested diploid fibroblasts. NF-jun shares several biological features with the ubiquitous transcription factor NF-κB, including its subcellular localisation which predisposes NF-jun to function as a molecule which transduces signals from the cytoplasm to the nucleus. We have cloned the NF-jun encoding gene by screening an expression library. Prelimiary sequence data indicate that the genes encoding NF-κB or various other members of the c-rel family and the NF-jun encoding gene are distinct. The NF-jun is transcribed into a 2.4 kB mRNA which is constitutively expressed by various human myeloid leukemia cells. NF-jun transcript levels in these cells are augmented by TNF-α treatment. In contrast, peripheral blood monocytes and non proliferating diploid fibroblasts fail to synthesize NF-jun transcripts irregardless of the presence or absence of TNF-α. Taken together, our data indicate that NF-jun acts as a sequence specific transcription factor regulating proliferation-associated expression of the c-jun gene in myeloid leukemia cells.

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PROLONGATION OF SURVIVAL OF HUMAN POLYMORPHONUCLEAR NEUTROPHILS BY GRANULOCYTE-MACROPHAGE COLONY STIMULATING FACTOR IS DUE TO INHIBITION OF PROGRAMMED CELL DEATH

Marion A. Brach, Sven deVos, Hans-Jürgen Gruss, and Friedhelm Herrmann

Polymorphonuclear neutrophils (PMN) play a central role in inflammation. During the inflammatory response PMN leave the circulation upon appropriate stimulation and enter the inflamed area to exert their biological function. A variety of chemotactic factors, including N-formylated bacterial peptides, C fragment C5a, Transforming Growth Factor-β, and IL-8 are responsible for recruitment of PMN at the site of inflammation. Various cytokines released during inflammation in an autocrine or paracrine manner may then regulate the survival of PMN in the lesion either by promoting or by inhibiting their death. It has been recently shown that aging PMN undergo characteric changes indicative of the so-called programmed cell death (PCD) or apoptosis, including cell shrinkage, nuclear chromatin condensation and DNA fragmentation into nucleosome-length fragments. The process of PCD may then allow recognition and ingestion of apoptotic cells by macrophages and thus may represent a mechanism by which PMN are cleared from the inflammatory site. We show that Granulocyte-Macrophage Colony Stimulating Factor (GM-CSF), but not the chemotactic factors formyl-methionyl-leucyl-phenylalanine (FMLP), recombinant human (rh) C5a, Transforming Growth Factor (TGF) -β1, and Interleukin (IL)- 8, or other cytokines including IL-3, IL-4, IL-6, G-CSF, maintains viability of PMN in culture by preventing these cells from undergoing PCD. Prevention from PCD by GM-CSF was associated with induction of RNA and protein synthesis in PMN. Inhibition of RNA and protein synthesis by Actinomycin-D (Act-D) and Cycloheximide (CHX), respectively impeded the protection of apoptosis by GM-CSF. Similarly, neutralization of GM-CSF biologic activity by a specific antiserum abrogated GM-CSF-mediated inhibition of PCD.

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RAF-1 IS A NECESSARY COMPONENT OF THE MITOGENIC RESPONSE OF THE HUMAN MEGAKARYOBLASTIC LEUKEMIA CELL LINE MO7 TO HUMAN STEM CELL FACTOR, GRANULOCYTE-MACROPHAGE CSF, INTERLEUKIN (IL)-3, AND IL-9.

Ulrich Brennscheidt, Detlev Riedel, Walter Kölch, Renate Bonifer, Roland Mertelsmann, and Friedhelm Herrmann

The product of the c-raf-1 proto-oncogene, Raf-1, is known to encode a 74-kd ubiquitously expressed cytoplasmic serine/threonine kinase. Various growth factors including epidermal growth factor (EGF), acidic fibroblast growth factor (a FGF), platelet-derived growth factor (PDGF), insulin as well as the hematopoietins granulocyte-macrophage colony-stimulating factor (GM-CSF) and interleukin-3 (IL-3) have been shown to induce phosphorylation of Raf-1 and therby activating Raf-1 kinase. Raf-1 may thus play a role in coupling some growth factor receptors to proliferation. We have examined the role of Raf-1 in the mitogenic response of factor-deprived human megakaryoblastic leukemia cell line MO7 to recombinant human (rh) GM-CSF, IL-3, IL-9, and stem cell factor (SCF) employing c-raf antisense oligodeoxyribonucleotide. Proliferation of MO7 cells (3H-thymidine incorporation) upon exposure to these growth factors was substantially reduced when c-raf antisense oligodeoxyribonucleotide which specifically decreases intracellular Raf-1 levels was added to cultures, but not in the presence of sense or nonsense oligodeoxyribonucleotides. C-raf-1 antisense failed, however, to alter DNA synthesis and proliferation of myeloid leukemia HL-60 cells known to be drivenby amplified c-myc and thus by an alteration downstream of c-raf-1.

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EXPRESSION OF TRANSFORMING GROWTH FACTOR-β_1 (TGF-β_1) AND INTERLEUKIN-10 (IL-10) mRNA INDICATE IMMUNOSUPPRESSIVE POTENTIAL OF AML BLAST CELLS.

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In acute myeloid leukemia cytotoxic activity of peripheral mononuclear cells is suppressed. This phenomenon might be due to synthesis and release of immunosuppresive agents by AML blasts. As TGF-β_1 is known to be an inhibitor of LAK cell activity and Il-10 has been characterized to suppress cytotoxicyty promoting cytokine release, the present study was designed to investigate the potential of AML blasts to synthesize TGF-β_1 and Il-10 on the mRNA level. To this end AML blasts were isolated from bone marrow or peripheral blood of patients in leukemic phase of AML and cellullar RNA was prepared. Northern blotting and PCR were performed for TGF-β_1 and Il-10. In 7 / 8 patients studied, specific transcripts for TGF-β_1 were detected by PCR. These results were confirmed by Northern blotting. When Il-10 specific mRNA was assessed in blasts by PCR in 4 / 4 preparations a specific band could be amplified, indicating the presence of Il-10 specific transcripts in these cells. Our observations suggest that the production and release of suppressive mediators to cellular effector phase within the AML microenviroment could play a crucial role in the inhibition of autologous cytotoxic mechanisms in these patients.

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CHEMOTHERAPY AND RADIOTHERAPY FURTHER INCREASE A HYPERCOAGULABLE STATE IN TUMOR PATIENTS

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Clinical evidence suggests that coagulation is frequently activated in cancer patients. In comparison to healthy volunteers or even other patients without a tumor diagnosis, thrombotic episodes are more frequently found in tumor patients and are demonstrated in up to 50 % of autopsies. Up to now the significance of these phenomena is largely unclear. Application of chemo- and/or radiotherapy (ct, rt) furthermore leads to a reinforcement of the thrombophilic development possible via release of procoagulatory factors.

We studied coagulation parameters in two groups of patients with a recently diagnosed malignant process, which, according to different protocols, were either treated with ct or, in the second group, solely underwent rt. The first group comprised 48 patients. In those patients blood samples were drawn directly before initiation of ct, 3 days afterwards as well as at the end of the course. The second rt group comprised 22 patients. Here the blood samples were drawn directly before and directly after the first radiation treatment (3 hours later) as well as on the second day of the complete radiation cycle. The most important results, especially in regards to new parameters, can be described as follows:

In rt patients TAT complexes remain within normal limits whereas in ct patients they were significantly elevated from the beginning. In both groups tat increased even further 3 days respectively 3 hours after initiation of therapy. A similar development was observed for D-dimers which were significantly elevated at beginning and rose even further during both regimens. Tissue plasminogen activator (t-PA) concentrations were elevated before therapy started, increased even further during therapy, but had dropped to significantly lower levels when the last blood sample was drawn.The acute phase proteins fibrinogen and factor VIII partially showed high activity during the course.FPA, plasminogen activators and PAI-1 did not show any significant changes.With these results we were able to show that before and at least during the first stages of ct respectively rt a significant higher level of the procoagulatory status is present and even reinforced.

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EXPANSION OF PURIFIED CD34$^+$ PERIPHERAL BLOOD HEMATOPOIETIC PROGENITOR CELLS IN VITRO.

W. Brugger, W. Moecklin, R. Mertelsmann, and L. Kanz

Peripheral blood progenitor cells were mobilised following standard-dose VIP chemotherapy and filgrastim (G-CSF) administration in 12 cancer patients with metastatic solid tumors. At day 8-12 after chemotherapy, CD34$^+$ cells were isolated from peripheral blood by using an avidin-biotin immuno-affinitiy column. Isolated CD34$^+$ cells were more than 90% pure as determined by morphology, flow cytometry and immunophenotyping of single cells. The cloning efficiency of CD34$^+$ cells in unseperated blood was 0.2% as compared to 5% (range 1.3-11) in purified CD34$^+$ cell populations. Purified CD34$^+$ cells were cultured up to 16 days in liquid culture and shown to proliferate when stem cell factor and interleukin-3 were present in the culture media. The proliferation of CD34$^+$ cells was highly variable and dependent on the day of progenitor cell collection and the number of CD34$^+$, lineage negative (CD33$^-$, CD38$^-$, HLA-DR$^-$) cells. Maximal proliferation occured at day 10-12 with a more than 500 fold increase in cell numbers. Expanded CD34$^+$ cells were clonogenic in vitro, producing large numbers of myeloid, erythroid, multilineage as well as blast cell colonies. The enrichment factor for clonogenic cells was up to 1000 fold. Replating assays of blast colonies revealed only myeloid progenitor cells. Our data indicate that chemotherapy + G-CSF mobilised CD34$^+$ cells from cancer patients can be enriched and succesfully expanded without loosing their clonogenic capacity in vitro. Clinical trials with purified CD34$^+$ cells as supportive agents following high-dose chemotherapy will be started.

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ACCELERATION OF THROMBOPOIESIS FOLLOWING HIGH-DOSE CHEMOTHERAPY BY VIP CHEMOTHERAPY PLUS G-CSF MOBILISED PERIPHERAL BLOOD PROGENITOR CELLS.

W. Brugger, H. Bertz, T. Hecht*, R. Mertelsmann and L. Kanz.

High-dose chemotherapy is potentially curative in some solid tumors and lymphomas, but is mostly limited by severe myelosuppression. In order to facilitate dose intensification, we used hematopoietic growth factors together with peripheral blood progenitor cells (PBPC) to shorten the period of severe pancytopenia after high-dose chemotherapy. PBPC were mobilised following a 1 day course of VIP chemotherapy and filgrastim (G-CSF) treatment (n = 40). PBPC were evaluated by the expression of CD34 antigens as well as their clonogenic capacity in vitro (CFU-GM, BFU-E, CFU-Meg, CFU-GEMM). A median of 415,000 $CD34^+$ cells/mL, 9,000 CFU-GM/mL, 3,500 BFU-E/mL and 200 CFU-GEMM/mL were recruited. One single leukapheresis yielded a median of 8 x 10^6 $CD34^+$ cells/kg body weight. Fourteen patients eligible for dose intensification were subsequently treated with high-dose VIP [cumulative doses of VP16 (1,500 mg/m²), ifosfamide (12 g/m²) and cisplatin (150 mg/m²)] with (n = 8) or without (n = 6) PBPC support in addition to hematopoietic growth factors (IL-3 and GM-CSF). Neutrophil recovery as well as platelet recovery were significantly faster in patients receiving PBPC support with a median of 6.5 days below 0.1 x 10^9 neutrophils/L and 3 days below 20 x 10^9 platelets/L, as compared to 10.5 days and 8 days in control patients receiving IL-3 and GM-CSF only ($p < 0.005$). These data demonstrate that G-CSF mobilised PBPC accelerate both, neutrophil and platelet recovery following high-dose chemotherapy.

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DIPEPTIDYL PEPTIDASE IV (DP IV, CD26) AND AMINOPEPTIDASE N (AP N, CD13) IN NATURAL KILLER (NK) CELLS. EXPRESSION AND FUNCTIONAL ROLE.

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Cell surface bound peptidases of immune cells have been found to be involved in regulation of activation and proliferation in vitro. In this paper the expression and activities of DP IV and AP N were studied by flow cytometric detection, measurement of hydrolysis of specific substrates and in situ hybridisation. It was shown, that both enzymes are present in NK cells. During incubation with IL-2 the expression of CD26 and the enzymatic activity was increased. The enzymatic activity of AP N was not affected under this condition. In presence of specific inhibitors of these enzymes and anti-DPIV antibodies the DNA synthesis was decreased and the cell cycle progression of NK cells was blocked at G1/S-phase. There was no difference in cytotoxic activities of DP IV-positive and DP IV-negative NK cells.
This results considered as basis of further investigations on the role of these peptidases in regulation of proliferation of malignant lymphoide and myeloide cells.

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PHARMACOKINETICS AND PHARMACODYNAMICS OF R-VERAPAMIL IN COMBINATION WITH CHEMOTHERAPY

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Doses of racemic verapamil required to achieve concentrations that reverse multi-drug-resistance (MDR) usually exceed therapeutic doses and are associated with severe cardiovascular side effects. R-Verapamil has less cardiovascular activity but is equipotent to racemic verapamil with regard to reversal of MDR. Therefore, R-verapamil might be superior to racemic verapamil in modifying MDR in cancer chemotherapy.
We have investigated the pharmacokinetics and cardiovascular effects of R-verapamil in 33 patients who were treated with chemotherapy. The R-verapamil doses ranged from 800 to 2400 mg per day. Maximal R-verapamil concentrations between 531 and 5489 ng/ml were achieved. The cardiovascular side effect limiting further dose escalation was arterial hypotension. In none of the patients did congestive heart failure develop. In all patients modest prolongations of PR-interval were observed, which were closely related to plasma concentrations. The R-verapamil concentration which elicited a 20 % prolongation of PR-interval was 630 ng/ml. Only 2 patients showed second-degree AV-block, one showed junctional rhythm. Most of the patients developed a significant increase in QT_c time, which was related to plasma concentration of R-verapamil.
In conclusion, R-verapamil concentrations achieved in vivo are in the same order of magnitude as those which reverse MDA in vitro. Compared to racemic verapamil, R-verapamil caused less severe side effects.

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ANTIBODIES RECOGNIZING DISTINCT EPITOPES OF C-KIT RECEPTORS MODULATE STEEL FACTOR-MEDIATED RECEPTOR DOWNREGULATION AND PROLIFERATION

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The combined regulatory effects of steel factor (SLF) and three monoclonal antibodies (Mabs) recognizing distinct epitopes of the c-kit receptor were investigated. Binding of biotinylated SLF to AML cells expressing high levels of c-kit receptors was abrogated by Mab SR-1 and reduced to about 50% by Mab YB5.B8, while Mab 17F11 did not significantly influence ligand binding. As a consequence of its blocking effect, Mab SR-1 inhibited SLF-mediated proliferation and receptor downregulation. Mab YB5.B8 blocked ligand-induced proliferation only at high titers (1:50 dilution of ascites) and did not significantly influence receptor downregulation. In contrast, Mab 17F11 caused a strong enhancement of SLF-mediated proliferation at low concentrations (1-2 μg/ml) and was almost as potent in its capacity to downregulate c-kit receptors as the ligand itself. Sequential incubation of AML cells with two different Mabs revealed that Mab YB5.B8 blocked the binding of Mab 17F11, but not vice versa. This might be explained by the possibility that Mab YB5.B8 induces conformational modifications of c-kit receptors and thereby eliminates the epitopes originally recognized by Mab 17F11. The binding of Mab SR-1 is not significantly affected by either antibody nor does Mab SR-1 influence the binding of Mabs YB5.B8 and 17F11.
In summary, three recently identified Mabs SR-1, YB5.B8 and 17F11, which recognize different epitopes of c-kit receptors, show functional heterogeneity: Mab SR-1 strongly inhibits receptor-ligand interactions, Mab YB5.B8 shows a less pronounced blocking effect, and agonistic Mab 17F11 synergizes with ligand activity.

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PHARMACOKINETICS OF 'ACTIVATED CYCLOPHOSPHAMIDE' IN HIGH-DOSE CYCLOPHOSPHAMIDE TREATMENT

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The induction of cyclophosphamide (CP) metabolism by itself is a well known phenomenon. It results in an enhanced metabolic clearance and a shortened half-life of cyclophosphamide on second and subsequent treatment days. To further elucidate this relationship, we studied blood levels of the 'activated cyclophosphamide' (4-OH-CP) in 10 patients undergoing allogeneic bone marrow transplantation. The conditioning regimen consisted of fractionated total body irradiation and CP, dose 60 mg/kg body weight, as 1-h-infusion. Blood levels of 4-OH-CP were monitored by means of a spectrofluorometric method. Individual blank values for each time point were used. Maximal care was taken to immediately proceed blood probes avoiding loss of instable metabolites.

Peak blood concentrations of 4-OH-CP were reached at 2 to 4 h after end of infusion on day 1, and between 0 and 1 h after end of infusion on day 2. Mean peak concentration was 4.6 (S.D 2.1) nmol/ml on day 1, and 13.1 (S.D. 5.0) nmol/ml on day 2, respectively ($p<0.001$, paired t-test). Area under the concentration-time curve was 41.2 (S.D. 17.8) nmol*h/ml on day 1 and 69.9 (S.D. 19.5) nmol*h/ml on day 2 ($p<0.001$). There was considerable interindividual variation: Peak blood levels of 4-OH-CP ranged from 2.2 to 7.9 nmol/ml and from 7.3 to 20.2 nmol/ml on day 1 and 2, respectively.

Neither peak blood levels on day 2 nor area under the curve were significantly associated to VOD, occurence or grade of GvHD or duration of mucositis.

As it is known that bioavailability of cyclophosphamide is only insignificantly increased on subsequent treatment days, our data suggest an altered metabolism or distribution of 4-OH-CP after the second dose. Whether this change of pharmacokinetic behaviour may be due to saturable metabolic processes is unclear. Pharmacokinetic studies of the active metabolite phosphoramide mustard are underway to further address this problem.

In the patient population presented up to now no influence was observed of blood levels of 4-OH-CP on several clinical parameters after bone marrow transplantation.

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HIGH-DOSE CYTOSIN-ARABINOSIDE IN ACUTE LEUKEMIA: PHARMACOKINETIC EVALUATION

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High-dose regimens of cytosin-arabinoside (Ara-C) are evaluated in the therapy of acute leukemias in order to overcome the problems resulting from resistent leukemic cell clones. It is expected that high plasma concentrations result in increased intracellular levels of the active metabolite. Therefore it is interest in evaluating the interpatient variability of Ara-C plasma concentrations and its metabolism.

To further elucidate Ara-C pharmacokinetic behaviour we analysed plasma concentrations of Ara-C and its inactive metabolite, Ara-U in 13 patients with acute leukemia. In the context of complex aggessive chemotherapy protocols three patients were treated with Ara-C, dose 1.0 g/m^2. In 10 patients Ara-C was applied at a dose of 3.0 g/m^2. Ara-C was given as 3-h-intravenous infusion, twice daily, on 3 or 4 days. Pharmacokinetic plasma samples were drawn into heparinised tubes containing tetrahydrouridine during and after the first treatment cycle. Concentrations of Ara-C and Ara-U were analysed by an own high-performance liquid chromatographic method with UV-detection.

Mean plasma concentration of Ara-C at steady-state was 8.1 µg/ml (S.D. 6.6, range 2.6 and 25.0), of Ara-U 31.5 µg/ml (S.D. 11.0, range 12.6 and 49.6). Mean area under the concentration-time curve during and post infusion (AUC) for Ara-C was 25.6 µg*h/ml (S.D. 17.2, range 8.7 and 64.6), for Ara-U 248 µg*h/ml (S.D. 110.1, range 84.3 and 455.7). Patients with a 3 g/m^2-regimen had significantly higher plasma levels and AUC of Ara-U ($p<0.05$, and $p<0.05$, respectively, F-test). No difference was observed for Ara-C concentrations or AUC. There was no statistically significant correlation of AUC (Ara-C) and AUC (Ara-U).

In our patients high-dose Ara-C treatment resulted in a considerable variation of Ara-C plasma levels, which were not correlated to dose. A strong correlation was observed between dose and concentrations/AUC of the incative metabolite Ara-U. These results are to be proved by greater patient numbers. Further studies are needed to define correlations of pharmacokinetic parameters and clinical outcome or toxicity.

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Human Epithelial Antigen (HEA) 125 - Positive Cells In The Bone Marrow Of Small Cell Lung Cancer (SCLC)

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The ability to accurately evaluate the process of tumor dissemination in the preclinical phase of malignancy is of utmost importance for proper diagnosis and therapy. The purpose of our study was to clarify the early diagnostic possibilities of the monoclonal antibody HEA 125 (Progen, Heidelberg, F.R.G.), when applied to bone marrow aspirates of SCLC. Ninety patients where included in our study (M: 74, F: 16; age: 58 years (40 - 77); stage: LD 47, ED 43). The bone marrow was removed from the iliac crest at the time of initial tumor staging. Specimens were evaluated utilizing both conventional methods (biopsy: undecalcified bone sections, Masson-Goldner, PAS, Giemsa; aspirates: May-Grünwald-Giemsa) and immunocytological tests. HEA 125 was selected for the immunocytological examination because results from previous studies had shown it to be the most effective on 4 different small cell tumor cell lines (SCLC 24, NCIN 414, SCLC 22 H, NCIH 69) from a variety of antibodies (Vimentine, Cytokeratine KL1, Synaptophysine, Chromogranine , NSE). HEA 125 has a broad bounding affinity for normal and neoplastic epithelia (Momburg, F et al., Cancer Res 1987; 47: 2883-2891). Using conventional methods, tumor cells were found cytologically in 4,4 % of the patients. Histological confirmation could be demonstrated in 8,8 %. With HEA 125, 80 % of the cases were positive. HEA 125 is clearly superior to conventional examination techniques in demonstrating the presents of epithelial cells in bone marrow aspirates. However, the clinical significance of these results for the therapy and prognosis of SCLC can only be judged after a long period of follow-up.

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EXPERIENCE WITH AUTOMATED CELL COUNTING AND DIFFERENTIATION IN A CLINIC OF HEMATOLOGY AND ONCOLOGY

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The Technicon H*1 hematology analyzer permits assessment of the patients hematological status through a combination of cell counting and differentiation, morphology flags, peroxidase and basophil/lobularity cytograms and red cell and platelets histograms.

Among 605 cases within 3 months 110 blood samples showed abnormal morphology and required a microscopic examination of the blood smears. In these 110 cases a direct association to one of the following groups of hematological disorders was possible.

1. Chronic lymphocytic leukemia	40
other low grade malignant lymphomas with blasts in the peripheral blood	6
2. Myelodysplastic syndroms	15
3. Acute leukemias	13
4. Myeloproliferative disorders	25
5. Others	11

We looked for the H*1-leucocyte diagrams and the light-microscopic differential blood pictures. The limitations and the profit of the analyse will be discussed.

The reports of the Technicon H*1 hematology analyzer are a quick and reliable sreening method.

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Interferon-α for the treatment of refractory idiopathic hypereosinophilic syndrome

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Idiopathic hypereosinophilic syndrome (IHES) is defined by unexplained eosinophilia (eosinophil count $>1.5x10^9/L$) lasting more than six months. Standard treatment regimens are based on prednisolone, vincristine and hydroxyurea. We initiated IFN-α therapy in three patients with IHES not manageable by conservative treatment. These patients had IHES according to standard criteria, and chromosomal abnormalities had been ruled out by repeated cytogenetic analyses. Organ involvement included restrictive endomyocardiopathy and right ventricular failure in all patients, one patient also developed recurring diarrhea interpreted as gastrointestinal manifestation of IHES. Therapy with interferon-α (0.5-1.0 $x10^6$U/d) was initiated after unsuccessful treatment with hydroxyurea and after the development of severe vinca polyneuropathy. All patients responded with decreasing absolute eosinophil counts, stable or rising platelet counts and improved clinical performance status. While two patients remain stable after more than two years on interferon-α, the third patient developed a clinical picture similar to CML blast crisis after one year. We conclude that interferon-α may benefit patients with some variants of IHES, particularly those who cannot be managed by conventional therapy.

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PLASMA CELL AND LYMPHOID MALIGNANCIES: ASSOCIATION WITH ASBESTOS EXPOSURE

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Increasing attention is now being focused on the possibility that asbestos is a lymphoid carcinogen. Supporting evidence came from case-control and animal studies. However, cohort studies have been inconsistent so far. This is explained by the use of the inadequate ICD classification in such studies, the rarity of lymphoid neoplasias, and the supposed only small increase (2-3fold?) of asbestos associated disease risk. Therefore interest has been concentrated upon analysis of casuistics.

From routine patient investigation, medical expert's reports, the German occupational asbestos cancer study and a Swedish pleural plaque cohort data of 81 patients were accumulated. They were suffering from plasmacytoma (23), CLL (16), nodal NHL (3), extra-nodal NHL (20), disseminated NHL (6) and NHL of yet unknown localisation (13). All patients were occupationally exposed to asbestos, usually over many years and in median 25 years ago (range 3-38 y). This remarkably high preponderance of initial extra-nodal solitary involvement among the lymphomas is also found in other casuistics. So far, data of at least 64 cases of lymphoplasmacellular malignancies have been reported in the literature (15 plasmacytomas, 13 CLL, 10 nodal, 16 extra-nodal, and 3 disseminated NHL and 7 of unknown localisation). Of together 58 NHL with known primary localisation 36 were extra-nodal. Of them 8 emerged in the lung and 5 were solitary lymphomas of the pleura, an otherwise extremly rare place of lymphoma origin, but one of other site-specific asbestos malignancy (mesothelioma) as is also the lung (carcinoma). 12 NHL occured in the orogastrointestinal tract, another region highly supected to be a target of asbestos carcinogenesis.

These observations and in particular the striking occurence of solitary pleural lymphomas substantially support present evidence that asbestos is an lymphoid, possibly site-specific carcinogen.

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IS IRRADIATION AS SINGLE THERAPY STILL AN ADEQUATE TREATMENT FOR STAGE IA AND IIA CB-CC LYMPHOMAS?

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Since 1976, all patients with newly diagnosed stage IA and IIA of CB-CC NHL have received extended field radiotherapy (EF-RT) only. In 1982, the protocol was changed. Primary chemotherapy (CHOP) was given to all stages IIA followed by EF-RT.

All patients with *stage IA* (n=42) attained a complete remission (CR). 13 relapsed, 12 outside of the irradiation field. The latest relapse occured after 7 years. 13 patients had an initial extranodal or large nodal (>10cm) disease. 6 of the relapses belonged to this group and 5 of them succumbed to their disease. 6 relapsed patients achieved a second persistent CR. The relapse-free survival curve falls to 54%, the survival curve forms a 7 to 15-years plateau of 81%.

33 of 34 patients with *stages IIA* treated with EF-RT only achieved a CR. 22 relapsed, the latest after 7 years. Only 3 of them arose within the irradiated field. 10 patients achieved a second persisting CR, 9 died. The disease-free survival probability falls to 29%, the survival curve has a 7 to 15-years plateau of 67%. Of the 26 stages IIA patients treated with CHOP and EF-RT, 25 attained a CR, 4 relapsed, only 1 patient with initial large tumour mass died. The survival probability curve forms a 4 to 10-years plateau of 90%.

These long-term results convincingly support the combined modality approach in stages IIA. EF-RT alone seems to be an adequate treatment for stages IA without extranodal or large initial tumor only.

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PATIENTS WITH CONGENITAL CLOTTING DISORDERS ARE FREQUENTLY EXPOSED TO PARVOVIRUS B 19 THROUGH CONTAMINATED CLOTTING FACTOR PREPARATIONS

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Multitransfused patients with congenital clotting disorders are at risk of being exposed to viruses contaminating plasma pools. Parvovirus B19 is a candidate virus since the prevalence of infection is high, most infections are asymptomatic, viremia exceeds 10^{12} genome copies/ml, and the virus is very stable. We analyzed 41 batches of factor VIII, factor IX, and PPSB from 5 manufacturers for B19 DNA by polymerase chain reaction (PCR). Viral DNA could be demonstrated in 18 samples (44%) at a concentration of 10^3-10^6 genome copies/ml. Positive batches came from each manufacturer and there was no obvious prevalence in a specific factor preparation. We are currently purifying viral particles to answer the question of infectivity in vitro. To address infectivity in vivo, single sera from 62 transfused patients with hemophilia or factor VII deficiency were analyzed. B19-DNA could be demonstrated in 12 sera (19%) at concentrations below 10^5 genome copies/ml. DNA could also be detected in 2 of 16 sexual partners suggesting intrafamilial transmission. None of 24 normal blood donors or 34 multitransfused patients was positive for viral DNA. Anti B19-IgG was present in 74% and antiviral IgM in 5% of the patients as compared to 42% and 0% in untransfused controls. There was no correlation of B19-results with the incidence of active HBV-, HCV-, or HIV-infection as analyzed by PCR (0% HBV-positive and 56% HCV-positive) or serology (16% HIV-positive). Retrospectively, there were no clinical symptoms of active B19 infection in any DNA-positive patient. This data suggest that these patients are frequently exposed to parvovirus B19 through contaminated factor preparations. If infections occur, they do not seem to cause significant disease.

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MAFOSFAMIDE PURGING IN AUTOLOGOUS BMT: RATIONALE FOR ADAPTED-DOSE PURGED FIRST REMISSION MARROW

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There is evidence that marrow-purging with mafosfamide (mafo) improves survival in AML patients (pts) autotransplanted in CR1, but not in CR2. Individually adapted mafo-doses appear to be superior to fixed mafo-doses. For adapted-dose purging the dose inhibiting the growth of 95 % of the individual patient's bone marrow CFU-GM (ID_{95}) is determined in vitro ≥ 2 weeks prior to bone marrow harvest. If ABMT in AML patients is not performed in first but in later remission, autologous bone marrow may be collected either prophylactically in CR1 or in later stages of disease before ABMT.
To evaluate whether conditions for mafo purging vary with stages of disease and accumulating pretreatment, we determined the ID_{95} in 3 different groups of a total of 21 pts: 1.) 7 pts with AML-CR1 prior to HDAC consolidation; 2.) 7 pts with AML after HDAC consolidation and/or in ≥CR2; 3.) 7 pts with non-myeloid malignancies (5 ALL and 2 NHL). The median ID_{95} was 140 μg/ml (range 110-190 μg/ml) in group 1, 75 μg/ml (range 55-110 μg/ml) in group 2, and 100 μg/ml (range 80-160 μg/ml) in group 3. For adapted dose purging significantly higher mafo doses could be used in patients with less pretreatment. Our purging experience in 18 autologous bone marrow harvests is as follows: The median CFU-GM growth after bone marrow purging was 4.2% for adapted dose (n=9, median mafo-dose 100 μg/ml, range 80-140 μg/ml); 24% for standard dose (n=4, mafo-dose 70 μg/ml); and 17,5 % in a heterogeneous group of 5 pts whose marrow was purged with a mafo-dose of 90-160 μg/ml, median 95 μg/ml, which was below the individual ID_{95}. Despite the higher median mafo-dose in the latter group, purging was less efficient than in the adapted dose group. Since early CR1 marrow allows the highest adapted mafo-doses, we conclude that purging is more effective, even if ABMT is performed in later stages of disease. This may offer an explanation why a clinical benefit of purging CR2-marrow has not yet been proven. Whether survival after ABMT in CR2 is improved by using adapted-dose purged CR1 marrow remains to be demonstrated.

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DECREASED LIPID PEROXIDATION BY ANTIOXIDANT THERAPY IN PATIENTS UNDERGOING CONDITIONING THERAPY FOR BONE MARROW TRANSPLANTATION

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Bone marrow transplantation (BMT) follows high-dose chemotherapy generally in combination with total body irradiation (TBI). This conditioning regimen approaches the limit of tolerance for several tissues. Lipid peroxidation has been shown to be significantly increased after conditioning therapy (1,2), while lipid-soluble antioxidants in blood exhausted (3). Those conditions implicate that peroxidation processes may be involved in organ toxicity of high dose chemotherapy and TBI. The present study was designed to investigate, whether a high dose supplementation of antioxidants can diminish conditioning therapy's toxicity. 17 consecutive patients (pts) undergoig BMT were studied and divided into two groups: 10 pts without (BMT-) and 7 pts with (BMT+) supplementation of 825 mg DL-α-tocopherylacetate (TOCα), 45 mg ß-carotene (CARß), and 450 mg ascorbic acid daily p.o. for the average period of 20 days between day -28 and day -7, e.g. 3 weeks before the beginning of conditioning therapy. Blood was analyzed on day -28, -7, 0, and 14. Measurements of antioxidants TOCα und CARß were performed by HPLC as previously described (3). Lipidhydroperoxides (LOOH) in serum as marker of total body lipid peroxidation were estimated by using a CHOD-Iodide method as described previously (1,2). Lipid-standardized plasma TOCα expressed as molar ratio with cholesterol was 2.6 fold higher in group BMT+ after antioxidant supplementation (day -7) compared with group BMT- without supplementation. In erythrocyte membranes a 2.2 fold increase was found for the molar ratio of TOCα with cholesterol. Lipid-standardized plasma CARß expressed as molar ratio with cholesterol was 8.3 fold higher in group BMT+ on day -7, whereas LOOH were 78% less in group BMT+ after antioxidant supplementation (day -7) compared with group BMT-. Following conditioning therapy TOCα in plasma and erythrocyte membrane and CARß in plasma decreased and LOOH increased in both groups BMT+/-. However, the loss of antioxidants and augmentation of lipid peroxidation was significantly lesser in group BMT+, as well as some side effects (mucositis, erythrocyte deformability, lipid abnormalities, and liver toxicity) .

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ANTIOXIDANT EFFECT OF TROLOX, N-ACETYLCYSTEINE AND THIOCTIC ACID ON LIPID PEROXIDATION OF ERYTHROCYTES

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Numerous investigations indicate the involvement of free radical reactions in the pathogenesis of hemolytic diseases; i.e. in vitamin E deficiency, abnormal glutathione metabolism, decreased NADPH production (e.g. glucose-6-phosphate dehydrogenase deficiency), catalase deficiency, sickle cell disease, thalassemia, and paroxysmal nocturnal hemoglobinuria (1). Other conditions leading to free radical mediated anemia may be the anemia of chronic renal failure and Fanconi's anemia. Many drugs induce oxidative hemolysis in both healthy individuals and in patients with a defect in red cell antioxidant capacity, such as G-6-PD deficiency and vitamin E deficiency. Clinical studies with the antioxidants vitamin E and deferoxiamine have been performed, however, results are conflicting. N-acetylcysteine and thioctic acid are two clinically used substances, which antioxidant properties emerged in the last years. The present study was designed to investigate their antioxidant capacity in an erythrocyte system, in which isolated cells are exposed to oxidant stress by acetylphenylhydrazine, phenylhydrazine, t-butylhydroperoxide, and cumene hydroperoxide. Lipid peroxidation in the membranes following oxidant stress has been monitored by measurement of loss of membrane polyunsaturated fatty acids (PUFA) and α-tocopherol (vitamin E). Oxidation of the intracellular compartment has been monitored by the measurement of loss of reduced glutathione (GSH). N-acetylcysteine efficiently protected GSH against oxidant stress in a dose dependent manner, however it did not influence the extent of membrane lipid peroxidation (loss of PUFA) and loss of α-tocopherol, respectively. Thioctic acid exhibited no protective effect against membrane peroxidation or GSH oxidation. Trolox exhibited a very efficient protection against membrane peroxidation. Erythrocytes obtained from G-6-PD deficient subjects have been efficiently protected against GSH oxidation by N-acetylcysteine, whereas this antioxidant was not able to protect the membrane of those patients against peroxidation. In summary, N-acetylcysteine exhibited the best antioxidant property in respect of GSH oxidation, which implicates a potential role for this clinically well-known drug in the treatment of hemolytic crisis in G-6-PD deficient subjects and other conditions with oxidation-related hemolysis.

(1) M.R. Clemens. Adv Exp Med Biol **264**, 423-433, 1990

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MOLECULAR PATHOLOGY OF BERNARD-SOULIER SYNDROME AND GLANZMANN'S THROMBASTHENIA

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Genetic defects in the platelet glycoprotein (GP) Ib/IX/V complex are the cause of some rare bleeding disorders. The best known of these are the Bernard-Soulier syndrome (BSs) and "platelet-type" von Willebrand's disease (PvWd). In classic BSS GPIbα, Ibβ, IX and V are missing from the platelets which fail to adhere to exposed subendothelium at high shear. A wide range of variants have been described, either with normal amounts of the GP and a functional defect due to a point mutation, or with drastically reduced but still detectable amounts of the GP due to a mutation in one of the chains. The origin of an example of classic BSs has not yet been described but this is understandable as it could be due to a defect in the gene for any of the chains or in its promotor. The genetic defect in PvWd lies in GPIbα causing spontaneous binding of von Willebrand factor to platelets and leading to platelet aggregation and thrombocytopenia. In extreme cases thrombi may form. All patients so far identified were heterozygotes and the defects were due to point mutations within the 40 amino acid disulphide-linked loop of GPIbα. Other rare bleeding disorders are linked to extreme forms of size polymorphism in GPIbα. Normal polymorphism in GPIbα leads to single, double, triple or quadruple copies of a 13 amino acid sequence in the O-glycosylated domain, each containing 5 putative O-glycosylation sites. Genetic defects in GPIIb/IIIa lead to the bleeding disorder, Glanzmann's thrombasthenia, with defective platelet aggregation. Variants have been described but the origin of the Type II where up to 20% of apparently normal GP are expressed is still unclear.

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CONTROVERSIES IN THE TREATMENT OF SEMINOMA STAGE I
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Standard treatment of seminoma stage I is adjuvant radiotherapy with usually 25-30 Gy in the retroperitoneal field. After this treatment, 95-100% of patients survive more than 5 years. Risk factors are described, such as lymphatic or vessel infiltration, testis histology, hCG levels, infiltration of epididymis or spermatic cord, or preceeding operations.In a current study of radiotherapy with 26 Gy, the influence of risk factors and field reduction to a paraaortic field is being tested in view of efficacy and side effects. Because of the high cure rate and possible side effects of radiotherapy, surveillance protocols are being carried out. With the nonseminoma patients, the calculated relapse rate is 10-40%, whereas for seminoma patients the relapse rate was observed at 20%. Yet the data is still in a preliminary stage and requires confirmation over a longer period of time. The risk of distant metastases and the need for intenisve treatment at relapse (radiotherapy or highly effective chemotherapy) has to be taken into account.
Combination chemotherapy with cisplatin is very effective in metastasized stage of seminoma, carboplatin monotherapy also achieves nearly 90% complete remissions. The two treatment protocols are currently being compared in a randomized German trial.
After preliminary reports of cisplatin therapy, in low stage seminoma (I) carboplatin studies are now in progress. In 2 trials, with a total of 37 patients, only 2 relapsed within 2 years. These results need further confirmation and should be the basis for a trilinear randomized study comparing carboplatin monotherapy with radiotherapy and surveillance strategy in seminoma stage I.

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PHASE I CLINICAL AND PHARMACOKINETIK TRIAL OF DEXTRAN CONJUGATED DOXORUBICIN (DOX-OXD, AD-70)
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We have performed a clinical phase I trial of doxorubicin (DOX) coupled to dextran (70000 m.w.) (IV every 21-28 days). 12 patients (pts) have received a total of 24 courses (median 2; range 1-3). The starting dose was 40 mg/m^2 DOX equivalent (DOXeq) corresponding to 1/10 LD_{10} in mice. Grade IV thrombocytopenia was noted in 2/2 pts. Grade IV hepatotoxicity and grade III cardiotoxicity were noted in a pt with preexisting heart disease. 5 pts were treated with 12.5 mg/m^2 DOXeq. Maximal toxicity was grade III thrombocytopenia and grade III vomiting in one pt each. 5 pts received 20 mg/m^2 DOXeq. Hepatotoxicity was noted in 5/5 pts (1 x grade IV, 1x grade III) and thrombocytopenia in 3/5 pts (1 x grade IV, 2 x grade III). One pt receiving 12.5 mg/m^2 of DOXeq showed stable disease for 4 months. Pharmacokinetic analyses of total and free DOX were performed in plasma and urine using reverse phase HPLC and gel permeation chromatography. A dose-dependent linear increase of mean peak plasma concentrations was observed for total DOX (2.9 -11.8 µg/ml) with <2% free DOX. AUC ranged from 33.9-83.6 µg/ml*h with dose-dependent elimination half lives ($t_{1/2\alpha}$: 0.21-0.64 h; $t_{1/2\beta}$: 2.86-10 h; $t_{1/2\gamma}$: 61.2-137.5 h). We conclude that the MTD of AD-70 using this schedule is 40 mg/m^2. The recommended dose for clinical phase II studies is 12.5 mg/m2 DOXeq.

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Nierenzellkarzinome und das von Hippel-Lindau Syndrom: Ein neues Tumor Suppressor-Gen auf dem Chromosom 3

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Das von Hippel-Lindau (VHL) Syndrom ist ein pleotropes, autosomal-dominant vererbtes Tumorsyndrom mit nahezu kompletter Penetranz. Genträger entwickeln sowohl gut-, als auch bösartige Tumoren in verschiedenen Organsystemen: ZNS-Neoplasien, einschließlich Augen-Tumoren und Phäochromozytome werden neben zahlreichen Zysten in parenchymatösen Organen gefunden. Außerdem erkranken zwischen 17 - 55% der VHL-Patienten an Nierenzell-Karzinomen, welche zu den ernsthaftesten Komplikationen dieses Syndroms mit häufig tödlichem Ausgang gehören. Wir konnten vor kurzem zeigen, daß sich die genetische Störung im VHL Syndrom in einer einzelnen chromosomalen Region lokalisieren läßt. Epidemiologische und von uns erhobene tumorgenetische Daten erlauben die Annahme, daß es sich bei dem VHL Gen um ein neues Tumor Suppressor-Gen handelt. Wir möchten in diesem Beitrag unsere jüngsten Daten der Gen-Kartierung und der tumorgenetischen Studien mittels klassischer und molekularer Zytogenetik darstellen. Fluoreszenz in-situ Hybridisierungen konnte erfolgreich bei der Suche nach chromosomalen Deletionen eingesetzt werden. Doppelmarkierungen mit differentiell fluorochrom-markierten DNA-Sonden ließen eine eindeutige Festlegung der Reihenfolge unserer das VHL-Gen am engsten flankierenden Marker zu. Auf dem Weg zur Klonierung des VLH Genes, konnten wir mittels 'genetic linkage' in den Chromosomenbanden 3p25-p26 einen Bereich von etwa 8 centiMorgan zwischen den flankierenden DNA-Markern abgrenzen. Da auch für sporadische Nierenzellkarzinome die am häufigsten beschriebene chromosomale Aberration Deletionen im kurzen Arm des Chromsoms 3 darstellen, ist es möglich, daß das VHL Gen auch für die viel häufigeren sporadischen Nierenzellkarzinome eine kausale Rolle spielt. Alternativ dazu könnten sich aber auch mehrere Tumor Suppressor-Gene auf 3p befinden. Dieses Problem wird sich erst klären lassen, wenn das VHL Gen (oder Gencluster) isoliert und näher charakterisiert ist.

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MURINE BISPECIFIC MONOCLONAL ANTIBODY AGAINST CD28 AND CD30. POTENTIAL USE FOR THE TREATMENT OF HODGKIN'S LYMPHOMA
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Specific activation of tumor infiltrating lymphocytes can be achieved by a combination of bispecific antibodies directed against specific tumor markers and against the CD3 and CD28 locus on resting T cells. To take advantage of the enhanced T-cell activation by CD28-specific Mab 15E8, the bispecific Mab 15E8/HRS-3 with reactivity to both CD28 and the Hodgkin-associated CD30 antigen was generated by fusion of iodoacetamide treated 15E8 hybridoma cells with the HAT-sensitive hybridoma cell line HRS-3. Jurkat cells (CD3+/CD28+) representing resting T cells, were specifically activated to produce IL-2 by cocultivation with the B cell line LAZ509 (CD30+/CD19+) only in the presence of the CD30/CD28 crosslinking biMab 15E8/HRS-3 and a second CD19/CD3 bispecific Mab (OKT3/6A4). Neither these crosslinking biMabs alone nor any combination of the parental Mabs induced a comparable IL-2 production by Jurkat cells in the presence of LAZ509. Our results indicate, that such bispecific Mabs may offer a new approach for specific immunotherapy of Hodgkin's lymphoma, which takes advantage of the cytotoxic capacities of T cells.

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LIF mRNA EXPRESSION IS REGULATED AT THE TRANSCRIPTIONAL LEVEL IN DIFFERENT MURINE BONE MARROW STROMAL CELL LINES.

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Recently established evidence proved an important role for leukemia inhibitory factor (LIF) as hematopoietically active cytokine. The present study utilized two different murine bone marrow stromal cell lines, +/+-1.LDA11 and MBA-13, to define regulatory mechanisms of LIF mRNA induction. LIF mRNA was not detected in uninduced stromal cells under serum free condition. Incubation with interleukin-1 (IL-1), tumor necrosis factor-α (TNF-α) or 8BrcAMP alone resulted in weakly induced LIF mRNA levels. Coincubation of combinations of the used stimuli increased LIF mRNA expression supraadditively. LIF mRNA stability, even after stimulation, was low with a half life of about 30 min, indicating a functional effect of known AU-rich motives in the 3' untranslated LIF mRNA region. Transcriptional activation was found to be the main mechanism leading to LIF induction by IL-1, TNF-α and 8BrcAMP. Induction of transcription was detected 45 min post stimulation and showed peak levels at 90 min. Kinetics of LIF transcriptional activation showed high similarities with the kinetics of the transcription factors jun-B and c-fos, suggesting a possible role for these proteins in signaling transduction leading to LIF expression.

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IN VITRO ELIMINATION OF HUMAN LUNG CANCER CELLS FROM HUMAN BONE MARROW BY PURGING WITH ETHER LIPIDS AND CRYOPRESERVATION

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Effective and safe depletion of lung cancer cells from normal bone marrow prior to cryopreservation appears to be important when applying autologous bone marrow transplantation in patients with lung cancer. Four cell lines of human lung cancer (HTB 53, HTB 56, and HTB 58, and CCL 185) were investigated for in vitro growth before and after incubation with the antineoplastic ether lipid ET-18-OCH_3 (ET) for 4 hours and at escalating concentrations (25, 50, 75, and 100 μg/ml), as well as with and without cryopreservation. The higher the drug dose, the higher was the antitumor effect when measured as cytotoxicity with the human tumor cloning assay in agar or methylcellulose, thus showing a steep in vitro dose response relationship. After a 4 hour-exposure to 75 μg/ml ET at a cell density of 2 x 10^5/ml the number of colonies of HTB 53 decreased from 57 ± $4/10^3$ cells (100 %) to 1 ± $1/10^3$ cells (1,8 %) and after subsequent cryopreservation to zero colonies. To simulate the clinical condition in autologous bone marrow transplantation (ABMT) normal human bone marrow cells were mixed with malignant CCL 185 cells at a ratio of 100:1. After incubation with 75 μg/ml ET for 4 hours and subsequent cryopreservation the colony formation of CCL 185 was reduced to zero, whereas nearly half of the normal human hematopoietic progenitors (48 %) recovered. We conclude that the ether lipid ET may have potential value in purging human bone marrow of micrometastatic lung cancer cells before reinfusion in ABMT. Supported by Wilhem Sander-Stiftung 88.028.2.

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FLUDARABINE PHOSPHATE IN THE TREATMENT OF RELAPSED OR REFRACTORY LOW GRADE NON-HODGKIN LYMPHOMA

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Fludarabine phosphate is a fluorinated adenine nucleoside which has major activity in patients (pts) with de novo or refractory B-cell chronic lymphocytic leukemia. In order to investigate this agent in other lymphoid malignancies, we have treated 18 pts with advanced stages of low grade non-Hodgkin lymphoma (NHL). Thirteen pts were male, and 5 were female, ranging in age from 38 yrs to 74 yrs (median 56 yrs). The histologic classification was as follows: immunocytoma (ic), n=4; centroblastic-centrocytic (cbcc), n=5; centrocytic (cc), n=7; MALTOM, n=1; unclassified, n=1. One pt had stage III, and the remaining 17 pts had stage IV disease. Three pts had one, 8 pts had two, and the other 7 pts had more than two prior chemotherapeutic regimens. LDH levels were <240 U/l in 6, and >240 U/l in 12 pts. Fludarabine phosphate was supplied by the Division of Cancer Treatment, NCI, Bethesda, MD; the compound was administered at a dosage of 25 mg/m^2 at a 4 week interval up to a maximum of 12 cycles. Two pts had intercurrent therapy with prednisone and cyclosporin A because of hemolytic anemia. Responses were seen in 5/18 (28%), including one CR (NHL ic) and 4 PR (NHL cbcc, n=3; MALTOM, n=1). Durations of response are 3m, 4m+, 5m+, 7m+ for the pts achieving PR, and 4m+ for the pt with CR. There was one early death (NHL cc) most likely due to a tumor lysis syndrome. The major hematologic toxicity was thrombocytopenia in 10/18 (56%) pts and leukopenia in 7/18 (39%) pts. Major nonhematologic toxicity was infection in 10/18 (56%) pts. In conclusion, fludarabine phosphate used as a single agent is active in some advanced-stage patients with low grade malignant lymphoma. Fludarabine deserves further investigation in pts with less prior treatment, and as part of combination chemotherapy regimens in pts with advanced stages of these diseases.

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DETECTION OF t(14;18)+ LYMPHOMA CELLS IN BONE MARROW AND PERIPHERAL BLOOD OF PATIENTS BEFORE AND AFTER AUTOLOGOUS BONE MARROW TRANSPLANTATION

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The t(14;18) translocation can be detected in about 85% of follicular lymphomas. Applying a two-step PCR with nested primers we were able to detect minimal circulating t(14;18)+ lymphoma cells in 35% (7/21) of patients with cbcc NHL being in CR 2-12 years after radiotherapy for localized lymphoma. Four patients who relapsed 4-6 years after initial radiotherapy(2) or chemotherapy(2) had t(14;18)+ cells in their peripheral blood before relapse. After induction chemotherapy they were treated with autologous bone marrow transplantation. Three patients were transplanted with an histologically uninvolved, PCR+ bone marrow,the forth received an immunopurged marrow because of histological involvment. One patient relapsed at day +280, t(14;18)+ cells could already be detected in the peripheral blood on day +192. The other three patients are still in CR(2) or stable PR(1): two are PCR negative 8 and 18 months after BMT, in one case t(14;18)+ cells reappeared again in the circulating blood 2 months after BMT during stable PR lasting for up to 7 months. At the molecular level the t(14;18) translocation remained stable during the complete observation period in all patients (4-8 years). PCR analysis of peripheral blood or bone marrow cells allows the detection of minimal residual lymphoma cells,their prognostic significance with regard to relapse has to be evaluated in long term follow-up studies on patients in CR after radiotherapy, chemotherapy or BMT.

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ANGIOIMMUNOBLASTIC LYMPHADENOPATHY (AIL):HISTOLOGY AND SURVIVAL TIME.

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At present AIL is assessed as T-cell lymphoma of low grade malignancy although the progress can be rapidly fatal. Therefore we proofed the question to what extent histologic criteria are of prognostic relevance.

Mat. and Meth.: Retrospectively 40 lymph nodes with initially manifestation of typical histologic picture of AIL were semiquantitatively analyzed with regard to amount of T-cells (UCHL 1), B-cells (L 26), CD 30 positive blasts, reticulum cells (MAC, S 100), eosinophilic leukocytes, venules, PAS-positive material and histologic subtypes. These parameters were correlated with survival times.

Results: Only 5 of 40 pats. were still alive after a median follow up of 25 months. The median survival time came to 17 months. The amount of venules, PAS-positive material, reticulum cells and T-cells was without correlation to survival times. Partially preserved sinuses, more than 5 % B-lymphocytes and the subtype of mixed cellularity gave a weak prognostic relationsship. Favourable survival data correlated significantly with CD 30 positive blasts less than 5 %, low mitotic counts, few eosinophils and absence of multinucleated cells.

Conclusions

1. The amount of blasts and mitoses are of prognostic relevance in AIL likewise in other NHL.
2. Nevertheless, already a relatively low number of blasts signalized an unfavourable course of AIL.
3. With respect of our results most cases of AIL progress rapidly like high grade malignant NHL.

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GM-CSF RELATED EOSINOPHILIA AND LOEFFLER'S ENDOCARDITIS

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We observed a patient with GM-CSF related eosinophilic leucocytosis for several months and the coexistence of endocarditis parietalis fibroplastica.

Case report: A 66 year old man presented 1986 with neutrophilic agranulocytosis, fever and polyclonal gammopathy without evidence of toxic or infectious aetiology. The agranulocytosis of bone marrow and peripheral blood continued to Sept.1990. In Oct.1990 the patient was taken in a phase II study with GM-CSF. Now the leucocytes increased to a maximum of 22.3 10^9/l but with a percentage of over 80 eosinophils.GM-CSF-therapy was continued until Febr.1991 without efficacy concerning the neutrophil line. At autopsy, bone marrow revealed a total neutrophilic agranulocytosis and a marked eosinophilia. The parietal endocardium of both ventricles was fibrous thickened and lined by large mural thrombi of different age. In addition foci of microscopic scares were detectable in the inner third of myocardium.The patient died in consequence of gangrenous pneumonia with excessive eosinophilia and clusters of Charcot-Leyden crystals. Several possibilities for the association of GM-CSF related eosinophilia and endomyocardial fibrosis are discussed. Firstly, it could be coincidental. Secondly, eosinophils may cause in some way necrosis of myofibers and endocardial cells. Thirdly, the activation of monocytes and macrophages may stimulate the production of fibroblasts also. Watchfulness about this constellation is necessary.

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INCREASED SERUM LEVELS OF SOLUBLE INTERLEUKIN 2 RECEPTORS INDUCED BY G-CSF IN AUTOLOGOUS BONE MARROW TRANSPLANTATION

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Human recombinant growth factors are increasingly used for stimulating hemopoietic recovery after chemotherapy. However, their biological effects are not completely understood. Recent reports indicate that GM-CSF rapidly and strongly elevates the serum levels of soluble interleukin 2 receptors (sIL-2R), suggesting lymphocyte activation mediated by this growth factor. In this context, we have studied the influence of G-CSF on sIL-2R levels in 19 patients who underwent autologous bone marrow transplantion (BMT) for treatment of Hodgkin's disease or non-Hodgkin's lymphoma. Twelve patients received granulocyte colony-stimulating factor (G-CSF) from day 0 or day +1 after BMT until the white blood cell count had been stable for nine days above 1/nl, the remaining 7 patients did not receive growth factors.

Results: The application of G-CSF after marrow infusion did not result in an immediate change of sIL-2R levels (median 204 pM before vs. 256 pM after start of G-CSF, not significant). However, in all G-CSF-treated patients the sIL-2R levels increased steadily in the early posttransplant course, even in the absence of infection. This increase was statistically significant two to four days prior to appearence of leukocytes in the peripheral blood (median 340 pM, $p<0.025$) and peaked with the appearence of first peripheral blood leukocytes (median 536 pM, $p<0.001$). Cessation of G-CSF administration resulted in a decline of sIL-2R levels. In contrast, 5 of 7 patients without G-CSF treatment did not exhibit an sIL-2R increase before or at the time of engraftment. Infection was associated with a rise of sIL-2R levels. A correlation between sIL-2R levels and total leukocyte count, lymphocyte count, or CD25+ lymphocyte count was not evident.

Conclusions: G-CSF induces increased sIL-2R levels preceding engraftment in autologous BMT, which occur independent of lymphocyte activation. This may be compatible with involvement of activated myeloid bone marrow cells in G-CSF-induced sIL-2R release.

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PHASE I CLINICAL TRIAL OF AN ORAL INTERFERON INDUCER

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The low-molecular weight compound R-837 is known to be an effective interferon-alpha (IFN-α) inducer both in vitro and in vivo. In a phase I study 14 patients with malignant diseases (10 disseminated malignant melanoma, 2 chronic lymphatic leukemia, 1 nasopharyngeal and 1 renal cell carcinoma) were treated with the oral interferon inducer R-837 (3M Medica St. Pauls, MN, USA). Interpatient dose escalation was performed with every other day dosages of 100mg, 200mg, 300mg and 400mg. Treament was continued until either intolerable side effects (WHO grade III, IV) or disease progression occurred.

Pharmacokinetic studies demonstrated peak levels of R-837 within 2 hours after oral administration and a serum half life of approximately 6 hours. In 10 patients plasma IFN-α level were detected; remarkably, 2 of these patients had endogenous levels of IFN-α ca. 4x higher than levels reported after s.c. injection of 10 mio. units of IFN-α. In all patients increased levels of the Mx-A protein were measured suggesting that the Mx-A protein level is a more sensitive parameter than IFN-α level in detecting IFN-production in vivo. The side effect profile of R-837 was different from that of IFN-α. The major dose limiting side effects were nausea and vomiting which led to interruption of therapy in three and dose reductions in six patients; these side effects occurred 2-4 hours after administration of R-837 and did not correlate to the plasma levels of IFN-α or Mx-A protein. Side effects typical for IFN-α (fever, flu-like syndrome, loss of appetite) were present in half of the patients and were usually mild (WHO grade I). No complete or partial response was observed; however in one patient with CLL peripheral blood leukocyte counts dropped by more than 50% after R-837.

These data demonstrate that R-837 induced IFN-α in all patients studied. It remains to be established whether this oral interferon inducing activity can be used clinically.

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NEW AGENTS AND APPROACHES IN AML
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Areas in which progress can be made in AML are in improving induction therapy as well as enhancing post-remission therapy. Recent developments may affect both phases of AML therapy. Idarubicin is a new anthracycline which has produced a higher response rate when used in combination with AraC, compared to Daunorubicin and AraC. Idarubicin also appears to affect overall outcome, with enhanced survival among idarubicin treated patients compared to Daunorubicin treated patients. This improved result may reflect the additional activity of the alcohol (idarubicinol) observed against leukemia. Idarubicin is currently being used as firstline therapy in the ongoing U.S. Intergroup AML study. Interleukin-2 (IL2),T-cell growth factor, stimulates killer-cell mediated tumor kills, activating T-cells, NK cells and LAK cells. Antileukemic activity has been observed in vitro when LAK cells or NK cells are utilized. IL2 is being explored as post-remission therapy to stimulate an immune response to minimal residual disease. It is also being explored post-autologous transplant in an attempt to induce graft versus leukemia effect. Preliminary data are encouraging. Data in support of these statements will be presented.

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THE BFM STRATEGY IN CHILDREN WITH ACUTE LYMPHOBLASTIC LEUKEMIA (ALL) QUALIFIED FOR ALLOGENEIC BONE MARROW TRANSPLANTATION IN FIRST REMISSION

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Close to 3000 children with ALL have been treated according to 6 consecutive BFM protocols over a period of two decades. This analysis focuses on the retrospective evaluation of 998 children with non-B-ALL in trial ALL-BFM-86. The intention was to define small subgroups of pts with a critically impaired prognosis as potential candidates for allogeneic BMT in first remission. Over 90% of children treated in this trial have cure rates in the range of 70-80%. There is, however, a group of patients (< 10%) with an unsatisfactory prognosis despite most intensive chemotherapy. This subgroup can be subdivided: 1) late- or non-responders not achieving remission within one month (EFS 19%), 2) t(9;22) ALL (EFS 31%), 3) inadequate response to initial steroid treatment, if combined with at least one additional factor such as: tumor burden > 1.7 (EFS 31%), T-ALL with at least one early T-marker (EFS 22%), coexpression of myeloid marker(s) (EFS 24%), and t(4;11) in infants with null/mixed-lineage-ALL (EFS 20%). The validity of these criteria has to be proven in the ongoing trial ALL-BFM-90. At present, 41 children fulfil these criteria. 14 of these (34%), who were not transplanted, already failed experimental chemotherapy compared to 13/474 failures (3%) in the complementary group. Whether allogeneic BMT can improve EFS in these children is being investigated under controlled conditions.

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CARBOPLATIN, ETOPOSIDE AND VINCRISTINE (CEV): AN ACTIVE REGIMEN IN EXTENSIVE DISEASE (ED) SMALL CELL LUNG CANCER (SCLC).

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Carboplatin (C), Etoposide (E) and Vincristine (V) are highly active single agents in SCLC. After a phase I/II trial with this combination (J Cancer Res Clin Oncol. 116, 1990) we initiated a phase II trial in patients (pts) with ED SCLC. Inclusion criteria: measurable/evaluable disease, WHO performance status (PS) $\leq$ 3, no prior chemo-/radiotherapy, normal liver-, renal-, cardiac- and bone marrow function. Treatment plan: C 300 mg/m^2 iv, d 1; E 140 mg/m^2 iv, d 1,2,3; V 1.5 mg iv, d 1,8,15,22 q d 28 x 6. Pts characteristics: male/female 75/19; PS 1 (0-3); age 59 yrs (39-77). Results: evaluable for response and toxicity 94: CR 26 (28% [19-37%]), CR/PR 66 (70% [61-79%]), NC 18 (19%), PD 8 (9%); median remission duration 8 months (mo) (CR 11 mo; PR 7 mo); median survival time 9 mo (1-45+) (CR 13 mo, PR 10 mo, NC 7 mo, PD 4.5 mo). Toxicity (WHO grade): leukocytopenia 2^0 27%, 3^0 36%, 4^0 5%; infection 2^0 9%, 3^0 4%; thrombocytopenia 2^0 16%, 3^0 20%, 4^0 7%; anemia 2^0 22% , 3^0 16%, 4^0 4%; 3 treatment related deaths: one due to myocardial infarction, two pts due to septicemia; allergic reactions to E 1^0 1 pt , 2^0 1 pt , 3^0 1 pt; transient rise of creatinin above 1.5 mg/dl 2%; nausea/vomiting 1^0 38%, 2^0 16%, 3^0 4%; alopecia 3^0 was observed in all pts. Conclusions: CEV is an active regimen in the treatment of ED SCLC and well tolerable with respect to gastrointestinal, renal and hematologic toxicity. However, because of V induced neurotoxicity, V had to be discontinued after a median of 10.5 mg total dose applicated.

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A PILOT STUDY OF AN INTENSIVE PREOPERATIVE (PREOP) CHEMO-RADIOTHERAPY (CTx/RTx) FOR LOCALLY ADVANCED NON SMALL CELL LUNG CANCER (NSCLC).

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Since 3/91, 26 patients (pts) with stage IIIa/b NSCLC (T3-sulcus superior tumors, T4, N2(>2 lymph node stations involved as defined by mediastinoscopy), N3), unlikely to undergo curative resection have been entered into an ongoing pilot study with an intensive preop CTx/RTx. Treatment plan: Cisplatin(P) 60 mg/m^2 iv, d 1+7; Etoposide(E) 150 mg/m^2 iv, d 3,4,5, q d 22 for 3 cycles, followed by RTx 45 Gy within 3 weeks (1.5 Gy 2x daily, 5x/week) plus simultaneous CTx (P 50 mg/m^2, d 2+8; E 120 mg/m^2 d 4,5,6) starting on d 2 of RTx, followed by surgery (S) 3-4 weeks after end of RTx. Pts characteristics: male/female 20/6; PS 90%(70-100); age 55 yrs(32-67). IIIa/b 12/14; squamous cell ca 15, adeno ca 7, large cell/anaplastic ca 4. Results: after CTx: too early 6, CR 3/20(15%), CR+PR 15/20(75%), MR 3/20, PD 2/20; after CTx/RTx: further significant tumor regression in 4 pts. 4 pts are off treatment (treatm) without S(2 non-responders to CTx received palliative RTx, 1 pt had CTx/RTx only because of apoplexia, 1 CR after CTx refused further treatment); after S (n=12): pCR 6, R0-resection 5 (3 with microscopic disease only), irresectable 1. Four relapses occured up to now (2 CNS, 1 liver, 1 local). Toxicity (WHO grade): of CTx: leukopenia 3^0 21%, 4^0 4%; infection 3^0 4%; thrombopenia 3^0 11%, 4^0 2%; n/v 2^0 29%, 3^0 14%; of CTx/RTx: leukopenia 3^0/4^0 75%; thrombopenia 3^0/4^0 44%; esophagitis 3^0/4^0 50%. No increased preop morbidity and no treatm related death were observed. All pts are still alive. Conclusions: This intensive preop multimodal treatm is feasible and highly effective for locally advanced NSCLC. Twelve of 15(75%) pts who are off treatm were clinically disease-free after CTx alone(1pt) or after S(6 pCR, 5 NED). Because of these encouraging results a randomized trial with this program versus S alone is planned for pts with clinically resectable stage IIIa

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BONE MARROW TRANSPLANTATION IN CHRONIC MYELOID LEUKEMIA UTILIZING UNRELATED DONORS.

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Twenty-five patients with chronic myeloid leukemia were transplanted from HLA-identical, MLC negative unrelated donors. Two patients were in refractory blast crisis, 6 in second chronic phase, 4 in accelerated phase and 13 in the first chronic phase. The age range was 8 to 55 years (median 29 yrs.). The conditioning protocol included fractionated TBI (12 Gy, lung shielding 10.5 Gy) and cyclophosphamide (+ etoposide in pts not in first chronic phase). Cyclosporin plus methotrexate was administered for GvHD prophylaxis; a murine monoclonal anti-IL2-receptor antibody (BB-10) plus corticosteroids was added later on. The incidence of GvHD grade >=II was 65%. Causes of deaths were: GvHD and infection (7), infections (4) and relapse (2). On the basis of these data and other published work, a donor search should be done in all patients with CML in chronic phase under the age of 50 years.

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INCREASED SERUM IL-8 AND G-CSF-LEVELS IN PATIENTS AFTER AUTOLOGOUS BONE MARROW (ABMT) OR BLOOD STEM CELL TRANSPLANTATION (ABSCT)

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Interleukin-8 (IL-8) belongs to a group of 8kD-peptides, all produced by various cell types. Based upon structural similarities they were grouped in the so-called "small-cytokine" (scy)-superfamily. IL-8 exerts strong proinflammatory effects such as neutrophil activation and chemotactic activities. We studied the biological role of IL-8 and G-CSF in patients following ABMT or ABSCT. Using enzyme-linked immunosorbent assays (ELISA) for IL-8 and G-CSF (Quantikine, R & D Systems), the median IL-8 serum level in 11 healthy volunteers was 33.8 pg/ml (range:19.5-50). G-CSF was detectable only in 7/11 volunteers with a median of 25 pg/ml (12.6-37.3). A total of 435 samples from 41 patients (13 female/28 male) was examined. Their median age was 38 yr (range:16-51). Autografting (29 ABMT/12 ABSCT) was performed in AML, ALL, Hodgkin's disease and Non-Hodgkin's lymphoma. A strong correlation between IL-8 and G-CSF serum levels ($R = 0.73$, $p < 0.05$) could be demonstrated. Previously we have shown that G-CSF and WBC are inversely correlated reflecting the central role of G-CSF for neutrophil homeostasis. A similar relationship - although less pronounced - was found between IL-8 and WBC. Prior to autografting the median IL-8 serum level was 68.3 pg/ml (range: 13.3-3654). During marrow aplasia, a median IL-8 serum level of 422.9 pg/ml (range: 77.7-2866 pg/ml) was observed. A median 1.4-fold increase of IL-8 levels could be demonstrated in patients with fever (>38.5°C) compared to non-febrile ones. The highest IL-8 levels ranging from 890.7 to 4150 pg/ml were observed in patients with extensive pulmonary infiltrates. In summary, the endogenous serum levels of IL-8 and G-CSF following autotransplantation are closely related depending on the absolute leukocyte count and intercurrent events such as fever and/or infectious complications. These data might reflect a synergy of the proinflammatory cytokine IL-8 with G-CSF on mature effector cells as well as on responsive hemopoietic progenitor cells. The latter hypothesis is supported by in-vitro studies of MIP-1 and MIP-2 demonstrating their colony-stimulating activity as members of the "scy"-superfamily.

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IMMUNE RESPONSE MODULATION BY PENTOXIFYLLINE IN VITRO

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Pentoxifylline (PTX) has recently been shown to modulate TNF-α production and to reduce the incidence and severity of all major complications after bone marrow transplantation (BMT), including mucositis, veno-occlusive disease, renal insufficiency, hypertension and graft-versus-host disease. In order to analyze in detail the effect of PTX on immune complications after BMT we investigated the modulatory effect of PTX on immune response in vitro.
Continuous presence of PTX significantly reduced the proliferative response of peripheral blood mononuclear cells to phytohemagglutinin stimulation and to alloantigens in a dose-dependent manner. Starting at concentrations of 100 µg/ml, PTX was able to inhibit and at 1000 µg/ml completely block mitogen-induced proliferation. Maximal inhibition of more than 90% (91±4%) was also observed at PTX concentrations of 1000 µg/ml in the mixed lymphocyte culture. However, lower but still significant suppression (13±7%) was achieved at concentrations of 10 µg/ml PTX. The inhibitory capacity of PTX was increased by monoclonal antibodies against TNF-α (34±5% additional suppression at 100 µg/ml PTX) and not reversed by the addition of rTNF-α. The effect of PTX on the generation of cytotoxic T-lymphocytes (CTL) in vitro was studied in the CML assay. PTX 100 µg/ml significantly inhibited (p=0.0178) the in vitro generation of CTLs, when PTX was added to the culture on day 0. PTX also showed profound modulatory properties in the NK assay with a reduction of 23±3% in specific lysis at 10 µg/ml PTX and maximal reductions of 88±3% at 1000 µg/ml PTX.
Immunomodulatory properties of PTX were not only associated with blockage of TNF-α as shown by decreased mRNA expression and TNF-α values in the culture supernatants, but also with an impaired production of other cytokines and secondary messages such as IFN-γ and neopterin. PTX treatment, however, did not affect IL-2, IFN-α, IL-1β or β-2 microglobulin production, IL-6 release was even increased.
PTX has therefore profound immunomodulatory properties in vitro, which are associated with selective inhition of cytokines release and can be enhanced by the addition of monoclonal antibodies against TNF-α but not reversed by the addition of rTNF-α.

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Chlamydia pneumonia (TWAR) as a possible cause of pneumonia in immunocompromised patients

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Chlamydia pneumonia (TWAR) is a recently identified Chlamydia that causes both upper and lower respiratory tract infection including pneumonia. The etiologic role of this organism in immunocompromised pts. with pneumonia is not clearly understood. Bronchoscopy with bronchoalveolar lavage was undertaken in 61 immunocompromised pts. with fever and radiologically confirmed pneumonic lesions for microbiological and cytological diagnosis. Chlamydia pneumonia was identified by a fluorescein-conjugated TWAR monoclonal antibody (Chlamydia TWAR-Antigen JF-Test Medac Diagnostika) in BAL of 13 pts. (6/21 acute leukemias; 4/14 other malignancies, 0/5 HIV-infections, other underlying diseases 3/21). Other organisms identified were gram-positive (n=52) and gram-negative (n=15) bacteriae, fungi (n=12) and viruses (n=3). The fatality rate of pts. with and without Chlamydia pneumonia was comparable (4/13 = 31 %; 17/48 = 35 %).
Conclusion: There is a strikingly high incidence of colonization with Chlamydia pneumonia in immunocompromised pts., its relevance as a causative agent of pneumonias has still to be clarified.

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Respiratory function tests after chemotherapy and/or radiotherapy in patients with Hodgkin's disease and Non-Hodgkin-Lymphomas

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Given the high cure rate of pts. with Hodgkin's disease and Non-Hodgkin-Lymphomas, the complications related to therapy take on great significance. We therefore studied 33 pts., median age 42 (13 - 65 years, Hodgkin disease n=15, Non-Hodgkin-Lymphomas n=18) for 12 to 36 months after polychemotherapy (n=13) and mantle irradiation (n=33) with pulmonary function tests (PFT), progressive exercise tests on a bicycle ergometer, arterial blood gas tests and diffusing capacity of carbon monoxide (DLCO). Mantle doses ranged between 26 and 40 Gy given at 1,5 Gy tumor dose per day. Irradiated lung volume was 30 - 45 %.

Results: In analysis of the PFT and exercise data, individual test results were compared with normal standards for that patient's age, sex and height are expressed as a function of percent change from predicted values.

Total Lung Capacity	104 ± 13 % (81 - 123 %)
Vital Capacity	94 ± 14 % (67 - 131 %)
Forced Exp. Volume	87 ± 11 % (57 - 119 %)
PaO_2 (Rest)	81 ± 8 (63- 93 mmHg)
PaO_2 (1 Watt/kg B.W.)	86 ± 9 (71-106 mmHg)
DLCO	85 ± 16 % (58 - 119 %)
Maximum Work	2,2 ± 0,7 W/kg (1,0-3,7 W/kg)
	85 ± 25 % (51-139 %).

Conclusions: Pts. tolerated chemotherapy and/or mantle radiotherapy remarkably well. No clinically significant effect on the pulmonary function and exercise test results had been observed.

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FREQUENCY AND LOCATION OF CMV- AND GVHD-MEDIATED INTESTINAL LESIONS AFTER BMT

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Diarrhoea in patients occurring later than 20 days after allogeneic bone marrow transplantation is most likely due to either graft-versus host disease or an infection of the lower gastrointestinal tract. Apart from bacterial and fungal infections, viral disease, especially CMV enteritis, is a major cause of severe gastrointestinal symptoms after BMT. To further analyze the influence of CMV infection and graft-versus host disease on intestinal dysfunction after BMT, intestinal biopsies obtained from 27 patients suffering from diarrhoea around day +30 after allogeneic BMT were analyzed for the presence of CMV and GvHD-typical histological and immunohistological alterations. To exclude the influence of other infectious agents intestinal biopsies were only studied when no bacteria and fungi nor other viruses apart from CMV were grown from the stool or the biopsy specimens.
During colonoscopy biopsies were obtained from different sites and analyzed for the presence of CMV and GvHD-associated histological and immunohistological alterations. In biopsy specimens from 8 out of 19 patients analyzed by PCR and in situ hybridization assays CMV could be detected. Only in 6 of these 8 cases CMV could also be demonstrated by immunohistology using 4 monoclonal antibodies directed against different CMV proteins. Only in 4 biopsies cytomegalic cells could be found. CMV as well as histological and immunohistological lesions described as typical for acute GvHD were most often detected in biopsy specimens obtained from the ascending colon.
All the patients with CMV infection of the lower gastrointestinal tract as well as the 12 patients with marked histological alterations (GvHD grade II-IV) suffered from severe diarrhoea. The finding of severe histological and immunohistological alterations described as typical for acute GvHD in patients with CMV-enteritis further indicates an interaction of virus and the local immune system of the lower gastrointestinal tract.

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MAGNETIC RESONANCE TO ASSESS HAEMATOPOETIC RECONSTITUTION AFTER BMT

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Magnetic resonance has become a powerful tool for diagnostic imaging of soft tissue. Bone marrow differs from all other tissues in one important quality: In healthy persons there are nearly equal fractions of water and lipids. Separation of lipid and water signals is possible by the chemical shift difference. The larmor frequency difference of 215 Hz at 1.5 T allows to record alterations of the lipid/water content by imaging and 1H localized spectroscopic methods. In our experience with several patients with different haematological disorders of low and high bone marrow cellularity the increase of lipids in the bone marrow in vertebral bodies and the decrease of cellularity of iliac crest biopsies showed good correlation.
Patients were analyzed following 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. Patients after autologous and allogeneic BMT were analyzed for haematological reconstitution. Additionally the influence of acute GvHD on marrow reconstitution was assessed by NMR spectroscopy of the bone marrow.
The peripheral haematological parameters and the NMR data were compared in these patients. Additionally in some patients marrow cellularity could be determined by marrow histology.
In spite of a rapid increase in the white cell count after autologous marrow transplantation, NMR spectroscopy revealed an marked increase in the lipid/water ration indicating a low cellularity in the early post-transplant period. In contrast after allogeneic marrow transplantation NMR analysis showed a normal or even decreased lipid/water ratio already in the early post-transplant period. Only in patients suffering from severe GvHD, especially patients after BMT from an unrelated donor, marrow cellularity remained low as indicated by an increase in the lipd/water ratio. Thus, magnetic resonance might allow rapid, non-invasive analysis of haematopoetic reconstitution after BMT using chemical shift selective imaging techniques and 1H spectroscopy.

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MARKED B-CELL DYSFUNCTION AFTER BMT IN CORRELATION WITH THE OCCURRENCE OF CMV INFECTION

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CMV infection and acute GvHD have been shown to be correlated with alterations of the reconstitution of the cellular immune system. Inconsistent data have been reported about the influence of GvHD and infections on the reconstitution of the humoral immune system after allogeneic BMT. In animal models GvHD as well as viral infections have been observed to be associated with the occurrence of autoantibodies after BMT. 71 patients undergoing allogeneic bone marrow transplantation were followed up for the occurrence of paraproteins as well as organ-specific and non-organ specific autoantibodies in the early post-transplant period. In serum samples of these patients autoantibodies could be detected in a significantly higher frequency than in normal control persons. No correlation could be shown between the occurrence of autoantibodies and the severity of acute graft-versus-host disease. Symptomatic CMV infection was significantly associated with autoantibody production. Naturally occuring antimitochondrial antibodies and cytoskeleton antibodies were only observed among patients with symptomatic CMV infection. Similarly monoclonal, biclonal and triclonal paraproteins were only detected in patients with CMV disease. In all of these patients CD4-lymphocytopenia indicated marked disturbance of cellular immune reconstitution. Oligo- and polyclonal stimulation of B-cells may thus be a sign of defective T-cell regulation of humoral immune responses during CMV infection after allogeneic BMT.

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ANALYSIS OF PARAMETERS PREDICTING THE OCCURRENCE OF CMV DISEASE AFTER BONE MARROW TRANSPLANTATION

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63 patients undergoing allogeneic bone marrow transplantation were followed up for the development of CMV infection and CMV disease. In 46 out of these 63 patients CMV could be detected in blood and urine samples a median of 29 days after BMT. In 36 of these patients the virus could be additionally cultivated from blood and/or urine samples a median of 45 days after BMT. CMV infection was associated with severe acute GvHD. 28 out of the 46 patients developed symptomatic CMV infection. All the patients with symptomatic CMV infection showed a drop in the lymphocyte count several days prior to the onset of CMV disease. Antiviral therapy of CMV disease was followed-up by virus culture and PCR technique. In successfully treated episodes of CMV disease clinical improvement was associated with an effective suppression of virus replication as demonstrated by negative culture and PCR technique. Apart from PCR-positivity at the end of antiviral therapy, CD4+-lymphocytopenia was found to be a bad prognostic parameter for the outcome of CMV infection after allogeneic BMT. To further analyze host- and virus-specific parameters for their influence on the development of CMV disease HLA-type and virus strain differences were analyzed in patients with symptomatic and asymptomatic CMV infection. Demonstration of mutations in the immediate early gene region and the a-sequence of the CMV strain in comparison to the CMV strain AD 169 could be found more often in patients with symptomatic than asymptomatic CMV infection. Due to the high rate of treatment failures in patients with CMV infection and persistent CD4+-lymphocytopenia adoptive immunotherapy should be considered in addition to treatment with nucleoside analogues.

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IS THE KARYOTYPE A PROGNOSTIC FACTOR FOR RESPONSE TO CYTOKINE-THERAPY IN PATIENTS WITH MYELODYSPLASTIC SYNDROMES (MDS)?

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To investigate the influence of the karyotype on the response to treatment with GM-CSF, G-CSF, Il-3 or Erythropoietin the karyotype of patients (pts) with MDS was related to the hematological changes induced by cytokine therapy. During the time period between 1985-1992 a total of 48 pts with MDS was evaluated retrospectively: 26 men and 22 women; median age of 61 years (range 32-78). Morphological subtypes were refractory anemia (RA) in 28, RA with ringsideroblasts (RARS) in 2, RA with excess of blasts (RAEB) in 11, RAEB in transformation (RAEB-t) in 6, and CMML in 1 pt. 33 pts (69%) had chromosomal abnormalities. Out of the total of 48 pts, 34 pts were treated with cytokines, 7 of them received several growth factors sequentially (Il-3: n=14; GM-CSF: n=18; EPO: n=4; G-CSF: n=7). Of the pts treated with Il-3, cytogenetic follow-up revealed evolution, i.e. appearance of further abnormalities or increase in percentage of abnormal metaphases in 3/6 pts. No new cytogenetic clones appeared in the other 3 pts. Cytogenetic follow-up was also done in 9/18 pts treated with GM-CSF. Of the 7 pts with abnormal karyotype the percentage of abnormal metaphases increased in 2 pts, remained unchanged in 2 pts and decreased in 3 pts. 4 pts treated with EPO retained their karyotypic pattern. 4/7 pts receiving G-CSF were followed cytogenetically and in 2 of these the percentage of abnormal metaphases decreased. There was no difference in the response pattern, i.e. increase of blood counts, in pts with or without abnormal karyotype. 6 pts (5 receiving GM-CSF, 1 Il-3) had progression to AML; out of these 3 had an abnormal karyotype. Cytokine treatment stimulates both, cytogenetically abnormal and normal cell clones. Presence of chromosomal aberrations was not predictive for progression to AML during cytokine treatment and did not preclude hematopoetic response, e.g. increase of neutrophils or platelets.

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PROGNOSTIC RELEVANCE OF INITIAL PARAMETERS IN HIGH-GRADE MALIGNANT NON-HODGKIN LYMPHOMAS: RESULTS OF AN INTERNATIONAL METAANALYSIS

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In the chemotherapy of high-grade malignant NHL a modification and intensification of standard regimens has led to an increase in complete remission rates achievable. However, relapse rates have not been reduced accordingly and a relevant improvement in survival is not yet discernible. A number of initial parameters present at the time of diagnosis influence significantly the long-term relapse-free and overall survival. Beside the general condition of the patient these factors include the pattern of involvement and the indicators of tumor volume and biological activity. Several models have been developed to quantify the individual risk. In order to increase the predictive power of such calculations for long-term prognosis, an overview analysis (16 centers from Canada, USA and Europe) with a total of 4,300 cases recruited between 1975 and 1987 and including the COP-BLAM/IMVP-16 study (BMFT) was performed. Parameters identified to retain highly significant prognostic value in multivariate analysis (age, performance status, stage, serum LDH, number of E-sites) were used to form a prognostic index defining four risk groups. Detailed results of the analysis (e.g. the persistent risk in the 'cured' patients) and an evaluation of the prognostic index in the COP-BLAM/IMVP-16 multicenter trial will be presented.

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IMMUNOTOXINS: NEW TOOLS FOR THE TREATMENT OF HUMAN HAEMATOPOITIC MALIGNANCIES?

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Since the first description of so called "Magic bullets (Zauberkugeln)" by Paul Ehrlich nearly hundred yers ago, oncologists have been searching for treatment modalities that would specifically kill the malignant cell thereby leaving normal tissue unharmed. However, it was not until the advent of monoclonal antibody (MoAb) technology that tumor selective reagents became available in limitless supply. A large number of MoAbs with selectivity for tumor cells have been prepared particularely for hematopoietic malignancies. Many of these were linked to the ribosome damaging A-chain of ricin or other toxins like abrin, saporin or diphteriatoxin to form immunotoxins which combine the selectivity of the antibody moiety with the potency of the toxin. The first generation of these immunotoxins showed impressive results *in vitro* but in most cases disappointing antitumor effects in in animal systems or patients. In contrast, the second generation of immunotoxins consisting of recombinant toxin molecules with ligands that are genetically engineered or ricin A-chain immunotoxins with a greatly improved stability and selectivity have been demonstrated to be extremely effictive in several animal models. The results of the current clinical trials in lymphoma patients suggest a possible clinical use of immunotoxins.

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INTENSIFIED CHEMOTHERAPY IN A PATIENT WITH POLYARTERITIS NODOSA

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We report on a 61 year old farmer who became sick with fever, want of appetite, vomiting, weight loss, head-ache, cough, myalgias, and anemia. First a malignancy was suspected. Inspite of intensive diagnostic procedures the specific diagnosis could not be obtained. Assuming an arteritis cranialis Horton steroids were given. This lead to a transient improvement. Because of adverse effects the patient discontinued the treatment and sought alternative therapies.
8 months after the initial onset of symptoms the patient was admitted to our hospital in a severely ill condition. A biopsy of the musculus suralis lead to the diagnosis polyarteritis nodosa.
Therapy with cyclophosphamide (800 mg / m^2 intravenously every three weeks) and steroids did not lead to a complete regression of clinical symptoms and pathological laboratory findings within three months. Thereafter, we started an intensified chemotherapy with cyclophosphamide and doxorubicine, 50 mg / m^2 every three weeks up to a total dose of 430 mg hoping that this would suppress the overwhelming immunological process.
The treatment was well tolerated; the main side effect was an alopecia grade III to IV. Shortly after starting the intensified chemotherapy a dramatic improvement occurred. Today, 20 months after discontinuation of treatment the patient is well. He is able to work on his farm again.

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LONGTERM REMISSION BY LOCO-REGIONAL BLEOMYCIN IN A PATIENT WITH PRETREATED MALIGNANT MESOTHELIOMA

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Diffuse malignant mesothelioma is a rare disease with heterogeneous course and an overall median survival < 12 months, not convincingly altered by different treatment modalities.
In 2/1982 diffuse malignant mesothelioma of the pleura was diagnosed in a 40-years-old man and treated by extrapleural pneumectomy and radiation therapy with 50 Gy. In 11/1989 massive ascites developped and the diagnosis of malignant mesothelioma was confirmed by cytology and histology, now confined to the peritoneum. Intraperitoneal therapy with 5 cycles of mitoxanthrone, 1 cycle of carboplatinum as well as two instillations of yttrium 90 did not controll the production of ascites. Repeated paracenteses had to be performed every three to six weeks. In 5/1990 the patient received two 30 mg doses (d1, d21) of bleomycin intraperitoneal. Thereafter no paracentesis was necessary. Without further treatment the patient is still in complete remission without ascites, metastases or signs of mesothelioma of the peritoneum in ultrasonography or CT scans
This case report demonstrates that individual patients may benefit from consecutive treatment attempts in malignant mesothelioma.

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EXPRESSION OF ADHESION MOLECULES LFA-1 / ICAM-1 AND C-MYC ONCOPROTEIN IN HUMAN PLASMA CELLS

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Signal transduction by LFA-1 and its ligand ICAM-1 and gene regulation by nuclear proteins like c-MYC might mutually interfere and control complex physiological processes e.g. intercellular communication, localization of tumour cells, interaction with stromal cells and proliferation. We analysed bone marrow plasma cells of 40 patients with multiple myeloma, MGUS or reactive increase in plasma cells for the expression of these proteins by applying a double sandwich immunoperoxidase method. In the majority of cases independent of diagnosis and proliferative state plasma cells showed a marked expression of ICAM-1, with membrane and characteristic intracytoplasmic distribution. LFA-1 staining was observed in 30% of cases and was confined to small plasmacytic cells and type I myeloma. Although *c-myc* is usually expressed in highly proliferative normal and neoplastic cell populations and downregulated during differentiation, human plasma cells representing a hardly proliferating, terminally differentiated stage of the B-cell lineage showed a distinct expression of this oncoprotein. Myeloma clones with a proportion of cells beyond the G_0-phase of the cell cycle (Ki67+ >1%) had high MYC values ($p<0,05$). But as (i) the percentage of MYC+ always exceeded Ki67+ myeloma cells, (ii) myelomas with low proliferative indices showed a broad range of MYC values and, (iii) non neoplastic as well as (iv) non clonal plasma cells also expressed the oncoprotein in some cases, one can postulate additional physiological functions for *c-myc* in human plasma cells which are different from proliferation and transformation. Correlation of MYC values to ICAM-1 expression ($p<0,05$) could indicate a regulatory interaction between these molecules. However an epiphenomenon still has to be ruled out and it remains to be determined whether *c-myc* is involved in differentiation or apoptosis of human plasma cells as was recently suggested for other cell types.

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ALLOGENEIC FAMILY UNRELATED BONE MARROW TRANSPLANTATION IN ACUTE AND CHRONIC MYELOID LEUKEMIA.

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Twelve patients, 19-53 years old, with acute and chronic leukemia (5 AML, 2 ALL, 5 CML), were given marrow transplants following intensive cytoreductive therapy (fractionated total body irradiation, and cyclophosphamide). The patients received an HLA-identical family unrelated donor marrow. The mixed lymphocyte cultures were either negative (7 patients) or slightly reactiv (5 patients). The prophylaxis for graft versus host disease (GvHD) consisted of cyclosporine (CsA) startet at day - 3 at a concentration of 2,5 mg/kg, i.v. twice daily over a period of 4 hours in combination with prednisone (0,5 mg/kg) given at day 7 post grafting. All patients tolerated the immunsuppressive therapy, however some complained about nausea and headaches. At present time 7 patients are alive and well. 2 patients developed chronic graft versus host disease, grade II. One patient with AML transplanted in a second partial remission relapsed after 11 months post transplant without any signs of acute or chronic graft versus host disease. One patient died at day 14 due the toxicity of the conditioning regimens. Two patients developed acute graft versus host disease at day 47 and day 68, respectively, and both died of uncontrolled aGvHD primarily with liver and bowel involvement. One patient with a very slow take developed chronic GvHD and died subsequently with uncontrolled cGvHD. The results suggest that bone marrow transplantation with HLA-indentical family unrelated marrow donors using a modified GvHD prophylaxis protocol might generate a similiar outcome compared with family related transplants.

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IMMUNOSUPPRESSIVE EFFECTS OF FLUDARABINE PHOSPHATE IN PRETREATED ADVANCED CHRONIC LYMPHOCYTIC LEUKEMIA

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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 19 patients with advanced and to the standard regimens resistant CLL, 17 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. 71 cycles of FAMP were administered. 12 of 19 patients (63%) achieved partial remission, 1 of 19 (5%) had stable disease, and 5 of 19 (26%) were found progressive (PD). In most cases, PR was achieved within 2 cycles of FAMP. The duration of partial remission until end of observation time, relapse or death were 2-14 months. 2 of the patients in PR relapsed after 14 and 9 months, 9 patients in PR died due to infection. Major toxic effects included severe infections in 11 patients and nausea in 8 patients. The development of frequent pulmonal, even opportunistic infections can be explained by FAMP-induced reduction of $CD4^+$ cells. Fludarabine is effective in patients with advanced CLL, but its immunosuppressing effects requires long-term antibiotic prophylaxis.

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MODULATION OF ADHESION MOLECULES BY IMMUNOTHERAPY AS A MECHANISM OF CYTOTOXOCITY IN AML, RENAL CELL CANCER, AND MALIGNANT MELANOMA.

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Adhesion molecules are a heterogenous group of surface receptors involved in cell-cell-interaction. Several cytokines including IL-1, IL-4, IL-7, TNFα, and IFNγ are able to upregulate the expression of adhesion molecules LFA1 and ICAM-1. The influence of IL-2 on the expression of these molecules is still uncertain.
In this study the effect of αIFN and IL-2 on the expression of LFA1α, LFA1β, VLA1, and ICAM-1 was investigated. In 4 Patients with metastatic renal cell cancer and 2 patients with malignant melanoma receiving αIFN 3 Mill. U/m^2 s.c. and IL-2 9 Mill. IU/m^2 i.v.(bolus) in a daily alternating schedule for 14 days the expression of adhesion molecules on peripheral blood lymphocytes was examined using flowcytometry. Same examinations were performed in 3 patients with AML in 2nd remission receiving IL-2 in a dosage of 9 Mill. IU/m 2 from day 1-5 and day 8-12. Soluble ICAM-1 was detected by ELISA. The cellular expression of LFA1α, LFA1β, VLA1, and ICAM-1 showed no changes during treatment, but was upregulated 7-14 days after each therapy. This corresponds to other biological effects of the IL-2/IFN therapy like rebound lymphocytosis, which occurred also 7-14 days following therapy. Soluble ICAM-1 serum concentrations increased in some cases with a maximum on day 8 of treatment. Following IL-2 bolus on first day of treatment sICAM-1 serum concentrations rose to a peak after 6-8 hours followed by a rapid decline. At this time the cellular expression of ICAM-1 remained unchanged. Immunotherapy with IL-2/IFN or IL-2 alone induces upregulation of adhesion molecules, which may induce antitumoral activity. Still uncertain is, whether IL-2 itself or through induction of secondary cytokines promote this effect.

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COMPLETE REMISSION OF AN ALLOGENEIC MALIGNANT MELANOMA ACQUIRED BY KIDNEY TRANSPLANTATION WITH IMMUNOTHERAPY OF INTERLEUKIN-2/αINTERFERON

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In our hospital immunotherapy with interleukin-2 (IL-2)/α interferon (αIFN) is in therapeutic use by patients with renal cell cancer and metastatic malignant melanoma. Here, we report of a complete remission in a patient with allogeneic malignant melanoma acquired by a kidney transplant.
The patient, a 26 year old female, was in chronic hemodialysis since 1987 due to a reflux nephropathia. In 10/90 transplantation with a renal cadaver transplant was performed. In 1991 a malignant melanoma with disseminated lymphomas in the abdomen, paravertebral, paraaortal, and the pelvis muscles was diagnosed. Chromosomal analysis of the tumor cells showed its origin from the transplant. So it was explanted in 8/91 and an immunotherapy with αIFN 10 Mill. U/m^2 s.c. and IL-2 18 Mill. IU/m ^{2} i.v.(bolus) in a daily alternating schedule for 14 days was given. After two courses of treatment the patient showed no more lymphomas in abdominal CT scans and only residual enhancement in one lymph nodus in an immunoszintigraph using the monoclonal anti-melanoma antibody K1. Since third cycle the patient is in continous complete remission.

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NEW ANTICANCER DRUG DEVELOPMENT: RESULTS OF A SIX-YEAR COOPERATIVE PROGRAM BETWEEN THE EORTC-NDDO AND THE FREIBURG PRECLINICAL ANTICANCER DRUG DEVELOPMENT GROUP

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Within the last six years, a cooperative program between the EORTC New Drug Development Office and the Preclinical Anticancer Drug Development Group at the University of Freiburg was set up in order to provide a rational basis for further development of experimental agents. Potential antitumor compounds acquired by the NDDO from various sources are submitted for evaluation of antineoplastic efficacy in a combined in vitro/in vivo test system developed in Freiburg.
New compounds are studied in vitro for anticancer activity in 6 human tumor xenografts using a modified colony forming assay. Agents demonstrating a cytotoxic effect on tumor cells are subsequently studied in a total of 20 xenografts in the clonogenic assay. For assessment of bone-marrow toxicity, the effect on colony formation (CFU-GM) of human bone marrow cells from healthy donors is evaluated. In parallel, the drug is studied in vivo using two or more in vitro sensitive xenografts transplanted subcutaneously into nude mice.
Overall, a total of 138 compounds of various classes, e.g., classical cytotoxics, antibiotics, natural products, lipids, biological response modifiers and rationally designed drugs, have been studied for antineoplastic efficacy. 38/138 compounds (28%) have been further evaluated in vivo, 10/138 drugs (7%) have shown reproducible activity (regression to ≤75% of the initial tumor size) in tumor-bearing nude mice. The data obtained are basic criteria with regard to the final decision of the New Drug Development Coordinating Committee on further development of the compounds tested. A total of 8 agents (6%) have been selected for clinical trials e.g. EO-9, Rhizoxin, Topetecan, Dabis maleat. Once preclinical activity is established, further steps covering formulation, animal toxicology and clinical trials are coordinated by the NDDO. This multicenter approach allows for highly effective evaluation of new anticancer agents and treatment strategies.

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INTERLEUKIN 10 IS HETEROGENEOUSLY EXPRESSED IN ACUTE AND CHRONIC LYMPHOCYTIC LEUKEMIA

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Interleukin 10 (IL-10), also known as cytokine inhibiting factor (CSIF) is a strong growth factor for activated human B lymphocytes. IL-10 has originally been identified as a product from T_H2 helper T-cells able to suppress IL-2 and Ifn-gamma production. Human IL-10 shows striking sequence homology to BCRF1, an Epstein-Barr virus (EBV) encoded lytic protein, and infection of Burkitt's lymphoma (BL) cell lines with EBV induced IL-10 production. Previously, it has been reported that murine B lymphomas and normal peritoneal B cells produce IL-10 which is largely secreted by Ly-1 B cells. It has been shown that Ly-1 pos. chronic B cell leukemias (CLL) are generated in early ontogeny and the human equivalent are CD5 pos. B-CLLs that are known to have defective T cell responsiveness. Because of its B cell growth promoting and CSIF capacity a possible role of IL-10 in lymphomagenesis has been suggested. We examined clinical specimen of patients with CLL as well as acute leukemias, and a panel of EBV positive and negative BL and lymphoblastoid cell lines (LCL) for the expression of IL-10 by reverse transcriptase (RT-) PCR. All BL and most LCL strongly expressed IL-10 regardless of the presence or absence of EBV. No correlation was seen to the degree of activation of the cells suggesting an important role of IL-10 as an autocrine B cell growth factor **in vitro**. Eleven from 20 CD5 pos. CLL and half of the ALL expressed IL-10. The strongest signal was obtained in the common-ALL subgroup showing about 10 fold higher levels as compared to CLL in dilution experiments. MOLT4 and Jurkat T cell lines were positive, and the myeloid derived cell lines HEL, K562, HL60 and all but one AML specimen were negative for IL-10 expression. Clearly, IL-10 expression is not restricted to B cells activated by EBV and can be seen even in the absence of CD40 **in vitro**. In contrast to previous findings in the murine system the human CD5 pos. B-CLL examined were strikingly heterogenous with regard to IL-10 production. Thus, this disease entity can be divided into two subgroups, however, correlation with clinical outcome remains to be shown. The same holds true for the different secretion of IL-10 by certain ALL but not others. IL-10 secretion by the malignant cells is likely to contribute to the genesis of certain lymphomas and leukemias.

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FLOW CYTOMETRIC STUDIES OF DNA ABNORMALITIES AND CELL PROLIFERATION IN BENIGN AND MALIGNANT COLORECTAL NEOPLASIA.

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Following Nowell's hypothesis of carcinogenesis as a result of genomic instability with the production of genetically abnormal clones of cells we performed flow cytometric studies in 16 colorectal adenomas (>1.5cm) and 14 carcinomas. To study the frequency and distribution of aneuploid populations as well as the proliferative pattern 4.6 (2-13) specimens (o 2-3mm) were taken from each neoplasm in a grid array for flow cytometric and parallel histologic analysis. This way, maps illustrating DNA content abnormalities, cell proliferation and histology were assessed throughout the entire neoplasms. Samples for flow cytometry were minced, stained with DAPI as DNA dye and analyzed on a PARTEC II. 13/57 adenomatous specimens without dysplasia revealed a single aneuploid population of cells. Tetraploidy (4N>15% of cells analyzed) was present in 13 of these 57 cases. Of 6 adenomatous fractions with high grade dysplasia 3 were aneuploid: two of them with multiple aneuploid lesions (D.I. = 1.13/1.24 and 1.20/ .1.70, resp.). Single (n=34) and multiple (n=6) aneuploid populations were detected in 40/70 (57.2%) carcinomatous specimens. Tetraploidy was present in another 8 samples. DNA mapping of adenomas and carcinomas revealed both distinct regions and extended areas of aneuploidy and tetraploidy, respectively. Overall, cell proliferation as determined by the percentage S-phase cells was significantly ($p< 0.05$) higher in carcinomatous specimens (13.9± 0.9%; x± SEM) than in adenomatous fractions (7.3±0.6%). The tumor adjacent colonic mucosa showed a S-phase of 5.0± 0.6%. These data showing DNA content abnormalities within the adenoma-carcinoma-sequence are consistent with Nowell's hypothesis of genomic instability leading to aneuploid clones in colorectal carcinogenesis.

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Transrepression of the interferon-alpha response by transient expression of an interferon-stimulated- response-element binding protein

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Interactions of cellular proteins with the cis-acting interferon (IFN)-stimulated-response-element (ISRE) have been implicated in the transcriptional regulation of IFN-responsive genes. We have isolated a mouse cDNA from an expression library by using the ISRE as a recognition site DNA probe. This cDNA encodes a novel polypeptide of 976 amino acids (predicted molecular weight 108,715 Da), designated ISRE-binding factor-1 (IBF-1). ISRE-binding characteristics in vitro of bacterially expressed IBF-1 fusion proteins indicate that the DNA binding domain of IBF-1 lies between amino acid residues 276 and 450. IFN-1 binds to the truncated ISRE motif, GAAANN. The IBF-1 cDNA hybridizes with an approximately 4.4 kb mRNA, constitutively expressed in different mouse cell lines. The IBF-1 mRNA is not induced by either IFN-alpha or IFN-gamma; however the level of mRNA is differentially regulated in virus infected cells. Cotransfection experiments reveal that transient expression of IBF-1 suppresses the IFN-alpha induced transcriptional activation of an ISRE-containing, heterologous promotor. The level of this transrepression can be reduced by treating the cells with increasing concentrations of IFN-alpha. These data indicate that IBF-1 may be a constitutively expressed repressor of IFN-inducible promotors.

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INFLUENCE OF INTERLEUKIN 4 ON CYTOKINE PRODUCTION BY B CELLS IN CHRONIC LYMPHOCYTIC LEUKEMIA

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Cytokines produced by the malignant cell clone may be involved in dysregulated growth control of lymphoid malignancies. Recent reports suggest that TNF alpha plays a role as an autocrine growth factor in progression of B CLL. In this study we investigated the production of cytokines by B-CLL cells and their regulation by crosslinking anti-IgM antibodies and by the cytokines IL-2 and IL-4. Highly enriched B-CLL cells were obtained from peripheral blood mononuclear cells by depletion of T cells and adherent monocytes. First, effects of the various stimulans on proliferation of B-CLL cells was studied. Optimal proliferation of the malignant cells was obtained by anti-IgM and IL-2. IL-4 inhibited IL-2 induced cell proliferation without decreasing viability of B-CLL cells. Furthermore, IL-4 upregulated expression CD23 on B-CLL cells suggesting that the B cell population remained functionally active during exposure to IL-4. Under our culture conditions TNF-a failed to stimulate proliferation of B-CLL cells but rather inhibited IL-2 induced proliferation at higher concentrations. In freshly separated B-CLL cells transcription of mRNA for TNF, IL-6 and IL-1 was absent or -for TNF- in a few patients very low. In vitro incubation for 8 hours in culture medium alone resulted in upregulation of message for TNF a, which was further enhanced by anti-IgM and/or IL-2. By contrast,mRNA for IL-1 and IL-6 are not detected in presence of medium or IL-2 alone. However, costimulation with IL-2 and anti-IgM or LPS induced transcription of the IL-1 and IL-6 genes. In presence of IL-4, TNF, IL-1 and IL-6 were all downregulated as compared to appropriate control cultures. Our study demonstrates negative regulatory signals by IL-4 in chronic lymphocytic leukemia comprising both cell proliferation and production of cytokines involved in B cell regulation.

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TREATMENT WITH A COMBINATION OF INTERFERON α-2B AND CYTOSIN-ARABINOSIDE OF CHRONIC MYELOID LEUKEMIA.

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Twenty-five patients (12 male, 13 female, mean age 42.9 yrs.; range 19.1 - 63.8 yrs.) with CML in the chronic phase have been treated with a combination of interferon (IFN) α-2b and cytosine-arabinoside (ara-C). 7 patients were pretreated, most of them with busulfan, and 18 patients were without pretreatment. In pretreated patients, the time from diagnosis to admission was 36.3 months (range 6.7 - 106.3 months). IFN was started at a dose of 3 MU/m² SC daily. From the second week on ara-C was added at a dose of 20 mg/m² SC on 5 days per week. Lateron, the doses were adapted to response and side effects. Between the second and the 12th month, mean doses of IFN α administered were from 3.3 to 3.5 MU/m² daily and mean doses of ara-c from 50 to 70 mg/m² weekly. Prevalent side effects consisted in anemia, mild thrombocytopenia, fever, fatigue, nausea, weight loss and a mild alopecia. There were no life threatening side effects. Ten patients had a complete hematologic response with normalization of the WBC and the differential. Nine patients had a partial hematologic response with WBC $<20.000/\mu l$ and $<50\%$ of the initial value. Five patients had no major hematologic response and another is too early to evaluate. Nine of 10 patients with complete hematologic response had had no pretreatment. Four minor (35 - 95 % Ph^1+ metaphases) and one partial (1 - 34 % Ph^1+ metaphases) cytogenetic responses have been observed. We conclude that the side effects of IFN α and ara-C are tolerable. Although the recruitment continues and therefore the observation time is short, preliminary results are promissing but limited to patients without pretreatment.

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CHARACTERIZATION OF B CELL CLONES IN NON HODGKIN´S LYMPHOMA BY FINE NEEDLE ASPIRATION CYTOLOGY AND AMPLIFICATION OF IMMUNOGLOBULIN GENES

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Clonal cell populations in tumor samples from patients with B cell Non Hodgkin´s lymphomas can be identified by unique rearrangements of immunoglobulin genes. Tumor cells can easily be sampled by fine needle aspiration (FNA). Since only small amounts of DNA can be extracted from FNA material, we have used polymerase chain reaction (PCR) to amplify junctional sequences of the third complementarity-determining region of immunoglobulin heavy chain genes. Cells were scraped from stained FNA slides up to 12 years old. After lysis of the cells and using β globin as a single copy control gene, DNA could be amplified in 68% of the samples. By phenol-chloroform extraction, the proportion of samples with suitable DNA could be increased to 93%. Among multiple combinations of primers complementary to sequences of V_H- and J_H- genes, we identified primer pairs that generated clonotypic bands in 72% of samples from 82 patients with Non Hodgkin´s lymphoma in a nested primer PCR. A distinct smear of bands derived from polyclonal B cells was seen in samples from 34 patients with nonmalignant lymph node hyperplasia. On polyacrylamide gels, more than one discrete band could be identified in 27% of the samples suggesting multiple clones or clonal evolution within single lymph nodes. Tumor cells from single sites showed a typical pattern of bands that could be distinguished from cells aspirated from other nodes at the same time or during followup. In the patients analyzed to date, direct sequencing of single bands suggests a sequence of somatic mutations over time. The combined use of FNA and PCR for analysis of rearranged antigen receptor genes or genes with possible function in oncogenesis will certainly help to clarify the natural history of Non Hodgkin´s lymphomas.

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DISCRIMINATION OF EARLY $CD34^+$ MYELOID PROGENITORS USING CD45-RA ANALYSIS AND CLONOGENIC ASSAY

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Mononuclear cells (MNC) isolated by density centrifugation of cord blood and normal bone marrow samples, and of peripheral blood (PB) from patients treated with GM-CSF, were double stained with anti CD34 MAb (8G12) versus CD45, CD45-RB, CD45-RO and CD45-RA, respectively, and analyzed by flow cytometry. In all specimen, $CD34^+$ MNC co-expressed CD45 at a low to very low level, while the expression of CD45-RB was similar or slightly higher. In contrast, CD45-RO and CD45-RA could subdivide the $CD34^+$ population into fractions negative, dim ($^+$) and normal positive ($^{++}$) for these subgroups. In bone marrow, the majority of the $CD34^+$ MNC was RA^{++} and RO^-, but there were also $34^+/RA^+$ and $34^+/RA^-$ cell fractions. In PB, most cells were $34^+/RA^-$ with varying proportions of $34^+/RA^+$ and $34^+/RA^{++}$ and a variable expression of RO. In cord blood, the hematopoietic progenitors were usually $34^+/RA^-/RO^-$. Semisolid culture of sorted $CD34^+$ MNC revealed that clusters and dispersed (late) CFU-GM originated from $34^+/RA^{++}$ cells, while the $34^+/RA^-$ MNC formed compact and multiple centre, white and red colonies derived from early progenitors. Addition of 20 ng SCF (Amgen) per ml of medium containing sorted $34^+/RA^-$ cord blood MNC led to a change of many BFU-E to CFU-mix which was not, to this extent, seen in blood and bone marrow. We conclude that early hematopoietic cells are $34^+/RA^-/RO^-$. Since this population excludes $34^+/19^+$ B cells which are RA^{++}, two colour flow cytometric analysis using CD34 and CD45-RA facilitates the quantification of early myeloid progenitor cells.

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CHARACTERIZATION AND ISOLATION OF CD34+ LYMPHOHEMATOPOIETIC PROGENITOR CELLS IN MULTIPLE MYELOMA

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The selection of CD34+ cells may be used as an alternative purging procedure for autologous transplantation in multiple myeloma (MM). However, normal hematopoietic progenitor cells can only be separated effectively from tumor cells if the latter do not express the CD34 antigen. As some reports suggested myeloma stem cells to be of pre-plasma-cell origin, we first investigated whether the CD34+ pre-B-cell compartment is expanded in MM. After informed consent bone marrow (BM) and peripheral blood (PB) was obtained from 14 MM patients and 5 healthy subjects. Specimens were subjected to ammonium chloride lysis or density gradient centrifugation and stained for the CD10, CD19, CD33, CD34, CD38 and CD45 antigens. Subpopulation analysis of gated CD34+ cells showed no significant difference between MM and controls. More CD34+/CD19+ cells were detected in BM (MM: 21% $\pm$ 13% of CD34+ cells, mean $\pm$ SD, n=14) than in PB (MM: 5% $\pm$ 4% of CD34+ cells, n=13). Using tricolor flow cytometry, in BM the CD19 expression on CD34+/CD10+ cells was found to increase continuously from very low levels, whereas B-lymphoid progenitors in PB were mostly CD34+/CD10+/CD19-. For the preclinical assessment of immunomagnetic CD34+ cell enrichment in MM, BM from 3 patients (2 IgG, 1 IgA) and 4 healthy controls was obtained. CD34+ cells were selected with the HPCA-1 antibody and detached from the beads with chymopapain. In MM the plasma cell content in the initial mononuclear cell preparation (MNC) was 3%, 10% and 63% as compared to <1% plasma cells and 89% $\pm$ 5% immature blasts in the CD34+ fraction. The plating efficiency was 9.7% $\pm$ 2.8% (n=3) in this fraction which was 51-fold higher than in the starting preparation. Results in the control group were comparable except that the mean colony recovery was 30% $\pm$ 19% (n=4) as compared to 19% $\pm$ 13% (n=3) in MM. This was due to a higher frequency of cell clumping in MM BM. Mobilized PB may be an alternative source of progenitor cells for CD34-selection in MM. For clinical application upscaling of the procedure is required.

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CONTROL OF PROGENITOR CELL PROLIFERATION BY ADHESION MOLECULE STRUCTURES

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A monoclonal antibody termed 7F7, which recognizes the intercellular adhesion molecule-1 (ICAM -1) , has been used in our experiments to modulate the influence of autologous T-lymphocytes on committed haematopoietic progenitor cells. Using a microagar culture system for human haematopoietic progenitor cells and for T-lymphocyte colony formation we demonstrate that BFU-E, CFU-Meg, CFU-GM derived colony formation was not directly affected by 7F7 antibody treatment . PHA-induced T-lymphocytic colony formation, however, was markedly suppressed by 7F7 mAb. In coculture studies between monocyte and T-lymphocyte depleted bone marrow and peripheral blood MNC and autologous T-lymphocytes preincubated with the 7F7 mAb we were able to demonstrate that ICAM-1 is involved in T-lymphocyte mediated modulation of BFU-E, CFU-Meg and CFU-GM proliferation . To further clarify the question if this effect on progenitor cell proliferation is mediated by cell adhesion blocking between T-lymphocytes and progenitor cells or by direct Ab-mediated effects on T-lymphocytes and monocytes, the 7F7 mAb was used to investigate the role of ICAM-1 in cytokine production by T-lymphocytes and monocytes. Production of TNF-alpha, IFN-gamma and IL-1 was significantly inhibited ($p<0.01$) by the incubation of mAb 7F7 with PHA activated blood MNC or isolated E rosette positive T-lymphocytes. The maximal level of inhibition was reached using saturating concentrations of 400 µl/ml of mAb 7F7 hybridoma supernatant corresponding to an inhibitory activity of 1µg of purified mAb. In contrast, GM-CSF release showed a heterogenous response over 5 experiments with an increase found in 3 experiments and a decrease in 2 experiments. Addition of increasing concentrations of supernatant or purified mAb to unstimulated MNC or T- lymphocyte cultures had no effect on cytokine release .
Our studies show that ICAM structures are involved in the T-cell mediated modulation of normal haematopoietic progenitor cells and that ICAM-1 exerts its regulatory effect via the lymphokine cascade.

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DYSHEMATOPOIESIS IN BONE MARROW ASPIRATES OF PATIENTS WITH ACUTE MYELOID LEUKEMIA AT DIAGNOSIS. B. Gahn, J. M. Bennett, W. Hiddemann, Th. Büchner, M. Unterhalt and B. Wörmann

Secondary leukemia is a negative prognostic factor in patients with acute myeloid leukemia. It's defined by an antecedent hematologic disorder in the patient's history or exposure to mutagenic agents. By light microscopy, the residual normal hematopoiesis shows characteristic features of dysplasia, defined by the FAB group for MDS. In the present study, the bone marrow aspirates of 101 patients with AML were evaluated by light microscopy of Pappenheim - stained slides for the presence of dysplastic cells. These were defined by the presence of karyorrhexis, megaloblastoid features, multinuclearity or nuclear fragments in $\geq$50 % of 25 red cell precursors; by the presence of agranular or hypogranular PMN, or Pseudo Pelger - Huet anomaly in $\geq$ 50 % of 100 myeloid cells; by the presence of micromegakaryocytes, multiple separated nuclei or large mononuclear forms in at least 3 of 6 cells of the megakaryocytic lineage. Morphologic evaluation was performed retrospectively by two of the authors (GB, JMB) without knowledge of the patient's history and of the clinical outcome. All patients were treated between 4/89 and 12/91 according to the protocols of German AML Cooperative Group. Dysgranulopoiesis was found in 55, dysmegakaryopoiesis in 61 and dyserythropoiesis in 64 of the 101 slides. Patients with dysgranulopoiesis had a 55 % chance for achievement of CR and of 30 % for continuous CR (CCR) at 30 months, significantly worse than for patients without dysgranulopoiesis, who had a 69 % chance for achievement of CR and of 43 % for CCR. No significant differences for CR were found for patients with dysplastic features in the other lineages, but a tendency for a shorter CR duration was visible. The results show that dysplastic features are common in bone marrow aspirates of AML patients at diagnosis. Dysgranulopoiesis was the most significant negative prognostic factor. Further cell biological analyses for the classificationof these cases as secondary leukemias. Light microscopic evaluation of the residual normal hematopoiesis provides additional informations for early identification of patients with unfavorable prognosis.

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FREQUENT DETECTION OF BCR-ABL SPECIFIC M-RNA AND EARLY DETECTION OF RELAPSE IN CML PATIENTS FOLLOWING NON T-CELL DEPLETED BMT USING THE PCR

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We have performed a two step PCR to detect bcr-abl specific m-RNA in 400 peripheral blood and/or bone marrow samples of 30 CML patients (median 10 samples, range 1 to 48 samples of each patient) following non t-cell-depleted allogeneic (n= 28) and syngeneic (n= 2) bone marrow transplantation (bmt). The mean observation time is 22 months. In 13 of our patients (43%) bcr-abl specific m-RNA could be detected following bmt. Pre and post bmt transcripts matched in all cases were pre bmt material was obtained. The bcr 2/abl II rearrangement was detected in 11 patients, the bcr 3/abl II rearrangement in 2 patients following bmt. In 6 patients after bmt bcr-abl positive cells were deteted frequently. Hematological relapse occured in 5 of these 6 patients. All of these 5 patients were analysed prospectively. First time of detection of serial PCR positivity was 4 months before relapse in UPN 99, 1 months in UPN 120, 2 months in UPN 123, 15 months in UPN 131 and 0,5 months in UPN 133. In 7 patients transient PCR positivity could be detected, none of these patients relapsed during a mean observation time of 22 months, all are in complete hematological remission. Bcr-abl positivity was rare in patients, who were in hematological remission for at least 2 years (3/14). Bcr-abl positivity in consecutive samples indicated a high risk of relapse (5/6), whereas the risk of relapse was low in patients, who had positive and negative results in consecutive samples (0/7). Bcr-abl positivity was detected more frequent in patients without GVHD (9/11), than in patients, who sufferred from GVHD (4/19). This data indicate, that transient bcr-abl positivity is usually not followed by hematological relapse, while patients, who are positive in serial samples have a higher risk of relapse.

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ALL-TRANS RETINOIC ACID (ATRA) AND GRANULOCYTE COLONY-STIMULATING FACTOR (G-CSF) IN THE TREATMENT OF MYELODYSPLASTIC SYNDROMES (MDS): A PILOT STUDY

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Differentiation induction therapy has been studied in patients (pts) with myelodysplastic syndromes in various forms, e.g. treatment with interferons, retinoic acid, vitamin D, low-dose ara-C, colony-stimulating factors (CSF). Since ATRA and G-CSF synergistically promote the differentiation of myeloid leukemic blast cells in vitro, we have started a pilot study of combined treatment with ATRA and G-CSF in pts with MDS, especially with refractory anemia without blast cells in the bone marrow. ATRA was given at 45 mg/m²/day PO from week 1-12 and G-CSF at 5 µg/kg/d SQ from week 5-12 with dose modifications according to the neutrophil counts (ANC). A total of 11 pts (median age: 66 years; range: 57-71) have been treated, 8 males and 3 females. During initial ATRA therapy, ANC increased in 3 pts, platelet count (plts) in 4 pts. During combined ATRA/G-CSF therapy, ANC increased in all pts, plts increased in 3 out of 10 evaluable pts but decreased in 1 pt. An increase in hemoglobin concentration occurred in one pt. In the bone marrow, the myeloid-erythroid ratio increased, but not the maturation index of myeloid cells. Cytogenetic analysis demonstrated the persistence of the abnormal clones in all pts. Adverse effects included dermatitis and cheilosis in most pts. and a drop in plt. counts in 1 pt. The pilot study demonstrates that the combination treatment with ATRA/G-CSF is well tolerated in most pts, leading to normalization of ANC in the majority of plts and in a subgroup of pts.

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IN VIVO EFFECTS OF RECOMBINANT HUMAN INTERLEUKIN-3 (IL-3) IN PATIENTS WITH MYELODYSPLASTIC SYNDROMES (MDS): COMPARISON OF TWO TREATMENT PROTOCOLS.

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Two different treatment protocols were used to study whether IL-3 can restore hematopoietic function in patients (pts) with MDS: Protocol A consisted of daily SQ administration of high dose IL-3 (250 µg/m² and 500 µg/m²) for 15 days. Pts entering prot. B received 60 µg IL-3/m² three times per week SQ from week 1-6 and 125 µg/m² three times per week from week 7-12. Nine pts were treated in prot. A (6x refractory anemia (RA), 3x RA with excess of blasts), and 12 pts in protocol B (11x RA, 1x RA with ring sideroblasts; 11 pts evaluable). Pronounced rises in total leukocyte counts, including neutrophils, eosinophils, basophils, monocytes, lymphocytes, were seen in prot. A, e.g. a significant increase in neutrophil counts in 7/9 pts. In only 1 pt treated according to prot. B, the neutrophil counts increased. Platelet counts increased in 6/9 pts in prot. A and in 6/11 pts in prot. B. Reticulocyte counts increased in 2 pts in both protocols, respectively, but in only 1 pt on prot. A transfusion requirements decreased transiently. In both treatment regimens, the rise in platelet counts correlated inversely with the initial TNF-α serum levels and induction of TNF-α during IL-3 treatment. In vitro growth of hematopoietic progenitor cells did not improve in any pt on either protocol. Headache and bone pain only occurred in prot. A, while low-grade fever was seen in 9/9 pts in prot. A and in 8/11 pts in prot. B. Progression of the disease with increase of blast cells was seen in 1 pt on prot. A, but not in prot. B. The data indicate that stimulation of thrombopoiesis can be obtained with both high-dose short-term (prot. A) and low-dose long-term (prot. B) IL-3 treatment. Since IL-3 mainly acts on progenitor and precursor cells, combination therapy of low-dose IL-3 and late-acting cytokines should be tested.

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THE FAB CLASSIFICATION: PEROXYDASE CYTOCHEMISTRY IN THE DIAGNOSIS OF ACUTE LEUKEMIAS

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The French-American-British (FAB) classification of acute leukemias requires more than 3 % of the blasts being positive for peroxydase for the morphological diagnosis of acute myelogenous leukemia (AML) type M1 or M2. The treshhold of 3 % of peroxydase positive blasts had been chosen though lymphatic cells are uniformly negative for this enzyme. The rationale behind is that a small number of residual nonleukemic neutrophil blasts being positive for peroxydase may be mistaken as leukemic cells leading to an eronneous classification of a patient.

From February 1th, 1988, to December 31st, 1991, smears of bone marrow and peripheral blood of 835 patients of the AMLCG and the German ALL study were examined prospectively by the central review institutions for immunological and for morpholoical/cytochemical workup. Immunological analysis established the diagnosis of AML in 347 patients. On morphological/cytochemical analysis, 96 of this 347 cases presented as poorly differentiated leukemias fulfilling the morphological criteria of AML M1(44 pts) or AML M0/ALL (52 pts) and do represent the basis of the analysis of peroxydase activity. The percentage of blasts being positive for peroxydase was: no unequivocally peroxydase positive blasts 33 pts, 0,2-1% positive blasts 14 pts, 1,1-2,9% 5 pts, 3-6% 5 pts, 7-10% 4 pts, 11-30% 9 pts, 31-50% 3 pts, 51-100% 23 pts. In all 19 patients with peroxydase positive blasts in the range from 0,2 to 3 %, the myeloid nature of the leukemia had been considered as certain on morphological grounds.

These data show: (1) The cut off limit for classification as AML of 3 % seems to be biologically irrelevant. (2) The absence of blasts being positive for peroxydase is no reliable indicator for the lymphatic nature of a leukemia, even in case the PAS reaction is typical for ALL. The morphological diagnosis of ALL needs confirmation by immunology in any instance. (3) The FAB type M0 is much more common than suggested previously by the FAB group.

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IMPAIRMENT OF HUMORAL IMMUNE RESPONSES IN ACUTE LEUKEMIAS

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Immune responses to Tetanustoxoid (TTx) and Polysaccharides (Pneumococci polysaccharide, PCP) were studied in 14 patients with acute lymphocytic leukemia (ALL) and 32 patients with acute myelogenous leukemia (AML) before and during chemotherapy by enzyme immuno assay. The immune response to TTx was significantly lower in ALL patients than in controls. This may be due to elevations of sCD8, the soluble equivalent of the CD8 receptor on cytotoxic/suppressor T-lymphocytes, in the serum of 12 of the 14 ALL patients. Patients with AML had normal immune responses before therapy and the average TTx antibody titers rose during chemotherapy, while average antibody titers to PCP remained constant. In AML patients with septic complications the increase of antibody titers to TTx was lower than in patients without sepsis. The average antibody titer to PCP decreased in patients with sepsis while it increased slightly in patients without sepsis. There was no correlation of immune responses with levels of IL-6 in the serum of AML patients, but increases of IL-6 levels during chemotherapy were related to acute phase responses due to sepsis. Our results confirm previous observations that the humoral immune response in AML in contrast to ALL is largely unaffected by the underlying disease and aplasiogenic therapy.

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MONITORING OF CYTOSTATIC DRUG RESISTANCE BY A COMBINED IN VITRO CYTOTOXICITY- AND PCR BASED ASSAY

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Measuring increasing unresponsiveness towards antineoplastic agents during therapy of certain malignant diseases should allow an individual treatment design.
Using 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide in tumor cell culture a colorimetric assay quantitates relative cytotoxicity of cytostatic drugs.
Furthermore, by rtPCR and consecutive ion exchange HPLC the transcription of the mdr1 gene was measured.
This combined method allows experiments, monitoring the kinetics of resistance development and identification of p-glycoprotein mediated phenomena, which can be influenced by resistance modifiers.

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REDUCED HEMATOPOIETIC COLONY-FORMATION IN LONG-TERM MARROW CULTURES OF PATIENTS WITH PROGRESSIVE HIV-1 INFECTION

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Besides other causes, deficiencies in bone marrow stromal cell structure and function have been considered as reason for bone marrow failure in progressive HIV-infection. Thus, in the present analysis the colony forming capacity of hematopoietic progenitor cells after long-term bone marrow culture (LTBMC) was compared in patients with AIDS and in healthy controls.
To this end, $4x10^5$ light-density bone marrow cells were incubated in a micro-LTBMC-system containing 300 µL Mc Coy 5A medium with 12.5% fetal calf serum, 12.5% horse serum, 10^{-6} M hydrocortisone, and 10^{-4} M α-thioglycerol per well in microtiter-plates. 50 % of the medium was changed weekly without irritating the stromal layer. At weekly intervals, individual wells were completely harvested for total cell count and enumeration of the colony-forming cells (CFC) in a methylcellulose culture containing IL-3, GM-CSF, G-CSF, and EPO.
As result, the stromal layers of HIV+ patients needed more time to form and contained fewer cells than that of the healthy controls. The total number of cells cumulated from HIV+ individuals ($2.7x10^6$) were significantly ($p<0.05$) higher than that from healthy controls ($1.5x10^6$). In contrast, as compared to initial colony growth (100%) the cumulated number of CFC after LTBMC was lower in samples from HIV-infected patients with regard to CFU-GM (HIV+ 19.9%, healthy 229.0%; $p<0.05$), BFU-E (HIV+ 2.3%, healthy 68,4%), and CFU-GEMM (HIV+ 4.8%, healthy 28.7%) during a 12-week-observation time.
In conclusion, the difference between the significantly increased cell numbers in LTBMC and the altered colony forming capacity of bone marrow samples from persons with AIDS as compared to uninfected persons suggest an uncoupling between proliferation and differentiation of hematopoiesis in this disease. Further studies have to show whether bone marrow stromal cells in HIV-infection are altered functionally due to viral infection, changes in the expression of adhesion molecules and/or of secretion of inhibitory/stimulatory cytokines.

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DELTA-TCS-1-POSITIVE CELLS INHIBIT HEMATOPOIETIC COLONY-GROWTH IN PATIENTS WITH SEVERE HIV-1-INFECTION

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The inhibitory effect of CD8+ lymphocytes on hematopoietic colony growth constitutes one of the pathophysiological mechanisms responsible for hematopoietic failure in HIV+ patients. In addition, the expansion of a CD8+ lymphocyte subpopulation, namely the cytotoxic delta-TCS-1+ gamma-delta-T-cells, has been observed in these patients. Aim of this study therefore was to further analyze the inhibitory role of this delta-TCS-1-subpopulation in severe HIV-infection.
A panning depletion of T-lymphocytic subpopulations from light-density non-adherent bone marrow cells of 6 HIV+ patients (5 patients CDC-4, 1 patient CDC-3, according to CDC classification), and of 6 healthy controls was carried out. For enumerating the number of colony forming hematopoietic progenitor cells, the remaining cells were incubated in a methylcellulose assay stimulated by IL-3, EPO, GM-CSF, and G-CSF.
As a result, the colony growth of HIV+ individuals was significantly ($p<0.001$) increased by depletion of CD8+ (CFU-GEMM 135.4%, BFU-E 127.1%, CFU-GM 136.8%), of gamma-delta+ (CFU-GEMM 156.7%, BFU-E 136.0%, CFU-GM 153.0%), and of delta-TCS-1+ (CFU-GEMM 237.2%, BFU-E 139.6%, CFU-GM 154.3%) as compared to control cultures without depletion (100%). No changes in colony formation was seen in the cell samples from healthy controls.
In conclusion, the mostly CD8+ delta-TCS-1+ subpopulation of gamma-delta-receptor+ lymphocytes can be considered an important inhibitor for hematopoietic colony growth in progressive HIV-infection. Further studies have to show if this subpopulation has a direct cellular and/or an indirect cytokine-mediated inhibitory effect on the hematopoiesis of HIV-infected persons.

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IL-6 INDUCED IGM SECRETION IN A HUMAN B CELL LINE IS MEDIATED BY ACTIVATION OF A TYROSINE KINASE, A H-7 SENSITIVE PROTEIN KINASE
AND THE PROTOONCOGENE C-FOS.

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Following bone marrow transplantation some patients have a decreased IgM production due to an intrinsic B cell defect. We investigated mechanisms by which IL-6 induces IgM secretion in human B cells. First we analyzed the role of the protooncogene c-fos in IL-6 induced IgM secretion. The human, EBV-transformed B cell line SKW 6.4 was incubated with medium or IL6 (5ng/ml). After 10 to 120 minutes total cellular RNA was extracted and examined by Northern blot analysis using a radio-labeled anti-sense specific RNA probe. Within 30 minutes IL-6 induced c-fos expression in SKW 6.4 cells, the maximum enhancement was detectable after 1 h with a 3,5 fold enhancement over background ($p< 0,01$) and concentrations returned to basal levels after 2 hrs. A possible link between IL-6 mediated c-fos expression and IL-6 induced B cell differentiation was evaluated using anti-sense oligonucleotides to c-fos. After 48 hrs of stimulation IgM secretion was detected by ELISA. Anti-sense oligonucleotides to c-fos but not control oligonucleotides blocked IL-6 induced IgM production in a dose-dependent fashion up to 55% ($p< 0,001$). Addition of Genistein (a specific tyrosine kinase inhibitor) or H7 (a protein kinase inhibitor that did not affect tyrosine phosphorylation in a mouse hybridoma cell (Nakajima et al., 1990)) 30 min prior to stimulation with IL6 significantly inhibited IL6-induced c-fos expression ($p<0.01$). In conclusion, these results indicate that IL6 induced IgM secretion is mediated by activation of a tyrosine kinase and a H7 sensitive kinase followed by expression of c-fos.These data may help to identify the molecular basis of intrinsic B cell defetcs in patients following bone marrow transplantation.

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RAPID AND CONTAMINATION-SAFE NESTED PCR AS A ONE-TUBE-REACTION WITH THERMOSTABLE RTTH-REVERSE-TRANSCRIPTASE/POLYMERASE AND CG-CLAMP PRIMERS

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RT-PCR is an effective tool in detection of chromosome translocations, e.g. mRNA transcripts of the bcr-abl chimeric gene. Although it represents a fast and sensitive method the major technical problem in handling this method still remains contamination. Especially nested PCR, which can be crucial in order to obtain specific results, bears a great risk of contamination.
Nested PCR so far is a three-tube-reaction consisting of the reverse transcription, a first and a second amplification protocol, the latter run with nested primers.
A method has been developed to perform RT-PCR as a one-tube-reaction using a previously described thermostable recombinant enzyme from Thermus thermophilus (rTth). RTth has both reverse transcriptase and polymerase activity depending on the cations added to the enzyme. To unify reverse transcription and amplification protocols and in order to achieve a one-tube-reaction, a pair of high-temperature-annealing primers with a CG-40mer attached to the 5´-end was used for reverse transcription and first amplification as well. A second amplification protocol was performed with low-temperature-annealing nested primers.
The CG-40mer, which does not anneal to mRNA, leads to amplification products containing CG-40mers at both the 3´-and the 5´-end. This results in the formation of double stranded cDNA amplificates with melting-resistant CG-clamps flanking a melting-sensitive inner region. Consequently, the concentration of CG-primers diminishes by synthesis of stable specific products in the course of amplification, thus abolishing dilution procedures commonly performed in classical RT-PCR between the first and the second amplification protocol. PCR is completed by a second amplification protocol at different temperatures with a set of low-temperature nested primers that anneal to the melting-sensitive inner region of the CG-clamp amplificates.
In Philadelphia chromosome diagnosis RT-PCR with rTth and CG-attached primers resulted in the formation of specific products. Altogether the above described method showed equal sensitivity and yield in comparison to classical RT-PCR and was also less time consuming. The risk of contamination is markedly decreased because of the reduction to a one-tube-procedure.

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LYMPHOKINE ACTIVATED KILLER CELLS SUPPRESS RESIDUAL ACUTE MYELOID LEUKEMIA CELLS IN REMISSION.

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We have developed a method to identify and quantify residual leukemia progenitor cells in complete remission of acute myeloid leukemic (AML) by immunologically staining clones cultured in agar employing an enzyme-immunoassay. Leukemic colonies (CFU-L) were specifically distinguished form normal clones by their positivity for "blast markers" like CD 10, CD 20, and CD 34. Clones of leukemic phenotype were found in all 32 cases of AML complete remission (CR) studied at a proportion of 5-40%. These included 14 cases of long-term remission (>24 months). CFU-L were prognostically relevant since patients relapsing within 5 months had significantly higher numbers (P=0.003). Lymphokine activated killer (LAK) cells were raised from AML patients in CR (n=9) and at relapse (n=3) by suspension culture of peripheral blood mononuclear cells with Interleukin-2 (IL-2, 1000 units/ml). Coculture of LAK cells with autologous bone marrow (BM) cells obtained at the same time significantly inhibited the growth of CFU-L at BM to LAK cell ratios of 1:2.5 and 1:5 during CR (P=0.01 at one-dimensional variance analysis) but not in relapsing cases (P=0.28). Normal myeloid clones (CD 15-positive) were inhibited at a lesser extent only. These data show that CR of AML is a balance of normal and leukemic hematopoieses rather than eradication of leukemia which is maintained by the cytotoxic action of LAK cells.

Lymphokin-aktivierte Killerzellen unterdrücken die Residualleukämie bei akuter myeloischer Leukämie in Remission.

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CHEMOTHERAPY OF ADVANCED METASTATIC NONSEMINOMATOUS GERM CELL TUMORS WITH ETOPOSIDE, CISPLATIN, BLEOMYCIN, AND CYCLOPHOSPHAMIDE

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Since treatment of advanced metastatic nonseminomatous germ cell tumors with cisplatin, vinblastine and bleomycin (PVB) led to unsatisfactory results, we started 1983 to treat poor-risk patients with an intensified chemotherapy consisting of etoposide 120 mg/m², cisplatin 30 mg/m², bleomycin 12 mg/m², and cyclophosphamide 300 mg/m² (days 1-4) and 15 mg bleomycin bolus injection (day 1). 50 patients had tumors of testicular and 11 of extragonadal origin (median age 26 years, range 16-65 years). Patients with testicular tumors had bulky retroperitoneal disease and/or advanced haematogenous dissemination. 10 patients had hepatic and 4 cerebral metastases, 18 patients presented with 3 or more metastatic sites. HCG was elevated in 47 patients (in 22 patients > 10000 U/l), AFP in 39 patients (in 25 patients > 1000 IU/ml). The majority of patients received 4 to 6 cycles of ECBC. All patients experienced leuco- and/or thrombopenia of WHO grade 3 and 4. 2 patients died from septic shock after the first cycle, 2 further early deaths were due to organ failure because of extremely advanced metastatic spread. 7 patients died from primary disease progression after few cycles, 7 patients relapsed and died after salvage chemotherapy. 2 patients are alive with progressive disease 3 years after diagnosis and initiation of chemotherapy. Of the remaining 41 patients, 38 are currently alive disease-free and 3 with marker negative stable disease (median duration 41 months).
We conclude that despite of its high bone marrow toxicity ECBC merits further clinical investigation in patients with advanced metastatic nonseminomatous germ cell tumors.

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Molecular alterations affecting drug-sensitivity in sensitive and resistant cells

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Primary and secondary resistance against cytostatics is a major obstacle in chemotherapy. Several independent molecular mechanisms have been proposed affecting all levels of the cell: transmembrane transport , enzymatic drug detoxification, vesicular trapping of drugs, increased DNA repair, altered susceptibility of drug target proteins (e.g. Topo II), and others. We examined topoisomerase II alterations, MDR1 gene-overexpression and MDR1 mambrane-protein expression in pairs of sensitive and resistant cells. The cells where derived from three different cell lines (mouse erythro-leukemia, human gastric-carcinoma and human leukemia HL-60 cells), from normal lymphozytes and a patient with acute myeloid leukemia. All examined cell lines are established and grow permanently in liquid culture and in some of the resistant gastric carcinoma subline intracellular compartmentalization of mitoxantrone has been described previously. In most examined resistant cells more than one molecular mechanism can be found which indicates that cellular resistance to cytostatics is multifactorial in these cases. We propose that alteration of topoisomerase II is a major reason for high resistance against topoisomerase II inhibitors and that additional mechanisms might contribute for multidrug resistance against drugs from different chemical classes.

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INDUCTION OF GRAFT-VERSUS-LEUKEMIA (GVL) ACTIVITY BY IL-2 PRETREATMENT OF BONE MARROW (BM) GRAFTS .

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Using a murine leukemia model, we investigated (1) whether ex-vivo incubation of BM-grafts with IL-2 can induce GVL activity against otherwise resistant targets, and (2) whether in-vitro cytotoxic activity correlates with GVL effects observed in vivo. Experimental model: WEHI-3 is a myelomonocytic leukemia cell line of Balb/c (H-2^d) origin. Balb/c mice were inoculated with $1x10^6$ WEHI-3 cells which invariably lead to death with a median survival time of 21 days (n=48). After lethal irradiation (7,5 Gy) at day 5 post leukemia injection, $2x10^7$ syngeneic or semiallogeneic (C57xBalb/c)F1 BM cells were transplanted either unmanipulated or after 24 hr incubation with 200U/ml IL-2. In-vitro cytotoxicity of donor strain cells was measured by a 4hr Cr-release assay. Results: Animals receiving unmanipulated syngeneic or semiallogeneic marrow showed a relapse rate of 37% (n=12) and 38% (n=17), respectively. Transplantation of IL-2-activated grafts resulted in significantly ($p<0.05$) reduced relapse rates of 20% for syngeneic (n=25) and 19% (n=20) for semiallogeneic donors. In vitro tests for NK activity demonstrated that syngeneic and semiallogeneic effector cells were not able to lyse WEHI-3 targets. Whereas a specific lysis of 2.5% (E:T ratio 100:1; n=6) was observed after incubation with unmanipulated cells, IL-2 activated effector (LAK) cells exerted a specific lysis 15.7% of (E:T ratio 100:1; n=6). Preliminary data suggest that the effect of IL-2 can be markedly enhanced by the addition of either INF-gamma or TNF-alpha. Conclusions: (1) There is a correlation between in-vivo GVL effects against leukemia cells and in vitro lytic activity of BM donors against these leukemia targets (2) in primarily NK resistant leukemias, antileukemic effects can be achieved by pretreatment of the graft with IL-2

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ULTRASOUND IN THE EVALUATION OF HEPATIC AND SPLENIC MICROABSCESSES IN THE IMMUNOCOMPROMISED PATIENT

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High-dose chemotherapy, especially bone marrow transplantation for malignant hematologic disease causes a great degree of immunosuppression and thus carries a great risk of invasive fungal infection. Although hepatic and splenic involvement in disseminated candidiasis is frequent, involvement of these organs is rarely appreciated antemortem. During the last decade focal hepatosplenic candidiasis has been recognized by ultrasound with increasing frequency.

We report the sonographic and clinical findings of 6 patients (4 AML, 2 NHL) who demonstrated multiple, small-noduled, hypoechoic lesions in spleen and liver after high dose chemotherapy. All cases were in complete remission. Septic fever was unresponsive to antibiotic therapy. Granulocytopenia ($\leq 1000/mm^3$) was seen over a period for at least 10 days. However, the manifestation of hepatolienal microabscesses became apparent only after the patients neutrophil count turned to normal in all patient. Microabscesses decreased or disappeared after antifungal treatment. Candida-infection was confirmed serologically. Sonographic guided abscess biopsy (n=3) revealed necrosis/abscess.

In summary hypoechoic microabscesses of the liver and spleen could not be differentiated from leucemic or lymphomatous involvement solely on the basic of sonographic findings. Clinical data, especially time of diagnosis, sonographic short-term follow up after specific treatment are necessary for final diagnosis of hepatolienal candidiasis.

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INFLUENCE OF DIFFERENT CYTOKINES ON STROMAL CELL DEPENDENT MURINE B PROGENITOR CELL LINES

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Stromal cell dependent long-term cultures of Whitlock-Witte type provide a unique opportunity to study pre-B cell proliferation at a relatively mature level. However, only a few models using virus transformed cell lines or fetal liver derived cells exist which allow to study the regulation of earlier events in B cell development. We describe the development and characterization of murine early B progenitor cell lines which are strictly dependent on direct stromal cell interaction for long-term proliferation. Initially, murine bone marrow cells were incubated in modified Whitlock-Witte cultures in presence of IL-4 which have previously been shown to promote the development of immature B lineage cells. Lymphoid cells derived from such cultures were transferred to a stromal cell line designated HS4-A8. This stromal cell line selectively enhances expansion of a subpopulation of immature cells which have been further characterized. These cells grow in close contact to the stromal cells and express the B lineage marker B220 whereas cytoplasmic μ-chains were not detectable. Southern blot analysis with a J-H probe and 5' D probes revealed an incomplete DJ-rearrangement of the Ig heavy chain gene. There is preliminary evidence for a differentiating capacity of these cells to more mature B lineage cells under vatious culture conditions. The pro-B cell line H20 has been studied for growth factor requirements in detail. H20 cells are strictly dependent on stromal cells for long-term proliferation. Addition of IL-7 to the stromal cultures enhances expansion of H20 cells. In short term proliferation assays in liquid culture limited proliferation of H20 cells can be observed with stem cell factor (SCF) and IL-7 whereas either factor alone fails to stimulate H20. In clonal assays in soft agar H20 cells can not be induced to colony formation by SCF and IL-7. However, in agar assays performed on top of a confluent layer of HS4-A8 cells clonal prolfieration can be observed in presence of IL-7, IL-3, SCF or in combinations of these factors suggesting the release of an additional stromal cell product with proliferating effects on early B lineage cells. Another cell line D4-C requires exogenous addition of SCF to the stromal cell cultures to maintain long term proliferation again demonstrating that other factors in addition to SCF and IL-7 are essential for early B progenitor cells.

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SUCCESSFUL CANNULATION OF THE INTERNAL JUGULAR VEIN USING SONOGRAPHY IN CANCER PATIENTS

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The internal jugular vein (IJV) is a common access route to the central venous system. Anatomical landmarks are normally used for localization of the IJV (group I). We have compared the approach between the two bellies of the sternocleidomastoid muscle with a method using sonography to identify the IJV and the carotic artery (group II). Central venous catheters were placed with the Seldinger technique in all cases.

Results of the IJV-punctures

Group I 1/88 - 12/90 (84 patients)		*Group II 1/91 - 4/92* (79 patients)
126	total number of punctures	110
9	unsuccessful punctures	1
5	punctures of carotic artery	0
4	hematomas	2
2	pneumothoraces	0

In accordance with previously published data, our results demonstrate that the puncture of IJV using sonography has a low rate of complications. Using sonographically-guided puncture the success rate even is increased. Based on these findings we propose to establish the use of sonography in cannulation of the IJV as a routine method.

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GASTRIC MUCORMYCOSIS FOLLOWING AUTOLOGOUS BONE MARROW TRANSPLANTATION (ABMT)

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A 47-year old patient with centrocytic non-Hodgkin's lymphoma of stomach, colon, lymph nodes and bone marrow was resistant to conventional chemotherapy (six courses), including CHOP-Bleo. He achieved a partial remission with high-dose Ara-C/Mitoxantrone (two courses). Following TBI (14.4 Gy) and Cyclophosphamide (200 mg/kg) he was autografted with bone marrow which was purged with monoclonal antibodies and immunomagnetic beads. Three days post transplant the patient developed fever on unknown origin which did not resolve under broad spectrum antibiotic therapy. Therefore, antifungal treatment was started with conventional Amphotericin B (d 4 - d 11) and liposomal Amphotericin B (d12 - 28, total Amphotericin B-dose 4,54 g). On day 29 the antifungal therapy was changed to Fluconazole (200 mg/d), which was continued till day 60 (total Fluconazole dose 6.2 g). Multiple microbiologic examinations of blood, urine, stool, sputum and pleural fluid failed to reveal any pathogens. On day 51 after ABMT the patient developed severe pain in the left upper abdomen and signs of a subileus which responded to Prostigmin. The patient died on day 71 posttransplant in cardiovascular failure. Hematological reconstitution (WBC $> 1.0 \times 10^9/l$, Plt. $> 20 \times 10^9/l$) had not been reached. At autopsy a circular, sharply demarcated necrosis of the stomach wall was found and a massive fungal infiltrate. Spreading from the stomach, involvement of the spleen, diaphragm, left basal lung and left adrenal could be demonstrated. Histologic examination revealed the typical non-septate hyphae of mucor characterized by vascular invasion and necrosis. No residual lymphoma was found. The clinical course in our patient illustrates the features of gastric mucormycosis. Results of conventional microbiological tests did not yield positive results. In the phase of missing hematological reconstitution early and intensive antifungal therapy was not successful.

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NEUROBLASTOMA IS A POTENTIAL TARGET FOR RICIN A-CHAIN IMMUNOTOXINS

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We screened monoclonal antibodies recognizing the GD2 antigen for their potential use as ricin A-chain immunotoxins against different neuroblastoma cell lines. Two antibodies, BW704 and 14G2a, showing high potency in an indirect cytotoxicity assay were subsequently linked via SMPT to deglycosylated ricin A-chain (dgA). BW704.dgA and 14G2a.dgA killed 50% of neuroblastoma cells *in vitro* at concentrations of 3×10^{-10}M and 2×10^{-10}M, respectively as assessed by their 3H-Leucin-uptake. The GD2 antigen is expressed uniformly on the surface of neuroblastoma tumor cells. Staining of normal tissues including liver, lung, pancreas, colon, kidney, spleen and thyroid has so far not revealed major crossreactivities apart from strong staining of cerebellum by antibody 14.18 (an IgG3 variant of 14G2a). We are currently investigating a variety of normal human tissues for further crossreactivities with the GD2 antibodies. To test the efficacy of the two immunotoxins *in vivo*, we established a tumor model of human neuroblastoma in SCID mice. Intravenously inoculated neuroblastoma cells showed a disseminated growth in 100 % of the animals. The growth pattern resembled that of neuroblastoma in man. GD2-ricin A-chain immunotoxins currently being tested in our SCID mouse model might be useful in the treatment of neuroblastoma in the future.

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T-CELL ALL TREATED WITH MONOCLONAL ANTIBODIES TC-12 and TH-69 IN VITRO AND IN VIVO

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Despite effective modalities for treatment of T-cell acute lymphocytic leukemia (ALL), monoclonal antibodies against T-cell lymphoblasts may be useful for bone marrow purging as well as for killing of residual tumor cells in vivo. In our laboratory several antibodies directed against T-cell ALL have been developed, namely TH-69 and TC-12. Antibody TH-69, detecting the CD7 molecule, showed extremely sufficient killing of T-ALL tumors in a nude mice xenotransplant model. This was in sharp contrast to 3A1, another antibody also of IgG_1 isotype detecting the same epitope. Both antibodies were internalized after binding to the CD7 structure. The surprising cytotoxicity seen with TH-69 in animal experiments was dependent on the presence of the Fc-portion of the immunoglobulin. Available data suggest complement mediated lysis. The antibody TC-12 without such endogenous activity was conjugated to cytostatic agents. Effective coupling of antibodies to cis-platin and mitoxantrone was achieved. TC-12 immunoconjugates demonstrated in vitro and in the xenograft model activity towards T-ALL cells. A high specific enrichment of the antibody in the tumor area was detectable by ^{131}I-immunoszintigraphy. Recent data obtained during CD3 treatment of a T-ALL patient refractory to conventional chemotherapy also suggest complement mediated killing for OKT 3 antibody. It appears, that while many antibodies require immunoconjugation for tumor cell killing, for some such as TH-69 or OKT3 this may not be necessary.

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DETECTION OF MINIMAL RESIDUAL DISEASE IN ACUTE LYMPHOBLASTIC LEUKEMIA BY CLONE-SPECIFIC T-CELL-RECEPTOR DELTA REARRANGEMENTS.

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Considerable progress has been made in the treatment of childhood and adult ALL due to induction therapy intensification based on the stratification of high risk ALL. Yet, about 25% of children and 40% of adults suffer ALL-relapse after successful induction chemotherapy. Sensitive detection of residual leukemic blasts may be of value for the prediction of clinical relapse and risk adapted post-remission therapy. We have initiated a retrospective study of pediatric and adult ALL (mean follow-up 50 months) in an attempt to correlate clinical outcome with the persistence of residual leukemic disease using the polymerase chain reaction for the detection of clonal T-cell receptor delta (TCRδ) gene rearrangements. In non-T, non-B-ALL, clonal TCRδ rearrangements frequently occur involving Vδ2 and Dδ3 which are highly diversified by the insertion of random N-nucleotides, P-nucleotides, exonucleolytic trimming of coding regions and additional diversity elements. 9 of 19 childhood and 4 of 9 adult ALL had monoallelic (all adults, 4 children) or biallelic (5 children) Vδ2-(D)-Dδ3 rearrangements . Synthetic oligonucleotides ("signature probes") complementary to the highly diversified clonal junctional regions of individual ALL were used for the detection of residual leukemic cells in remission marrows 24 months after diagnosis. Specificity of the oligonucleotides was demonstrated, and sensitivity of the method was determined to be below 10^{-3} in all cases. Remission marrows at 24 months after diagnosis of 3 children in continuous complete remission for 39, 50 and 89 months, respectively were found to be pcr negative. Results of the remission marrows of the other 10 informative patients three of which relapsed will be presented. In conclusion, our results of 28 childhood and adult ALL off therapy show that a) leukemic clone specific TCRδ rearrangements were informative in almost 50% and b) CCR correlates with residual leukemic cells being undetectable by pcr at a sensitivity of at least 10^{-3} in remission marrow at 24 months.

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PCR-ANALYSIS OF IMMUNOGLOBULIN AND T-CELL RECEPTOR GENES FOR LINEAGE AND CLONALITY IN LYMPHOPROLIFERATIVE DISORDERS

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Polymerase chain reaction (PCR) conditions and primer sequences for V and J regions of the immunoglobulin heavy chain (IgH) or T cell receptor γ chain gene (Tγ) were chosen according to Trainor et al. (Blood 1991; 78:192-196). DNA amplification products (AP) were separated by temperature gradient gel electrophoresis. Clonal B-/T-cell populations representing less than 1% of the polyclonal lymphocytes were recognized using DNA extracted. In 31 nodal and extranodal lymphoma cases PCR analysis was performed from fresh-frozen as well as paraffin-embedded tissues with identical results except for two specimens where clonality was detected in the DNA from fresh-frozen tissue only. Clonality and lineage were confirmed in 92% (n=38) of different subclasses of T-lymphomas and 80% (n=108) of different B-cell lymphomas. Three of eight cases of AILD-type T-lymphomas had clonal amplification products for both, Tγ and IgH, and immunohistochemically were shown to contain a monoclonal B-blast population. Among 19 paraffin-embedded specimens of large cell anaplastic lymphomas (LCAL), PCR studies confirmed clonality and immunohistochemically defined lineage in 12 of 13 cases, only one B-LCAL remained negative. Five of 6 LCAL without lineage-assignment had clonal amplification products either for IgH or Tγ (2 and 3 cases, respectively).

The results indicate that T- and B-cell genotyping in routinely processed specimens by PCR technique is efficient and reliable. It is a valuable adjunct in the diagnosis and disease monitoring of lymphoma patients.

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SEMIQUANTITATIVE ANALYSIS OF A 21 KD PROTEIN IN LIVER CANCER PATIENTS UNDER THERAPY

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A new 21 kD protein (p21) was found in patients with proliferating urogenital and gastrointestinal tumors as well as in teratocarcinomas. The objective of the present study is to evaluate the prognostic value of p21 protein level in patients with liver cancer (10 hepatocellular carcinoma, 1 cholangiocellular carcinoma, 1 carcinoid).
Methods: P21 serum levels were measured by an ELISA in 12 liver cancer patients (median age of 55 years, 6 male, 6 female) during a period of a median of 8 months. Blood was taken before and after therapy. The measured values of p21 were compared with clinical stage, progression and response to chemotherapy (4-Epirubicin/ combination).
Results: In 5 patients p21-level decreased under therapy (median 23%). Progress of disease correlated with increased levels of p21 in 4 patients (median 50%). Almost constant levels (+ 7%) were observed in 3 patients with stable disease. Highest amounts of p21 were mesured in patients with active, advanced disease.
Conclusion: The amount of p21 protein correlated with the clinical course of disease under therapy. P21 may be a useful parameter to control the outcome of treatment in liver cancer patients.

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INVOLVEMENT OF NUCLEAR-FACTOR-κB IN INDUCTION OF THE INTERLEUKIN-6 GENE BY LEUKEMIA INHIBITORY FACTOR

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Recent studies have indicated that the Leukemia Inhibitory Factor (LIF) induces secretion of IL-6 in myeloid cells. We here show that synthesis of IL-6 by human mononuclear phagocytes exposed to recombinant human (rh) LIF is preceeded by an increase of IL-6 transcript levels as a result of transcriptional activation of the IL-6 gene. Analysis of deleted fragments of the IL-6 promoter showed that transcriptional activation of the IL-6 promoter was associated with enhanced binding activity of the transcription factor nuclear factor (NF)-κB. Binding of activation protein (AP)-1 and NF-IL-6, also known to transcriptionally activate the IL-6 promoter, was not inducible by LIF. Furthermore, introduction of the NF-kB sequence, but not of AP-1 and NF-IL-6, confered inducibility by LIF to a heterologous promoter. Taken together, our results show that rhLIF induces IL-6 gene expression in mononuclear phagocytes through transcriptional gene activation involving NF-κB.

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THE SIGNIFICANCE OF SERUM LEVELS OF SOLUBLE 60KD RECEPTORS FOR TUMOR NECROSIS FACTOR IN PATIENTS WITH HODGKIN'S DISEASE.

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Soluble forms of the two molecular species of the cell surface receptors (Rs) for Tumor Necrosis Factor (TNF) have been detected in normal urine and serum including type I and type II TNF-Rs. Using enzyme-linked immunosorbent assay (ELISA) we have determined type I 60 kD sTNF-R levels in the sera of 45 age- and sex-matched healthy subjects and 106 patients with Hodgkin's disease (HD). HD patients were either previously untreated (n= 76) or were in complete remission for at least 3 years after remission induction treatment (n= 30). The mean ± SD concentations of the 60 kD type sTNF-R were significantly higher in HD patients than in ~~healthy controls~~ (1,32 ± ~~0,19 ng/ml versus~~ 0,6 ± 0,13 ng/ml; p<0,001). The extent of increase correlated with the disease stage. Soluble 60 kD TNF-Rs were found to be significantly higher in stage III and IV (1,42 ± 0,21 ng/ml) than in stages I and II (1,08 ± 0,15 ng/ml). Patients with B-symptoms (n= 33) had higher levels (1,67 ± 0,20 ng/ml) than patients without systemic symptoms (1,02 ± 0,11 ng/ml; p< 0,001). In 52 patients evaluable for response, the complete remission (CR) rate of patients with 60 kD sTNF-Rs < 1,2 ng/ml was higher (88%) than in those with 60 kD sTNF-Rs > 1,2 ng/ml (64%; p≤ 0,01). A significant increase in serum levels of 60 kD sTNF-R levels was also observed in HD patients in long standing CR (1,04 ± 0,10 ng/ml). Our data suggest that the pretreatment serum concentration of 60 kD sTNF-Rs in HD may bear prognostic relevance. Increased 60 kD sTNF-R levels seen in HD patients in remission may point to the defect in cellular immunity characteristic of HD patients.

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DIFFERENT SUPPRESSIVE EFFECTS OF CANINE BONE MARROW CELLS

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Suppression of allogeneic lymphocytes is important for the induction and maintenance of transplant tolerance. Bone marrow contains cells that suppress autologous and allogeneic immune reactions against allogeneic cells (natural suppressor cells) and allogeneic immune reactions against autologous cells (veto cells). Unseparated bone marrow cells and marrow cells separated using immuno-magnetic beads were added to mixed lymphocyte cultures (MLC) and cell mediated lympholysis (CML) assays. Two monoclonal antibodies to human HLA-DR and CD6 that were crossreactive with canine cells were used. Marrow cells depleted of DR-negative cells exhibited a veto effect in both MLC and CML. Marrow cells depleted of CD6-positive cells exhibited a non-specific suppressor effect. CD6- and DR-negative marrow cells may be useful for prevention of marrow and organ transplant rejection.

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EXPRESSION OF THE RECEPTOR FOR GRANULOCYTE-MACROPHAGE COLONY STIMULATING FACTOR (GM-CSFR) IN ACUTE UNDIFFERENTIATED LEUKEMIA

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In 13 cases of acute leukemia with a undifferentiated phenotype after extensive morphological, cytochemical and immunophenotyping studies (POX$^-$, CD 1$^-$, CD 2$^-$, CD 3$^-$, CD 10$^-$, Cμ^-, SMIG$^-$) the expression of GM-CSFR and peroxidase activity at the electron microscopical level (POEM) were analyzed. Human GM-CSF (Behringwerke, Marburg) was iodinated using Jodogen^R reactant (Pierce^R) with a specific activity of 60-110μCi/μg and a biological activity of more than 80%. After T-cells/monocytes were depleted, all samples consisted of >90% leukemic blasts and were subsequently incubated for 1h with 500000 cpm radioiodinated GM-CSF. Ultrastructural demonstration of myelo- and platelet peroxidase was performed by incubating unfixed leukemic cells in a medium containing diaminobenzidine and H_2O_2 as previously described. In 3/13 samples specific binding of ^{125}J-GM-CSF (>50%) was detected. The immunophenotype of these cases was CD19$^+$/CD 33$^+$; CD 7$^+$/CD33$^+$ and in one case no lineage associated antigens were found. By the ultrastructural demonstration of myeloperoxidase activity in the leukemic cells, an early myeloid differentiation of these 3 cases could be clearly demonstrated. In 3 POEM positive cases, no GM-CSF receptors could be detected (CD 24$^+$/CD 13$^+$; CD 19$^+$/CD 65$^+$; CD 7$^+$/CD 13$^+$). In another 3 cases with a POEM$^+$/GM-CSFR$^-$ phenotype, expression of platelet peroxidase was found by ultrastructural peroxidase cytochemistry, indicating the megakaryoblastic nature of the blasts. The lack of GM-CSFR on megakaryoblasts in our cases is in contrast to other findings, probably due to the very immature differentiation stage. None of the 4 POEM$^-$cases showed GM-CSFR, despite the expression of myeloid lineage associated surface antigens in 3 cases. Taken together, GM-CSFR was expressed in minimally differentiated acute myeloid leukemias. It remains open whether the lack of GM-CSFR expression in some cases is due to the malignant transformation of the blasts. POEM$^+$/GM-CSFR$^-$ and POEM$^+$/GM-CSFR$^+$ cases may also reflect different maturation stages within the myeloid differentiation pathway.

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SEVERE TETRAPARESIS AS PRIMARY SYMPTOM OF A LOW GRADE NON-HODGKIN LYMPHOMA

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A 78 year old woman was in good health before she presented with sensory loss of the lower extremities. After a few days the disease progressed to incomplete tetraparesis. On physical examination, no lymphadenopathy and no hepatosplenomegaly was found. There were no signs of a infectious or tumorous process. A cranial computered tomogram was normal, and so were all laboratory tests performed on cerebrospinal fluid. Neurological examination led to the diagnosis of a polyradiculitis with severe tetraparesis. Inspite of administration of high dose methylprednisone and plasmapheresis symptoms persisted. White blood cell count was 10,4 G/l. Examination of a peripheral blood smear by a hematologist revealed a moderate lymphocytosis with atypical lymphocytes with only moderate chromatin condensation and rare nucleoli. Bone marrow cytology and histology showed a diffuse infiltration of the marrow by lymphoid cells, immunophenotyping revealed kappa light chain restriction and absence of the CD 5 antigen. The diagnosis of an immunocytoma was made and Chlorambucil 0,1 mg/kg and prednisone 2 mg/kg was administered orally in two weekly intervals. The neurologic symptoms disappeared rapidly and the patient was able to carry out normal activities again. 4 months after termination of the cytotoxic therapy, she recognized again paresthesia of both legs. Physical examination revealed moderate lymphadenopathy. Chlorambucil was reinstituted for 3 months and the paresthesia disappeared promptly. The patient is now feeling well for one year. To our knowledge, tetraparesis as the primary symptome of an immunocytoma was not reported so far. In elderly patients with unclear neurologic symptoms atypical manifestation of a low grade NHL should be considered.

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RAPID AND SUSTAINED ENGRAFTMENT WITH rhG-CSF-MOBILIZED BLOOD STEM CELLS FOLLOWING MYELO-ABLATIVE CONDITIONING THERAPY

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Autografting with blood-derived stem cells (ABSCT) following high-dose conditioning therapy was performed in a group of 7 patients with malignant lymphoma in sensitive relapse (5 Hodgkin's disease, 2 non-Hodgkin's lymphoma; 6 male/1 female; median age 38 years, range 27-51). All patients were heavily pretreated (on average: 9 cycles of 4 different chemotherapeutic regimens) and 5 had previous radiotherapy. To enforce the chemotherapy-induced rebound of hemopoietic progenitors during leukocyte recovery, we adminstered rhG-CSF (Neupogen^R) subcutaneously (5 μg/m^2) starting 24 hours after salvage therapy. Using the total number of nucleated cells (TNC) as an endpoint for blood stem cell collection (at least 0.6 x 10^9/kg bw), the quantity of TNC was between 0.6 - 0.95 x 10^9/kg bw (median 0.69 x 10^9). However, this parameter proved to be not useful and even misleading, because the content of hemopoietic progenitors of the autografts varied considerably reflecting the unpredictable differences of the individual hemopoietic reserve in extensively pretreated patients. The conditioning regimen consisted either of TBI (14.4 Gy)/Cyclophosphamide or of the CBV regimen (Cyclophosphamide, VP-16 and BCNU). All patients achieved complete engraftment with a median of 14 days for 1.0 x 10^9/l WBC, 20 days for 1.0 x 10^9/l PMN and 13 days for 20 x 10^9/l platelets. The number of CFU-GM/kg bw infused was predicitive for neutrophil and platelet recovery and a strong correlation was found between the number of CD34+ cells/kg autografted and platelet recovery ($R = -0.85$, $p < 0.02$). Patients transplanted with a quantity of > 5 x 10^6 CD34+ cells/kg bw achieved platelet recovery in less than 13 days. No late graft failure has occured. In summary, the quality of blood stem cell autografts is most reliably reflected by the quantitiy of CFU-GM or CD34+ cells with predicitve capacity for hematological reconstitution. Without additional bone marrow support rhG-CSF mobilized blood stem cells are capable of restoring long-term hemopoiesis following TBI-containing high-dose conditioning regimens.

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INDUCTION THERAPY WITH IDARUBICIN / CYTOSINE ARABINOSIDE AND MITOXANTRONE / ETOPOSIDE FOR DE NOVO ACUTE MYELOGENOUS LEUKEMIA.

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Since 1/89, 56 patients (p) (31 female / 25 male) with de novo AML were included into the study. Their median age was 43 years (range 15-60) with a distribution according to FAB as follows: 6 M1, 14 M2, 4 M3, 19 M4, 9 M5, 2 M6, 2 M7. The IDAC regimen consisted of: Idarubicin (12 mg/m^2/d i.v., d 1-3) and Ara-C (100 mg/m^2/d cont. i.v., d 1-7). Patients achieving complete remission (CR) or partial remission (PR) received another cycle of IDAC followed by NOVE (Mitoxantrone, 10 mg/m^2/d i.v., d 1-5 and Etoposide, 100 mg/m^2/d i.v., d 1-5). 54 p are evaluable for response (one toxic death and one early withdrawal). After 2 cycles of IDAC, 42 patients had attained a CR (78%). Of the 12 initial nonresponders to IDAC, 4 p obtained CR after NOVE. Thus, 46 of 54 p (85%) achieved CR after sequential induction therapy with IDAC and NOVE. In the last 14 p who achieved CR or PR after the first cycle of IDAC, rhGM-CSF (3 ug/kg/d, ESSEX) was administered for 6 days starting 3 days prior to the second cycle of IDAC. For the consolidation cycle with NOVE, rhGM-CSF was administered with identical dose schedule. The WBC showed a 3.4-fold increase after 72 hours of rhGM-CSF treatment, without the appearance of myeloblasts in the peripheral blood. During the sequential induction chemotherapy, no significant complications were observed. Patients received either post-remission therapy consisting of BMT or high-dose Ara-C/Mitoxantrone or were followed without any further treatment. Out of 46 p evaluable for disease-free survival, 21 p (51%) are in unmaintained CR after a median follow-up of 15 months (range: 3 - 38). The response-adapted treatment with IDAC/NOVE is effective and very well tolerated. RhGM-CSF administration is safe after response to induction therapy. To define the therapeutic impact of rhGM-CSF a higher number of patients and a longer follow-up are needed.

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CYTOGENETIC FINDINGS IN 179 PATIENTS WITH MYELODYSPLASTIC SYMDROMES (MDS)

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Chromosome analyses of bone marrow and/or peripheral blood specimens were performed in 179 patients with myelodysplastic syndromes. According to the FAB-classification 44 patients had RA, 16 had RARS, 34 suffered from RAEB, 21 from CMML and in 26 patients RAEB-T was diagnosed. 9 additional patients displayed a MDS secondary to an exposition to cytostatic or other mutagenic agents. In 29 patients no FAB-classification was available.

Clonal chromosome anomalies werde observed in 92 (51.3 %) patients. An AN-status (mosaik of normal and abnormal metaphases) was present in 58 patients, while 34 patients showed an AA-status (only abnormal metaphases). An as yet unpublished phenomenon of increased sporadic chromosome breakage was uncovered in 19 (10.6 %) patients. Three of them developed clonal anomalies during the course of their disease.

The most frequent chromosome anomalies were as follows: 5q- (27.2 % of 92 abnormal cases), -7/7q- (26.1 %), trisomy of 1q (14.1 %), +8 (13 %), -20/20q- (8.7 %), -X/Y (6.5 %), + 21 (4.3%). Thirteen patients (14.1 %) displayed complex karyotype anomalies and in 17 patients (18.5 %) a karyotype evolution could be uncovered.

In 53 patients sequential chromosome analyses were performed, ranging from two to six examination dates. The observation period varied between 1 an 62.5 months with a mean of 9.9 months. Thirty-one of these patients showed no karyotypical change, while 18 of them displayed a cytogenetic progression. In 4 patients a cytogenetic regression was documented. One occurred spontaneously, three were due to therapeutical intervention.

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FACTOR XII DEFICIENCY: A RISK FACTOR IN THE DEVELOPMENT OF THROMBOEMBOLISM - INCIDENCE OF F XII DEFICIENCY IN OUTPATIENTS SUFFERING FROM RECURRENT VENOUS AND ARTERIAL THROMBOEMBOLISM

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One hundred and three patients suffering from recurrent venous thrombosis (DVT), recurrent arterial thromboembolism (ATE) and/or recurrent myocardial infarction (MI) and 50 healthy subjects were tested for Hageman factor (F XII) activity and antigen. Among the 103 patients we identified 15 subjects with F XII deficiency (15 %), 3 with protein C deficiency (3%) and 3 with protein S deficiency (3%). The 103 patients were divided into subgroups according to the kind of thrombotic complication. Of the DVT-group 8 % ($p < 0.153$) of the patients were deficient in F XII. Among patients suffering from recurrent ATE and/or MI, the incidence of F XII deficiency was significantly higher (20 %, $p < 0.003$). In 67 % of the patients with F XII deficiency a positive family history of thrombosis could be established. In contrast, only 32 % of all DVT and 28 % of all ATE/MI patients had a positive family history. We believe that reduced levels of F XII should be considered as a risk factor in the development of thromboembolism. Consequently, more attention should be payed to the measurement of F XII when evaluating thromboembolic risk factors especially in cases of recurrent ATE and/or myocardial infarction.

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INTERFERON-γ INCREASES THE EXPRESSION OF THE GENE ENCODING THE β SUBUNIT OF THE GM-CSF RECEPTOR

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Granulocyte-macrophage colony stimulating factor (GM-CSF) activates a broad range of myeloid cells through binding to high affinity receptors (GM-CSF-R) consisting of at least two distinct subunits, GM-CSF-R_α and GM-CSF-R_β. The genes of these GM-CSF-R subunits have been identified recently, but little is known about the regulation of their expression. We investigated the expression of the GM-CSF-R subunit genes in freshly isolated normal human monocytes. Most cytokines and factors examined had no effect on GM-CSF-R_α and GM-CSF-R_β mRNA. However, interferon-γ (IFN-γ) increased the GM-CSF-R_β mRNA expression 3 to 6 fold with no effect on GM-CSF-R_α mRNA. Maximal effects occured 2 to 4 hours after stimulation with 500-5000 U/ml IFN-γ. Nuclear run-on assays and mRNA half-life studies revealed that IFN-γ increased the expression of the GM-CSF-R_β gene mainly by stabilizing the GM-CSF-R_β mRNA expression. The presence of cycloheximide did not abrogate the increase of GM-CSF-R_β mRNA expression induced by IFN-γ, indicating that de novo protein synthesis was not required for this activity. When monocytes were exposed to IFN-γ for 6 to 24 hours, the number of high affinity GM-CSF-R per cell was increased modestly (+79.3% of controls), whereas the receptor affinity remained unchanged. These data indicate that the GM-CSF-R expression in monocytes may be up-regulated by IFN-γ via an increased expression of the β subunit gene, involving predominantly posttranscriptional mechanisms.

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COMPARATIVE ANALYSIS OF THE SIGNAL TRANSDUCTION PATHWAYS OF STEEL FACTOR (C-KIT LIGAND) AND GM-CSF

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Steel factor (SF) supports the growth and differentiation of human progenitor cells in synergy with a variety of hemopoietins including GM-CSF. In an effort to characterize the mechanism of interaction of these factors, we have investigated their effects on the human factor-dependent cell line MO7. Proliferation of this cell line is induced by both GM-CSF and SF alone, but the maximum rate of proliferation is enhanced synergistically (3-6 fold) with combinations of SF and GM-CSF as compared with optimum concentrations of either factor alone. This synergy was not due to an SF-induced increase in GM-CSF receptor number or affinity. The effects of SF and GM-CSF on the activation of several intracellular signal transduction pathways were therefore assessed. Both cytokines induced a rapid, transient and concentration-dependent tyrosine phosphorylation of a number of substrates. GM-CSF stimulated tyrosine phosphorylation of several phosphoproteins with mol. weights of 150, 125, 93, 63, 55, 44, 42 kDa; proteins phosphorylated in response to SF had mol. weights of 140-150, 116, 94, 63, 44 and 42 kDa. A potential overlap between GM-CSF and SF was observed for 42, 44, and 63-kDa proteins. Some of the substrates phosphorylated in response to these growth factors were identified by immunoblotting with monospecific antibodies (Table 1). Additionally, the induction of the transcription factors c-myc and c-fos, and of two G1 cyclins, cyclin D2 and D3, by either factor was assessed by northern blotting (Table 2).

Table 1. Protein tyrosine phosphorylation induced by GM-CSF and/or SF

Signal transduction intermediate	mol.w. (kDa)	GM-CSF	SF	GM-CSF+SF
c-kit	145	-	+	+
phospholipase C-γ (PLC-γ)	145	-	+	+
mitogen associated protein kinase (MAPK)	42,44	+	+	+
Raf-1 (serine phosphorylation only)	70-75	+	+	+

Table 2. Induction of mRNA by GM-CSF and/or SF

	GM-CSF	SF	GM-CSF+SF
c-myc	+	-	+
c-fos	+	+	++
cyclin D2	+	-	+
cyclin D3	+	+	+

Co-stimulation with SF and GM-CSF resulted in at least additive effects on c-fos mRNA levels. In conclusion, the synergy between SF and GM-CSF may result from the activation of common signal transduction intermediates like MAPK, Raf-1, c-fos, and cyclin D3, as well as of unique pathways such as c-kit, PLC-γ, c-myc, and cyclin D2.

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DIFFERENTIATED BLAST CELLS DO NOT INDUCE T LYMPHOCYTE-MEDIATED INHIBITION OF ERYTHROID PROGENITORS GROWTH IN ACUTE MYELOID LEUKEMIA

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In our previous studies we have demonstrated that a subset of radioresistant, HLA-DR-positive helper T cells from blood of patients with acute myeloid leukemia /AML/ inhibits the clonal proliferation of autologous marrow-derived erythroid progenitors /BFU-e and CFU-E/ via the release of interferon-gamma. This T cell-mediated inhibitory effect was not observed during complete remission /CR/, however, T lymphocytes from CR-patients could be induced to become suppressor for the erythroid progenitors growth after incubation with blast cells. The results of these experiments clearly show that activation of HLA-DR-positive subset of CD4+ T cells by blasts is an indispensable stage for the induction of the above described suppression of erythropoiesis in AML. In further sudies it was shown that only undifferentiated blast cells have such an ability. Blast cells after incubation with GM-CSF, phorbol esters and DMSO had a no influence on the autologous T lymphocytes. The results of these experiments reveal that the blast cells induced into differentiation lose their ability to activate T cells and in turn to suppress the formation of BFU-E- and CFU-E-derived colonies.

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EVALUATION OF THE CYTOTOXIC ACTIVITY OF INTERFERON α IN COMBINATION WITH DIFFERENT CYTOSTATIC DRUGS IN HUMAN GASTROINTESTINAL TRACT CANCER CELL LINES

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The combination of fluorouracil and interferon α has shown considerable antitumor activity in metastatic colon carcinoma. Little is known about the activity of interferon in combination with other drugs in GI tract cancer. We therefore evaluated the interaction of interferon with different antineoplastic drugs in 2 human colon carcinoma (HTB 138, CCL 29) and 1 human gastric carcinoma (M2) cell lines. Interferon was given at a fixed dose of 100 U/ml continuously. The drugs were added for 96 hours. Cytotoxicity was evaluated in the SRB-assay. The fractionated survival product method was used to characterize the nature of drug interactions. FU and interferon were synergistic in HTB 138 and additive in CCL 29 and M2 cells. The interaction of interferon and ifosfamide was additive in M2 and CCL and less than additive in HTB 138. Doxorubicin and interferon were synergistic in HTB 138 and additive in M2 and CCL 29. Etoposide and interferon were less than additive in all 3 cell lines.

From these data the combinations of 5-FU and interferon and doxorubicin and interferon appear promising in GI malignancies, since synergism was observed in at least one test system. On the other hand the combination of etoposide and interferon showed less than additive interactions in all 3 cell lines and therefore seems to have no therapeutic benefit.

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IN VITRO ANTITUMOR ACTIVITY OF LOBAPLATIN (LOB) IN CISPLATIN (DDP) SENSITIVE AND RESISTANT HUMAN CARCINOMA CELL LINES (HCCL).

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Lobaplatin is a new platinum compound with apparently no nephro- and neurotoxicity, currently undergoing clinical phase I- and II- studies. We evaluated the activity of this new compound in a variety of HCCL in comparison to cisplatin and carboplatin, using 96 h continuous drug exposure in a SRB-assay. In 3 cisplatin sensitive testicular carcinomas and 1 ovarian carcinoma LOB and DDP had comparable antitumor activity (IC 50 DDP: 0,1 - 0,4 μM; IC 50 LOB: 0,25 - 0,5 μM), whereas carboplatin was significantly less active (IC 50: 8 - 20 μM). In order to assess the cross resistance between LOB and DDP, both drugs were evaluated in 5 HCCL with acquired DDP resistance. Complete cross resistance was seen in 2 gastric carcinomas (3,3 and 9 fold DDP resistant, 3,1 and 6,5 fold LOB resistant) and 1 ovarian carcinoma (15 fold DDP and 10 fold LOB resistant). However LOB was active in a 10 fold DDP resistant testicular carcinoma and a 20 fold DDP resistant ovarian carcinoma cell line. LOB possesses high antitumor activity in cisplatin sensitive tumors and appears to be only partly cross resistant to the parent compound. Given its favourable toxicity profile this drug could develop into a very valuable clinical agent.

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ELEVATED RISKS FOR ACUTE MYELOGENOUS LEUKEMIA (AML) AND CHRONIC MYELOGENOUS LEUKEMIA (CML) IN A GERMAN COUNTY CONTAMINATED WITH TRINITROTOLUENE (TNT)

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The relationship between exposure to benzene and leukemia is well known. The benzene derivate TNT had been widely used for producing explosives during World War II. Due to inappropriate handling of waste the soil in a densely populated area of Middle Hesse, where a former important TNT producing plant had been located, is still contaminated with TNT and numerous of its derivatives. We assessed the relative risk (RR) of leukemia in a county contaminted with TNT as compared to the risk in a neighboring county lacking contamination with TNT. In the most contaminated zone the RR of AML was 3.5 (95% confidence interval (CI) 1.4-8.5) in males and 3.2 (95% CI 1.4-7.2) in females. The RR of CML was 9.1 (95% CI 3.5-23.4) in males and 1.3 (95% CI 0.2-10.3) in females. Our data indicate, that chronic exposure to TNT of its derivatives might be a risk factor for AML and CML.

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PREFERENTIAL MUTATIONS OF P53 IN LIVER METASTASES OF COLORECTAL CANCER

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Mutations of the p53 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 tumor progression and metastasis we analyzed 28 metastases of colorectal carcinomas (24 liver, one lymph node, one lung, one omentum, one skin metastasis) with respect to mutational changes and expression of the p53 tumor suppressor gene. Total RNA was extracted from fresh frozen material, reverse transcribed into cDNA, and cDNA sequences corresponding to the four mutational "hot spots" of the gene were PCR-amplified and directly sequenced. 18 of 28 metastases (64%) showed point mutations in the p53 gene. Interestingly the frequency of mutations at amino acid positions commonly mutated in primary colon cancer differs significantly in the metastases compared to the data published for primary tumours. For instance, whereas mutations at amino acid position 175 in "hot spot B" are a relatively frequent event in primary colon cancer (18%), we found no liver metastasis with a mutation at this position. Taken together the data indicate a preferential occurance of mutations in metastases in the "hot spots" C and D compared to the distribution of mutations along the four "hot spots" in primary colon cancer. Thus we suggest that cells carrying mutations in the "hot spots" C and D may have a selective advantage to metastasize.

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EFFECTS OF DEXTRAN-CONJUGATED DOXORUBICIN (DOX-OXD, AD-70) ON CLONOGENIC GROWTH OF FRESHLY EXPLANTED HUMAN TUMORS

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DOX-OXD is a high-molecular-weight conjugate of doxorubicin (DOX) and dextran (70000 m.w.) via a Schiff's base. The rationale for this construct is to enhance antitumor activity of DOX by alteration of drug disposition and increased tumor selectivity. We have studied the effects of DOX-OXD on in vitro colony formation of freshly explanted human tumors using a capillary soft agar cloning procedure and have compared this compound's activity to 4 other anthracyclines (doxorubicin, mitoxantrone, epirubicin, daunorubicin). At present, 12/32 (38%) tumor specimens are evaluable (4 gastric, 3 lung, 2 renal, 1 colorectal, 1 melanoma, 1 lymphoma) with a median colony formation of 19.4/capillary in controls (range 4.2-88.0). At clinical achievable concentrations, DOX-OXD was as active as daunorubicin (colony growth ≤50% x control in 5/12 specimens), less active than mitoxantrone (10/12) or epirubicin (10/12), but slightly more active than DOX (3/12) alone. Head-to-head comparisons of DOX-OXD and DOX using equimolar DOX concentrations showed similar activity of DOX-OXD (2/12 specimens) as compared to DOX (3/12 specimens). In one gastric cancer specimen DOX-OXD was the only active drug out of the 5 agents studied. We conclude from these preliminary data that DOX-OXD may be at least as active as DOX against clonogenic cells from freshly explanted human tumor specimens in vitro.

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DECREASED FUNCTIONAL ACTIVITY OF ALPHA-1-ANTICHYMOTRYPSIN IN A PATIENT WITH HIGH GRADE MALIGNANT NON-HODGKIN LYMPHOMA

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Alpha-1-antichymotrypsin is a 68,000 mw glycoprotein, belonging to the SERPIN family (*ser*ine *p*roteinase *in*hibitors) of plasma proteinase inhibitors. Being a major acute phase reactant, its plasma concentration can drastically increase within hours after injury. Although the interrelationship of Alpha-1-antichymotrypsin and the leukocyte proteinase cathepsin G is well established, the physiological role of this system is not known. With immunological methods, heterozygous deficiency states have been described without severe clinical sequelae. However, no deficiencies with less than 50% of normal have been seen so far. With our novel assay for functional activity (Clin Chem 36:1990, 2077-81), we now found six patients with malignant lymphoma in whom the activity of Alpha-1-antichymotrypsin was decreased beyond the 50% margin (21-45% of normal). This opens the question whether impaired activity of Alpha-1-antichymotrypsin is a permissive pathogenetic factor for the acquisition of malignant lymphoma.

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HIGH-DOSE CYTARABINE AND DAUNORUBICIN POSTREMISSION CHEMOTHERAPY FOR THE TREATMENT OF ADULTS WITH DE-NOVO ACUTE MYELOGENOUS LEUKEMIA (AML)

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In a prospective trial of the "Süddeutsche Hämoblastosegruppe" 146 patients aged 15 to 50 years (mean =36 years) with de-novo AML (FAB M1-M6) received two cycles of a three drug combination (DAV I/II) including cytarabine (100 mg/m², continuous infusion, 8 days) daunorubicin (60 mg/m² daily for 3 days) and VP 16-213 (100 mg/m² daily, 5 days) for remission induction. Patients in complete remission received one additional course of the DAV combination therapy (DAV III) as early consolidation after recovery of the neutrophil count to over 1000/µl and thrombocytes to over 100.000/µl. In 104 out of 149 patients (70%) a complete remission (CR) was achieved. Partial remission was achieved in 7 patients (5%) and 14 patients (9%) died within the first 6 weeks during remission induction. 24 patients (15%) were treatment failures and in 2 patients (1%) the result of induction therapy could not be evaluated. Sixty-one patients of the 104 complete responders underwent planed late consolidation therapy with high-dose cytarabine (3 g/m², every 12 hours for 12 doses) followed by daunorubicin (30 mg/m² daily for 3 doses). High-dose cytarabine/daunorubicin therapy was started 4 weeks after recovery from early consolidation. 43 patients did not receive late consolidation therapy owing to excessive toxicity of induction therapy, early relapse, selection for bone marrow transplantation or refusal.At a median follow up of 3.4 years the five year actuarial leukemia-free survival was 34% for all patients achieving CR and 38% for the patients who received late consolidation therapy. Despite the fact that this form of consolidation therapy may represent an improvement over conventional therapy, relapses were common even after planed therapy indicating that post-remission therapy remains further to be improved.

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GM-CSF IN A DOUBLE-BLIND RANDOMIZED, PLACEBO CONTROLLED TRIAL IN THERAPY OF ADULT PATIENTS WITH DE-NOVO ACUTE MYELOID LEUKEMIA (AML)

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This multicenter trial initiated in 5/90 addressed the question whether the addition of rhu GM-CSF to a standard cytotoxic regimen prolonges event free survival of AML patients. Furthermore we wished to reduce the incidence of infections by shorting the duration of critical neutropenia. Induction and early consolidation therapy included cytarabine (Ara-C, 100 mg/m^2, day 1 -8 civi), daunorubicin (60 mg/m^2, IV, day 3 - 5,) and etoposide (100 mg/m^2, day 4 - 8, 2 h IV infusion) with reduced dosages in the second induction and early consolidation course. Late consolidation included one cycle with high-dose Ara-C (3g/m^2, 12 doses) and daunorubicin (30 mg/m^2, day 7 - 9) for patients aged 50 years and younger, whereas patients over 50 years received a reduced dose of Ara-C (0.6g/m^2, 12 doses). Patients were randomized after the first induction course to receive either rhu GM-CSF (E. coli, 250 μg/ m^2/ day, s.c.) or placebo. GM-CSF/placebo therapy was started 48 hours prior to the second induction and the subsequent courses and was stopped after chemotherapy induced aplasia at absolute neutrophil count > 500/μl. 80 out of 84 patients (median age = 50 years) included into the study could be randomized. The remaining four patients could not be randomized due to death during the first induction course prior to randomization (n = 2), alternate therapy (n = 1) or refusal (n = 1). In 51 out of 65 patients (79%) evaluable so far a complete remission (CR) could be induced. 12 patients (18%) were treatment failures and another 2 patients (3%) died during the first induction course. 14 patients suffered a relapse after a median remission duration of 2.8 months (range 1.2 to 9.5 months). 20 out 84 study patients died including the two patients who died during induction therapy. Seven patients died as treatment failures and another 11 patients died in relapse. These preliminary data with a rather high CR rate together with the low induction death rate compare favorably with those obtained after intensive chemotherapy regimen, while the influence of GM-CSF on the treatment outcome remains open till unblinding of the study.

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SERUM PHARMACOKINETICS OF LIPOSOMAL AMPHOTERICIN B (AmB) (AMBISOME^R)

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Twentyone patients treated on an intensive care unit received liposomal amphotericin B (Ambisome^R) at a median dose of 3 mg/kg (range 1.1 - 3.75 mg/kg) once daily. Serum concentrations of AmB were analyzed before (trough) and one hour after end (peak) of a 1-h infusion of Ambisome^R. AmB concentrations, as determined by HPLC, showed a great interpatient variability. Median initial trough and peak concentrations of AmB were 0.8 μg/ml (range 0.2-3.5 μg/ml) and 5.9 μg/ml (range 1.8-21.8 μg/ml). The median overall half-life ($T_{\frac{1}{2}}$) of AmB in serum was 10.4 h (range 6.9-16.5 h).
A longitudinal analysis of serum AmB concentrations was performed in 15 patients for a median duration of 10 d. Two groups of patients could be identified with regard to serum $T_{\frac{1}{2}}$ and maximal trough -and peak concentrations of AmB. Median $T_{\frac{1}{2}}$ in group A (n=7) was significantly lower with 8.7 h (range 6.9-10.3 h) than in group B (n=8) with 12.4 h (range 10.4-16.0 h). Accordingly, serum peak and trough concentrations of AmB remained steady in group A, and increased cumulatively in group B. Group A achieved maximal trough and peak concentrations of 1.7 μg/ml and 9.5 μg/ml respectively, while significantly greater concentrations were reached in group B with maximal trough and peak concentrations of 5.4 μg/ml and 19.3 μg/ml respectively. We conclude that a patient entity defined by prolonged elimination of liposomal AmB from serum might rather profit from a modified schedule of Ambisome^R administration.

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2',2'-DIFLUORODEOXYCYTIDINE (GEMCITABINE^R): SELF-POTENTIATIVE INTERACTION WITH CELLULAR NUCLEOTIDE AND DEOXYNUCLEOTIDE METABOLISM

V. Heinemann*, and W. Plunkett**

Gemcitabine^R (dFdC) is an antimetabolite which after intracellular phosphorylation to the active 5'-triphosphate (dFdCTP) exerts severalfold greater cytotoxicity than comparable dCyd analogs. dFdC acts as a relative chain terminator and induces cytotoxicity by inhibition of DNA synthesis. Through inhibition of ribonucleotide reductase (RR), dCMP deaminase (dCMPD), and CTP synthetase (CTP-S) dFdC metabolites modulate cellular NTP and dNTP metabolism to an extent which increases phosphorylation of dFdC , and prolongs retention of dFdCTP. dFdCDP acts inhibitory to RR and consequently depletes the dCTP pool. This reduces feed-back inhibition on dCyd kinase and so augments the activity of this rate-limiting enzyme for dFdC phosphorylation. Depletion of dCTP also diminishes competition of dFdCTP with dCTP at DNA polymerases which increases incorporation of dFdC into DNA. dFdCTP-mediated inhibition of dCMPD decreases the katabolism of intracellular dFdCTP to deaminated, nontoxic metabolites. Consequently, intracellular dFdCTP retention and with it cytotoxicity may increase with rising dFdCTP concentrations. The activity of dCMPD is further diminished as dCTP, the allosteric activator of the enzyme, is depleted. Cellular activity of CTP-S is inhibited at high cellular dFdCTP concentrations. Depletion of CTP is inhibitory to RNA synthesis and, by mass action, results in dCTP depletion. We conclude that the increased cytotoxicity of dFdC may be explained by its self-potentiative mechanism of action.

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CYTOKINE INTERACTIONS AND SIGNALING IN NORMAL AND LEUKEMIC HEMATOPOIESIS: INPLICATIONS FOR NEW TREATMENT STRATEGIES IN AML.

F. Herrmann.

Like normal hematopoietic progenitor cells (PC), leukemic PC of patients with acute myelogenous leukemia (AML) have retained the ability to respond to hematopoietic growth factor (HGF) stimulation with IL-3, SCF, GM-CSF and G-CSF being the most potent stimulators. However, in contrast to normal PC leukemic PC show only little, if any, maturation under the influence of these regulatory molecules. The inability of AML cells to mature towards terminally differentiated non-dividing elements prevents the progression of these cells to the stage where extinction of clonogenicity and ultimately apoptosis occurs. Thus, exposure of AML-PC to HGFs favours mainly induction of proliferative events and self-renewing. AML cells not only respond to exogenous HGFs, about 20-30% of AMLs are also capable of producing their own growth promoting molecules either in an autocrine or paracrine fashion requiring the presence of hematopoietic stroma cells to interact with. Autocrine and paracrine loops whereby AML-PC may escape their physiological growth restrictions are mostly mediated by multiple factors that synergistically interact. This synergism takes place at the level of receptor transmodulation and of complementary signal transmission. This presentation will focus on the possibilities to eliminate clonogenic leukemia growth in vitro that may lead to possible in vivo treatment strategies as well, including strategies aimed at blocking ligand/receptor interactions (receptor antagonists, soluble non-antagonistic receptors), aimed at interrupting autocrine and paracrine growth stimulatory loops (neutralyzing anti-cytokine antibodies, oligonucleotides, anti-signaling compounds and antagonistic cytokines such as IFN-gamma, TGF-beta, IL-4, IL-10, and finally aimed at targeting the molecular mechanisms that interfere with the prevention of apoptosis.

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MODULATORS OF P-GLYCOPROTEIN (PGP) ASSOCIATED DRUG RESISTANCE

R. Herrmann

Overexpression of the MDR1 gene product pgp, a transmembrane protein, has been found to be responsible for resistance to a variety of antineoplastic agents, so called classical multidrug resistance (MDR). In vitro as well as in vivo pgp may be overexpressed following exposure drugs (induced overexpression). Likewise, overexpression may be present in tumors not previously exposed to drugs (de novo overexpression).The mechanism of this type of resistance is likely to be increased outward transport of the drug. Several substances have shown to competitively inhibit the function of pgp. These include calcium chanel blockers, antiarrhythmic agents, trifluoperazine, detergents, antiestrogens, cyclosporin A and monoclonal antibodies to pgp. For these agents high blood levels are required which in most instances cause unacceptable side effects, analogues have been or are beeing developed with improved therapeutic index. Experimental studies as well as early clinical trials have pointed to a number of problems regarding the use of pgp modulators: MDR1 is just one of several mechanisms of drug resistance, the concentrations found to be effective in vitro cannot always be achieved in vivo, some of the modulators have antineoplastic activity of their own which cannot be differentiated from a modulating effect, modulators are likely to inhibit pgp function in normal cells which may increase toxicity and cause new types of toxicity. Thus, the evaluation of a potential benefit of the addition of pgp modulators to chemotherapy requires carefully designed and executed clinical trials.

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IMMUNOPHENOTYPICAL AND HAEMATOLOGICAL CHANGES IN PATIENTS RECEIVING INTERFERON α AND DONOR BUFFY COAT TRANSFUSIONS FOR TREATMENT OF CML RELAPSE AFTER ALLOGENEIC BONE MARROW TRANSPLANTATION.

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Treatment of relapsed CML after BMT using IFN α and donor buffy coat transfusion was recently described by Kolb et al.. We evaluated this treatment modality in 6 patients (3 female, 3 male, age 25-56 ys) relapsing after T-cell depleted (n=3) or conventional BMT (n=3). In 5/6 patients treatment respose could be demonstrated by cytogenetic analysis, RFLP-testing or blood group typing. One patient showed no response. GvHD occured in the 5 responders resembling typical grade II aGvHD of the skin in two patients and showing features of acute and chronic GvHD in 3 patients. Bone marrow hypoplasia, being severe in 4 cases was observed in all responding patients and was cause of death in 3 cases (infection n=2, bleeding n=1). Prior institution of treatment lymphocyte subsets (double IF-labeling using anti CD3, CD4, CD8, CD19, HLA-DR, CD56 and CD16 antibodies) were normal in 5 patients, in one patient inversion of the CD4/CD8 ratio was present. Following buffy coat treatment increase in the proportion of CD8 cells was seen in 5 cases, most markedly in the patients developing typical aGvHD. No changes were observe within the NK subset. This data indicate that treatment with buffy coat transfusions results in clinical response in the majority of patients but has substantial toxicity due to BM-aplasia. Phenotypical analysis may indicate that T-cells and not NK cell play a major role in the mechanism of this treatment.

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EXPRESSION OF GROWTH REGULATORY GENES IN POLYCYTHEMIA VERA

Georg Hess, Heinold Gamm, Christoph Huber, Barbara Seliger

Polycythemia vera is a monoclonal disorder of hematopoietic stem cells, characterised by extensive proliferation of granulocytic, erythroid and megakaryocytic cells in the bone marrow and frequent deletions of chromosome 20q. We here analyse the expression of growth regulatory genes e.g. oncogenes and tumor suppressor genes of peripheral blood samples from 25 PV patients, who were diagnosed according to the PCV study group. The oncogenes trk, fyn, myc and p53 were differentially expressed in the patients. Furthermore, overexpression of the RB gene was detected when compared to normal peripheral blood cells. Polymerase chain reaction (PCR) revealed in 1 out of 25 cases either a loss of exon 5 or 8 of the p53 gene and in 3 out of 25 cases a loss of ras tumor suppressor gene NTS-1. Furthermore, single strand conformation polymorphism (SSCP) showed a mutation of the GNAS-1 gene, which is like NTS-1 located on chromosome 20q. In addition, we are characterising the erythropoietin (EPO) system in PCV patients. In some patients low epression of Epo was detected using reverse PCR. Interestingly the patients produce differential amounts of Epo. Detailed analysis of structural alterations of the Epo system, oncogenes and tumor suppressor genes is in progress.

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SEVERE HYPERCALCEMIA IN A PATIENT WITH ADVANCED B-CLL

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The onset of hypercalcemia in Chronic Lymphatic Leukemia (CLL) is infrequent. It is usually due to lytic bone lesions based on B-cell mediated local or wide spread osteoclastic activation. We observed a severe hypercalcemic crisis in a 56 years-old patient with a 10 years history of B-CLL, who did not develop demonstrable bone lesions. The patient was admitted to our clinic with recurrent pneumonia. At admission serum creatinine, calcium and phosphorus levels were normal. Antibiotics were started, and he recovered slowly. Within 8 days, however, serum calcium continuously rose to 4.19 mmol/l, and phosphorus values declined to 0.70 mmol/l. The patient became symptomatic presenting restlessness, confusion and polyuria. Moreover, we found an increase of serum creatinine (3.0 mg/dl), high urine calcium levels (6.2 mmol/l), and reduced calcitriol values (< 15 pg/ml). Primary hyperparathyreoidism was not present (Parathormone 11 ng/ml). Other common causes of hypercalcemia (e. g. lytic bone lesions, 2nd malignancies) were excluded later by autopsy. Hypercalcemia was successfully treated by calcitonin and clodronat infusions. However, renal function declined, and the patient died. We believe that a humoral factor with PTH like activities was responsible for the hypercalcemia in our case. This is indicated by the laboratory data pattern and by exclusion of other causes. Such a factor is rare in CLL and is usually associated to a large tumor burden and a bad prognosis.

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DUAL LUMEN GROSHONG CATHETERS FOR PERMANENT CENTRAL VENOUS ACCESS

J. Heymanns, H. Köppler, K. Havemann

The Groshong catheter is a long term central venous catheter with a pressure sensitive three position valve placed near the closed tip. Therefore heparin flushing and external clamps are not necessary. Since February 1991 dual lumen Groshong catheters were inserted in 34 patients, in 13 of them prior to autologous bone marrow transplantation. Patients diagnoses included malignant lymphoma (n=13), acute leukaemia/CML-blast crisis (n=12), plasmocytoma (n=3) and miscellaneous malignancies (n=6) requiring intensive therapy. Generally catheters were inserted under local anaesthesia into the right subclavian vein. Operative placement was performed in the first six patients, subsequently catheters were inserted by hematologists using the Seldinger-technique and a peel-away introducer sheath. In two patients subclavian vein could not be cannulated and catheter was placed into the right internal jugular vein. X ray was used to verify correct position of the catheter. Besides one accidental arterial puncture there were no other insertion complications. Duration of use ranged from 33 to 306 days (mean 112 days calculated as of April 30, 1992). The complications experienced are as follows: Exit site infections in 4 patients and fever/bacteremia in 8 patients with positive blood cultures, all were treated with antibiotics and catheters could left in place; progressive infection of the subcutaneous tunnel in spite of antibiotic treatment in 3 patients where catheter had to be removed; three clotted catheter lumen. No thoracic vein thrombosis was observed. In 18 patients catheter was removed at the end of therapy, 4 patients died. The advantages of the dual lumen Groshong catheter include small outer diameter, a relatively simple and quick insertion procedure, and time saving and cost effective maintenance procedures.

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IL-2, IFN-GAMMA AND IL-3 DIFFERENTLY AFFECT CIRCULATING PRECURSORS OF CTL (CTL-P) IN MAN

F. Hladik, K. Kolbe, E. Irschick, J. Aman, C. Peschel, W.E. Aulitzky, and C. Huber

Synergism of specific antigen and cytokines for clonal expansion and differentiation of CTL-p has generally been accepted. However, the primary effect of cytokines on CTL-p without challenge by specific antigen remains unclear.

We investigated, wether systemic treatment with cytokines affects the clone size of circulating alloreactive CTL-p in man. We used limiting dilution analysis to determine CTL-p frequencies in patients with non-hematologic diseases before and after three days of subcutaneous application of either IL-3 (2.5, 5.0 or 10.0 μg/kg/d), GM-CSF (5 μg/kg/d), IFN-gamma (400 μg/d), IFN-alpha ($5x10^6$ IU/d) or IL-2 ($4.8x10^6$ IU/m^2 bd). Simultaneously clone sizes of circulating autoreactive CTL-p were determined by split well analysis.

Our data suggest only minor influences of low and intermediate dose IL-3 on CTL-p frequencies. Further escalation of dose interestingly led to significant expansion of these cells. Significant expansion was also observed under systemic treatment with IL-2. In contrast IFN-gamma markedly diminished the clone size of circulating alloreative CTL-p. GM-CSF and IFN-alpha did not exhibit measurable effects. Furthermore we could not find autoreactive CTL-p at any time tested.

In conclusion systemic application of IL-2, IFN-gamma or IL-3 differently affects circulating precursors of CTL in man. This may have impact on the responder status to specific antigens in vivo.

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HUMAN E-CADHERIN AND TSH-RECEPTOR GENE: EXPRESSION LEVELS AS MARKERS OF DIFFERENTIATION IN NORMAL THYROID AND THYROID CANCER

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TSH receptor (TSH-R) mRNA is persistently expressed in all differentiated thyroid tumors and low in hyperfunctioning adenomas (Mol Cell Endocrin 82:R7, 1991). E-Cadherin, an epithelium dependent cell-cell adhesion molecule, has expressed in non-invasive and is lost in invasive metastatic tumors (J Cell Biol 113:173, 1991). We used northern blotting to analyse mRNA of E-cadherin and of TSH receptor transcripts in patients operated for follicular (FTC,n=5), papillary thyroid carcinoma (PTC,n=7) or anaplastic thyroid carcinoma (ATC), in patients with benign nodular goitre (NG,n=2) and in normal controls (C,n=7). E-cadherin protein levels were measured by immunostaining. TSH-R and E-cadherin mRNA were strongly expressed in C and in NG, but greatly reduced in ATC. In PTC and FTC the TSH-R and E-Cadherin transcripts varied from normal to reduced levels. C and NG showed positive immunostaining for E-cadherin, variable staining was detected in PTC and FTC and no staining was found in ATC. These data demonstrated a parallel change of TSH-R and E-cadherin mRNA levels in differentiated and a reduction in undifferentiated tumors. The potential role of E-cadherin for metastatic growth of thyroid tumors as suggested for other epithelial tumors requires further longitudinal investigation.

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ADVERSE EVENTS OF HYDROXYUREA IN THE TREATMENT OF CHRONIC MYELOGENOUS LEUKEMIA Update October 1992

A.Hochhaus, R.Hehlmann, H.Ansari, J.Hasford, S.Mende and the German CML-Study Group

201 consecutive hydroxyurea (HU)-treated patients (100 male, 101 female, age between 8.1 and 86.9 years) with chronic myelogenous leukemia (CML) were followed prospectively from 1983 to 1992. The mean time of observation under therapy was 772 days. The occurence of adverse events (AE) was routinely checked and documentated at standardized intervals.

38 (19%) of the HU-treated patients showed AE; in only two cases the therapy had to be changed to busulfan. This was due to the occurence of septic temperatures 4 to 6 hours after the intake of HU. After the exclusion of other causes of fever and positive reexposition-trials the fever was designated as a "drug-fever".

The most frequent AE of HU were 19 gastrointestinal AE (gastritis, gastric and duodenal ulcers, nausea, vomiting), 8 neurological disturbances (paraesthesias, headache, tremor, dizziness) and 7 dermatological AE (hyperpigmentation, atrophy of dermis, alopecia).

In comparison, 64 (32%) of 197 busulfan-treated patients of the same trial showed AE, 28 patients suffered from gastrointestinal AE, 24 patients showed neurological AE, 15 patients dermatological AE and 25 patients had an increased activity of liver enzymes.

The size of the cohort, the total observation time of 9 years, the uniformity of the disease and the uniform treatment as a monotherapy renders the present study a sound basis to evaluate the frequency of AE of HU versus busulfan. Comparable studies are not known in the literature.

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CHRONIC MYELOGENOUS LEUKEMIA AS A SECOND NEOPLASIA IN THE COURSE OF CHRONIC LYMPHOCYTIC LEUKEMIA

A.Hochhaus, E.Lengfelder, M.Simon, Ch.Fonatsch, and R.Hehlmann

We report on a 68-year-old man who developed Philadelphia (Ph)-positive chronic myelogenous leukemia (CML) two years after the onset of B-cell chronic lymphocytic leukemia (CLL). When CML was diagnosed, both malignant cell populations were detected in the bone marrow and in the blood. Peripheral leukocytes were fractionated by Ficoll density gradient, and cytogenetic and molecular genetic studies were performed on mononuclear cells and granulocyte-enriched populations. The bone marrow metaphases of the myeloid line were 100% Ph-positive, the metaphases of the lymphoid line after the stimulation of mononuclear cells with phytohemagglutinine and Epstein-Barr-Virus were Ph-negative. Immunocytological investigations showed the B-cell type of the lymphocytes in the peripheral blood. A bcr-rearrangement was observed in DNA from the granulocyte-enriched fraction. The results indicate that the two neoplastic populations originated independently.
The CLL had been treated with chlorambucil for two years. After the diagnosis of CML treatment was changed to busulfan. After acceleration of CML the therapy was changed to hydroxyurea. The following myeloid blast crisis had been treated with low dose cytosine arabinoside s.c. The patient died in blast crisis four years after the diagnosis of CLL and two years after the diagnosis of CML.
Possible causes for the development of a myeloproliferative disorder in a patient with a pre-existing lymphoproliferative disorder will be discussed.

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CLINICAL AND PROGNOSTIC FEATURES OF PHILADELPHIA CHROMOSOME-NEGATIVE CHRONIC MYELOGENOUS LEUKEMIA

A.Hochhaus, R.Hehlmann, H.Ansari, J.Hasford, C.R.Bartram, B.Heinze, D.K.Hossfeld, H.Heimpel, H.-J.Kolb, A.Georgii and the German CML-Study Group

From July 1983 to May 1992 72 (41 male, 31 female) patients with Philadelphia (Ph) chromosome-negative chronic myelogenous leukemia (CML) were recruited for studies designed to compare the effects of hydroxyurea and interferon alpha in mono- and combination therapy, and busulfan on the duration of the chronic phase and on survival.
64 patients are evaluable for treatment response and survival. 24 patients were treated with hydroxyurea, 18 with busulfan, 18 with interferon alpha and 4 with a combination of hydroxyurea and interferon alpha. Ph chromosome-negativity was observed in 11% of a total of 650 evaluable CML-patients. The median survival of Ph-negative CML-patients by now is 1.2 years compared to 4.1 years for Ph-positive patients. Ph-negative CML is characterized by lower blood cell counts (WBC 103,000 vs. 168,000/µl, platelets 370,000 vs. 495,000/µl, hemoglobin 11.3 vs. 12.1 g/dl). Ph-negative patients as a group are older (63.5 vs. 47 years) and more ill (Karnofsky index 80% vs. 90%, initial fatigue and general ill-feeling 78% vs. 65%) than Ph-positive patients.
In 13 (18%) out of the 72 Ph-negative patients a cytogenetic aberration other than the Ph-chromosome was observed, in 7 of these a trisomy 8. 6 of the patients with other cytogenetic aberrations died, 3 of them (all with trisomy 8) died in the first year of observation. 3 out of 14 other (except one) examined patients showed the bcr/abl rearrangement. The Ph-negative, bcr-positive cases have a disease that is probably identical to Ph-positive disease and respond well to treatment. All 3 bcr-positive patients of our series are alive 13 to 33 months after diagnosis. Ph-negativity, bcr-negativity and trisomy 8 may be considered to be a typical constellation for myelodysplastic syndrome.
We confirm that Ph-negative CML may be a separate disease entity on the basis of survival, age maximum, clinical and hematological characteristics. In addition, it appears to be a heterogeneous group, in so far as it includes some patients with an obscured Ph-chromosome, cases which obviously belong to the myelodysplasias and a further group of not yet sufficiently characterized CML-like disease.

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FIRST LINE TREATMENT OF aGVHD FOLLOWING ALLOGENEIC BMT WITH CORTICOSTEROIDS: CLINICAL RESULTS, CYTOKINE MODULATION AND IMPLICATIONS FOR FUTURE STRATEGIES

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The role of donor T-cell activation in aGvHD is documented by clinical and experimental results, but there is increasing evidence for the additional involvement of cytokines like TNFa. As corticosteroids are widely used for first line treatment of aGvHD, we now analyzed retrospectively clinical results as well as modulation of systemic and cellular cytokine release in 42 pts receiving prednisolone as primary treatment of aGvHD. Clinical results: after 30d of treatment 33% of pts showed a PR and 26% a CR. At 90d after BMT, 24% had a CR and only 7% showed a sustained PR. Cytokines: 55% of pts had increased TNF levels in the week prior to GvHD II. Suppression of systemic TNF activity required at least 48-92h of prednisolone treatment. In addition, pts with refractory or relapsing aGvHD had higher TNF levels at d15 and d22 after initiation of prednisolone treatment.
These results suggested that immediate neutralization of TNFa might improve results of first line treatment. Therefore, a pilot study combining monoclonal anti-TNFa with steroids was started resulting in a permanent response at d90 in 4/6 pts treated so far. This approach will now be investigated in a phase II multicenter trial.

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LONG TERM SIDE EFFECTS OF CISPLATIN CONTAINING CHEMOTHERAPY FOR CURATIVE TREATMENT OF NON SEMINOMATOUS GERM CELL TUMORS (NSGCT)

Ch.Hoetzl, Ch.Clemm, H.Lauter, A.Gerl, W.Wilmanns

We evaluated 107 patients receiving cisplatin containing treatment for NSGCT between 1978 and 1988 in a retrospective analysis. The median time of observation was 78 months. Treatment consisted of PVB (68 pts), PEB (17 pts) and ECBC (22 pts). Neurological side effects (paraesthesia, areflexia) were present in 40-50% of pts after the first year of therapy. In the PVB treated group, these side effects were noted in 60-70%. Paraesthesia persisted in 30% of the PVB treated group versus 20% of the PEB/ECBC treated group. Vascular side effects like the Raynaud phenomenon were observed in 34% of the PVB treated group and in 18% of the PEB/ECBC treated group. No significant reversibility was seen. One patient died of a major vascular event (media-infarction) shortly after completion of therapy. Chronic pulmonary symptoms were clinically absent, but in x-ray and CT in 15% of the pts some densification in the basal parts of the lung was demonstrable, irrespective of the regimen. The renal function was impaired in 25% of the pts with an elevation of the serum creatinine. There was no reversibility after 5 years. Chronic side effects without reversibility include the Raynaud phenomenon and the elevation of the serum creatinine as mild manifestations of long term toxicity. In our patient group we did not observe the development of second malignancies after intensive chemotherapy for NSGCT.

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NEARLY FATAL TOXICITIES FOLLOWING ACCIDENTAL SHORT TERM INFUISION OF HIGH-DOSE 5-FLUOROURACIL

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Ardalan et al (JCO 9, 1991, 625-630) recently reported on high-remission rates in patientswith metastatic colorectal carcinoma using a weekly 24 hour continuous infusion of high-dose 5-fluorouracil (2,6 g/m^2) and folinic acid (500 mg/m^2). Stimulated by these results we have treated several patients with the same regimen in our department. In two patients 5-FU accidentally was administered as a 1 hour infusion instead of a 24 hour continuous infusion. Patient 1: a 71 year old patient with metastatic colon carcinoma had tolerated the first two treatments without any side effects. The third infusion was given accidentally within 1 hour instead of 24 hours. 6 days later mucositis and diarrhea grade 2 developed. Within 3 days severity of mucositis and diarrhea increased to grade 4 and the patient was hospitalized. At admission leukopenia grade 4, thrombocytopenia grade 3 and fever grade 2 were also diagnosed. The patient's condition was critical. These clinical conditions necessitated total parenteral nutrition and antibiotic therapy. The patient recovered within 7 days. After recovery 3 further chemotherapy courses as 24 hour infusions were given without any complication. CT-scans performed after the 6th course showed a partiell remission. Patient 2: A 30 year old patient was diagnosed to have peritoneal carcinosis of a adenocarcinoma of unknown orign. 3 high-dose 5-FU cntinuous infusions over 24 hours were well tolerated. The 4th infusion was accidentally given within 1 hour instead of 24 hours. Severity and duration of the side effects were roughly identical to patient 1. After recovery he received 4 additional courses as a 24 hour infusion without any side effect.
Both of these accidental applications of high-dose 5-FU/FA demonstrate the critical importance of time-schedule in high-dose chemotherapy. 5-FU, 2,6g/m^2, given as a 24 hours infusion is well tolerated while the same dose given as an 1 hour infusion may result in life-threatining toxic side effects.

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DEVELOPMENT OF GRAFT-VERSUS-HOST REACTIONS AFTER INJECTION OF PERIPHERAL BLOOD LYMPHOCYTES FROM EBV$^-$ HUMAN DONORS IN SCID MICE

G. Hoffmann-Fezer, C. Gall, B. Kranz, U. Zengerle and S. Thierfelder

Amazingly little graft-versus-host disease has been observed in scid mice injected intraperitoneally with human blood lymphocytes (PBL-hu-scid), which has raised the question whether GVHD in scid mice injected with human PBL takes a mild course or does not occur at all. We followed the development of chimerism in PBL-hu-scid by immunohistochemistry as well as by an immunocytochemical slide method for minute cell numbers which allow us to follow the chimeric status of individual mice by multimarker phenotyping of weekly mouse tail bleeds. About half of the mice showed less than 0.5% chimerism and were free of human cells when studied by immunohistochemistry. The other mice developed a remarkable chimerism and high mortality. Immunohistology revealed proliferation of activated human T-cells in lymph nodes and splenic white pulp. From about 4 weeks post injection human T-cells infiltrated the red pulp of spleen, thymus, and bone marrow, and also the portal triads of liver and intertubular areas of kidney. Comparison with different mouse GVHD models displayed a very similar pattern of donor cell localization with the exception of epithelial infiltration in dermis, tongue and intestines. PBL-hu-scid could thus be identified which furnish a model for analysis and manipulation of human T-cell-induced GVHD.

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BLOOD STEM CELL COLLECTION WITH rhG-CSF IN PATIENTS WITH MALIGNANT LYMPHOMA

S. Hohaus, R. Haas, R. Ehrhardt, M. Pfoersich, U. Birkenstock, and W. Hunstein

Recent studies suggest that by using blood stem cells hematological recovery following high-dose conditioning therapy can be achieved more rapidly than with bone marrow-derived progenitor cells. We treated a group of 7 patients (p) with malignant lymphoma (5 Hodgkin's disease, 2 non-Hodgkin's lymphoma) with following second-line regimens: Dexa-BEAM (5 p), HAM (1 p), and a modified COP-BLAM protocol (1 p). The patients received rhG-CSF (5 μg/kg/d s.c., Neupogen^R) to shorten the phase of neutropenia and to enhance the chemotherapy-induced rebound of hemopoietic progenitor cells. Leukaphereses (LP) were started as soon as a WBC $>1.0 \times 10^9$/nl was achieved. A median of 6 LP (range 6-11) was performed over 8 days (median, range 9-15). The individual peak of CFU-GM and CD34+ cells in the peripheral blood (PB) varied substantially from patient to patient. Compared to baseline a median 194-fold increase of CFU-GM/ml of PB was observed. The expansion of CFU-GM was paralleled by a rise of circulating CD34+ cells. During steady-state hemopoiesis, virtually no CD34+ cells could be detected, whereas at the time of peak increase circulating CD34+ cells ranged from 2×10^3 to 47×10^3/ml of PB. Although a comparable quantity of TNC/kg bw was harvested, we observed a striking patient-to-patient variation with respect to the yield of CFU-GM and CD34+ cells/kg bw. Based on the analysis of 53 leukapheresis products, a strong correlation could be found between the number of hemopoietic progenitors (CFU-GM/BFU-E) and CD34+ cells collected ($R = 0.8$, $p < 0.01$). In contrast, the absolute number of mononuclear cells in PB correlated only weakly with the quantity of harvested progenitor cells. The CD34+ cells coexpressed HLA-DR (> 99%) and CD33 with a varying percentage (22-98%). The pattern of coexpression did not change significantly during the collection period for a given patient. Two types of G-CSF-induced mobilization kinetics emerged: patients characterized by an early and relatively brisk hemopoietic progenitor overshoot and patients with a gradual and rather late increase during leukocyte recovery. Therefore, to avoid unneccessary leukaphereses, sequential CD34 assessment in PB is essential to ensure optimal blood stem cell collection.

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IFOSFAMIDE, MITOXANTRONE AND ETOPOSIDE AS SALVAGE THERAPY IN NON HODGKIN LYMPHOMAS

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Although several therapeutic approaches for conventional salvage therapy in non Hodgkin lymphoma have been made, successful treatment is still lacking. The combination of etoposide, ifosfamide and mitoxantrone showed a high effectiveness in relapsed and refractory NHL.
During 1986 and 1992, 56 patients (36 males, 20 females) with a median age of 66 years (range 18-89 years) were treated with a combination of etoposide (100 mg total dosage), ifosfamide (1g total dosage) and mitoxantrone (3mg/m^2) given on three consecutive days. Mesna was given as uroprotector.

Stages according to the Ann Arbor classification were I/7, II/3, III/6 and IV/40 patients.
33 patients suffered from high grade, 23 from intermediate grade non Hodgkin lymphoma.
Toxicity according to the WHO recommendation was as follows: Anemia grade I was observed in 10 patients. Leukopenia grade I/2 patients, grade II/1 patient and grade IV/4 patients.
Thrombocytopenia was not observed.
Overall response was 32% (9 CR and 9 PR). High grade NHL showed a better response rate (18/33 patients) compared to the intermediate grade NHL's (7/23 patients). Bulky disease was a significant adverse prognostic factor, response was observed in 3/15 cases. It seems noteworthy that 15/31 cases refractory to previous treatment responded.
The combination of ifosfamide, mitoxantrone and etoposide is active in pretreated refractory and relapsed patients with non Hodgkin's lymphoma and has tolerable toxicity.

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ELDESINE PLUS BLEOMYCINE AS SALVAGE TREATMENT IN NON HODGKIN LYMPHOMAS.

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Though great achievement in the treatment of NHL has been made, salvage therapy is still necessary for patients having a refractory or relapsed course of disease. Most published schedules have considerable toxcity and poor long term outcome. So we deviced a regimen of comparable low toxicity, consisting of bleomycine (15 mg) and eldesine (5 mg) combined with irradiation (16 pts.) or cytostatics non cross resistant to firstline therapy (asparaginase and methotrexat, 56 pts.). Most patients were pretreated with regimens including anthracyclines.
Bleomycine and eldesine were given as a 2 - 24 hours continuous infusion. Influence of the different infusion durations will be discussed. Prophylactic antipyretics were given in all patients.
The medical records of 100 patients, 55 male and 45 female with a median age of 59 years (range 21-88) treated at our department between 1.1.1985 and 1.5.1992 were analysed retrospectively. Ann Arbor stage was I/5, II/8, III/17 and IV/70 patients. 6 patients suffered from low grade, 48 from intermediate grade and 46 from high grade non Hodgkin lymphomas.
Overall response was 38% (13% CR, 25% PR). There was no difference in the response between the histological subtypes.
Toxicity measured according to the WHO-recommendation was as follows: Leukopenia grade I/5 patients, grade II/8 patients and grade IV/7 patients.
Thrombocytopenia grade I/2 patients, grade II/4 patients and grade III/1 patient. Anemia grade I was seen in one patient, grade II/3 patients, grade III/1 patient and grade IV/1 patient. In contrast to published data short time infusion of bleomycine/eldesine was superior and therefore this treatment can be easily applied in ambulatory patients.

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MULTIPLE MYELOMA: GD-DTPA ENHANCED MRI

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Plain radiographs are currently used to screen for osteolytic changes in patients with multiple myeloma, as bone scintigraphy has limited sensitivity. To determine a possible role of MR imaging, 34 patients with multiple myeloma were studied prospectively. Opposed-phase gradient-echo imaging (GE, TR 400 msec, TE 22 msec, 5 mm slice thickness, 4 NEX, coronal or saggital orientation) were acquired before and after 0.1 mmol Gadolinium-DTPA / kg b.w. using a body or spine coil. Plain films and CT scans of selected cases were available in all patients. Evaluation was done by two blinded observers.
Body coil screening detected 35 lesions in 19 patients; in 3 patients suffering from pain, MRI demonstrated large bone marrow lesions which were not detected on plain films. In an additional 15 patients where plain films of the spine demonstrated diffuse demineralization only, MRI detected diffuse or focal bone marrow involvement of the axial skeleton in 14. CT scans confirmed the presence of osteolytic lesions in areas where MRI had shown cellular marrow infiltrates. In one patient open biopsy of a lesion demonstrated by MRI disclosed replacement of normal marrow by an infiltrate consisting of plasma cells.
In conclusion, MRI can directly demonstrate focal or diffuse infiltration of bone marrow by multiple myeloma even in those cases where lytic destruction of bone is to discrete to be detected by radiography.

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EFFECT OF INTERFERON $ß_{ser}$ ON INTERLEUKIN 6 PRODUCTION AND ON TRANSCRIPTION OF THE NUCLEAR FACTOR IRF-1 IN HUMAN MYELOMA CELL LINES

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Recent data indicate that the use of Interferon (IFN) in the therapy of patients with multiple myeloma is of benefit. The precise mechanism of action of IFN's is still unknown. Possible interactions with interleukins, in multiple myeloma especially with interleukin 6 (IL 6), are discussed. We examined 4 human myeloma lines (OCI-My1, 3, 4, 5), which are heterogeneous with respect to their requirement and production of IL 6.

Cell line:	OCI-My1	My3	My4	My5
IL 6 dependent growth	–	–	+	–
mRNA for IL 6	+	+	–	–
IL 6 production	–	+	–	–

Cells from each line were incubated with different concentrations of $IFNß_{ser}$ in liquid suspension cultures. Supernatants were collected, RNA was extracted, and Northern blot analyses were performed with probes for IL 6, interferon regulatory factor 1 (IRF-1) and IFNß. IL 6 concentrations were determined in supernatants by ELISA. Only OCI-My3 showed an upregulation of IL 6 mRNA levels after incubation with 10^4 IU/ml $IFNß_{ser}$. This upregulation of IL 6 mRNA corresponded to a dose-dependent elevation of IL 6 protein production with an almost 4-fold increase above the negative control after incubation with 10^4 IU/ml $IFNß_{ser}$. Supernatants from the other examined cell lines did not contain detectable amounts of IL 6 protein. Untreated cells of all 4 lines displayed IRF-1 mRNA. Incubation with $IFNß_{ser}$ led to a dose-dependent increase of IRF-1 mRNA in each line. Basal expression levels of IFNß mRNA, examined in OCI-My1 and My3, remained unchanged during $IFNß_{ser}$ incubation.
We conclude that $IFNß_{ser}$ incubation increased IL 6 production only in the line that is already able to produce IL 6 constitutively. However, IRF-1 mRNA was upregulated in all 4 lines whereas IFNß mRNA levels remained constant in 2 examined lines. The latter finding makes an autocrine loop in IFN regulation rather unlikely.

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ROLE OF FcγRII IN FcγRIIIb-DEFICIENT NEUTROPHILS FROM PATIENTS WITH PAROXYSMAL NOCTURNAL HEMOGLOBINURIA

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The pathogenesis of paroxysmal nocturnal hemoglobulinemia (PNH) can be explained by the deficiency in expression of glycosyl-phosphatidylinositol (GPI) anchored proteins on the cell surface. The lack of the GPI-linked complement regulatory proteins lead to the typical symptoms of hemolysis and thrombosis. But functional consequences of the defect in GPI-linked immune control proteins, important for cell function and activation, are not clear. Therefore, we investigated the deficiency of Fcγ receptors (FcγR) on PNH neutrophils. Normal neutrophils express constitutively two low-affinity FcγR, FcγRII (CDw32) with moderate and FcγRIIIb (CD16) with high density. FcγRII is a transmembranous protein, FcγRIIIb is attached to the lipid bilayer through a GPI-anchor. Both can induce the release of calcium from intracellular stores and the production of hydrogen peroxide after specific cross-linking with monoclonal antibodies. Using an IgG,κ cryoglobulin complex it previously could be demonstrated that the GPI-linked FcγRIIIb is the predominant receptor for physiologic ligands. On PNH neutrophils the density of FcγRIIIb is much lower than of FcγRII. Therefore, stimulation via specific crosslinking of FcγRIIIb induced only minimal increase of cytoplasmic calcium and production of hydrogen peroxide. But cryoglobulin complex stimulation was diminished by 25% only, and this activation could be demonstrated to be FcγRII dependent for H_2O_2 production and calcium release. Therefore, a shift of FcγRIIIb functions to FcγRII in PNH neutrophils has to be postulated. Whether the defect in production of GPI-molecules leads to different intracellular signal transduction needs to be further investigated.
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ANTI-MHC-CLASS II ANTIBODY TREATMENT ALTERS GROWTH FACTOR mRNA EXPRESSION IN CANINE MARROW STROMAL CELLS

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Dogs given 920 cGy of total body irradiation (TBI) and autologous marrow infusion uniformly show hemopoietic reconstitution. However, if treated with an anti-MHC-class II monoclonal antibody (MAB) postgrafting, they develop marrow aplasia. Experimental data are consistent with a growth factor deficiency. For example, hemopoietic reconstitution can be partially restored by a short course of recombinant canine stem cell factor (SCF = c-kit ligand). Serum levels also indicate reduced production of G-CSF. Preliminary data suggest, furthermore, an altered interaction of hemopoietic precursors and stromal cells. Therefore, we established stromal cell lines from long term bone marrow cultures as an in vitro system to determine the effects of irradiation and MAB administration on the expression of mRNA of various growth factors and MHC molecules. Cytoplasmic RNA was isolated from canine stromal cells and from various cell lines at different time intervals following treatment with anti-MHC-class II MAB or irradiation. The RNA was hybridized with canine cDNA probes where available or with cross-reactive human cDNA probes, using slot-blot technique and Northern blot analysis. Within hours of anti-MHC-class II treatment or irradiation there was a decline of TGF-ß mRNA. There was also a slight decrease in mRNA expression for G-CSF and SCF after MAB application in parallel with an increase in message for MHC-class II. GM-CSF mRNA declined initially but showed a prominent rise after 48 hours. In conclusion, anti-MHC-class II MAB treatment and irradiation affect mRNA transcription in stromal cells in a complex way. The emerging pattern suggests that growth factor expression after TBI may indeed be altered by MAB administration and may contribute to the development of aplasia in our model.

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THERMOCHEMOTHERAPY FOR HIGH-RISK SARCOMAS.

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From July 86 to August 91, 88 patients with deep-seated, advanced sarcomas were treated at the University of Munich in two consecutive protocols consisting of regional hyperthermia (RHT) combined with systemic chemotherapy. Sixty-five patients (43 soft tissue-sa., 12 Ewing's-sa., 7 chondro-sa. and 3 osteo-sa.) received ifosfamide (1,5 g/m2, day 1-5) and etoposide (100mg/m2, day 1,3,5) with RHT given at day 1 and 5 in repeated cycles every four weeks. RHT was produced by an electromagnetic deep regional heating device (BSD-System). Detailed thermal mapping by invasive thermometry was performed. In 61 patients evaluable for tumor control (62 % pretreated with ifosfamide containing drug regimens) the objective response rate including 9 CR, 4 PR and eight patients with favourable histologie response (8 FHR) was 34 % and confirmed the results of an previous interim-report on 40 patients (Issels et al. J. Clin. Oncol Vol 8, 1990). The time-averaged temperatures of all RHT treatments calulated for 20 % (T20), 50 % (T50) or 90 % (T90) of measured tumor sites differed significantly between responders (CR + PR + FHR, 21 patients) and non-responders (PD, 18 patients), respectively.

To test further the potential for RHT combined with chemotherapy we have started in Nov. 90 a pilot study (RHT-91) for patients with-high risk non-metastatic sarcomas (tumor diameter $\geq$ 8 cm and/or non-resectabel without mutilation or local recurrence after initial treatment). The patients (20 soft tissue-sa., 3 chondro-sa.) were scheduled to receive ifosfamide (1,25 g/m2, day 1-4), adriamycin (50 mg/m2, day 1), and etoposide (125 mg/m2, day 1 + 4) with RHT given at day 1 and 4 in repeated cycles every three weeks. After preoperative thermochemotherapy (2-5 cycles) conservative surgical resection of the tumor could be performed in 10 patients (cut off-date: August 30 th). Six of these patients showed an objective response and before (2 PR) or at the time of surgery (4 FHR). In the other four patients no tumor regression has been observed by CT/MRI or pathohistological evaluation. All 23 patients on study are alive but three show tumor progression during the short observation time.

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Transfusional iron overload in patients with myelodysplastic syndromes

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The main causes of death in patients with myelodysplastic syndromes (MDS) are infections and hemorrhages due to progressive bone marrow failure. During the course of disease multiple red cell transfusions may lead to relevant iron overload. Reliable data on this complication are largely lacking.

Patients and results: This retrospective study covers a period from 1975 through 1991. Patients were followed until death or through December 1991. Among a total of more than 500 cases of MDS, 56 patients could be identified who had received more than 50 blood transfusions during the course of disease (RA n=16, RARS n=19, RAEB n=7, RAEB/T n=7, CMML n=7; mean age 66,5 years, mean survival time 41 months, mean number of blood units 91). In 32 of them (RA n=8, RARS n=15, RAEB n=4, RAEB/T n=3, CMML n=2; mean age at diagnosis 66,8 years), followed up between 12 and 159 months (mean survival time 48,5 months) multiple blood transfusions (mean number 96) caused secondary haemochromatosis. The heart was the most frequently affected organ (n=29), while liver damage was of minor importance (n=17). Nine patients developed diabetes mellitus. Four patients complained about severe hypohidrosis. Polytransfused patients with RARS were particularly affected by the development of secondary haemochromatosis. There were 26 deaths among the 32 haemochromatotic patients. At least 15 patients died from refractory heart failure, whereas none died from hepatic cirrhosis.

Conclusions: Secondary haemochromatosis is relatively common in polytransfused MDS patients. Among these patients, FAB-subtypes with favourable prognostic features are particularly frequent. Therefore in cases with high transfusion requirement and otherwise good prognosis (RA and RARS) iron chelation therapy should be commenced. The development of a new oral iron chelator (L1 or 1,2-dimethyl-3-hydroxypyrid-4-one) offers a promising approach to prevent severe complications from iron overload without major compliance problems.

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INTERFERON-α (IFN-α) INDUCED TRANSCRIPTION OF TRANSFORMING GROWTH FACTOR-$ß_1$ (TGF-ß) IS IMPAIRED BY INTERLEUKIN-2 (IL-2) IN VIVO.

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Interleukin 2 (Il-2) in combination with interferon-α has been shown to have significant antitumoral effects in some patients with advanced renal cell cancer or malignant melanoma. As it seems possible that this therapy is modulated by secondarily induced cytokines, we investigated transcription of TGF-ß in patients undergoing immunotherapy combining daily alternating application of IFN-α and IL-2 for 14 days. When gene transcription of TGF-ß was analysed by Northern blotting an induction of TGF-ß gene transcription in peripheral mononuclear cells (PMN) was seen 12h after subcutaneous IFN-α application. The following intravenous Il-2 administration diminished the amount of TGF-ß specific mRNA. In contrast to first dose effect subsequent reapplication of IFN-α did not enhance TGF-ß gene transcription. Only at the end of treatment course at d15 and d22 to d28 TGF-ß transcription reappeared. Our results indicate a complex regulatory effect on secundarily induced cytokines as TGF-ß by immunotherapeutic approaches. As TGF-$ß_1$ is supposed to inhibit LAK cell function, the diminuition IFN-α induced TGF-$ß_1$ mRNA by Il-2 may represent a positive immunregulatory effect on cytotoxic cells. Furthermore a modulating effect on proliferation of neoplastic tissues during combined immunotherapy may be considered.

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$CD3^+CD4^+$ T-CELLS AS EFFECTOR CELLS OF AUTOLOGOUS BLAST SPECIFIC CYTOTOXICITY IN ACUTE MYELOCYTIC LEUKEMIA

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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. Sixtythree resulting cell lines and clones were phenotypically characterized by mAbs (CD2, CD3, CD4, CD8, DR, CD56, TCRα/ß, TCRγ/δ). All cells stained positively for CD2, CD3 and DR. The vast majority of cells stained positiv for CD4 (56/63) and a few for CD8 (5/63). In one patient 3 clones with TCRγ/δ 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 TCRγ/δ+ clones from 2 patients were chosen for functional studies in regard to cytokine release and cytotoxic activity. No significant lysis of K562 (NK) nor Daudi cells (unspecific LAK) could be detected. However, all clones tested exerted a cytotoxic effect on autologous blast cells. The data indicate that the mechanism underlying immunotherapeutic approaches in AML treatment with Il-2 may be the in vivo activation of blast specific cytotoxic lymphocytes.

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SUCCESFUL TREATMENT OF SEVERE APLASTIC ANAEMIA WITH ANTIHYMOCYTE GLOBULIN /ATG/

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The case of a 18-years old patient with severe aplastic anaemia is reported. There was no response to high dose corticosteroid /methylprednisolone/ and to oxymetholone. Bone marrow transplantation could not be performed since he had no histocompatible donor. Antithymocyte globulin treatment had been, wich resulted in a complete clinical and nearly complete haematological remission.

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HUMAN LEUKAEMIA CELL LINES EXPRESS DIFFERENT INSULIN-LIKE GROWTH FACTOR BINDING PROTEINS

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Conditioned media from eight leukaemia cell lines (2 CML, 4 AML, 1 ALL, 1 Lymphoma) were examined for the presence of insulin-like growth factor binding proteins (IGFBP). Cells growing under serum-free conditions released binding proteins with m.w. of 26-38.5 kDa when analyzed by a ligand blotting method under non-reducing conditions. In order to identify the IGFBPs from these cell lines the RNA was analyzed by Northern blot analysis with the probes for six different IGFBPs (IGFBP-1, -2, -3, -4, -5, -6). Hybridization signals could be detected with the probes encoding for IGFBP-2, IGFBP-4 and IGFBP-5 to varying degrees by all cell lines. These findings document the ability of leukaemia cells to secrete a heterogenous mix of low molecular weight, high affinity IGF-selective binding proteins, which modulate IGF-peptide and receptor interaction. Preliminary results show a marked growth inhibition by IGFBP-1 and -2 suggesting that IGFBPs are important in controlling growth of leukaemia cells.

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PLASMIN (PL) MODIFIES PLATELET RESPONSIVENESS TO THROMBIN AND PROSTACYCLIN (PGI2)

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The question as to whether PL modulates platelet function has been addressed in several in-vitro studies with conflicting results. In our hands, at low temperatures (20°C) PL caused time- and dose-dependent platelet activation (EC_{50} 0.04 CU/ml). PL-induced platelet activation could be inhibited by SQ 29548 and iloprost but not by the thrombin inhibitor MCI 9038. After incubation of washed platelets with PL at 20°C, platelet responsiveness to thrombin was considerably impaired. Flow cytometry studies using Mab AN51 and P2 excluded that this might simply result from proteolytic degradation of platelet glycoproteins (GP IIb/IIIa, Ib). In addition, binding studies with ^{3}H-SQ 29548 demonstrated that neither PL, t-PA or streptokinase interfere with the TXA_2/endoperoxide-mediated signal amplification at the receptor level. Thus, PL-mediated activation of protein kinase C and the subsequent modification of postreceptor responses (PLC-activity) might provide a more likely explanation. This mechanism depends largely on platelet activation that particularly occurs at low temperatures and is no longer demonstrable when incubation is carried out at 37°C. In further experiments PL was shown to pontentiate the antiaggregatory effectiveness of PGI_2 without any modification of platelet PGI_2-receptor surface expression. The finding that plasmin causes an increase of basal cyclic AMP in ^{3}H-adenine prelabelled platelets points towards a modification of postreceptor responses (e.g. G_i/cyclase-interaction) and deserves further investigation. In the light of these observations we conclude that PL under "physiological conditions" (37°C, 3mM Ca^{2+}) does not modify the platelet responsiveness to thrombin. However, PL acts synergistically with PGI_2 to inhibit platelet activation. Thus, PL may efficiently operate together with EDRF and PGI_2 to localize platelet activation in vivo.

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FISH OIL PREPARATIONS CONTAINING DIFFERENT DOSES OF EPA AND DHA MODIFY PLATELET RESPONSIVENESS TO U 46619

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Changes in the receptor microenvironment and membrane microviscosity regulate the accessability of ligands for its corresponding binding sites. In previous experiments (Jaschonek et al., Thromb Res 1989/1991) we demonstrated that platelet-PGI2 receptors behave as syndromic proteins: an increase in membrane microviscosity induced by cholesterol decreased whereas fluidizing fatty acids increased platelet surface PGI2-receptor expression. There is some evidence from in-vitro studies that TXA2/PGH2-receptors are regulated in a opposite fashion. In this study, we investigated the effects of supplementation with either 4.5 g eicosapentaenoic (EPA) and 3.35 g docosahexaenoic (DHA) acid (group I, n=6; EPA/DHA = 1.33) or 3.5 g EPA and 6.4 g DHA (group II, n=6; EPA/DHA=0.544) on platelet thromboxane (TXA2) synthesis and platelet responsiveness to the stable endoperoxide-analogue U 46619. Dose-response-curves (DRC) of U 46619-induced aggregation were analyzed by computerized nonlinear curve-fitting. The synthesis of TXB_2, TXB_3 and PGE_2 by platelets was measured after incubation with thrombin (5 U/ml) by HPLC. In group I, the dose of U 46619 required for half-maximal platelet aggregation (K) remained unchanged, whereas the Hill-coefficient decreased from 6.2 to 3.3 ($p<0.02$). In group II, characterized by a high intake of DHA, a considerable increase of K from 0.3 to 1.4 μM was found ($p<0.02$). These results suggest different effects of EPA and DHA on the platelet thromboxane/endoperoxide-amplifying system. After the EPA-rich preparation, the reduced cooperative effect found presumably is simply the result of the reduced endogenous thromboxane synthesis. However, the considerable shift of the DRC in group II suggests an effect of DHA on the presentation of endoperoxide receptor and/or postreceptoral events.

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CYTOKINES AND SOLUBLE FORMS OF ACTIVATION ANTIGENS IN THE SERUM OF PATIENTS WITH HODGKIN'S DISEASE

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In a search for specific serum markers with prognostic impact, we evaluated the clinical significance of several cytokines (IL-1ß, IL-2, IL-3, IL-4, IL-6, IL-7, IL-8, G-CSF, GM-CSF, TNF-α) and soluble forms of membrane-derived antigens (sCD4, sCD8, sCD23, sCD25, sCD30) in the serum of patients with untreated Hodgkin's lymphoma (HD). Elevations of three groups of serum factors were observed: 1st, elevations of the hematopoietic cytokines GM-CSF (detected in 39%), IL-6 (57%) and IL-3 (13%), which occurred simultaneously in the majority of the cases; 2nd, simultaneous elevations of the inflammatory cytokines TNF-α and IL-1ß (detected in 7%); and 3rd, elevations of membrane-derived activation antigens sCD8, sCD25, and sCD30. While the cytokine levels were not correlated with other obvious parameters, the membrane-derived activation antigens sCD8, sCD25 and sCD30 were associated with a poor prognosis. Only sCD30 correlated with disease activity and holds promise for the follow-up of patients in remission. Further investigations of these parameters at the cellular level might help to elucidate the enigmatic biology of HD.

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DIFFERENTIAL EXPRESSION OF RETINOIC ACID RECEPTORS IN HUMAN LUNG CANCER CELL LINES

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Retinoic acids have an effective chemopreventive potential in a subgroup of lung cancers. They act via nuclear receptors which belong to the family of steroid hormone receptors. To select lung cancer subtypes that may be responsive to the treatment with retinoic acid human lung cancer cell lines were studied immuncytologically with specific polyleonal antipeptide antibodies directed against the retinoic acid receptors alpha, beta and gamma for presence of retinoic acid receptors. Specificity was demonstrated by inhibition of immunoreaction after presaturation with the corresponding peptide as well as by immunoblotting. Immunoreaction was restricted to cell nuclei. Small cell lung cancer cell lines (SCLC)(4/4) and non - small cell lung cancer cell lines (NSCLC)(7/8) reacted with the anti - alpha receptor antibody. However in 2 of 4 SCLC lines and 5 of 8 NSCLC lines the beta receptor could not or only faintly in less than 50% of cells be demonstrated. Among the NSCLC lines those derived from adenocarcinoma(2) exhibited a strong reactivity, squamous cell carcinoma lines (3) displayed no or faint reactivity. Reaction pattern with the anti-gamma receptor antibody was faint in SCLC (2/3) and NSCLC(4/8). Our data suggest that in some lung tumors predominantly in squamous cell carcinomas the beta receptor is not expressed. Studies are in progress to determine receptor expression after preincubaiton of cells with retinoic acid.

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PREVENTION OF INTERSTITIAL PNEUMONIA CAUSED BY CYTOMEGALOVIRUS AFTER ALLOGENEIC BMT FOR HEMATOLOGICAL MALIGNANCY

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Between IX/83 and III/92, 96 consecutive allogeneic bmt recipients transplanted for HM (AML n=37, CML n=32, ALL n=18, Hodgkin`s disease n=1, Non-Hodgkin lymphoma n=3, myelodisplasia n=5) were studied. During conditioning therapy, all pts received TBI: single dose TBI (1000 rad, cobalt-67-source, lung shielding at 800 rad) plus chemotherapy (CT) n=71, fractionated TBI (1200 rad, lung shielding at 1000 rad, Mevatron) plus CT n=25. GVHD prophylaxis consisted of Methtrexate (Mtx) n=38, Mtx plus CsA n=51 and CsA plus prednisone n=7. For prevention of CMV-IP, only CMV-negative platelet concentrates were administered; red blood cells were filtered; CMV hyperimmunoglobuline (Cytotect, 1ml/kg from day -1 to 100 in 2 week intervals) was given; and the dose rate used in single dose TBI was <5 rad/min. Twenty two of the 96 pts died before day 100 from other cause than IP and were excluded from further analysis. Of the 74 remaining pts, 53 (72%) are currently alive at a median of 35 months (5-105). Relapse occured in 14/74 pts (19%). CMV-IP was seen in 1/74 (1%), occuring on day 274 while the pt received immunosuppressive treatment for extensive chronic GVHD; 1 pt died from idiopathic IP on day 61. CMV-disease was see in 20/74 pts. (27%).Acute GVHD grade II-IV was seen in 27/74 pts (36%). These data show that our combined modality approach was effective in prevention of CMV-IP without increasing the risk of relapse.

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TRANSPLANTATION OF HODGKIN'S LYMPHOMA INTO SEVERE COMBINED IMMUNODEFICIENT MICE.

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The scarcity of tumor cells has hampered the genotypic and karyotypic characterization of Hodgkin's lymphoma. Xenotransplant tumor models allow to study the nature of malignant cells and to identify conditions necessary for their malignant proliferation. We reasoned that the SCID mouse might provide an appropriate microenvironment for the growth of primary Hodgkin (HD)- and Reed-Sternberg (RS)-cells.
Fifteen biopsy specimen of Hodgkin's lymphoma from 13 patients were transplanted into the subrenal capsule or into the liver of untreated SCID mice. In two cases human tumors developed in the mice within a latency period of 3-5 months. Tumor growth was observed at the primary transplantation site as well as in liver, lymph nodes, thymus and spleen. Three histological types of EBV positive human lymphomas were found in the mice: 1. immunoblastic lymphoma, 2. anaplastic large cell (ALC) lymphoma, 3. Hodgkin-like lesions. Minor cytogenetic aberrations were found both in the grown tumor specimen and in their EBV-positive B cell lines recultured in vitro. The Reed-Sternberg cells were free of EBV in one of the two original biopsy specimen leading to EBV positive tumor growth in the mice. Therefore, cytogenetically abnormal EBV positive cells in the surrounding of the Hodgkin cells may be those that proliferate into ALC lymphoma lesions in SCID mice. Such cells might possibly be precursors of the malignant cell clone in Hodgkin's disease.

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INTERFERON-ALPHA ENHANCES THE NEU-ONCOGENE PRODUCT SECRETED BY THE BREAST CARCINOMA CELL LINE SK-BR-3

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The neu-oncogene (HER-2, c-erb-B2) codes for a transmembrane tyrosine kinase, commonly referred to as p185, sharing extensive homology to the epidermal growth factor receptor. The extracellular domain of p185 is released from the surface of the human breast carcinoma cell line SK-BR-3 (J Biol Chemistry: 266, 1716, 1991). Using a double monoclonal antibody capture enzyme-linked immunosorbent assay (ELISA) for p185 (DIANOVA, Hamburg), we measured the extracellular domain of p185 secreted by SK-BR-3 cells into conditioned medium after three and five days of incubation with interferon-alpha-2b (ESSEX, München). Comparing 10 and 100 Units interferon-alpha-2b/ml medium (RPMI with 10% FCS; cell density $1.25 \times 10^4/cm^2$; replenishment of interferon-alpha-2b after 48 hours; collecting conditioned medium every 48 hours for p185 ELISA) we observed a mean growth inhibition of 16% after three and five days, respectively. However, a mean increase of 12% after three and 19% after five days in secretion of the extracellular domain of p185 occurred. Thus, the relative increase per cell in secretion of the extracellular domain of p185 was 31% after five days. We conclude, that similar to the upregulation of the epidermal growth factor receptor by interferon-alpha (Cancer Research: 51, 1294, 1991) an increase in secretion of the extracellular domain of p185 occurred. The mechanisms altering the growth factor receptor expression need yet to be elucidated.

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Interdisciplinary treatment of metastatic melanoma: Surgical resection of residual disease following IL-2 based immunotherapy - a curative approach?

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Surgery of advanced metastatic melanoma is of limited value and usually not recommended. Immunotherapy using high dose IL-2 is effective in a substantial proportion of patients, however, the duration of responses is limited, and benefits in survival are not yet proven. Maintainance therapy with IL-2 has not been established and would hardly be tolerable because of toxicity.
This evaluation was done to determine the value of resection of residual tumor lesions following succsessful immunotherapy. 54 patients with progressive metastatic melanoma have been enrolled in immunotherapy trials including IFN-α and high dose IL-2 in our hospital since 1987. 21 patients showed antitumor response (3 CR, 11 PR, 8 SD >3 months). In patients responding to immunotherapy, residual lesions were resected, whenever technically possible and patients agreed to surgery (8 patients).
12 of the 13 patients without surgery relapsed, the median time to progression was 5 months (range 2-14), most relapses occured locally, 7 patients died so far. 8 patients underwent surgery, and histology revealed vital tumor cells in all resected specimens. Two of these 8 patients relapsed (one locally, 5 months after surgery, one CNS, 9 months after surgery), six are still free of recurrence (6+,7+,9+,10+,20+,23+ months after surgery). All 8 patients are still alive.
Surgical reevaluation and resection of residual lesions should be considered in patients with partial response or clinical complete remission after immunotherapy, and in selected cases also with stable disease. This approach offers the chance for extended disease free survival, and may be curative in certain patients.

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Regional immunotherapy: Perfusion of liver metastases with LAK cells

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A regional approach of adoptive immunotherapy with interleukin-2 and lymphokine activated killer cells for the treatment of liver metastases is reported. The treatment consists of continuous infusion of interleukin-2 i.v. or into the splenic artery, and transfer of ex vivo generated lymphokine activated killer cells into the portal vein or the hepatic artery.15 patients with malignant melanoma, 2 with renal cell carcinoma, and 1 with thyroid carcinoma have been treated. All had progressive liver metastases. In 9 patients with liver metastases of cutaneous melanoma, 2 CR (24 and 13+ months), 2 SD (10 and 9+ months), and 2 PD were observed, three are too early to evaluate. No responses were observed in 6 patients with liver metastases of ocular melanoma, suggesting an immunologic difference between these two melanoma subtypes. 1 PR (6 months) and 1 SD (10 months) were achieved in 2 patients with renal cell carcinoma, and 1 SD (6+ months) in the patient with thyroid carcinoma. Trafficking studies using indium-oxine labelled cells revealed that >80% of the LAK cells remained in the liver after regional adoptive transfer.
Evidence for the crucial role of regional cell transfer is provided by the observation in a patient with an anatomic variation of hepatic blood supply in whom we achieved complete and durable tumor regression. In this case anti-tumor responses were only observed in anatomic areas of the liver which were perfused with LAK cells.

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THROMBOEMBOLIC RISK IN CANCER AND CHEMOTHERAPY

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More than a century ago Trousseau recognized the association of thromboembolic disorders and cancer. Today the mechanisms leading to thromboembolic complications in cancer patients are known to be multifactorial, but are not yet completely understood. 5 - 15 % of cancer patients suffer from thromboembolic events and in more than 90 % of cancer patients altered coagulation parameters were measured: predominantly elevated levels of fibrinogen, fibrinopeptide A and TAT-complexes. None of these parameters however is predictive by itself or pathognomonic for thromboembolic events. The individual thrombotic risk for cancer patients depends upon:

1. The tumor type: lung and pancreatic carcinoma and myeloproliferative disorders are known to have a high thrombotic risk.
2. Local complications induced by the tumor itself such as compression of vessel walls or penetration and destruction of vessel walls.
3. An imbalance of the coagulation system due to increasing procoagulatory or decreasing anticoagulatory active coagulation factors and/or an impairment of the fibrinolytic system can induce a hypercoagulable state leading to thromboembolic complications.
4. Local venous irritation may occur induced by indwelling catheters or chemotherapy: a variety of chemotherapeutic drugs are known to cause thrombotic complications. Furthermore, antioestrogens, estrogens and corticosteroids enhance the risk to suffer from thromboembolic complications.
5. Surgery, immobilization and hyperviscosity further enhance the risk to suffer from thromboembolic complications.

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ENHANCED PRODUCTION OF IL-6 AFTER EXPERIMENTAL INFECTION OF MICE WITH SCHISTOSOMA MANSONI

R. M. Khalil[1,2], L. Hültner[1], R. Mailhammer[1], A. Luz[3], M.-A.. Hasslinger[4], S. Omran[2] and P. Dörmer[1]

Interleukin 6 (IL-6) is a cytokine playing a major role in the regulation of the haemopoietic and immune system. Moreover, the production of IL-6 in response to viruses and bacteria indicates a central role in host defense mechanisms. Manoukian et al (1984) found that serum acute phase protein concentrations increased in murine schistosomiasis. These proteins are known to have important functions, namely confining the inflammation, limiting the autolytic processes and repairing damaged tissues. Therefore we expected IL-6 to play a role during the course of schistosomiasis, particularly in regulating the hepatic part of acute phase responses. BALB/c mice were subcutaneously infected with a dose of 50 S.mansoni cercariae per mouse to study the kinetics of IL-6 production. At different time points from 1 to 28 weeks following infection 5 to 10 mice per group were examined for IL-6 levels in sera and pokeweed mitogen-stimulated spleen cell-conditioned media (SCM) using an IL-6 specific indicator cell line (7TD1) in a colorimetric bioassay (MTT test). IL-6 was specified using the neutralizing monoclonal anti-mouse IL-6 antibody 6B4. In addition the livers of these animals were analyzed histopathologically. We found that IL-6 levels in SCM samples were significantly increased starting at week 6, reaching a peak at week 10 and remaining significantly elevated till week 20 after infection. In infected mice serum IL-6 levels followed similar kinetics with the difference that increased concentrations were first observed at week 8, reached a peak at week 12 and were still significantly higher than those of uninfected controls till the 24th week after infection. IL-6 peak values of both SCM and serum samples were increased by a factor of 8 as compared to uninfected controls. The expression of IL-6 mRNA in spleens and livers correlated with the serum IL-6 levels, whereas the number of liver granulomas formed in the early stage of the disease correlated with the IL-6 levels in the corresponding SCM samples. These results strongly suggest a biological role of IL-6 in S. mansoni infection, which should be elucidated in future experiments using *in vivo* neutralizing anti-IL-6 antibodies and/or antibodies blocking IL-6 receptors.

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INVESTIGATIONS ON THE GENETIC BASIS OF FACTOR XII DEFICIENCY

B. Kempter, S. Rüth and P. Lohse

Factor XII deficiency exists in two main forms: patients with immunological crossreacting material CRM^+ and patients without CRM^-. For one case of CRM^+ an amino acid substitution (Cys^{517} -> Ser) has been described. We are currently investigating two families of Hagemann CRM^- trait with the typical hemostaseological pattern: one member with virtually no factor XII activity and antigen, whose sisters and children have about 50% of activity and antigen. To elucidate the genetic defect, primers were synthesized for both exon-intron borders for each of the 14 exons. Polymerase chain reaction (PCR) was performed for each single exon and for any two exons in a row with the intron in between (double-exon-screening). We were able to demonstrate that the homozygous patients do not have major rearrangements, deletions or insertions of the factor XII gene. To localize the molecular defect, the complete gene was sequenced by cloning the PCR products into pBS. In one patient, a single base deletion in exon 12, leading to a nonsense protein, was detected. The propositus was shown to be heterozygous for this defect by a deletion specific restriction fragment length polymorphism of the PCR product.

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ARTERIAL THROMBOPHILIA

J. Kienast

The term "thrombophilia" applies to familial or acquired disorders of the haemostatic system which are likely to predispose to thrombosis. In the narrow sense of the definition, there are only few thrombophilic disorders that are particularly associated with an increased risk of arterial thrombosis. These include rare congenital abnormalities such as homocysteinaemia and possibly dysfibrinogenaemia or acquired disorders such as antiphospholipid antibody syndromes or thrombocythaemia. In classical thrombophilic states, e.g. congenital deficiencies of natural anticoagulants, arterial events account for less than 5 % of thromboembolic episodes. However, numerous epidemiological studies have prospectively demonstrated that variations of haemostatic factor levels even within currently recommended "normal" ranges are positively linked to the incidence of coronary or cerebrovascular ischaemic events. Most convincingly, fibrinogen levels have been shown to be positively associated with the risk of myocardial infarction or stroke. In addition evidence has been presented for a role of factor VIIc levels, reduced fibrinolytic activity and platelet hyperreactivity in predicting the individual coronary risk. These findings gave rise to the hypothesis that an increased activity of coagulation proteins or platelets might serve as a marker of, or even directly contribute to the thrombotic component of ischaemic arterial disease. Using activation peptide assays such as prothrombin fragment 1+2 measurements, ongoing studies address the relationship between coagulation system activity and ischaemic heart disease.

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THE SITE OF THE t(9;22) *bcr/abl* TRANSLOCATION - A FACTOR OF PROGNOSTIC VALUE?

K.P. Kister, W. Seifarth, R. Hehlmann

The significance of the breakpoint location in the *bcr/abl* translocation for the prognosis of chronic myelogenous leukemia (CML) is still being discussed despite numerous publications in the field. The question of whether or not the location of the breakpoint in bcr has any impact on the survival of patients suffering from CML has not yet found a conclusive answer.

We are attempting to investigate this question in a controlled fashion by analysing patients who are (for other reasons) included in the German multicenter study on the treatment of CML. The advantages of this approach are: i) all patient data are immediately accessible, ii) a continual follow-up is ensured, iii) no preselection of patients will occur.

We are also interested in refining the established method with respect to the preparation of the DNA. Using double-digested samples and smaller probes will probably give more detailed data on the location of the breakpoint and possibly also the putative splice site of the *bcr/abl* mRNA. The results of these experiments will be included in the evaluation of the data collected in the CML study.

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SEQUENTIAL ANTIMICROBIAL THERAPY FOR THE TREATMENT OF INFECTIONS IN NEUTROPENIC PATIENTS WITH HEMATOLOGIC MALIGNANCIES

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The present study investigates a three step interventional antimicrobial strategy in neutropenic patients (pts) with fever of unknown origin (FUO) and clinically and/or microbiologically documented infections. Including criterias are: Absolute neutrophile count (ANC) < 500/qmm and temperature > 38.5 °C. Between January 1991 and May 1992 60 pts aged 17-77 (median 41.4 yrs) were included into the study. The diagnosis were AML 33 pts (55%), ALL 6 pts (10%), NHL 19 pts (31.7%), CML 2 pts (3.3%). During phase I pts received the combination Piperacillin (3x4g/d) and Netilmycin (1x400mg/d). Pts with persisting temperature > 38.5 °C more than 72 hrs entered phase II and were treated additionally with Teicoplanin (1x400mg/d). After another 72 hrs failures of phase II entered phase III in which the current antibiotic regimen was substituted by Ceftazidim (3x2g/d) and Amphotericin B (0.5-1g/kg/24h). The majority of pts got a selective oral decontamination with Ofloxacin (2x200mg/d) and Fluconazole (1x400mg/d). 18 pts (30%) had FUO and 42 pts (70%) documented infections. These infections were: pulmonary infiltration 9 pts (21.4%), microbiologically verified infection (blood culture) 21 pts (50%) and both type of documented infection 12 pts (28.6%). For 37 pts (61,7%) antimicrobial treatment was finished within phase I with a response rate (RR) of 81.6%, for 15 pts (25%) in phase II with a RR of 75% and for 8 pts (13.3%) in phase III with a RR of 66.7%.Cumulative RR was 95%. Among the 18 pts (30%) with FUO cumulative RR was 100% and among the 42 pts (70%) with documented infections cumulative RR was 92.9%. 33 pts (55%) with microbiologically verified infection in blood cultures had the following predominant mocroorganisms: gram-positive 60.5%, gram-negative 32.6% and fungal infection (Candida albicans) 6.9%. It is to be noted that in 43 pts (71.7%) neutropenia persisted more than another 3 days after response to the antimicrobial treatment. This data indicate a high cumulative RR (95%)to the applied sequential antimicrobial strategy in FUO and documented infections. Only 8 out of 60 pts (13.3%) needed antimycotic treatment. The selective oral decontamination with Fluconazole might have played a role.

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HUMAN BONE MARROW STROMAL CELLS PRODUCE BASEMENT MEMBRANE COMPONENTS

G. Klein, S. Beck-Gessert, R. Timpl* and C.A. Müller

The composition of the extracellular matrix in the human bone marrow microenvironment is still poorly defined, although the matrix has been shown to be of critical importance for support of hematopoiesis in vitro. We have studied the expression of the extracellular basement membrane components laminin, nidogen, BM-40 (SPARC), type IV collagen and low density heparan sulfate proteoglycan (HSPG) in human long term bone marrow cultures (LTMC) by immunofluorescence and immunoblotting/immunoprecipitation analysis. A laminin isoform consisting of only B1 and B2 chains is synthesized by the stromal cells and secreted into the medium. Neither the laminin A chain nor the homologues merosin and s-laminin could be detected. When LTMC were incubated with the cytokine G-CSF, an extracellular deposition of laminin was found, untreated cultures did not deposit laminin extracellularly. It is still an open question whether the cytokine treatment can alter the expression of the different laminin chains. The strongest expression and extracellular deposition was seen for HSPG, which can be detected very early during the establishment of the adherent stromal layer. Type IV collagen, BM-40 and nidogen were also found in an extracellular meshwork suggesting the the stromal cells in vitro, which are largely composed of fibroblasts, produce significant amounts of basement membrane components. To address the question whether these components are involved in establihing the intimate cell contact required for hematopoiesis in vitro we performed cell attachment assays to plastic immobilized proteins. Several hematopoietic cell lines were tested for their ability to bind to the laminin/nidogen complex, to type IV collagen and to matrigel, a solubilized tissue basement membrane. None of the cell lines tested adhered to the laminin/nidogen complex, and only K562 attached to type IV collagen. In contrast, all cell lines strongly adhered to matrigel consisting of a mixture of native basement membrane components. Further studies are aimed to characterize the individual components responsible for hematopoietic cell attachment.

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TENASCIN IS A MAJOR EXTRACELLULAR MATRIX PROTEIN OF THE HUMAN BONE MARROW

G. Klein, S. Beck-Gessert and C.A. Müller

Tenascin, a large glycoprotein of the extracellular matrix, has a restricted tissue distribution in normal adult tissue, but seems to be prominently expressed in regenerative organs and regenerating tissues. The expression of tenascin was analysed in the human hematopoietic system. Long term bone marrow cultures ((LTMC) and cryostat sections were analysed by indirect immunofluorescence with different tenascin specific antibodies. Tenascin was always found to be strongly expressed by the adherent stromal cells of the LTMC and deposited in an extracellular network. Northerrn blot anlysis revealed the expression of two mRNA splice variants of 6 and 8 kb. In an immunoblot analysis a prominent band of 275 kD could be detected together with a smaller band of 220 kD which was more weakly expressed. By immunofluorescence staining of cryostat sections of bone marrow a strong expression of tenascin could be detected in the microenvironment surrounding the maturing hematopoietic cells. An adhesive function of tenascin in the bone marrow could be demonstrated by an adhesion assay between bone marrow stromal cells and hematopoietic progenitor cells. The anti-tenascin antibody showed an inhibitory effect on cell binding of myelomonocytic progenitor cells to the stroma. These data suggest an involvement of tenascin in binding of hematopoietic progenitor cells to the stroma.

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STEM CELL FACTOR (SCF) CAUSES STIMULATORY EFFECTS ON B-CLL-CELLS IN SERUM FREE SUSPENSION CULTURES

H.-D. Kleine, H. Hinrichs, E. Lux, G. Döring, H. Poliwoda, and M. Freund

Direct effects of Stem Cell Factor (SCF) on peripheral B-CLL-cells were studied in vitro by bromodesoxyuridine/propidiumiodide (BrdU/PI) cell-cycle-analysis. Peripheral blood cells from patients with B-CLL were prepared as follows: After Ficoll centrifugation and lysis of monocytes by leucine-methyl-ester (LME) T-lymphocytes were depleted with a CD3 monoclonal antibody by magnetic cell sorting. Cells were grown in serum free cultures (CG-medium) containing 10 µmol/l BrdU, 100 ng/ml SCF. Controls were grown without cytokines. Samples were drawn repeatedly between 20 and 140 hours. Cell-cycle-analyses were performed after double DNA staining with propidiumiodide and staining with anti-BrdU-antibodies (B44, Becton Dickinson) and determinated by flow cytometry. Stimulatory effects of SCF could be demonstrated in proliferation kinetics.

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HIGH EFFICIENT IMMUNOMAGNETIC T-CELL DEPLETION FOR CLONOGENIC AND FUNCTIONAL ASSAYS

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Immunomagnetic separation techniques are useful to deplete cytokine producing cells from samples for clonogenic assays or other functional assays depending on proliferation. The depletion is not as effective as sorting with a flow cytometer, but faster by several times. One method is the "Magnetic Cell Separator" (MACS) utilizing a biotin-avidin-system bound to a polysaccharide coated superparamagnetic nucleus (size 50-150 nm). A alternative method for high efficient T-cell depletion using the "Magnetic Cell Separator" is described. T-lymphocytes were depleted from peripheral blood samples of 37 patients with chronic lymphocytic leukemia (CLL). The flow speed in the MACS column was adjusted by a syringe-driver allowing a constant flow speed of about 100 µl/minute. The mean content of T-cells after depletion was 0.17 % (SD ± 0.27 %). The effectiveness of depletion was calculated to a mean 93.7 %. There was no correlation between the proportion of CD3-positive cells before and after depletion.

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G-CSF-TREATMENT OF SEVERE INFECTIONS IN PATIENTS WITH ACUTE LEUKEMIA

K.O. Kliche, A. Wehmeier and W. Schneider

Patients suffering from acute leukemia often acquire severe infections in the phase of therapy-induced bone marrow aplasia. Among these infectious episodes pulmonary affections are most threatening.
Recently, granulocyte-stimulating-factors have become available for clinical use thereby opening the chance of shortening neutropenic phases.
We report our experience with 21 patients receiving G-CSF (Neupogen®) in therapeutical manner after developping infectious complications in post-therapeutic bone marrow aplasia.
In our group there were 12 female and 9 male patients, 18 suffering from acute myelogenous leukemia (AML), 5 of them with secondary leukemia emerging from myelodysplastic syndrome (MDS), 1 advanded MDS and 2 patients with acute lymphoblastic leukemia (ALL). 7 patients were treated for relapse, 2 had been non-responsive to induction chemotherapy.
All of them had been leukocytopenic posttherapeutically for 8 until 30 days when G-CSF was started. In 15 cases the site of infection was in the lung, 4 patients suffered from ARDS, 1 patient had hepatolienal candidiasis and 1 candida septicaemia. G-CSF was predominantly given subcutaneously at a dosage of 300-600 µg/day. Antimicrobial and further supportive therapy was employed as usual.
Under these conditions 14 patients have survived, 7 have died. 11 complete hematological remissions were achieved, 2 patients had partial remissions, 1 patient was non-responsive. G-CSF treatment was well tolerated, discontinuation of therapy was not necessary.
More detailed analysis reveals some noteworthy treatment results, i.e., 2 of 4 patients with ARDS survived and another 2 patients, who were non-responsive to initial therapy, achieved complete remission. On the other hand the limitations of G-CSF-treatment became apparent, when patients had to be treated early in aplasia and needed up to 23 days for regeneration despite continued stimulation.
In summary we conclude that G-CSF represents an useful novel element in supportive therapy for leukemic patients experiencing severe infections, especially at the end of bone marrow aplasia.

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EOSINOPHILIC ENCEPHALITIS AS A CAUSE OF DEATH IN IDIOPATHIC HYPEREOSINOPHILIC SYNDROME

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Idiopathic hypereosinophilic syndrome encompasses a heterogenous group of disorders. Besides persistent eosinophilia greater than or equal 1500/µl and lacking underlying cause for eosinophilia signs of organ involvement are postulated for diagnosis. Especially neurologic manifestations often lead to a fatal course of the disease. However the role of eosinophilic granulocytes in the development of tissue damage remains to be clarified. Furthermore from a hematologist's point of view often the distinction between a reactive versus autonomous character of the disease is difficult to be made.
We report the clinical course of a 34-year old female, who was admitted to our hospital after a four weeks period of continued malaise and complex neurologic symptomatology. She was found to have an excessive leukocyte count of 550.000/µl, 95 % of which were eosinophils. Bone marrow examination revealed a nearly complete infiltration by mature eosinophils with a partial suppression of normal hematopoiesis and a normal blast relative count. Immediately leukapheresis was started and within 48 hours leukocyte counts could be lowered until 20-30.000/µl. Unfortunately the patient required mechanical ventilation because of an acute respiratory failure, additionally multiple biventricular cardiac thrombi and at least two intracerebral lesions had been diagnosed meanwhile. Finally the patient died in central dysregulation after massive intracerebral hemorrhage.
Autoptically typical features of Löffler's endocarditis were found, further eosinophilic infiltrates could be demonstrated in lung, spleen and kidney. Neuropathological examination showed expanded intracerebral hemorrhage thrombotically occluded vessels and hitherto undescribed perivascular as well as intraparenchymatous infiltrates of eosinophils. The latter finding could support the hypothesis of eosinophils contributing to cerebral tissue damage, e.g. by their granule substances. Furthermore the present case illustrates once again the difficulties in diagnosis and therapy of hypereosinophilic states.

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SERIAL CYTOKINE PLASMA LEVELS IN ACUTE AMPHOTERICIN-B-TOXICITY

K.O. Kliche, A. Wehmeier and W. Schneider

Cytokines represent important mediators of the acute-phase-response. Detailed analysis of the function and mutual interaction of these factors is essential to the understanding of the complex cytokine network. However these investigations are hampered by the short half-life of cytokines in the circulation, the presence of soluble inhibitors and the ill-defined beginning of acute-phase-reactions.
Therefore we have chosen the acute toxicity after intravenous amphotericin B (Am B) application, characterized by fever, severe chills and hypotension as a model for an acute-phase reaction. Amphotericin B is believed to induce its adverse reactions via liberation of pro-inflammatory cytokines, e.g. Tumor-Necrosis-Factor α (TNF α) and Interleukin 1, from cells of the monocyte/macrophage system.
We have determined plasma levels of TNF α, Interleukin-6, soluble-TNF-receptor (s-TNF-r) and Interleukin-1-receptor-antagonist (IL-1-RA) from 6 patients with acute leukemia and systemic fungal infections. Serial samples of EDTA blood were obtained before and up to 6 hours after start of Am B infusion. Samples were immediately centrifuged and the supernatant was stored at - 40° C until analysis by ELISA (Medgenix and R&D Systems).
In patients with adverse reactions TNF α reached peak plasma levels 90-180 minutes after starting Am B. Peak s-TNF-r levels were observed about 30 minutes after TNF α, whereas IL-1-RA reached its maximum thereafter. Most patients tolerating Am B well did not show increased TNF α levels.
We conclude that adverse Am B reactions in humans provide a suitable model for studying time dependent interactions between inflammatory cytokines and their natural inhibitors.

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CARBOPLATIN,METHOTREXATE AND VINBLASTIN (MV-CARBO) FOR ADVANCED UROTHELIAL CANCER

J.Klocker, M.v.Trotsenburg, J.Schumer, M.Kanatschnig, K.Henning*

Due to considerable toxicity of established combination chemotherapy as M-VAC or M-VEC regimen a prospective phase II trial was initiated to evaluate both efficacy and toxicity of carboplatin in combination with methotrexate and vinblastin. Treatment consisted of monthly cycles of 300 mg/m² carboplatin, 30 mg/m² methotrexate and 3 mg/m² vinblastin and the same dosage of methotrexate and vinblastin on days 15 and 22. After evaluating 15 patients in 1989 21 new patients received this regimen. All patients suffered from locoregional or metastatic transitional cell cancer and were pretreated locally. 13 patients had impaired renal function due to obstructive uropathy. Of 15 patients evaluable for response, 4 patients achieved partial remission, 8 patients stable disease and 2 patients had progressive disease. Mean duration of response was 8.33 month (range from 3^+ to 29^+). Subjective tolerance was excellent though considerable bone marrow toxicity but no deterioration of renal function was noticed. In all responding and all patients experiencing no change there was remarkable improvement of life quality measured by an increase of the Karnofsky index.
In conclusion, the regimen appears to be a well-tolerated palliative treatment for patients with unfavorable prognosis.

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IN VITRO AND IN VIVO EFFECTIVENESS OF FLUDARABINE IN B-CELL CHRONIC LYMPHOCYTIC LEUKEMIA (B-CLL)

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Fludarabine, a nucleoside analogue, is reported to be highly effective in low grade Non-Hodgkin's lymphoma. Patients (pts) with B-cell chronic lymphocytic leukemia (B-CLL) were therefore consecutively enrolled in a phase II/III trial and received 2-6 courses (median 5) with fludarabine 25mg/m², day 1-5, monthly. In these pts, we tested the in vitro antiproliferative activity of fludarabine, mitoxantrone, and chlorambucil. Peripheral blood was obtained from nine pretreated pts with advanced stage B-CLL before initiation of systemic therapy. Mononuclear cells were isolated by Ficoll-Hypopaque gradient. Triplicates of $5x10^4$ cells were seeded in 96-well microplates. Cells were cultured in 10% FCS containing medium and exposed to fludarabine, mitoxantrone, or chlorambucil at concentrations of 0.05-50.0 μg/ml. In addition, combinations of fludarabine and mitoxantrone were examined. Incubation time with drugs was 48 h at 37°C. Cytotoxicity was assessed using a modified colorimetric tetrazolium salt assay (MTT assay). In order to quantify additive or even synergistic drug interactions the median effect equation and combination index was applied (Chou and Rideout, 1991). B-CLL cells from all the nine pts were found to be sensitive to fludarabine in vitro with IC_{50} values ranging from 0.5-10.0 μg/ml. Five of these pts responded also in vivo. One patient achieved a complete remission and four pts a partial remission, whereas two pts had stable disease and another two pts had progressive disease. The mean IC_{50} value of responding pts was 4.75 μg/ml and of non-responders 8.5 μg/ml. Furthermore, all pts demonstrated in vitro responsiveness to mitoxantrone with IC_{50} values ranging from 0.125-1.0 μg/ml, and to chlorambucil with IC_{50} values varying from 1.5-10.0 μg/ml. In five pts tested so far, the combination of fludarabine and mitoxantrone showed additive interactions and enhanced cell kill rate by 1-2 logs. In summary, our results indicate the in vitro and in vivo activity of fludarabine in pretreated pts with B-CLL. The combination of fludarabine and mitoxantrone revealed additive cytotoxicity in vitro. Therefore, clinical trials are warranted to assess the clinical efficacy of this combination.

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SERUM LEVELS OF sCD25 AND sCD8 IN B-CELL CHRONIC LYMPHOCYTIC LEUKEMIA AND CORRELATION WITH DISEASE ACTIVITY

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Soluble forms of CD25 and CD8 (sCD25/sCD8) are detected at low levels in the serum of healthy subjects. Elevated levels are found in patients (pts) with active autoimmune disorders as well as with malignant lymphomas. Therefore, we examined sCD25 and sCD8 in pts with B-cell chronic lymphocytic leukemia (B-CLL) in order to assess their role as indicators of disease activity. 47 pts with B-CLL were studied so far. Staging was performed according to the classification systems of Rai and Binet, respectively. Serum samples were freshly stored in liquid nitrogen until further processing. Levels of sCD25 and sCD8 were measured by a sandwich ELISA technique using commercially available assays (Biermann, Germany). Advancing Rai stages were associated with a progressive increase ($p<0.01$) of sCD25 from 1412±680 U/ml in stages 0/I, to 5730±2117 U/ml in stage II, and to 7125±2873 U/ml in stages III/IV. This progression of sCD25 was also evident when the Binet classification was applied ($p<0.01$). Levels of sCD8 were found to increase from 471±287 U/ml in Rai stages 0/I to 1028±570 U/ml in stage II ($p<0.05$), but without further progression in stages III/IV with 1183±778 U/ml. Applying the Binet classification, sCD8 was shown to be 589±484 U/ml in stage A, but 1114±481 U/ml in stage B ($p<0.05$). Again, no further increase was found in Binet stage C. High levels of both sCD25 and sCD8 were associated with the occurrence of B-symptoms ($p<0.05$). Moreover, sCD25 and sCD8 were preferentially elevated in pts with a lymphocyte doubling time of < 12 months, but this tendency was not statistically significant. In summary, (1) progressive serum levels of sCD25 and sCD8 correlate with advancing stages of disease in B-CLL. (2) B-symptoms were associated with high levels of sCD25 and sCD8. (3) We found sCD25 to be the more sensitive marker of disease activity than sCD8. Thus, sCD25 may be useful in monitoring pts with B-CLL.

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MAGNETIC RESONANCE IMAGING OF BONE MARROW IN CHRONIC LYMPHOCYTIC LEUKEMIA - CORRELATION WITH RESPONSIVENESS TO FLUDARABINE

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Fludarabine, a nucleoside analogue, is currently investigated in phase III trials on chronic lymphocytic leukemia (CLL). It is reported to induce complete remissions (CR) in some patients (pts). Magnetic resonance imaging (MRI) was found to detect bone marrow (BM) infiltrations by malignant lymphomas with high sensitivity. Therefore, we used MRI to examine BM infiltration in CLL and to assess the responsiveness to treatment with fludarabine. Seven conventionally pretreated pts with Binet stage C and one patient with Binet stage B disease were studied so far. They received at maximum six courses of fludarabine 25 mg/m^2 x 5 d monthly. Before initiation of treatment, and after the third and the sixth course of therapy, BM of the lumbar spine and the pelvic region was examined by MRI using a 1.5 tesla imager, "Magnetom" system (Siemens, Germany). Areas of abnormal signal intensities were determined both by visual criteria and quantitavely. As internal standard we chose subcutaneous fatty tissue. While three pts remained refractory to fludarabine treatment, four other pts turned to Binet stage A with normal blood counts but slight lymphocytosis in the BM. One patient had a mixed response with normal leucocyte counts but persisting anemia and thrombocytopenia. Before therapy, signal reduction in t1 weighted images and prolonged t1 relaxation times indicated lymphocytic infiltration in large BM areas in all pts. No change was observed in pts refractory to fludarabine. In pts with clinical response, signal elevation and shortened t1 relaxation times indicated regrowth of hematopoiesis. However, some BM areas remained unaltered. In summary, the degree of BM infiltration in CLL can be estimated by MRI. Thus, MRI may contribute to redefine remission criteria in CLL.

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ATTENUATION OF THE HEMOSTATIC DISORDER IN ACUTE PROMYELOCYTIC LEUKEMIA BY TREATMENT WITH ALL-TRANS RETINOIC ACID.

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Acute promyelocytic leukemia (APL) is associated with disseminated intravascuar coagulation (DIC), which can be accelerated by induction of cytostatic chemotherapy and causes a markedly risk for (intracerebral) hemorrhage. Complete remissions of APL can be achieved by differentiation-inducing therapy with all-trans retinoic acid (ATRA). We studied activation markers of the coagulation system (thrombin-AT III complex (TAT), D-Dimer) in 3 patient with APL treated with ATRA. All patients had signs of DIC before induction of therapy (low plasma fibrinogen, low platelet-count) and moderate to severe bleeding tendency, activation markers were elevated (TAT >15 μg/L, D-Dimer >1500 μg/L). DIC was treated with fresh frozen plasma (3-9 U/d), no heparin was administered. APL was treated with ATRA (45 mg/m^2/d). D-Dimer levels decreased rapidly to values <300 μg/L, TAT <8 μg/L within 1 week of ATRA therapy, after 2 weeks activation markers were in the normal range (D-Dimer <100, TAT <3 μg/L). No platelet substitution was necessary during ATRA therapy, FFP substitution could be stopped after at least 4 days. Our data show, that treatment with ATRA can reduce the coagulation disorder and the risk for severe bleeding in patients with APL.

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Detection of chromosomal translocations t(14;18) within the major and minor cluster region in formalin-fixed paraffin embedded lymphomas

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Follicular lymphomas (centroblastic-centrocytic lymphomas, cb/cc NHL) show in 80% a specific chromosomal translocation t(14;18) when DNA from unfixed, fresh tumor biopsies is analyzed. Polymerase chain reaction amplification (PCR) of chromosomal rearrangements from DNA of formalin-fixed paraffin-embedded material is hampered by DNA degradation. We analyzed paraffin-embedded specimens of 21 lymphomas (13 cb/cc, 2 centroblastic, 4 immunoblastic, 2 MALT lymphomas) by PCR using single pairs of primers for the major breakpoint (mbr) and the minor cluster (mcr) region. Seven cb/cc NHL showed translocations and 2 centroblastic lymphomas had gene rearrangements. In one cb/cc lymphoma a translocation within the mcr was observed. Specificity of amplification products was shown by Southern blot analysis with an internal bcl-2 oligonucleotide probe for all PCR products and by direct DNA sequence analysis of junctional regions in two cases. The data show that even in formalin-fixed paraffin-embedded material PCR analysis provide an additional molecular tool for the diagnosis of cb/cc NHL in small histologic specimens with degraded DNA.

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CELL CYCLE LINKED EXPRESSION OF THE PROTO-ONCOGENE BCL-2

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Resting T and B blood lymphocytes were stimulated toward blast activation and mitogenesis using phytohaemagglutinin short term culture. The transition to S phase of cell cycle was reversibly blocked for 48 hours by Methotrexate and released by cold thymidine together with crude supernate of control cultures. The detection of bcl-2 RNA was performed by Dot blots with the bcl-2 probe. This was a 4,0 kb Hind III-Hind III insert from probe "b" obtained from Y. Tsujimoto, labeled with digoxygenin-dUTP. Obtained findings indicate that the bcl-2 gene is operative in a cell-cycle linked manner being induced as early as after 1-3 hours after mitogen addition. The peak expression was observed after one day followed diminishing signals detectable up to 6 days in cultured lymphocytes. Most important time course points of bcl-2 gene expression were 1. the growth induction period followed by blastic development and 2. the accumulation of lymphoblasts. MTX block reversal with subsequent proliferative period initiated by DNA synthesis was not accompagnied by further significant changes in bcl-2 gene expression. From simultaneously performed clinical trials it is evident that the bcl-2 gene expression is slighly elevated in CLL-blood lymphocytes, in umbilical blood mononuclears and also in neoplastic human myeloid cells. Above data are in accord with present suggestions that the proto-oncogene bcl-2 is related to genes implicated in cell cycle control in haemopoietic cell lineage.

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HEPATITIS B REACTIVATION AFTER HIGH DOSE CHEMOTHERAPY AND SUBSEQUENT AUTOLOGOUS BONE MARROW TRANSPLANTATION RESULTING IN CHRONIC HEPATITIS B - A CASE REPORT

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Reactivation of hepatitis B virus (HBV) as a complication of immunosuppression is possible both in asymptomatic HBsAg carriers and in HBsAg negative patients with antibodies to HBV as a result of previous infection. HBV reactivation is a potential complication of autologous bone marrow transplantation (autologous BMT) and may result in chronic hepatitis B.

Case report

A 24-year-old man received combined radiochemotherapy for hodgkin's disease, achieving a complete remission. Two years later, a thoracic relapse of preexistent hodgkin's disease was diagnosed. High dose chemotherapy with subsequent autologous BMT was performed. Investigations revealed anti-HBs and anti-HBc IgG antibodies indicative of prior exposure to HBV. The transaminases were within normal limits pre-transplant. After autologous BMT, substitution therapy for erythrocytes and thrombocytes was necessitated throughout the whole treatment procedure (28 units of platelet concentrates, 13 units of filtered packed red blood cells). One week after autologous BMT, a slow but progredient increase of transaminases was noted. Six months later, conventional HBV serology showed both HBsAg and HBeAg in the serum. Additional serology demonstrated anti-HBc (IgM), anti-HBc (IgG+IgM) and a maximum alaninaminotransferase (ALT) of 545 IU/l confirming the diagnosis of subacute hepatitis B. In the further course, chronic hepatitis B developed.

Discussion

We report a case of HBV infection following autologous BMT. Since the patient was immune to HBV prior to transplant it is almost certain that he reactivated HBV as a result of immunosuppression associated with hodgkin's disease and its therapy. Disappearance of anti-HBc and anti-HBs antibodies and reactivation of HBV with transient HBs antigenemia after immunosuppression has been observed repeatedly. Acquired blood-borne HBV infection is unlikely since all blood products tranfused demonstrated an HBV serology completely negative at repeated testing. All blood and platelet donors were followed carefully, none showed a positive HBV serology at subsequent donations. Maximum liver damage in this patient correlated with immunological reconstitution post-transplant. This is consistent with the finding that the target for cytotoxic T cells in chronic hepatitis has been identified as HBcAg expressed on the hepatocyte membrane. Patients at risk of HBV reactivation should be identified by conventional serological screening before transplant. Patients so identified could then be introduced into controlled trials of HBV immunization pre-transplant or therapy with interferon alfa post-transplant. Anti-HBs hyperimmune globulin may be effective in limiting liver damage by preventing HBV replication.

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COMPARISON OF TWO QUANTITATIVE ASSAYS FOR MEASURING TUMOR CELL ADHESION TO EXTRACELLULAR MATRIX PROTEINS

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Adhesion of tumor cells to extracellular matrix (ECM) components is considered to be an important step for the development of metastases. Several limitations of current in vitro adhesion assays such as the use of radioactivity, low reproducibility, and time consuming read-out steps have led us to develop a new assay and to compare it to an established technique using radioactively labeled cells.

Methods: In both assays, $25x10^3$ cells of the colon cancer cell line HT 29 are incubated serum-free for 90 min at 37°C in microtiter wells precoated with individual ECM proteins or BSA. Non-adherent cells are removed by repeated washing. After subtracting nonspecific binding to BSA, specific adhesion is calculated as % of totally added cells . For the fluorescence method, adherent cells are incubated with methylumbelliferyl heptanoate (MUH). Fluorescence intensity as a function of cell numbers is quantitated using a microtiter plate reader (Titertec Fluoroscan II). For the radioactive method, cells are labeled with ^{51}-Chromium (^{51}-Cr) before adhesion. Adherent cells are lysed and the radioactivity is measured in a gamma counter.

Results: Specific adhesion (data from 8 experiments, given in % ± SD)

Method	Collagen IV	Fibronectin	Laminin	Vitronectin
MUH	22±6	15±8	8±3	2±2
^{51}-Cr	28±6	20±4	17±5	11±5

Variation between different experiments and between replicate measurements within individual experiments is higher with the MUH method.

Conclusions: 1. Both assays demonstrate high adhesion of cancer cell line HT 29 to collagen type IV, moderate adhesion to laminin and fibronectin, and low attachment to vitronectin. 2. The ^{51}-Chromium assay consistently showed higher adhesion to all ECM proteins tested. This difference may be partly due to the requirement of active esterases for the formation of the fluorescent metabolite, whereas the radioactive signal of ^{51}-Chromium labeled cells is independent from such enzymatic function. 3. While the MUH assay is a rapid and non-radioactive method to quantitate tumor cell adhesion, it has a lower reproducibility when compared to the conventional ^{51}-Chromium assay.

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THE SIGNIFICANCE OF MONOCLONAL GAMMOPATHY DETECTED IN ROUTINE SCREENING EXAMINATIONS OF NATIONAL PENSION INSURANCE CONTRIBUTORS

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In the years from 1987 to 1991 45821 patients were examined in routine screenings concerning applications for rehabilitation and the premature drawing of pension. The screenings were conducted by doctors working von behalf of the national pension insurance institution (Landesversicherungsanstalt Berlin, LVA).

The purpose of this study was to look at the percentage of monoclonal gammopathy (MG) detected and its clinical relevance to the patients. Of all 45821 patients examined by the national pension insurance institution proteine-electrophoretic analyses were made. If there was any suspicion of an existing MG (even with extremely low M-components), an immuno-fixation-electrophoresis was conducted to confirm or reject the presence of MG.During the period 1987 - 91 a total of 304 patients with MG were detected. The mean age of these patients was 55 years, with a relation of 2/3 : 1/3 in favour of males.

In the last 5 years 42 patients out of the 304 with MG were admitted to our hospital in order to conduct a complete staging. The mean age and the immunologic classification of all gammopathies, including the biclonal gammopathies, of this sample was in direct proportion to the consistency of the whole group of 304 patients. The staging examinations led to the following results: in 33 patients a MGUS was diagnosed, and regular testing by their GPs recommended. In 3 patients early infiltration of the bone marrow by a multiple myeloma could not be ruled out; they were classified as smouldering multiple myeloma (SMM). In these cases rigid supervision by a haematologist was recommended. In 2 patients a MM with confirmed infiltration of the bone marrow was found. In 1 of these patients we diagnosed a stage 1a according to SALMON, in the other a stage 2a. The latter was referred to chemotherapy treatment as advanced osteolytic destruction was present. In 4 patients with IgM-gammopathy a lymphoplasmocytic immunocytoma was found. At the time of diagnosis no treatment was required.

A rate of 21% malignant MGs out of all the detected MGs in routine screenings is a very high proportion compared to other findings in literature. It has to be assumed that there is a relation between the malignant illness and the desire for rehabilitation or premature retirement.

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SUCCESSFUL TREATMENT OF ASPERGILLUS PNEUMONIA/ASPERGILLOMA WITH LIPOSOMAL AMPHOTHERICIN B IN TWO PATIENTS WITH ACUTE MYELOGENOUS LEUKEMIA

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Two neutropenic patients who developed a pulmonary infiltrate during induction respectively consolidation therapy for acute myelogenous leukemia (AML), who did not respond to broad spectrum antibiotics were treated with liposomal amphothericin B. In both patients a bronchoalveolar lavage had not revealed a pathogen and all serological investigations for virus- or fungi infection were negative. Nevertheless a systemic antimykotic therapy was empirically started. Liposomal amphothericin B, a preparation lacking the nephrotoxic and pyrogenic side effects of standard amphothericin B, was chosen because both patients had an impairment of renal function with C_{Krea} < 30 ml/min. due to prior aminoglycoside treatment in one patient and preexisting impairment in the other patient. Both patients were treated with 4 mg/kg/d intravenously and become afebrile within 72h. The pulmonary infiltrates showed a regression and then changed to the classical aspect of aspergilloma with a ball of hyphae within a cavity. At that time IgG antibodies to aspergillus antigens were detected in both patients sera. Systemic treatment was continued for a total of 16 weeks in one and 12 weeks in the other patient. During this time the cavities with fungus balls showed a continuous regression and disappeared in both patients. One patient showed a complete clearance of infiltrates on chest x-ray. A small dense consolidation remained in the area of the fungus ball in the second patient. This patient is still alive and without evidence of relapse of the aspergillus infection 12 months after stopping intravenous liposomal amphothericin B. The other patient received a second course aggressive chemotherapy as consolidation for his AML without relapse of the pulmonary aspergillus and is off therapy for 6 months. We conclude that liposomal amphothericin B is active and well tolerated in neutropenic patients with pulmonary aspergillus/aspergilloma and concomittant renal insufficiency.

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HIGH DOSE CYCLOPHOSPHAMIDE, ETOPOSIDE AND BCNU FOLLOWED BY NON-CRYOPRESERVED AUTOLOGOUS BONE MARROW TRANSPLANTATION FOR POOR PROGNOSIS HODGKIN'S DISEASE

H. Köppler, K.-H. Pflüger, J. Heymanns and K. Havemann

We treated 17 patients with relapsed Hodgkin's disease with high dose cyclophosphamide (2 x 60 mg/kg), etoposide (3 x 700 mg/m^2) and BCNU (300-600 mg/m^2) followed by non-cryopreserved autologous bone marrow transplantation (ABMT). 8 patients were in second or subsequent complete remission (CR), 6 patients had a relapse responding to conventional chemotherapy and 3 patients had resistent relapses. 4 out of 9 patients who were not in CR at the time of ABMT entered CR after high dose chemotherapy and ABMT. With a median follow up of 8 months (range 1-38 months) the event free survival (event defined as disease-progression or death) is projected to be 61 % at 2 years. Main non-hematologic side effects were severe mucositis in 6 patients and congestive heart failure WHO Grade 3 in one patient. All patients had a full hematologic reconstitution. However one patient was still dependent on platelet transfusions 6 months after ABMT when she died of progressive disease. We conclude that high dose cyclophosphamide, etoposide and BCNU followed by non-cryopreserved ABMT is a efficacious approach for poor prognosis Hodgkin's disease. The procedure is straight foreward to perform and less cost effective than cryopreservation.

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SEQUENTIAL CHOEP VS ALTERNATING HCHOP/IVEP: A MULTICENTER RANDOMIZED TRIAL FOR HIGH GRADE NON-HODGKIN'S LYMPHOMAS

H. Köppler on behalf of members of the study group.

185 patients with high grade non-Hodgkin's lymphoma stages II-IV were randomized to receive either four cycles of CHOEP (cyclophosphamide 750 mg/m^2 i.v. d 1, doxorubicin 50 mg/m^2 i.v. d 1, vincristine 2 mg i.v. d 1, etoposide 100 mg/m^2 i.v. d 3-5, prednisolone 100 mg p.o. d 1-5) (treatment arm A), or four cycles of chemotherapy with hCHOP (cyclophosphamide 1,200 mg/m^2 i.v. d 1, doxorubicin 40 mg/m^2 i.v. d 1+2, vincristine 2 mg i.v. d 1, prednisolone 100 mg p.o. d 1-5) alternating with IVEP (ifosfamide 1,500 mg/m^2 i.v. d 1-5, vindesine 3 mg/m^2 i.v. d 1, etoposide 120 mg/m^2 i.v. d 3-5, prednisolone 100 mg p.o. d 1-5) in treatment arm B. After four cycles of chemotherapy an involved field irradiation with 35 Gy was given to all patients in complete or partial remission. 173 patients were evaluable for response and survival. A complete response (CR) was seen in 148/173 patients (85%) with 86% CR in arm A vs 84% CR in arm B. With a median follow-up of 35 months (range 17-61) the overall projected survival at 48 months is 61% vs 65% for arm A and B, respectively. Event-free survival is projected to be 57% in arm A and 47% in arm B at 48 months. So far, the differences in CR, survival and disease-free survival are statistically not significant. Factors associated with poor survival were advanced stage (III, IV) and an LDH > 250 U/l. Toxicity of all regimens was acceptable. Main side effects were mild nausea/vomiting, leukopenia and fever/infection associated with leukopenia. Two patients died of infectious events and one from hemorrhage giving a toxic death rate of 2%. In conclusion, both treatment modalities produced high complete remission rates and survival data indicate that the majority of patients will be longterm survivors.

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12 CASES OF BILATERAL TESTICULAR GERM CELL TUMORS

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In the last two decades we treated more than 500 patients with germ cell tumors (GCT), 13 of whom presented with bilateral testicular tumors, accounting for about 2.5 %. In 3 patients (pts) the tumor was diagnosed simultaneously and in 10 pts metachronously. In the pts with metachronously diagnosed GCT age at diagnosis of the first tumor ranged from 16 to 36 years (ys) (median 24 ys), at diagnosis of the second tumor it ranged from 24 to 54 ys (median 34 ys). The interval between diagnosis of first and second GCT ranged from 20 to 206 months (mts) (median 85 mts). Histology of the first tumor was non-seminomatous (NS) in 6 pts, and in 4 pts it was a seminoma (S) Histology of the second tumor was NS in 4 pts and S in 6 pts. Treatment of the first tumor consisted of radiation in 6 pts (4 S, 2 NS), chemotherapy in 2 pts (both NS) and only surgery in one pt (NS).Treatment of the second tumor consisted of radiation in 4 pts (3 S, 1 NS) and chemotherapy in 3 pts (1 S, 2 NS). 'Wait and see'strategy was performed in one pt (NS) in the first, and in 3 pts (2 S, 1 NS) in the second tumor. At diagnosis of the second tumor 7/10 pts underwent follow-up examinations. Therapy of first and second tumor resulted in a complete remission in all pts. 3 to 209 mts after diagnosis of the second tumor (mean 55 mts) 7/10 pts currently are alive without disease. 3 pts are lost for follow-up. In the 3 simultaneously diagnosed pts age at diagnosis was 24, 27 and 31 ys. Histology of one of both tumors was MTU in all pts and that of the second tumor was seminoma, MTI and MTD, respectively. 2 pts presented with advanced metastatic disease and died within few mts after onset of chemotherapy. One pt (stage IIA) received 4 cycles of adjuvant chemotherapy and remains disease-free 84 mts after diagnosis.

We conclude, that bilateral testicular GCT is an extremely rare disease. Due to combined modality treatment and careful follow-up examinations pts suffering from bilateral testicular GCT have a definite chance of cure.

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EFFECTS OF SEQUENTIAL THERAPY WITH IL-3 AND GM-CSF ON HEMOPOIESIS IN PATIENTS WITH MALIGNANT DISEASES ON HIGH RISK OF RELAPS

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The mobilisation of circulating hemopoietic progenitor cells (HPC) by preceding chemotherapy with or without hemopoietic growth factors is a well established method. Recently it has been demonstrated in a monkey model that the sequential administration of IL-3 and GM-CSF caused a marked increase in HPC without prior cytostatic treatment.

Based on these data we initiated a prospective, non randomised phase I/II trial in patients with breast cancer, testicular cancer, and high grade NHL in complete remission and high risk of relapse.Primary aim was to evaluate the optimal dose and time schedule of IL-3 in combination with GM-CSF for maximal stem cell yield thus providing superior conditions for stem cell transplantation in chemotherapy sensitive relapse.

16 patients have been treated with various doses of IL-3 (2,5 ug, 5 ug, 10 ug/kg) for 3 days or IL-3 5 ug/kg for various days (3, 7, 14) followed by administration of GM-CSF for 5 days. As a control GM-CSF (5 ug/kg) alone was given for 5 days. The combination of IL-3 (2,5 ug - 10 ug/kg) for 3 days did not show any further increase of HPC compared to GM-CSF alone. A marked additive effect was observed when the treatment time with IL-3 (5 ug/kg) was raised to 7-14 days. In all cases HPC disappeared from peripheral blood immediately after IL-3 therapy thus indicating an effect on the expression of special adhesion molecules. Concerning cytokine induction there are preliminary data that GM-CSF seems to upregulate IL-8 expression whereas IL-3 was without any influence. In this part of the study the sequential administration of IL-3 and GM-CSF was safe and well tolerated. On this basis we plan to further escalate the dose and treatment duration of IL-3.

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ALL TRANS RETINOIC ACID TREATMENT +/- CHEMOTHERAPY IN ACUTE PROMYELOCYTIC LEUKEMIA

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Acute promyelocytic leukemia (APL), a distinct subtype of AML, is frequently associated with disseminated coagulopathy (DIC) causing a high incidence of fatal bleeding complications. APL is cytogenetically characterized by the translocation t(15;17) involving the region of the retinoic receptor α gene. In vitro all trans retinoic acid (ATRA) exhibits a differentiation-inducing and antiproliferative capacity on APL-cell-lines. Similar effects were observed when ATRA was administered to APL-patients, who achieved complete remission without any cytostatic therapy. We have treated 10 patients with APL (5 males and 5 females, median age: 36 years, range 22 - 60 years). The cytogenetic translocation t(15;17) was observed in all patients, 3 patients had additional karyotypic abnormalities. ATRA was administered as capsules (45mg/m^2 divided in two daily doses) for 90 - 106 days. 4 primarily untreated patients, 1 patient in 1st relapse after autologous transplantation and one patient with a chemotherapy-resistant relapse received ATRA without any cytostatic treatment. Two patients were treated with a combination of ATRA and "3+7" induction (60 mg/m^2 Daunorubicin d1-3, 100 mg/m^2 Ara-C, d1-7) and two patients received additional chemotherapy (4 x 3g/m^2 Ara-C) due to excessive hyperleukocytosis developing during ATRA-treatment. Clinical and laboratory signs of DIC (fibrinogen level, fibrinogen degradation products) improved rapidly in all patients. Complete hematological, immunological and cytogenetic remission was obtained in all patients within 4 to 12 weeks therapy. Hyperleukocytosis developed in 3 patients and was treated in 2/3 with 4 x 3g/m^2 Ara-C. Other ATRA side effects (dryness of skin, alopecia, bone pain) were mild but improved during continuing ATRA-treatment. 7/10 patients are still in remission for 1+ to 8+ months after starting treatment. 3 patients receiving ATRA as salvage therapy after treatment failure relapsed after 2 - 7 months in complete remission. Our results indicate that ATRA therapy for APL is safe and highly effective.

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STANDARD HEPARIN AND LOW MOLECULAR WEIGHT HEPARIN EFFECT ON SPONTANEOUS PLATELET AGGREGATION MEASURED IN WHOLE BLOOD AND IN PLATELET-RICH PLASMA

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The aim of the study is the evaluation of both standard heparin (SH) and low molecular weight heparin (LMWH) (Fraxiparine) impact on spontaneous platelet aggregation assessed in the whole blood and in platelet-rich plasma (PRP). Venous blood samples were obtained from 10 healthy volunteers and collected into plastic tubes containig citric acid. SH or LMWH were added to the whole blood and PRP. After sample stirring, the percentages of the remaining platelets were calculated. The results obtained reveal that SH enhance spontaneous aggregation in a similar degree both in the whole blood (65.5 ± 3.05, control 78.8 ± 4.1) and in PRP (66.5 ± 2.7,control 86.5 ± 3.5). LMWH significantly intensifies spontaneous aggregation in the whole blood (53.1 ± 3.05, control 78.8 ± 4.1) but not in PRP (80.5 ± 4.2, control 86.5 ± 3.5).

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PRIMARY BLADDER LYMPHOMA

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The authors present the case of a 76-year-old man in whom bladder tumor was detected the reason for haematuria. The histological investigation of trans urethral resected pattern showed lymphocytic lymphoma. In pocession of the histological finding detailed haematological examination has been carried out, the patient was without evidence of lymphoma dissemination. He received multichemotherapy /6 cources/ and locoregional cytostatic therapy and complete clinical remission was detected. The authors report our experience with extranodal bladder lymphoma included diagnosis and therapeutical notes as well as review of the relevant literatura.

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OBSERVATIONS ON THE LONG TERM SURVIVAL IN HODGKIN'S DISEASE (HD).

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Out of 61 patients (41% of those diagnosed before Dec. 31,1981) surviving more than 10 years from diagnosis of HD, 46 (28 women and 18 men) are alive showing mean survival time 172.8 months (mo), SD 47.7 (range 121-335) median 159.9 mo and 15 have died (7 women, 8 men) 120-216 mo after diagnosis, mean 154.4, SD 32, median 144 mo. Mean age and range were similar in both groups but advanced clinical stages were more common in the patients dead (11/15 v. 25/46).

Among 46 pts. alive 26 are in CCR whereas 20 experienced recurrences, in 2 as late as after 10 years since dgn. Among patients who have died, only 2 were in CCR and the remaining ones relapsed: 4 in first 5-yrs period, 4 in the second and 5 in the third after dgn; the latest relapse 15.5 yrs after dgn showed a complete insensivity to therapy. Progressive or recurrent HD was the only cause of death in 9 pts, concommitant with infections in 3 (1 case of cryptococcal meningitis) and in 3 (among them in 2 being in CCR) - secondary neoplasms.

The authors conclude that the main cause of death in over ten years survivors remains the underlying disease and long term survival not in all pts is equivalent with the cure of HD.

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Immuno-electron-microscopical investigation of ICAM-1 and LFA-1 antigen surface expression on aggregating human monocytes

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Interferon-γ induced aggregation of human monocytes has been shown to be inhibited by monoclonal antibodies against the integrin LFA-1 (CD11a/18). To further investigate the role of this antigen and its physiological ligand ICAM-1 (CD54) in mediation of cell-cell-contacts between human monocytes we investigated antigen expression and surface distribution of LFA-1 and ICAM-1 on monocytes stimulated with interferon-γ by immuno-electron microscopy. Human monocytes were obtained by plastic adherence of Ficoll Hypaque isolated human peripheral blood mononuclear cells of healthy donors. The adherent cells were detached from the plastic and after washing in PBS resuspended in RPMI + 10% FCS. After that, the cells were seeded for 30min onto glass coverslips covered with melamine resin foils. After adherence to the foils, the monocytes were incubated in RPMI + 10% FCS with interferon-γ in a concentration of 200U/ml. Immuno-electron microscopical analysis was performed after 1h and after 18h of incubation: The cells were rinsed with PBS, prefixed with 0.2% glutaraldehyde, incubated with the mouse monoclonal antibodies against LFA-1 (IOT16) or ICAM-1 (WEHICAM1) for 1h, washed again and incubated for 1h with a goat-anti-mouse antibody coupled to 30nm gold colloids (GAM gold 30, Janssen). After that, the cells were fixed in 2,5% glutaraldehyde and air dried. The melamine foils with the adherent cells were detached from the glass and mounted on coppergrids. These preparations of the whole cells were viewed at with an electron microscope at 100kV. After 1h of adherence, the cells expressed only low levels of LFA-1 and ICAM-1. However, they had started to spread over the substrate surface and formed membrane structures such as filopodia, which sometimes reached the neighbour cell and formed cell-cell-contacts. Neither LFA-1 nor ICAM-1 was accumulated on these membrane structures. After 18h of incubation, most of the cells had formed cell-cell-contacts with their neighbour cells. Moreover, the cells were intensively stained for ICAM-1 and LFA-1 indicating antigen upregulation during incubation. In several cell-aggregates the antigens were found to be accumulated in the cell-cell-contact areas. These morphological data suggest that aggregation of interferon-γ stimulated monocytes is a process consisting of several steps including development of membrane projections, antigen upregulation and redistribution and that LFA-1 and ICAM-1 are probably not involved in the early steps of cell-cell-interaction of monocytes.

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CYTOKINE-MEDIATED REGULATION OF INTEGRIN RECEPTORS CAN INFLUENCE THE ADHESION OF TUMOR CELLS TO EXTRACELLULAR MATRIX PROTEINS

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Integrin receptors play a critical role in mediating adhesion to extracellular matrix proteins and invasion of tumor cells thus influencing their metastatic potential. In order to elucidate the impact of rIFN-α and rTNF-α on the regulation of integrin receptors, we studied constitutive and inducible $ß_1$-, $ß_2$-, and $ß_3$-integrin expression on breast cancer (MCF 7), colon cancer (HT 29), and glioblastoma (U 87) cell lines by flow cytometry using monoclonal antibodies specific for α-subunits ($α_1$-$α_6$, $α_V$) and ß-subunits ($ß_1$, $ß_2$, and $ß_3$). Adhesion to extracellular matrix proteins as laminin, vitronectin, fibronectin and collagen IV was measured by a recently established microwell fluorometric assay. Synthesis of extracellular matrix proteins of the tumor cells was measured by northern blots. $ß_1$-integrin receptors ($α_1$-$α_3$, $α_6$, $α_V$) were heterogeniously expressed on MCF 7, HT 29, and U 87 cell lines. $ß_2$-receptors (LFA-1, Mac-1, p 150, 95) were undetectable. The classical vitronectin receptor $α_Vß_3$ was only expressed in U 87 glioblastoma cell line. Both, rIFN-α and rTNF-α could selectively regulate integrin expression in all cell lines tested. Adhesion of tumor cells to extracellular matrix proteins showed striking differences. Attachment of breast cancer cell line MCF 7 to vitronectin was 4.5 ± 1.2 %, to laminin 13 ± 4.3 %, to fibronectin 22 ± 8.8 %, and to collagen 30 ± 9.8 %. In contrast, glioblastoma cell line U 87 expressing the vitronectin receptor showed marked attachment to vitronectin. Adhesion could be reduced by rIFN-α and significantly increased by rTNF-α. Only breast cancer cell line MCF 7 revealed significant synthesis of vitronectin. These results demonstrate: 1. $ß_1$- and $ß_3$- integrin receptors are heterogeneously expressed in tumor cell lines tested. 2. $ß_2$-integrin were not detectable. 3. rIFN-α and rTNF-α can selectively regulate integrin receptors and influence adhesion to extracellular matrix proteins.

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EVALUATION OF LATE CARDIOTOXICITY BY PULSED DOPPLER ECHOCARDIOGRAPHY IN PATIENTS TREATED FOR HODGKIN'S DISEASE

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Only limited data is currently available about therapy-related cardiac toxicity on hemodynamic parameters probably reflecting their clinical significance. The impact of valvular, myocardial and pericardial abnormalities on cardiac hemodynamics in patients treated for Hodgkin's disease with COPP/ABVD ± radiation was determined by submitting 49 patients to cardiac evaluation 2-12 years (median 5.37 years) after they had completed induction therapy. Diagnostic procedures to evaluate cardiac functions consisted of history, physical examination, bicycle exercise stress test, M-mode, 2D- and pulsed Doppler echocardiography, including the peak and integrated early (E, Ei) and late (A, Ai) diastolic flow velocities across the mitral and tricuspid valve as well as their ratios (E/A and Ei/Ai). The assessment also covered the diastolic filling period and the deceleration times of early diastolic transmitral, transtricuspid, and hepatic-vein flows. Doppler indices were compared to those of 25 age- and sex-matched control subjects. No patient reported symptoms related to cardiomyopathy or coronary heart disease. Pericardial thickening was demonstrated in 19/49 (38.8%), valvular thickening in 21/49 (42.9%) and reduced fractional shortening in 9/49 (18.4%) patients. The Doppler-derived mean E and A (± SD) of transmitral flow were 0.75 ± 0.14 m/s and 0.56 ± 0.09 m/s respectively in patients receiving chemotherapy and 0.81 ± 0.19 m/s and 0.63 ± 0.20 m/s in those with additional mantle field irradiation. There was no difference between mean E and A of transmitral flow of patients treated for Hodgkin's disease and control subjects. Furthermore, the transtricuspid and hepatic-vein flow velocities did not differ significantly. Although the present study demonstrates a high frequency of pericardial and valvular thickening in patients treated for Hodgkin's disease with the COPP/ABVD regimen ± mediastinal irradiation, these findings had no impact on cardiac flow velocities and may be thus of minor clinical relevance in cured patients.

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HOW MUCH ARA-C IS NECESSARY FOR A THERAPY OF AN ACUTE LEUKEMIA

H. Kreutzmann

A 29 year old woman came to me in May 1988 with a hemolytic anemia and an idiopathic thrombocytopenia (Evans-Syndrom) after a 2-month holiday in Chile, where a shigellosis was diagnosed. She had got antibiotics in Chile. For this Evans Syndrom she got 1oomg Corticosteroids a day and an increase of thrombocytes and Hb was observed.
In July 1988 she developed fever and a salmonella sepsis was diagnosed.
In Dec. 1988 she developed an acute leukemia M4. After 3xTAD chemotherapy and 1x HAM she had a complete remission, after another HAM-consolidation therapy a slight increase of blasts and a relapse was observed.
At 2o.7.89 she had got a bone-marrow transplantation from her brother, a complete remission was diagnosed at the 31.8.89. The relapse was seen at the 12.1o.89.
At the 13.11.89 a therapy with 2x2omg AraC for 5 days every 4 weeks was started, the thrombocytes raised within 6 weeks from 19.ooo to 93.ooo and a complete remission developed.
This therapy was continued till August 1991.
The patient is still in complete remission.
The results and mechanism of Ara C will discuss.

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MONOCLONAL ANTIBODIES AGAINST HUMAN THIOL-PROTEINDISULFIDE OXIDOREDUCTASE IN IMMUNOPHENOTYPING OF LEUKEMIAS AND LYMPHOMAS

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The thiol-proteindisulfide oxidoreductase (TPO, EC 1.8.4.2, proteindisulfide isomerase, EC 5.3.4.1) catalyzes interchanges of disulfide bonds mediated by low molecular weight thiols and is thought to be involved in the posttranslational processing of disulfide containing proteins. Using polyclonal and monoclonal antibodies against human liver TPO we could show that the main part of this enzyme is localized in the cytoplasm of cells producing disulfide containing proteins. Double staining experiments in flow cytometry and immunoprecipitation revealed, however, that this enzyme or an unknown homologeous protein is expressed on the plasma membrane of B lymphocytes, too. Immunophenotyping studies showed that the cytoplasmic TPO is expressed particularly in normal and neoplastic plasma cells. Furthermore the enzyme was found in different types of monocytes, fibroblasts, endothel cells of spleen and epithel cells of mucous membranes. In contrast to the widely distributed cytoplasmic form of the TPO, the plasma membrane TPO was found exclusively only on B lymphocytes and not on T or NK cells. All cells from B cell leukemias studies as yet did express this antigen. In conclusion , this new antigen seems to be a helpful marker in diagnosis of malignant lymphomas and leukemias.

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DETECTION OF MINIMAL MALIGNANT CONTAMINATIONS IN BONE MARROW AND PERIPHERAL BLOOD STEM CELL PREPARATIONS

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High-dose chemotherapy and subsequent autologous bone marrow transplantation (ABMT) or peripheral blood stem cell transplantation (PBSCT) have become a salvage therapy for patients with malignant lymphoma. The value of ABMT and PBSCT for women with high-risk breast-cancer (BC) is under investigation. Marrow involvement in NHL and BC is associated with a poor prognosis. The meaning of minimal malignant contaminations in autografts is not yet clear, but there is some evidence that it may influence the outcome of patients with NHL and BC.

We used a long-term liquid culture system for detection of occult tumor cells in BM and PBSC [1]. Samples were cultured in supplemented media for seven weeks. Each week half of the culture was removed, cytospin slides were made and stained with Wright-Giemsa stain. The remaining culture was supplemented with fresh media. In cases of BC, additionnally slides were made for immunostaining with antiepithelial antibodies. The system showed a sensitivity of about one in a million in dilution experiments of NHL or BC cell lines in marrow samples of vounteer donors. Samples from healthy donors were always negative. Malignant cells were cultured from BM with known involvement. We were able to culture suspected cells from cytologically and histologically normal BM and PBSC of patients with NHL and BC prior to ABMT or PBSCT without prior marrow involvement. Due to the short follow-up and the small number of patients, the clinical significance of these results is not evaluable at this time, but we hope that the system will be helpful to predict relaps in BC and NHL patients after autologous transplantation.

1: JOSHI, S.S., NOVAK, D.J., MESSBARGER, L., WEISENBURGER, D., AND SHARP, J. G.. *Bone Marrow Transplantation* 1990, **6**: 179-183.

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COMPARATIVE PHARMACOKINETICS OF IFOSFAMIDE (IF), 4-OH-IFOSFAMIDE (4-OH-IF) AND CHLOROACETALDEHYDE (CAA) IN PATIENTS ON FRACTIONATED I.V. IF THERAPY

V. Kurowski, T. Wagner

The initial metabolism of IF consists of 2 different pathways: enzymatic hydroxylation forming the cytostatically active metabolite 4-OH-IF and side chain oxidation resulting in the liberation of CAA, a compound with possible neurotoxic properties. We studied the pharmacokinetics of IF, 4-OH-IF and CAA in 11 patients with bronchogenic carcinoma receiving IF in a divided dose schedule (1.5 g/m² per day over a period of 5 days). Blood samples were drawn on day 1 and on day 5 up to 24 h after start of IF infusion. IF determination was performed by means of gaschromatography (NPFID). 4-OH-IF was measured using a HPLC assay with fluorometric detection of 7-OH-quinoline, which is formed by condensation of 4-OH-IF derived acrolein with m-aminophenol. CAA was quantified by gaschromatography with EC detection. On day 5 $t_{1/2}$ and AUC of IF were reduced by 30 %, a known phenonemen which is explained by self-induction of hepatic IF metabolism. This was accompanied by increased metabolite levels. For 4-OH-IF and CAA the mean c_{max}(day 5) : c_{max}(day 1) ratios were 1.94* and 2.05*, the AUC (day 5) : AUC (day 1) ratios were 1.51* and 1.29, respectively (*significantly different from 1, $p < 0.05$). These data reveal that fractionated IF doses increase the cancerotoxic effect of the drug; with higher IF doses also the neurotoxic effect of CAA may be evident after repeated IF application. With the IF dose examined in our study no change in the ratio of the 2 metabolic pathways was observed on day 5 (as compared to day 1).

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PCR-DETECTION OF t(15;17) POSITIVE CELLS IN PROMYELOCYTIC LEUKEMIA

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The t(15;17) which juxtaposes the PML gene with the gene encoding the retinoic acid receptor alpha (RARalpha) is the chromosomal hallmark of acute promyelocytic leukemia (M3). The translocation creates PML-RARalpha fusion RNAs which can be specifically detected by a polymerase chain reaction (PCR) using 5'primers from PML and 3'primers from RAR (deThe et al., Cell 66: 675-684, 1991). We have used this technique to analyze samples of three patients with M3 and t(15;17) before, during and after treatment with all-trans retinoic acid (ATRA) and conventional chemotherapy. PCR was positive at diagnosis in two of the three patients. One of the two PCR-positive patients had a short clinical remission, but relapsed early and died of a cerebral hemorrhage due to DIC after 6 months. Residual t(15;17) cells could be detected in blood and bone marrow samples of this patient at any time. The other patient is still in remission after 6 months. Interestingly, t(15;17) cells disappeared from the peripheral blood of this patient after chemotherapy, at least at a detection level of approximately $1/10^4$ cells. Our data indicate that (1) t(15;17) PCR can be used for the diagnosis of M3 in many, but not all patients, probably due to a certain heterogeneity of breakpoint location; (2) it is useful for the detection of minimal residual disease, especially since there may be a group of patients whose blood samples become negative after treatment.

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MUTATION AND EXPRESSION OF P53 IN TUMOR CELL LINES: COMPARISON OF IMMUNOLOGICAL METHODS AND NON-ISOTOPIC DNA SEQUENCING

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Mutation in the p53 gene has been reported to be involved in malignant transformation. Point mutations in the p53 gene are the most frequently identified genetic change in human cancer. The overexpression of p53 in tumor cells appears to reflect the accumulation of mutationally inactivated forms of the protein. In normal cells however, wild-type p53 has a short half-life and is generally not detectable. In order to study the sensitivity and specificity of different p53 specific monoclonal antibodies (PAb 1801, PAb 240, PAb 1620, PAb 421) we analyzed p53 expression by flow cytometry using Triton X-100, immunoblotting and immunofluorescence in colon cancer cell lines HT 29, NMG, LS 180, LoVo.
Furthermore, direct non-isotopic solid-phase sequencing of p53 cDNA was applied to detect point mutations in the highly conserved domains spanning from exon 4 to 9 of the p53 gene. All of the cell lines tested expressed high levels of p53 using PAb 1801 and PAb 240 in flow cytometry studies. In contrast, p53 expression could not be detected by the monoclonal antibody 1620. To exclude unspecific fluorescence induced by Triton X-100 additionly immunofluorescence and immunoblots were done demonstrating overexpression of p53 in all cell lines.
By direct non-isotopic solid-phase sequencing of p53 cDNA using the polymerase chain reaction HT 29 and LoVo showed G to A transitions resulting in missense mutations in aminoacids highly conserved in evolution. In conclusion, overexpression of p53 can be demonstrated by flow cytometric analysis using Triton X-100 in tumor cell lines and mutation of the p53 gene is associated with excess of p53 reflecting stabilization of the protein within the tumor cells.

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HICKMAN CATHETERS FORPARENTERALTHERAPY IN PATIENTS WITH MALIGNANTTUMORS

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One hundred and sixty patients with a median age of 38 years (range, 2 months to 84 years) having silicone rubber central venous access catheters for long-term parenteral nutrition or chemotherapy were studied prospectively. Two different types of catheters were used, the Broviac-type 'life-cath™ (Vygon, Aachen) and the Groshong™-catheter (Boehringer, Ingelheim). Intraoperative complications were not noticed. Parenteral therapy was performed in 124 patients (81%) without any complications. After a mean postoperative intervall of 36 weeks there were 34 catheters removed because of end of therapy. Seventy-eight patients died with the catheter in place. Fourty catheters had to be removed before end of therapy due to catheter-related complications. Occlusion of the lumen occurred in 9 patients after an intervall from 3 to 20 weeks. Explantation of catheters due to a suspected catheter-related sepsis was performed in 17 patients. Both types of catheter showed a high cumulative patency rate of 90% after a twelve months period. However, there were catheters that had to be removed before planned end of therapy. Thus, the cumulative rate of functioning catheters is lower (44% of all catheters).
In conclusion, Hickman type catheters are important devices for parenteral nutrition, chemotherapy and even bone marrow transplantation in patients with malignant tumors.

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INHIBITION OF CELL PROLIFERATION OF K 562 CELLS BY BCR/ABL TARGETED SYNTHETIC RIBOZYMES.

Winand Lange, Jürgen Finke, Gottfried Dölken.

In about 95% of patients chronic myelogenous leukaemia (CML) is characterized by the presence of the Philadelphia chromosome which is caused by a reciprocal translocation of chromosomes 9 and 22. The result of this event is a novel BCR/ABL fusion gene which codes for the K-28 and the L-6 type mRNA, both of which are translated into a protein of 210 kD; unique is that transcription and translation products can only be found in malignant and not in non-malignant cells. We therefore designed synthetic ribozymes which are capable of exclusively cleaving the BCR/ABL K-28 type mRNA without altering any normal cellular transcript. First cDNA segments carrying the BCR/ABL junction of the two target mRNAs and as controls cDNA of the ALL-type RNA as well as the normal ABL RNA were cloned into Bluescript. Transcripts were then incubated with different ribozymes that were directly transcribed from short ribozyme genes. K-28 type RNA could only be cleaved by the correct ribozyme and not by any of the controls. The K-28 type directed ribozyme on the other hand did not cleave any of the control RNAs. We then introduced the different ribozymes by lipofection into K562 cells which express only the K-28 type mRNA. A marked inhibition of proliferation was observed only with K-28 directed ribozymes; controls showed the same proliferation rate as lipofection agent alone or Hepes buffered saline. The effective delivery of ribozymes into living cells and their intracellular localization were shown by direct fluorescent microscopy; slot blot analysis revealed an intracellular stability of the ribozyme core sequence of at least 12 hours. The effective in vivo cleavage of K-28 mRNA was proved by primer extension assay. We conclude that ribozymes are able to inhibit the proliferation of malignant cells and that they might be possible therapeutic agents in CML patients.

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SEQUENCING OF REARRANGED IMMUNOGLOBULIN HEAVY CHAIN GENES OF LEUKEMIC CELLS

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A technique to sequence rearranged immunoglobulin heavy chain genes of leukemic cells was established. DNA was prepared from Ficoll-Hypaque seperated lymphocytes from peripheral blood samples from patients with chronic lymphocytic leukemia (B-CLL) and acute lymphoblastic leukemia at presentation (B-ALL) and amplified by polymerase chain reaction (PCR) in an automated thermal cycler. Using six different V_H-family-specific primers based on conserved 5'-leader sequences and a consensus J_H primer allowed amplification of almost the entire rearranged VDJ gene. By in vitro amplification of DNA prepared from 20 B-CLL and 7 B-ALL patients a DNA fragment of about 500 bp was obtained, which could not be seen with DNA from 6 healthy donors. Employing modified primers with restriction enzyme recognition sites incorporated we cloned several of the PCR fragments. Sequence analysis revealed homologies to V_H and D_H sequences known to be frequently involved in rearrangements of leukemic cells at least.
There are several applications of this PCR and sequencing strategy. Incidence of the various V_H-genes and V_H-gene-families in rearranged VDJ can be determined. PCR results have been compared to genomic southern blot analysis with a J_H-probe. In a number of B-CLL cases these studies revealed differences between the number of complete rearrangements as detected by the PCR described and higher numbers of non-germline-bands on southern hybridizations, indicating that a proportion of the latter might represent incomplete DJ rearrangements, translocations or deletions. Currently we are investigating the feasibility of this sequencing strategy to detect minimal residual disease in ALL.

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STRUCTURAL ALTERATIONS OF THE AMINOPEPTIDASE N (CD13) GENE IN MONONUCLEAR CELLS OF PATIENTS SUFFERING FROM LEUKEMIA OR LYMPHOMA

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The Aminopepetidase N (APN, CD13, EC 3.4.11.2) is a well established marker in the diagnosis of the myelomonocytic lineage leukemias. By means of both Southern analysis and PCR analysis of DNA isolated from mononuclear cells of peripheral blood or bone marrow aspirate structural aberrations in the APN-gene were observed in AML and NHL. These alterations were not found in mononuclear cells derived from normal probands. These findings could be of interest for understanding the molecular events provoking both diseases and might proof suitable as an additional diagnostic tool. We suppose a possible involvement of an APN-disregulation in the multistep tumorogenesis process in certain immuno-proliferative disorders.

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PROGNOSTIC FACTORS IN MYELODYSPLASTIC SYNDROMES

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The data of 69 patients with myelodysplastic syndromes (MDS) diagnosed from 1985 to 1991 were retrospectively analysed. 24 patients had RA, 8 patients RAS, 15 patients CMML, 10 patients RAEB and 12 patients RAEBt. Patients with RA or RAS and most patients with CMML only received transfusions. 14 of 22 patients with RAEB or RAEBt were treated with low dose cytosine arabinoside and 3 with aggressive combination therapy. According to Kaplan-Meier the median survival time of the combined RA/RAS group (36±17,5 months) differed significantly (p<0,05) from the combined RAEB/RAEBt group (9±7,5 months) and was not yet reached in the CMML group (medium observation time 20.4 months). The prognostic importance of 6 parameters at the time of diagnosis was evaluated for the RA/RAS group and for the RAEB/RAEBt group: Hb <10 g%, leucocytes <3000/µl, platelets <100000/µl, LDH >240 U/l, BKS >50mm first hour, splenomegaly. In the RAEB/RAEBt group none of these parameters was of prognostic importance. In the RA/RAS group Hb (p=0,006), platelet count (p=0,01) and splenomegaly (p=0,004) showed a significant influence on survival (Cox model, confidence interval 95%). The median survival time even of the unfavourable RA/RAS patients was still better than of the RAEB/RAEBt patients.

Conclusions: For all MDS-patients initial bone marrow blasts >5% signalize the worst prognosis. In the RA/RAS group initial hemoglobin, platelet count and splenomegaly have prognostic value. These parameters have no prognostic relevance in the RAEB/RAEBt group.

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MONOCYTOID B-CELL LYMPHOMA - A CASE REPORT

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Information on the clinical course and the prognosis of monocytoid B-cell lymphoma is limited. We observed an 58-year-old male patient with a very unfavourable course of this rare type of Non-Hodgkin's lymphoma. 20 years earlier a Hodgkin's disease had been successfully treated with splenectomy and combined radio-chemotherapy. The monocytoid B-cell lymphoma was diagnosed in 10/1991. The manifestations in cervical lymph nodes, stomach, ascites and pleural effusion were histologically and cytologically confirmed. Bone marrow and CNS were not involved. After chemotherapy with 3 courses of CHOP complete remission was achieved. After 2 additional courses the patient relapsed with an involvement of bone marrow, CNS and liver. Cytologically there was no evidence of a progression to a high grade lymphoma. The patient was then treated with a regimen including high dose methotrexate and died 8 days later in postcytostatic neutropenia (3/92).
This case confirms the already described tendency of the monocytoid B-cell lymphoma to manifest simoultaneously in peripheral lymph nodes and stomach. It further demonstrates that despite of its low malignant histology the monocytoid B-cell lymphoma can show the clinical feature and course of a high grade lymphoma.

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LOW-DOSE rIL-2 THERAPY IN MULTIPLE MYELOMA: CURRENT RESULTS OF A PHASE II TRIAL.

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Previous experiments raised evidence for the involvement of T-lymphocytes in control mechanisms of myeloma tumor cells. We could demonstrate in vitro that the growth of human myeloma tumor cells was downregulated by purified autologous CD3+ lymphocytes. This suppression could be enhanced by the addition of rIL-2 to the cultures. Using a cytotoxicity assay we demonstrated that peripheral blood mononuclear cells showed cytotoxic activity against autologous multiple myeloma (MM) tumor target cells. Based on these data we have initiated a phase II study in melphalan resistant progressive multiple myeloma patients with low dose rIL-2 on an outpatient basis. rIL-2 was administered at an initial dose of 9×10^6 IU/m^2 s.c. twice a day for 2 days followed by weekly administration of 0.9×10^6 IU/m^2 s.c. twice a day during 5 subsequent days until day 56 (q day 85). So far 18 patients have been treated for 1 - 30 months. In all patients the number of CD25+, CD3+, CD4+ lymphocytes increased (mean: 11% up to 24%) during therapy. NK-cell activity, determined by standard cytotoxicity assays, was augmented, and serum concentrations of sIL-2R rose as measured by an ELISA (mean: 2.5 up to 11.2 ng/ml). The observation time of 5/18 patients is too short for response evaluation. In 2/18 patients rIL-2 treatment induced tumor mass reduction, 5/18 patients achieved stable disease, and 6/18 continued with serologic progress during treatment. Side effects included local infiltrations at the inoculation sites, fever at the beginning of each treatment cycle, eosinophilia, temporary enlargement of the spleen, and slight liver enzyme elevations. These results show, that low dose rIL-2 application can augment suppressive immune functions, and that long term rIL-2 application may be a useful new therapeutic approach in multiple myeloma.

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TUMOR NECROSIS FACTOR-ALPHA MODULATES VINDESINE-RESISTANCE OF MULTIDRUG-RESISTANT PLEURAL MESOTHELIOMA CELLS

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Tumor necrosis factor-alpha (TNF) has been shown to synergistically potentiate the cytotoxicity of several anti-neoplastic agents. To examine whether multidrug-resistant cells are also targets of TNF, and whether TNF modulates chemoresistance, a pleural mesothelioma cell line, PXF1118L, and two chemoresistant sublines selected by vindesine (VDS) were used. As compared to the parental line, resistance was enhanced 40-fold resp. 170-fold. P-glycoprotein expression was increased in resistant cells as demonstrated by immunocytochemical peroxidase-anti-peroxidase staining with monoclonal antibody MRK16. Chemoresistance in these cells was confirmed to result from P-glycoprotein expression by decreased [^{3}H]-vinblastine accumulation that was reversed by a chemosensitizer, verapamil (VER), and enhanced VDS toxicity in the presence of VER. Parental and VDS-selected cells showed little, but comparable sensitivity to TNF alone. The combination of TNF with VDS or, to a lesser extent with doxorubicin, but not with cisplatin was more cytotoxic in multidrug-resistant cells than each compound alone. A synergistic interplay of TNF and VDS is thus suggested. In the sensitive parental cells, however, TNF had no synergistic or additive effect. Binding of [^{125}I]-TNF to sensitive and resistant cells was similar, thus the observed differences cannot be attributed to alterations of TNF binding sites. Since [^{3}H]-vinblastine accumulation was not altered by TNF, the mechanism by which TNF augmented the cytotoxicity of VDS is apparently different from that of VER. In addition, the chemosensitizing effect of the combination of TNF and VER was stronger than that of either TNF of VER alone. In conclusion, our study shows that TNF increased the antineoplastic activity of VDS in multidrug-resistant, but not in chemosensitive pleural mesothelioma cells by a mechanism that is different from chemosensitization by calcium antagonists. Further studies will be needed to prove whether the effects observed are restricted to certain tumor entities or not.

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TREATMENT OF POLYCYTHEMIA VERA (PV) BY ISOVOLEMIC LARGE VOLUME ERYTHROCYTE-APHERESIS (EA): SUPPRESSION OF RED BLOOD CELL (RBC) REGENERATION. THE POSSIBLE ROLE OF IRON.

T. Liersch, K. Vehmeyer, and U. Kaboth

EA is a very effective and well-tolerated treatment modality for RBC depletion in patients with PV [*]. Its long lasting effect (about 6 months) may, at least in part, be due to the massive loss of iron. Hematopoietic progenitor cells, as do all proliferating cells, need iron for their growth. Characteristically, in PV the erythropoietin (EPO)-independent, spontaneous growth of erythroid progenitor cells (BFU-E) is enhanced.

In this study we investigated the effect of EA in 6 cases of PV. Hematological parameters and the growth of hematopoietic progenitor cells (BFU-E, GM-CFU) were monitored. EA resulted in a profound reduction of the RBC count, hematocrit (Hct), and hemoglobin (Hb) in all cases. RBC parameters were before/after EA (mean values): RBC 7.74 / 5.97 x10^6/ml; Hct 54 / 41 %; Hb 16.3 / 12.5 g/dl and remained at these levels for several weeks.

In all patients a significant inhibition of the growth of BFU-E was detectable after EA while granulocyte-macrophage progenitor cells (GM-CFU) remained nearly unchanged. Within 3 to 6 weeks, the inhibition of endogenous BFU-E amounted to between 53% and 100% and of EPO-dependent BFU-E to between 31% and 74%. The inhibition of BFU-E after EA was completely abolished by in vitro addition of $FeCl_3$. On the other hand, in vitro exposure of progenitor cells to an equivalent concentration of the iron chelator DFO (Deferoxamine Mesylate) resulted in a total disappearance of progenitor cell growth.

The iron deficiency after EA was expressed less markedly in the reduction of the serum iron level (approx. 31% less than before EA) than in the reduction of the iron storage protein ferritin (approx. 41% less). These findings were accompanied by an about 2-fold enhancement of the expression of transferrin receptors on peripheral mononuclear cells, measured by flow cytometry.

Our data suggest that the beneficial effect of EA is not only due to the high volume removal of RBCs but also to the inhibition of the growth of EPO-independent and -dependent BFUs-E. The reduced proliferative capacity of erythroid progenitor cells is apparently maintained by the considerable loss of iron during the EA.

[*] Kaboth U., et al (1990) Klin Wochenschr 68: 18-25

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EMPIRIC ANTIMICROBIAL THERAPY IN FEVER AND NEUTROPENIA THE FIRST STUDY OF THE PAUL-EHRLICH-SOCIETY (PEG)

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In this prospective randomized trial different treatment strategies for patients with granulocytopenia $< 1000/\mu l$ and fever $\geq 38.5°C$ after chemotherapy were compared. 1573 of 1770 patients were evaluable with a median age of 48 (15-84) years, 90.2% of whom had acute leukemia or high grade malignant lymphoma. The initial treatment consisted of acylaminopenicillin (Pen) plus aminoglycoside (AG) or third generation cephalosporin (Ceph) plus AG or Pen plus Ceph. In 848 patients with fever of unknown origin (FUO) the response rates were: 70.8%, 69.8%, 65.1% total 68.4%. Patients (n=155) not responding received Pen/-Ceph/Vancomycin or Pen/Ceph/AG: response rates 50.7% and 50.0%, total 50.3%. If fever did not resolve the patients (n= 99) received either Pen/Ceph or Imipenem/Cilastatin in combination with Amphotericin-B/5-Flucytosin-/Rifampicin, resulting in response rates of 62.5% and 79.7%, total 72.7%. No significant differences between the treatment modalities compared were found. Analyzing all phases together 91.3% of patients with FUO were cured. The response rate in patients with gram positive bacteremia (n=183) was 82.5%, with gram negative organisms (n=145) 78.6%, in fungemia (n=51) 43.1% ($p<0.001$), with lung infiltrates (n=269) 61.3% ($p<0.001$), in clinically documented infections (n=198) 84.4%, in clinically and microbiologically documented infections (n=84) 82.1%. If infections were diagnosed after at least 5 febrile days, more lung infiltrates and fungal infections occurred ($p<0.001$). Leukocytes rising above $500/\mu l$ predicted better response rates ($p<0.001$): in FUO 97.8% vs 86.5% and death rates 1.5% vs 8.5%. In documented infections the response rates were 89.9% vs 62.3% and the death rates 7.0% vs 20.5%: Therapy of neutropenic fever and infections must be risk factor adapted and include early empiric antifungal therapy. The therapeutic and prophylactic use of hematopoietic growth factors to overcome neutropenia should be evaluated.

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G-CSF FOR TREATMENT OF FEBRILE NEUTROPENIA IN ACUTE MYELOBLASTIC LEUKEMIA AND LYMPHOID NEOPLASIAS

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If the neutrophil count does not rise at the onset of neutropenic fever, despite adequate antibiotic therapy, the risk of death is 8% in unexplained fever, 20.4% in documented infections and 26.8% in patients with pneumonia, as found in 1573 neutropenic episodes. The aim of this I/II phase trial was to determine the optimal dose of G-CSF in order to accelerate the regeneration of granulopoiesis to a maximum, when used therapeutically in fever with neutrophils less than $500/\mu l$. All patients were treated with standard combination of antibiotic therapy. 6 patients were treated with G-CSF, each at a dosage of 2, 5 and 10 μg/kg, and 7 patients with 20 μg/kg until a neutrophil count of $500/\mu l$ was reached. Glycosylated G-CSF was genetically engineered and derived from CHO-cells given as 30 min i.v. infusion. Only patients without leukemic cells in the bone marrow smear were entered into the trial (average age - 47 years; 16 male and 12 female). 21 patients had AML, 3 ALL, 1 high grade lymphoma, 1 hairy cell leukemia and 1 multiple myeloma. When treating with 2 μg/kg G-CSF the maximal neutrophil count was $6,500/\mu l$ and with 5, 10, or 20 μg/kg 19,700 - $28,500/\mu l$. There was an inverse relationship between the duration of aplasia before the use of G-CSF and the duration of G-CSF-therapy. One patient died from cerebral hemorrhage, one from congestive heart failure and one from interstitial pneumonia. At 2-10 μg/kg no side effects were observed, at 20 μg/kg one patient sufferred from fluid retention possibly due to G-CSF. No progression of leukemia was documented. We conclude, that G-CSF can safely be used at 2-10 μg/kg. The optimal dose for stimulation of neutrophil regeneration was found at 5 μg G-CSF/kg. G-CSF might be helpful for the treatment of febrile neutropenic patients with AML and lymphoid neoplasias, by enhancing neutrophil regeneration and thus improving the outcome of severe infections.

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CYTOMEGALOVIRUS ANTIGEN EXPRESSION IN LEUKOCYTES AFTER BONE MARROW TRANSPLANTATION

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Cytomegalovirus infection after bone marrow transplantation (BMT) is a major cause of lethal complications. Early and rapid diagnosis may enable early specific therapy before progression of the disease. We analyzed the number of leukocytes expressing the CMV pp65 antigen with specific monoclonal antibodies (Clonab, Biotest®), visualized by the APAAP technique. Cytospins with 20,000 leukocytes per glass slide were prepared regularly up to day 100 after BMT from 56 patients after allogeneic and 18 patients after autologous BMT. Standard methods for CMV diagnosis were performed weekly until discharge, then every other week. A CMV-infection was considered as symptomatic in the presence of clinical symptoms and a positive conventional test. Results: 18 symptomatic infections were observed (allo:14/56=25%, auto: 4/18=22%). Conventional methods had a sensitivity of 100% and a specifity of 23%. CMV pp65 expression had a sensitivity of 67% and a specifity of 57%. After allogeneic BMT a high number of pp65 positive cells was associated with the severity of CMV-disease. In CMV-asymptomatic patients, the maximal number (>0.1%) of pp65-positive cells correlated with the severity of aGvHD: aGvHD 0°: 22% of patients; I°: 31%; II°:36%; III°/IV°:50%, autologous BMT: 21%. In ten further patients the immunological subtype of immunomagnetically separated mononuclear blood cells expressing CMV-immediate early antigen (CMV-IEA, Clone E13, Biosoft®) was determined additionally. CMV-IEA was expressed earlier than CMV-pp65 in total leukocytes. In general CD4+, CD8+ and CD14+ cells were CMV-IEA-positive. CD56+ cells were rarely infected. CD19+ were not detctable within the first 10 weeks after BMT. The CD14+ cells had the highest percentage of CMV-IEA positive cells, ranging from 14-95%. Conclusions: This rapid method correlated well with the severity of CMV-infection and aGvHD in allogeneic BMT. CMV-IEA-expressing leukocytes could be detected earlier than pp65 positive cells. Monocytes were the predominantly infected mononuclear cells, arising early during the course of the CMV disease.

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LONGTERM FOLLOW UP OF SALVAGE TREATMENT IN RELAPSED AND REFRACTORY NSGCT USING ULTRAHIGH DOSE CARBOPLATIN, ETOPOSIDE AND CYCLOPHOSPHAMIDE WITH ABMT

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From March 1988 to September 1991 41 patients with relapsed or refractory non seminomatous germ cell tumors (NSGCT) were treated with carboplatin 2000 mg/sqm, etoposide 1500 mg/sqm and cyclophosphamide 120 mg/kg followed by retransfusion of bone marrow and/or peripheral blood stem cells (ABMT). All patients were deemed incurable with conventional therapy after second line cisplatin-based treatment. At time of ABMT 63 % of the pts. presented with advanced disease (Indiana staging > 6). Regarding response to prior chemotherapy pts. were either absolute refractory, progressive (unstable disease, increase of markers < 4 weeks) or refractory (stable disease, marker plateau, never CR or PR marker neg.) or had relapsed (CR or PR marker neg. > 4 weeks). 10/15 (67 %) pts. with refractory disease were responders (3 PR, 7 CR lasting 37+,36+,7,8,18+,17+,16+ months). 11/17 (64 %) pts. transplanted in relapse responsed (5 PR, 6 CR lasting 43+,28+,11,11+,10+,8+ months). 6 pts. with absolute refractory disease did not achieve CR with a survival not longer than 5 months. 10/13 pts. having achieved CR (median observation time 22 months) are still in continuous complete remission up to 43+ months without any further treatment. 2/3 pts. having relapsed 2 and 5 months after CCR hat primary extragonadal disease. This longterm follow up demonstrates that this regimen of ultrahighdose carboplatin, VP 16 and cyclophosphamide represents a curative option in heavily pretreated pts. with NSGCT.

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DETECTION AND CLINICAL RELEVANCE OF GENETIC ABNORMALITIES IN PEDIATRIC ALL: A COMPARISON BETWEEN CYTOGENETIC AND PCR ANALYSES

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Certain subtypes of ALL with a t(1;19)(q23;p13) or a t(9;22)(q34;q11) have been associated with poor prognosis. To test whether the employment of PCR improves the detection rate of these clinically relevant genetic anomalies we have developed a multiprimer-PCR protocol which facilitates the detection of each of the four chimeric E2A/PBX1 and BCR/ABL mRNAs in a single reaction. This protocol was used for the evaluation of bone-marrow or blood samples from 251 children with ALL in whom cytogenetic analyses had been performed. Twenty one patients carrying the E2A/PBX1 rearrangement and three with the BCR/ABL transcripts were detected by PCR. Twelve of these cases had escaped the detection by conventional cytogenetic analysis. In two of twelve patients with a typical t(1;19)(q23;p13), no E2A/PBX1 transcripts were identified by PCR, thus suggesting the presence of different molecular rearrangements. Residual leukemic cells were detected by PCR in five of eight patients who were followed during complete clinical remission. We conclude that the routine use of PCR may have an important impact on both clinical diagnosis and monitoring of minimal residual disease in patients with B-cell precursor leukemia who carry the E2A/PBX1 or BCR/ABL fusion genes.

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EARLY DETECTION OF RELAPSE BY QUANTITATIVE POLYMERASE-CHAIN REACTION IN PATIENTS WITH CHRONIC MYELOGENOUS LEUKEMIA AFTER BONE MARROW TRANSPLANTATION

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In patients with chronic myelogenous leukemia (CML), allogeneic bone marrow transplantation (BMT) is regarded as the only therapeutic approach with curative potential. However, disease recurrence after BMT remains a major clinical problem. Early identification of patients who will eventually relapse is therefore an important challenge in the follow-up of patients after BMT. The detection of residual leukemic cells carrying the bcr/abl rearrangement by highly sensitive techniques such as *qualitative* polymerase chain reaction (PCR) was shown to be of limited value in predicting disease progression. We have adapted the PCR for *quantitative* assessment of bcr/abl rearranged cells and applied the new technique to the monitoring of residual disease in CML patients after BMT. This approach was designed to provide information on the proliferative activity of the residual leukemic cells. Twenty six CML patients were monitored by qualitative and/or quantitative PCR during a follow-up period of up to 7 years after BMT. In the majority of these cases, enzymatic amplification of the bcr/abl rearrangement by PCR turned negative within 4 months posttransplant. In four cases, increasing numbers of leukemic cells were detected by *quantitative* PCR thus indicating the presence of a proliferating clone. So far, three of these patients experienced a relapse during the posttransplant course. The *quantitative* PCR technique may therefore play a prognostic role in the monitoring of residual disease by facilitating early detection of incipient relapse.

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REGIONAL HYPERTHERMIA IN RECURRENT DEEP SEATED PELVIC TUMORS: A STUDY OF FEASIBILITY AND RESPONSE

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This work is supported by Deutsche Krebshilfe (Grant M6/90/Fe6)

In the period from 1/89 to 6/91 25 patients with advanced recurrent pelvic tumors have been treated in about 100 regional hyperthermia (RHT)- sessions in the SIGMA-ring of the BSD-2000 system. Most of the patients had recurrent rectal or cervical cancer. In 16/25 patients RHT has been combined with radiotherapy. In 9/25 patients a bimodal or trimodal approach with chemotherapy or chemotherapy/radiotherapy has been performed. Temperature measurements were taken in a closed- end catheter which has been implanted under CT- scan guidance or fluorscopy from the vagina.

As a predominant side effect of RHT, some local discomfort or pain occured during or immediately after RHT in 70% of patients. These complaints are specific and will be discussed. The planned RHT courses were not accomplished in 6/25 patients: 2 cases of claustrophobia or psychic disorders; 2/25 tumor related necrosis; 2/25 thermal blisters in the rima ani.

The average SAR (specific absorption rate) in the target point of the tumor was in the range of 20-30 mW/g, the intratumoral temperature > 42 centigrade degree was achieved in 67% of patients. Overall objective response for these in most cases preirradiated patients was about 30%. However, a subjective palliative effect (e.g. pain decrease) was achieved in 70% of patients. Conclusions for further studies are extracted from the clinical results and will be discussed.

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EXPRESSION AND STRUCTURE OF THE p53 GENE IN ACUTE LEUKEMIA CELLS

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The 53 kDa phosphoprotein termed p53 acts as a tumor suppressor in vitro. Different forms of p53 altered by point mutations in portions of the coding region have frequently been detected in numerous types of human solid tumors. Finally, loss of one normal allele of the p53 gene is considered an important step in the development of several cancers. Promyelocytic HL-60 cells have complete loss of one p53 allele and gross alterations of the remaining allele. We examined p53 expression and gene structure in cells from 25 patients with AML and 30 patients with ALL. Using RT-PCR, p53 transcript segments of 445 bp (spanning codons 78-227 of exons 4-7) and 289 bp (spanning codons 214-310 of exons 6-9) could be amplified from total RNA from all ALL samples and from 18/25 AML samples. Structural analysis of the p53 gene locus by Southern blot using EcoRI, HindIII, BglII and BamHI revealed presence of a known BglII polymorphism in 4/36 patients and heterozygous loss of an EcoRI site 2.3 kb upstream of the promoter region in one case of ALL. This alteration might be due to a point mutation or small deletion. Control hybridization with a probe for M-CSF (located on the long arm of chromosome 1) did not indicate loss of a p53 allele in all cases studied. Direct DNA sequencing of PCR products was performed on 41 patients; however, no mutations were detected in any of the samples. Fine-mapping of the p53 gene rearrangement in HL-60 cells revealed at least three breakpoints, one in the central region of intron 1, the others 5′ of exon 4 and 3′ of exon 9, respectively. Transcript fragments encoded by the remaining exons could be detected by RT-PCR. Conclusions: active transcription of p53 can consistently be detected in primary cells from patients with ALL and frequently but not always in AML. Mechanisms that have been implicated in the pathogenesis of solid tumors, i.e. p53 point mutations in the highly conserved regions between codons 78 and 227, loss of alleles or gene rearrangements occur infrequently in the hematological malignancies examined. However, alterations may be complex, as exemplified by several rearrangements and deletions within the single allele of p53 in HL-60 cells.

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THE HUMAN LYSOZYME GENE IS DIFFERENTIALLY REGULATED DURING INTERMEDIATE AND LATE STAGES OF MYELOPOIESIS

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Lysozyme (LZM, EC 3.2.1.17) is a muramidase that is stored and secreted by mature myeloid cells and thought to be primarily involved in host defense. We have previously shown that LZM is differentially expressed and methylated in acute myeloid leukemias of different FAB subtypes (Lübbert et al., Onkologie 14 [suppl.]:100-101, 1991). In the present study, we examined patterns of LZM mRNA expression and its regulation during normal and leukemic myelopoiesis. The LZM gene was actively transcribed in cells of both promyelocytic (HL-60) and myelomonocytic (U-937) phenotype. In both cells, in vitro induction of macrophage differentiation using 12-0-tetradecanoyl-phorbol acetate (TPA, 10^{-7}M) resulted in rapid and drastic downregulation of expression. In contrast, treatment of HL-60 with interferon-gamma, Tumor Necrosis Factor alpha, dimethyl sulfoxide or cycloheximide did not result in significant alterations of transcript levels. Analysis of mRNA stability using actinomycin D did not reveal posttranscriptional means of TPA-induced downregulation of LZM in HL-60. This suggests a predominantly transcriptional mode of regulation which is being investigated using nuclear run-off. Treatment of normal human bone marrow cells with recombinant human Granulocyte-Macrophage Colony-Stimulating Factor (25 ng/ml) for 3 days resulted in an at least five-fold upregulation of LZM expression. In contrast, no transcription of LZM was detectable in early myeloblastic KG-1 cells using nuclear run-off, and no mRNA accumulation was detectable in either untreated cells or after treatment with TPA for various times. Also, LZM expression was undetectable in normal peripheral blood lymphocytes and lymphoid cell lines. In these different systems, expression of LZM strictly correlated with at least partial demethylation of a SmaI restriction site located 450 bp 5′ of exon 1 of the LZM gene; however, the cell-specific methylation status of this gene locus was stable during induction of terminal differentiation and concomitant regulation of expression. In conclusion, in the cell systems chosen, expression of lysozyme is differentially regulated during different stages of myelopoiesis. LZM gene expression and maturational arrest are associated with a variable degree of gene hypomethylation that is stable during in vitro differentiation.

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INDUCTION OF APOPTOSIS IN CHRONIC B CELL MALIGNANCIES

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Apoptosis is a distinct form of cell death characterized by chromatin condensation, membrane blebbing, the appearance of apoptotic bodies and nuclear fragmentation. Apoptosis leads to the activation of an endogenous endonuclease, which results in oligonucleosomal DNA cleavage. We studied the induction of apoptosis in chronic B cell malignancies by triggering the APO-1 antigen. In BCLL cells in vitro activation with Staphylococcus aureus cowan I (SAC) or Interleukin 2 (IL 2) resulted in APO-1antigen upregulation. Co-stimulation with SAC plus IL 2 resulted in a dramatic synergistic increase in APO-1antigen expression. BCLL cells stimulated with SAC plus IL 2 became sensitive for the apoptosis inducing effect of monoclonal anti-APO-1, whereas SAC or IL 2 stimulated cells were not susceptible to anti-APO-1 dependent apoptosis. In contrast apoptosis could be induced without prior in vitro stimulation in hairy cell leukemias revealing a constitutive expression of APO-1 antigen. Thus APO-1 antigen expression alone is not sufficient for the induction of apoptosis. We believe that SAC plus IL 2 stimulation prepared BCLL cells for the apoptosis inducing effect of anti-APO-1.This might be due to the processing of intracellular signals.

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A HISTOPATHOLOGIC SCORING SYSTEM FOR RISK ASSESSMENT IN MYELODYSPLASTIC SYNDROMES (MDS).

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Histopathological criteria for predicting survival in MDS patients are not well established. To identify those histological features which are most important for predicting life expectancy in MDS patients, bone marrow biopsies of 384 patients with MDS were evaluated in a retrospective study, and histopathologic parameters were correlated with clinical data and outcome of the disease.

A scoring system with 13 histological parameters was established, which allowed a discrimination of 3 patient groups with a mean survival of 34.6, 13.8, and 6.1 months, respectively (p-value = 0.00001).

Applying this scoring system to patients classified as RA and RARS according to FAB only, it was possible to distinguish 3 groups with different survival (A with 34.8 months, B with 20.3, and C with 7.0 months; p = 0.0001).

Meticulous evaluation of the histopathology of MDS in plastic-embedded bone marrow biopsies therefore seems to be of great value for classifying different risk groups of MDS patients, which provides a reasonable basis for clinical decisions regarding an individual risk-adapted therapy and helps to select those patients who would benefit of a more aggressive and special protocol.

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HISTOPATHOLOGY AND CLINICAL COURSE OF THERAPY-RELATED MDS (th-MDS). - A RETROSPECTIVE STUDY OF 384 MDS PATIENTS.

Hansjörg MASCHEK, Ralf GORMITZ, Vassiliki KALOUTSI, Axel GEORGII.

Among 384 patients with MDS, 30 patients with therapy-induced MDS (th-MDS) could be ascertained (15 women and 15 men). Median age was 63 years (minimum 44.4, maximum 79.0 years) in th-MDS. The most frequent basic diseases were carcinomas of the thyroid (6 cases), Hodgkin's lymphomas (5 cases), and mammary carcinomas (5 cases). 4 cases of thyroid carcinoma were treated with radio-iodium therapy alone, 10 of the 30 patients received chemotherapy only, 8 radiotherapy only, and 7 a combined chemo-radiotherapy. The minimum latency period between diagnosis of the primary disease and manifestation of MDS was 12 months, the maximum latency period amounted to 24 years; the medium latency period is thus 7.2 years. 37 % of the secondary myelodysplasias developed overt acute non-lymphatic leukemia (ANLL) in the course of their disease. - Classification of these 30 cases according to the FAB system revealed 10 patients with RA, 10 patients with RAEB, 4 patients not otherwise classified, 3 patients with CMMoL, 2 patients with RAEB-T, and 1 patient with RARS. Evaluation of the histopathology showed that sclerosis and fibrosis of the bone marrow occurred more frequently (33 % versus 17 %) in secondary than in primary MDS. Atypias of erythropoiesis can likewise be found twice as frequently as in primary MDS patients, whereas dysplastic changes of megakaryopoiesis and granulopoiesis did not show conspicuous differences in frequency and grade among patients with primary and with secondary MDS. Cases with increased siderin deposits in the reticulum of the bone marrow are observed significantly more rarely in secondary MDS than in primary MDS. - In contrast to other authors, we did not observe hypoplasia of hematopoiesis more often in th-MDS than in primary myelodysplasias.

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TREATMENT OF LUNG INFILTRATES IN PATIENTS WITH SEVERE NEUTROPENIA - RESULTS IN 269 PATIENTS FROM A MULTICENTER TRIAL.

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Patients with severe neutropenia after aggressive chemotherapy for high grade malignant hematologic disease who develop fever and lung infiltrates have a considerably poor outcome under conventional antibiotic treatment. Response rates of 30% are reported for this subgroup of patients from multicenter trials. As part of the first Interventional Antimicrobial Strategy Study of the PEG, patients with pulmonary infiltrates were initially randomized for a ß-lactam-aminoglycoside or a double ß-lactam combination plus rifampin and in case of non-response for amphotericin B plus 5-flucytosine either in combination with the former antibiotics or with imipenem/cilastatin already on day 4 to 6 of the study.
269 patients were evaluable for response, 71% of which had only clinically and 29% also microbiologically documented infiltrates. There was no difference in response rates between these two subgroups. As etiologic pathogens, fungi dominated with 43%, followed by gram-negative (31%), gram-positive (22%) pathogens and Pneumocystis carinii (4%). The overall response rate was 61.3% which was significantly lower than for other documented infections (82.9%) or for unexplained fever (91.3%)(p<.001). Mortality was 21.6% compared to 6.1% in FUO and 9.5% in other documented infections (p=.001). 44 patients had confirmed pulmonary mycosis. Their proportion on documented infections increased from 31% in the first week to 56% in 2nd and 3rd week with a mortality of 22% within the first 5 days. Only 27% of lung infiltrates could be cured by antibiotics alone.
Initially randomized combinations of antibiotics did not show significant differences in response rates. The empirical addition of rifampin had no beneficial impact on treatment outcome. Prophylactic oral antimicrobial treatment had no influence on response rates. The application of systemic corticosteroids did not improve treatment results. The trend in neutrophil counts and age were the only significant prognostic factors. Teatment results for neutropenic patients with lung infiltrates might be improved by early initiation of systemic antifungal therapy. Microbiologic diagnostics in neutropenic patients with lung infiltrates require further improvement.

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Successful treatment of chronic myeloid leukemia relapse after allogeneic bone marrow transplantation with α-interferon and transfusion of blood leukocytes of the bone marrow donor

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About 20% of patients with chronic myeloid leukemia (CML) develop hematological relapse after allogeneic bone marrow transplantation (aBMT). A new conception of relapse treatment studied by Kolb et al. (Munich) is based on the "graft-versus-leukemia" (GvL) reaction of immunocompetent bone marrow donor cells directed against the leukemic cell clone of the recipient. For this purpose, peripheral blood leukocytes of the donor are transfused to the patient not requiring immunosuppression because of persisiting immunotolerance due to mixed chimerism after aBMT. Additionally, α-interferon (α-IFN) is given to enhance the antileukemic effect .
We describe a patient with hematological relapse of Philadelphia chromosome positive (Ph+) CML one year after aBMT who was treated with α-IFN (5-8 x 10^6 U/day) and 10 single leukocyte transfusions ($\Sigma = 1.8 \times 10^{10}$ leukocytes) of his female donor. Four weeks later, he developed severe hepatic, intestinal and cutanous graft-versus-host disease (GvHD) requiring immunosuppressive treatment to avoid liver failure. Finally, not only hematological, but complete cytogenetic and molecular remission was achieved. A relapse constellation (46, XY, Ph+) was changed into cytogenetic remission with complete chimerism (46, XX, Ph-), and bcr-abl translocation could not be detected even by the polymerase chain reaction (PCR) method.
Successful treatment of leukemia relapse after aBMT with α-IFN and donor leukocyte transfusions reflects the antileukemic potency of this therapy, and further patients will be treated equally to determine its curative potency.

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HAEMATOLOGICAL AND INFECTIOUS COMPLICATIONS DURING CYTOSTATIC TREATMENT FOR SMALL CELL LUNG CANCER (SCLC) PATIENTS

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In clinical studies, during cytostatic therapy of SCLC-patients, hematopoietic growth factors are used to prevent the induced bone marrow toxicity. A detailed analysis of the hematological and infectious complications without growth factors is not available.
In a prospective study we evaluated the complications in the course of 107 patients who had been treated according to the EORTC-Trial: Alternating vs sequential chemo-radio-therapy and to the Havemann-Protocol: Etoposid/Vincristin vs AIO and Carboplatin/Vepesid. We documented hematological toxicity WHO-grade 3 and 4: Leukopenia 77%, thrombozytopenia 13%, anemia 8%. If during the treatment granulocytes decreased below 1000 x 10^6/l we used ciprofloxacin (2 x 500 mg p.o.) for infection prevention (65% of the patients). Fever episodes were seen in 78 patients (73%). In 65 cases (61%) infections were diagnosed clinically (62%) and microbiologically (34%). In 4% of the patients infections were suspected.
Manifestation of infections were pulmonal (purulent bronchitis 19%, bronchopneumonia 12%, pleural empyema 2%), sepsis 10% (bacteriemia 6%), oropharyngeal (25%) in the urinary tract (17%) and on the skin (15%). The prevailing germs were: Staph. aureus (21%), Strept. pneum. (11%), enterococci (11%), gram-negative cocci (18%).
The antibiotic response rate was 79%. 9 patients died during infectious episodes, 4 patients with an advanced tumor stage died as a result of the chemotherapy, 5 patients due to their underlying disease.
This analysis shows the necessity to improve the infection control during the intensive cytostatic treatment of SCLC-patients.

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WT1 EXPRESSION IN ACUTE LYMPHOBLASTIC LEUKEMIA

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The Wilms tumor suppressor gene (WT1) has been isolated and characterized recently. It is located at the human chromosome 11 band p13. The WT1 mRNA is expressed in a limited range of normal cell types, predominantly in kidney and spleen during early development. It was also found expressed in several sporadic Wilms tumors and some human tumor cell lines including the hematopoetic cell lines K562 and CEM.
The WT1 gene product includes a zinc finger region, potentially conferring DNA binding. It is suggested that the WT1 gene product functions as a transcription factor, being involved in differentiation.

Here we show WT1 transcription in a variety of immunologically well characterized human actue lymphoblastic leukemias (c-ALL, pre-pre B-ALL, pre T-ALL, T-ALL) utilizing RNA-PCR (polymerase chain reaction). Of 51 acute lymphoblastic leukemias (ALL), WT1 transcripts were detected in 30 cases (56 %). No correlation between WT1 expression and immunophenotype of the blast cells was found so far. In two cases one allele of the zinc finger region is altered. The precise genetic alteration was determined by automated solid-phase sequencing. Our data show a different expression pattern of the WT1 gene in ALL. As most of the ALL-patients are treated according to the AUL/ALL-study protocol, the expression of WT1 will be correlated to clinical risc factors, remission duration and overall survival.

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FREQUENCY ANALYSIS OF HUMAN STEM CELLS INDUCING LONG-TERM HEMOPOIESIS

H.-G. Mergenthaler and P. Dörmer

Long-term in vitro hemopoiesis strictly depends on the interaction of a heterogeneous population of adherent cells with very primitive hemopoietic stem cells (PSTC) from which clonogenic cells, e.g. granulocyte-macrophage colony-forming cells (GM-CFC), can be derived for many weeks. The stem cells inducing long-term in vitro hemopoiesis differ from clonogenic cells by their resistance to 4-hydroperoxycyclophosphamide and by differences in their antigen expression. In order to analyze the frequency of PSTC normal human bone marrow cells (BMC) were seeded onto subcultured and irradiated allogeneic adherent marrow layers in a wide concentration range. Thereafter, the cultures were incubated with weekly changes of half of the medium. After 5 weeks the cells were harvested and GM-CFC were determined individually from each microculture. The frequency of PSTC in the initial BMC sample was determined by employing Poisson statistics and regression analysis of the fraction of GM-CFC-non-producing cultures. In 6 different samples appx. 1 PSTC was found per 69,000 unseparated normal BMC resulting in a frequency of 14.5 ± 8.1 PSTC per 10^6 BMC. Our results demonstrate that the frequency of human PSTC can be analyzed in vitro which offers an interesting new tool for quantitative analyses at the PSTC level in the clinical bone marrow transplantation setting as well as in pathophysiological studies of stem cell diseases.

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CLINICAL ASPECTS ON NON-CUTANEOUS T-LYMPHOMAS

P. Meusers and W. Siegert

In chronic lymphocytic leukemia of T-cell type most cases exhibit T-cell receptor ß gene demonstrating predominantly large granular lymphocytes with granulocytopenia, associated with autoimmune disease and unlike the 'knobby' and pleomorphic subtypes a low frequency of skin infiltration and benign clinical course. Patients with the prolymphocytic leukemia of T-cell type unlike the B-cell equivalent, frequently have lymphadenopathy and skin infiltrations. Lymphoepithelioid lymphoma (Lennert lymphoma) with its histology suggestive of Hodgkin's disease and clinical presentation of a generalized non-Hodgkin's lymphoma (NHL) is of moderate aggressiveness. T-zone lymphoma exhibits a generalized lymphadenopathy, infiltration of lung and pleura and rarely of bone marrow. Angioimmunoblastic lymphadenopathy (AILD, LgrX) -type T-cell lymphoma is a distinctive disease complex including constitutional symptoms, generalized lymphadenopathy, rash, hepatosplenomegaly and autoimmune anemia. According data of a prospective study AILD behaves like a NHL of high-grade malignancy. The same is true for the T-cell counterparts of immunoblastic and the large cell anaplastic lymphoma (Ki-1 lymphoma). Data of retrospective and a few prospective studies seem to demonstrate that peripheral T-cell lymphomas of high-grade malignancy are associated with a poorer prognosis than their B-cell equivalents. Patients with adult T-cell leukemia/lymphoma (ATLL) ± HTLV 1 demonstrate skin involvement in more than 60-70% of cases, bone lesions often accompanied by hypercalcemia and exhibit a median survival probability of less than 6 months.

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MITOXANTRONE METABOLISM USING PRIMARY CULTURES OF RAT HEPATOCYTES

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The metabolism of mitoxantrone has been studied using hepatocytes in primary cultures freshly prepared from rat liver. Cells have been incubated for 24h with concentrations ranging from 10-100µM mitoxantrone. The metabolic pattern has been evaluated by high performance liquid chromatography using a method specifically developed for mitoxantrone and its thioether metabolites. By this technique three of the five metabolites occuring in intra- and extracellular compartment have been identified as a mono-L-cysteine conjugate, a monoglutathione conjugate, and a naphthoquinoxaline derivative of mitoxantrone. These metabolites have been independently prepared by chemical synthesis and their structures have been established by NMR spectroscopy and mass spectrometry.

The formation of these metabolites can be explained by cytochrome P-450 mediated oxidation of the phenylenediamine substructure to an intermediate quinonediimine. Our results emphasizes the importance of an oxidative biotransformation of mitoxantrone in cells to a quinonediimine intermediate characterized by a high alkylating potential.

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CLINICAL RESULTS AND BIOLOGICAL EFFECTS OF TWO DOSE MODIFIED DAILY ALTERNATING SCHEDULES WITH INTERFERON-α AND INTERLEUKIN-2 IN ADVANCED RENAL CELL CANCER

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Both, interferon-α (IFN-α) and interleukin-2 (IL-2) have some efficacy in advanced renal cell cancer (RCC) as single agents and act synergistically on activation of cytotoxic cells in vitro. In order to improve clinical results without more toxicity, we treated patients with advanced RCC in a daily alternating schedule of $10x10^6$ U/m² rIFN-α s.c. (Essex-Pharma, Munich) and $18x10^6$ IU/m² rIL-2 (EuroCetus, Frankfurt) as 1h infusion for 14 days. This cycle was repeated after a rest of 3-4 weeks up to a maximum of 4 cycles. In a following phase II study we reduced the dosages of IFN-α to $3x10^6$ U/m² and rIL-2 to $9x10^6$ IU/m².

In the first study, totally 36 patients were entered, 30 were evaluable for response. 2/30 achieved CR and 7/30 PR (CR+PR=30%) with a remission duration of 3-25+. One CR is still ongoing.

In the second study 15 patients are entered so far, 12 are evaluable for response. Up to now the response rate in this low dose study is only 8% with 1 PR.

In both schedules no grade IV toxicities were observed, the side effects were tolerable. No major difference between the higher and lower dosages were noted. In contrast to CIVI application of IL-2 no or only a mild capillary leak syndrome was observed.

High amounts of endogenous cytokine induction as IFN-γ, TNF-α and Il-6, sRIL-2 and an upregulation of endogenous IL-2 mRNA were detected. In the hiher dose study the lymphopenia was less pronounced than in the low dose study. Between responders and non-responders the only significant difference was a longer persisting lyphocytosis in responders. The preliminary data suggest a dose dependent reponse in RCC.

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ETOPOSIDE AND EPIRUBICIN CONTAINING CHEMOTHERAPY PLUS RADIOTHERAPY IN THE TREATMENT OF EARLY STAGES OF HODGKIN'S DISEASE (HD).

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ABVD seems to be at least as effective as MOPP in the treatment of HD with minimal risks of male sterilization and secondary ANLL when compared to MOPP. In an attempt to reduce toxicity the ABVD regimen was modified by replacing DTIC with etoposide, vinblastine with vincristine to avoid serious myelotoxicity and adriamycin with epirubicin to reduce the risk of cardiotoxicity. The regimen consisted of Epirubicin (E) 30 mg/m², Bleomycin 8 mg/m², Vincristin (O) 1.4 mg/m² i.v. d 1+8, Etoposide 100 mg/m² i.v. d 1-4 and Prednisone 40 mg/m² p.o. d 1-8 (EBOEP). Treatment was repeated every 3 weeks for 4 cycles followed by IF or EF RT with 30 Gy.

Fifty previously untreated patients (pts) with stages I-IIA,B with unfavourable prognostic factors, mainly mediastinal bulky disease or IIIA,B and 6 pts in first relapse following RT are so far evaluable. Twenty-four pts had B-symptoms. Nodular sclerosis (37/56) and mixed cellularity (14/56) were the predominating histologies. All pts entered complete remission with a median duration of 35+ months. The three year failure free survival is 90% for the previously untreated pts and 50% for the pts treated at first relapse at 3 yrs. Acute toxicity was minimal. Grade 3/4 toxicity was noted in 22% for leukocytes and in 3.5% for plateles of the chemotherapy cycles. The myelotoxic agents epirubicin and etoposide have been administered at the calculated doses in >90% of all courses. Severe nausea and vomiting (2%) were infrequent. Spermatogenesis was preserved in male pts. Cardiac and lung function testing did not reveal serious complications. Thus, EBOEP seems to be an effective regimen with the minimal acute toxicity, which should be introduced in the treatment of more advanced stages of HD.

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ETOPOSIDE AND INTERMEDIATE HIGH-DOSE ARA-C IN THE TREATMENT OF FIRST RELAPSE OF DE NOVO AML

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Combination chemotherapy of AML usually contain anthracyclines, which may interfere with bone marrow transplantation because of their cardiotoxicity. In a pilot study (unpublished results) the combination of etoposide (E) and intermediate high-dose Ara-C (I-HIDAC) demonstrated substantial activity in AML and has been introduced in the treatment of relapsing AML patients.

Patients: 29 pts with first AML relapse treated in 5 clinics are evaluable for response to treatment. The median age was 53 yrs. FAB-Classification: M1, M2 11, M3, M4,5 14, M7 2 pts. The median duration of the preceding first remission was 14 months.

Chemotherapy consisted of Ara-C 600 mg/m² every 12 hrsx8 and etoposide 100 mg/m² daily days 1-7. Pts received 2 courses of induction followed by an additional consolidation course. The study design of postremission treatment with Interleukin-2 and autologous bone marrow transplantation will be presented elsewhere.

Results and side effects: Twenty out of 29 pts (69%) achieved complete remission (CR), 2 PR, 2 were early deaths and 5 have been resistant to chemotherapy. The median duration of the second CR is 8+ months. In 5 pts (25%) the second CR was longer than the first CR. Four additional pts. are alive in CR 1-20 months after treatment. Infectious (FUO, pneumonia, septicemia) were the most frequent side effect. Chemotherapy was well tolerated.

Conclusion: Although preliminary, the results are encouraging indicating that aggressive chemotherapy followed by immunotherapy may substantially prolong second remission in pts. with relapsing de novo AML.

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APO-1/Fas, a new activation antigen and member of the NGF/TNF receptor family, in normal and neoplastic B cells. Co-regulation with ICAM-1 (CD54) in peripheral B cells and co-ordinate expression in a subset of follicular B blasts.

Peter Möller, Christof Henne, Frank Leithäuser, Anette Eichelmann, Silke Brüderlein, Jens Dhein, and Peter H. Krammer

APO-1/Fas is a 50kDa transmenbrane glycoprotein belonging to the NGF/TNF receptor family of surface molecules. Cross-linking of APO-1/Fas molecules via antibodies against this structure induces apoptotic cell death in sensitive cells.

Here we show that APO-1/Fas is an activation molecule on B cells. It could be induced/enhanced on dense and buoyant tonsillar B cells, respectively, through surface immunoglobulin cross-linking in combination with interleukin-2 or by interferon-γ together with tumor necrosis factor-α. These conditions also increased the amount of intercellular adhesion molecule-1 (CD54) on these cells. Epstein-Barr virus transformants of peripheral B cells co-expressed APO-1/Fas and CD54 at very high levels. Immunohistologically, Apo-1/Fas was detectable at low levels in a subpopulation of follicular center B blasts and, at higher levels, in sinusoidal B cells while follicular mantle B cells and plasma cells were negative.

Acute B lymphoblastic and chronic B lymphocytic leukemias and Burkitt's lymphomas were immunohistochemically devoid of both APO-1/Fas and CD54. At the individual level expression strictly corresponded in mediastinanal B cell lymphomas and statistically correlated in follicular center cell lymphomas ($p<0.0019$) but was less stringently associated in hairy cell leukemia. No association was found in plasmacytomas.This was in line with the differential expression of these molecules found in reactive plasma cells.

The co-ordinate expression of APO-1/Fas and CD54 might suggest that the receptor function of APO-1/Fas might be influenced by accesory molecules.

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INCREASED INCIDENCE OF HTLV1 REACTIVITY IN THE SERUM OF PATIENTS WITH MYELODYSLASTIC SYNDROMES

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We investigated patients with hematologic malignancies for evidence of HTLV1 in the serum. We included patients with the following diagnoses:
Acute myeloid leukemia, acute lymphatic leukemia, chronic myeloid leukemia, acute myeloid leukemia, myelodysplastic syndrome.
Additionally obtained sera from healthy platelet donors during the same period were also tested. None of the patients tested had a known risk for acquiring retroviral infection.
The results showed a high incidence of reactivity by both indirect immunfluorescence and Western Blot in the group of patients with myelodysplastic syndromes. Reaction was found in the group of patients with AML but to a lesser degree in the group of patients with chronic lymphatic leukemia, chronic myeloid leukemia and in the group of healthy platelet donors no reactivity was found.
The screening for HTLV1 which we carried out two years ago showed similar results.
Therefore we have to discuss: Whether the high occurrence is a results of the intensive supportive care required by the group of patients with MDS or whether the virus plays a role in the etiology of the disease.

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RESULTS OF DIAGNOSIS AND THERAPY OF PRIMARY EXTRANODAL NON-HODGKINS-LYMPHOMAS (NHL) OF THE GASTROINTESTINAL-TRACT (GIT)

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In this paper an account of results obtained by treatment of NHL-patients with primary manifestation into the GIT.
Sequentiell diagnostic programs and appropriate therapy were applied from January 1978 to December 1991 to 60 patients with primary GIT-NHL out of a large group of 952 NHL-patients.
All patients were examined and treated at the Department of Hematology, Clinic of Internal Medicine, Medical Academy Magdeburg.
The histological sub-types were diagnosed using KIEL-classification. Clinical staging was performed by the aid of modified ANN-ARBOR-classification. Survival courves have been calculated in accordance with product limit methods of KAPLAN-MEYER.
33 men and 27 womwn fell ill, most of them at an age beetwen 41 to 70 years.
A high-grade malignant sub-type was detected in 37 cases and 23 patients showed a low-grade malignant sub-type.
Long-term survival rates and diagnostic and therapeutic possibilities for the general improvement of therapeutic results will be discussed too.

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PHARMACOKINETICS OF HIGH-DOSE ETOPOSIDE IN CONDITIONING REGIMENS USED IN BONE MARROW TRANSPLANTATION PROCEDURES

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OBJECTIVES: VP-16 is one of the drugs being used for ablative chemotherapy prior bone marrow transplantation. We investigated the pharmacokinetics of VP-16 and studied the effects of VP-16 in bone marrow *in vitro* by measuring colony forming units of granulocyte or macrophage (CFU-GM) which have been shown predictive for hematopoietic reconstitution capacity.

PATIENTS, MATERIAL, and METHODS: 25 patients received VP-16 (30 - 60 mg/kg) in combination with either TBI or busulfan and cyclophosphamide prior BMT. Two days after the end of VP-16 administration the BMT was performed. Complete pharmakokinetics of VP-16 was established. Plasma samples containing VP-16 from different time points were used for the *in-vitro* cell culture experiments.

RESULTS: At the time of BMT significant amounts of VP-16 were detectable ranging from 60 - 1630 ng/ml. The terminal half-lives were significant longer than described in the literature ranging from 6 - 100 hours. The plasma samples from the day when BMT was carried out showed a significant inhibition of the CFU-GM in those samples with high VP-16 plasma levels.

CONCLUSION: The BMT procedure should be delayed if high plasma levels of VP-16 are detected. A drug monitoring procedure of the cytostatic agents being used in the conditioning regimen is recommended or if not possible, there should be at least an interval 72 hrs between VP-16 administration and BM infusion.

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RANDOMIZED PHASE II STUDY WITH EPIRUBICIN +/- VERAPAMIL IN ADVANCED BREAST CANCER PATIENTS

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INTRODUCTION: Anthracyclines are the most active anticancer agents in breast cancer patients. Drug resistance is the most important problem in metastatic breast cancer. Drug resistance modulation with high-dose verapamil (VPL) and intensive dose epirubicin (EPI) was applied to breast cancer patient with metastasis.

PATIENTS and METHODS: 51 patients entered the study, 26 were treated with EPI 40 mg/m^2 d3 iv bolus injection and 25 patients were treated with EPI (same dose and schedule) plus 4 x 120 mg VPL po d4. Response evaluation was carried out after three cycles. Study endpoints were the determination of the objective response rate and the overall survival time.

RESULTS: In the group of patients with EPI + VPL the objective response rate was 33 %, 38 % had an NC and 29 % a PD. The results in the EPI group were 33 %, 28 % and 40 % respectively. The median survival time was 8.9 month in the EPI + VPL group and 7.4 month in the EPI group. There was no statistical significant difference in clinical outcome and overall survival time.

CONCLUSION: The objective response rate was lower than expected. A resistance modifying action was not observed. VPL is not a good candidate for such trials and EPI at higher dose is as effective as at lower dose.

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PHARMACOKINETICS AND METABOLISM OF INTENSIVE DOSE EPIRUBICIN AND INTENSIVE DOSE VERAPAMIL

K.Mross, K.Hamm, D.K.Hossfeld

INTRODUCTION: Experimental data suggests that multi-drug resistance in cancer may be overcome by increasing the dose of anticancer agents in combination with a drug resistance modifying agent. We studied the pharmacokinetics and metabolism of epirubicin (EPI) as well as of verapamil (VPL) during a randomized clinical phase II study in breast cancer patients.

PATIENTS, MATERIAL, AND METHODS: Totally 11 patients with advanced breast cancer were treated either with EPI 40 mg/m^2 d3 or EPI (same dose) combined with VPL 4 x 120 mg po d4. EPI and metabolites and VPL and nor-VPL were determined by use of a HPLC assay procedure.

RESULTS: The c(t)-curves of EPI were identical in both treatment groups but the AUC of EPI was larger. Vdss, Clp and MRT were similar. The AUC's of E-GLU, EOL-GLU, EOL, AOLON, 7d-AOLON and 7d-AON were larger in the EPI+VPL group. The VPL + nor-VPL plasma levels varied between 100 and 1000 ng/ml (0.2 - 2 μmol)

DISCUSSION: The pharmacokinetics of EPI was altered by co-administration of VPL. The AUC's of the metabolites were higher in case of VPL administration. Enzyme induction or inhibition of excretion may offer an explanation. The VPL plasma levels varied in a wide range. Resistance modulation with VPL seems to be difficult under such circumstances.

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CMV INFECTION IN CORRELATION TO THE DEVELOPMENT OF GVHD MEDIATED SKIN LESIONS AFTER BMT

C.A.Müller, H. Schmidt, M. Steidle, J. Markovic-Lipkovski, H. Einsele

Since similar dermal lesions have been described to characterize human cytomegalovirus infection (HCMV) and GvHD related skin manifestations, a precise analysis of the relationship between HCMV infection and the development of cutaneous GvHR was performed on 118 skin biopsies obtained from 44 patients with and without GvHD after BMT, who were transplanted from HLA-identical sibling donors. Sensitive virus detection by PCR-DNA amplification was used and correlated to immunohistological and clinical alterations of skin GvHR to evaluate local HCMV involvment. 9 (29%) of 31 patients revealed presence of HCMV-DNA already before BMT in comparison to 3 of 20 (15%) controls of untransplanted patients with renal diseases. During the first 30 days after BMT a rise of skin HCMV infection was observed to 60% of the analysed patients in correlation with the development of cutaneous grade II-IV aGvHD. Sequential immunhistological staining in correlation to PCR analysis of skin biopsies in five patients with clinical signs of aGvHD after BMT revealed presence of HCMV before the development of abnormal expression of HLA-class II antigens on keratinocytes and of T-cell infiltrates representing established immunhistological criteria of dermal GvHD. Thus CMV may participate in tissue lesions not only by augmenting, but also by inducing aGvHD of the skin in humans.

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HLA-DP MATCHING IN BONE MARROW TRANSPLANTATION

C.A. Müller, G. Bitzer, G. Ehninger, H. Schmidt

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 still 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 74 patients (30 with CML, 6 with SAA, 35 with acute leukemia, 1 with myelodysplasia and 2 with lymphoma) and their bone marrow donors were determined by oligotyping. 62 times donor and recipient were HLA identical siblings, in 7 transplants the donor was a not completely HLA-A,-B,-DR matched relative and in 5 transplants a 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 6 out of 12 (50 %). Mixed lymphocyte cultures had been performed in all patients. The number of patients with GvHD index < 0.1 % was significantly higher in HLA-A,-B,-DR and -DP matched siblings compared to the pairs with a HLA-DPB mismatch. Preliminary results indicate even that patients with completely matched donors might suffer from less severe GvHD. Therefore it seems advisable to do HLA-DP typing especially since this difference could not be detected by mixed lymphocyte culture.

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CLINICAL OBSERVATIONS IN 23 PATIENTS WITH HEREDITARY THROMBOPHILIA

H. Müller, M. Oethinger, M. Grießhammer, E. Seifried

Hereditary causes of thromboembolism are becoming more evident since assays for antithrombin III (AT III), protein C, protein S and plasminogen are more widely available.
From 1982 to 1990 hereditary thrombophilia was diagnosed in 23 persons (mean age 28.6 years, 8 female, 15 male) of 14 families in our hospital. 15 patients presented with AT III deficiency, 2 patients had an abnormal low protein C level and 2 a decreased protein S level. Two patients presented with hypoplasminogenaemia and 2 with hypo-/dysfibrinogenaemia. Diagnosis of thrombophilia was evident in 21 cases after thromboembolism; thrombosis was determined in 19 patients under 40 years. Apart from 14 patients (60.8 %) with deep vein thrombosis (60.8 %) and 7 patients (30.3 %) with pulmonary embolism, some rare thromboembolic complications were observed: 1 case of mesenteric vein thrombosis and 4 cases of arterial thrombosis. In 3 cases the precipitating factor for thrombosis was simple trauma and in 4 cases surgical procedures, pregnancy in 3 cases and infection diseases in 4 cases. In 10 patients no such factor could be elicited. Two patients were asymptomatic. In 6 patients several thromboembolic complications occurred simultaneously. Coumarin treatment was started or continued in 16 cases. In one woman intraabdominal bleeding complications occurred during coumarin therapy. 7 patients were not started on oral anticoagulants for various reasons. A recurrent thrombotic event was seen in 3 patients, in whom the INR was not in the therapeutic range, and in 4 patients after cessation of anticoagulant therapy. One patient died from mesenteric vein thrombosis shortly after discontinuation of coumarins. One woman was successfully treated with AT III substitution during pregnancy without complications.
In conclusion, our data indicate that continuous oral anticoagulation may be beneficial in patients with hereditary thrombophilia.

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DECREASE OF PLATELET MEMBRANE INTEGRINS IN PATIENTS WITH SYSTEMIC HAEMATOLOGICAL DISEASES

H. Müller, I. Tüner, E. Gunsilius, H. Heimpel, E. Seifried

Introduction:

Acute leukemia (AL) and chronic myeloproliferative syndromes (c-MPS) are systemic haematological diseases, in which besides infection hemorrhagic complications are a major finding.Apart from disturbances of the hemostatic system the pathophysiological mechanism for bleeding complications is due to platelet dysfunction in these patients.
In the present study the expression of platelet membrane glycoproteins (GP) was investigated in 56 patients with acute myeloid (AML), or lymphoblastic leukemia (ALL) and c-MPS by flow cytometry using monoclonal antibodies.

Results:

Antibodies	GP Ib	GP IIb/IIIa	GP Ia/IIa	GP IV
AML n = 29	53 ± 24*	75 ± 25	53 ± 24	70 ± 29*
ALL/AUL n = 13	66 ± 23*	78 ± 24	58 ± 34	77 ± 28
c-MPS n = 14	48 ± 25*	76 ± 16	58 ± 16	68 ± 21*
controls n = 24	98 ± 11	97 ± 14	58 ± 11	98 ± 12

* $p < 0.05$ Mean ± SD

Conclusion:

The results suggest that in AML-, ALL- and c-MPS patients as compared to controls membrane glycoproteins, in particular GP Ib and GP IV, are decreased. This effect is most pronounced in AML- and c-MPS patients. Two possible explanations for these results can be discussed: Firstly, changes of proteolytic activity in plasma might degrade the membrane glycoproteins. Secondly, a pathological clone of megacaryocytes , in which distinct platelet membrane glycoproteins are absent, might be produced.

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Zielorientierte zytostatische Therapie mit Zytostatika-Hormonkonjugaten am Beispiel der Prednisolon- und Östrogenester des Chlorambucils

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Mit der Konjugation des Chlorambucils (CLB) an Steroidhormone wurde das Ziel einer größeren Tumorselektivität des CLB verfolgt.
Prednimustin, ein oral wirksamer Prednisolonester des Chlorambucils zeigte beim metastasierten Mamma-Carcinom sowie beim niedrig malignen NHL in verschiedenen Studien eine verbesserte Responserate und Verträglichkeit im Vergleich zur Therapie mit den Einzelkomponenten Chlorambucil + Prednisolon (Loeber et al. 1983; Möller 1985). PM zeigte darüber hinaus Wirksamkeit auch bei vorbehandelten Patienten, welche auf vorausgegangene Therapie mit Alkylantien + Corticosteroiden nicht angesprochen hatten (Pedersen-Bjeregaard et al. 1980).

Der Vergleich der Pharmakokinetik des PM versus CLB + P in aequihämatotoxischer Dosis (PM 300 mg - CLB 30 mg, P 50 mg) ergab jedoch auf eine aequimolare Chlorambucil-Dosis berechnet eine so viel kleineres Konz.-Zeit-Integral (AUC) des CLB für PM (15%) als nach Gabe von CLB, mit welcher die höhere cytostatische Aktivität des PM nicht zu vereinbaren ist.

Entsprechend der Grundidee, daß PM durch den Steroidanteil eine bevorzugte zelluläre Aufnahme in rezeptorpositiven Tumorzellen erfährt, haben wir in vitro wie auch in vivo Studien zur intrazellulären Pharmakokinetik des PM durchgeführt.
Die in vitro Experimente an verschiedenen Tumorzellinien ergaben intrazellulär hohe Konzentrationen an nicht gespaltenem PM, welches mittels FAB-Massenspektroskopie identifiziert wurde. Darüber hinaus unterschieden sich die mit PM inkubierten Tumorzellinien durch einen wesentlich verzögerten intrazellulären Konz.-Zeit-Verlauf von dessen Spaltprodukten von entsprechenden Tumorzellen, die mit den Einzelkomponenten CLB + P inkubiert wurden.

Auch die zelluläre Pharmakokinetik in vivo zeigte ähnliche zelluläre chromatographische Ergebnisse, wobei hier an der Strukturaufklärung noch gearbeitet wird.

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IN VIVO CYTOKINE INDUCTION BY LONG-TERM HIGH-DOSE RECOMBINANT HUMAN IL-3 IN A PATIENT WITH SEVERE APLASTIC ANEMIA

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We describe the course of a 37-year-old woman with severe aplastic anemia (SAA), who relapsed six years after anti-lymphocyte globulin (ALG) therapy. Treatment with intravenous recombinant human IL-3 (rhIL-3, Sandoz, Basel, Switzerland) at a dosage of 4 µg/kg/d for 21 days resulted in a moderate, but only transient hematological response. Subsequently, long-term therapy with subcutaneous rhIL-3 at the highest dose level tested so far (16 µg/kg/d) was initiated in order to maintain growth factor response. Therapy had to be discontinued ON day 73 for progressive thrombocytopenia and increased bleeding.
Intravenous rhIL-3 given as a 24-hour continuous infusion led to an increase in leukocytes from 1.5 to 2.3 G/l, due to an increase in monocytes, eosinophils and lymphocytes. After discontinuation of treatment, blood counts returned to pretreatment values. During subcutaneous treatment, leukocytes increased from 1.9 to 3.2, due to a 9.5-fold increase in monocytes, a twofold increase in neutrophils and a dramatic, but asymptomatic increase in eosinophils (13-fold) on day 66. rhIL-3 had no effect on hemoglobin, reticulocyte counts, basophils, bone marrow cellularity or the proliferative capacity of hematopoietic progenitors. Side effects of both treatments were mild and did not exceed WHO grade II.
During intravenous treatment, serum IL-3 increased from undetectable levels before treatment to > 1 ng/ml. rhIL-3 treatment slightly induced serum interferon γ (IFN-γ) and tumor necrosis factor α (TNF-α), although pathological values were not reached. No other secondary cytokines (interleukin 1, IL-1, interleukin 6, IL-6) were induced at this dosage. Subcutaneous high-dose rhIL-3 resulted in IL-3 serum levels > 2 ng/ml. However, steady-state serum concentrations were achieved for the first time on day fourteen. They declined after day 35, probably due to the induction of neutralizing antibodies. Subcutaneous rhIL-3 induced IL-6, soluble IL-2 receptor (sIL-2R) and, to a lesser extent, neopterin. In contrast, secretion of TNF-α, as observed during low-dose intravenous application, was not induced by subcutaneous rhIL-3. IFN-γ, IL-1 or granulocyte-macrophage colony-stimulating factor (GM-CSF) were unmeasurable in the patient's serum.
Results indicate that (1) in vitro-effective serum concentrations of IL-3 are achievable by intravenous (4 µg/kg/d) as well as subcutaneous (16 µg/kg/d) administration, (2) despite subcutaneous administration, high-dose treatment resulted in markedly higher serum levels of IL-3, (3) only minimal side effects were observed during high-dose treatment, (4) cytokine pattern induced by rhIL-3 was dose- and route-dependent, leading to induction of TNF-α and IFN-γ at low concentration and of IL-6, sIL-2R and neopterin at high concentration, (5) despite treatment, IL-3 serum levels disappeared after three weeks, suggesting an induction of neutralizing antibodies. Determination of IL-3 serum levels and IL-3-induced cytokines during treatment may be useful in establishing optimal treatment protocols.

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IMMUNOGLOBULINS ALTER IMMUNE CELL ACTIVATION BY SELECTIVE CYTOKINE MODULATION

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Passive immunotherapy with intravenous immunoglobulin (IVIG) has been successfully employed in primary and secondary antibody deficiency syndromes. In more recent years IVIG has been shown to also be of benefit in the treatment of various autoimmune diseases including immune thrombocytopenic purpura (ITP) and Kawasaki syndrome. In order to better elucidate the mechanisms involved in the immunomodulatory activity of IVIG, we investigated the influence of Gamimune (7S-IgG; Cutter), Gamma-Venin (5S-F(ab')$_2$; Behring) and heat-stabilized Fc fragments (Behring) on in vitro proliferative and cytotoxic immune cell response, focusing primarily on the action of IVIG on cytokine release.
Intact immunoglobulins (Gamimune, 1-10 mg/ml) reduced alloantigen-induced proliferation of peripheral blood mononuclear cells (PBMC) by more than 60% in a dose-dependent manner, whereas F(ab')$_2$ fragments and Fc fragments suppressed mixed lymphocyte reaction (MLR) only at the highest dose level tested (10 mg/ml). In a similar fashion, IVIG suppressed lectin-induced proliferation of PBMC, interferon-induced MHC antigen expression on a colon carcinoma cell line and interferon-induced macrophage activation. IVIG preparations containing the Fc fragment of the immunoglobulin molecule also inhibited the cytoloytic activity of NK cells. Immunosuppressive activity of IVIG was mediated by selective cytokine modulation. Secretion of T cell-derived cytokines such as interleukin 2 (IL-2) and granulocyt-macrophage colony stimulating factor (GM-CSF) was significantly inhibited in the presence of IVIG, whereas monocyte/macrophage-derived tumor necrosis factor a was induced. Release of IL-1, IL-6 and interferon-g during MLR was not significantly affected by IVIG.
We conclude that immunoglobulins have potent immunomodulatory properties mediated in part by selective inhibition/stimulation of endogenous cytokine production.

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A POSSIBLE ROLE FOR MITHRAMYCIN IN THE TREATMENT OF OSTEOMYELOFIBROSIS: INTERFERENCE WITH SYNTHESIS OF EXTRACELLULAR MATRIX (ECM) IN PRIMARY HUMAN FIBROBLASTS THROUGH ALTERATION OF THE ECM SYNTHESIS/DEGRADATION BALANCE.

M.C. Nehls, D.A. Brenner, H.-J. Gruss, H. Dierbach, R. Mertelsmann, and F. Herrmann.

The synthesis and deposition of extracellular matrix proteins is highly regulated and plays a pivotal in vivo role during tissue development, homeostasis, wound healing, and inflammation. Overexpression of ECM proteins may lead to tissue fibrosis with severe impairment of organ function. Therefore, the accumulation of the ECM proteins collagen type I and fibronectin is a pathological hallmark of several fibrotic diseases including osteomyelofibrosis. Collagen type I is a triple helical molecule composed of two protein chains of the α1(I) and one of the α2(I) collagen gene. Progress has recently been made in the identification of important regulatory elements of the human α1(I) gene. Several binding sites for the GC-box binding transcription factor Sp1 were located in the first intron of this, to which enhancer-like activities were ascribed. We have functionally characterized four highly conserved GC-rich elements located in the α1(I) promoter. The two most proximately to the TATA-box (Footprint I and II, FPI,II) contain mutually exclusive binding sites for the transcription factors Sp1 and NF-I, thereby forming two direct repeats of NF-I/Sp1 switch elements. These two elements control most of the activity of 3.5 kb of 5' regulatory region of the murine α1(I) gene. We could demonstrate that the affinity of Sp1 to the two switch elements co-increases with α1(I) mRNA levels in a model of experimental fibrosis. Using the GC-binding antibiotic mithramycin we show that the
GC-rich elements play a crucial role in determining the high basal transcriptional activity of the human α1(I) gene in primary fibroblasts. Low concentrations of the DNA binding drug completely block collagen α1(I) gene transcription by directly interfering with transcription factor binding to GC-rich regulatory elements as shown by DNAse I protection and mobility shift assays. The activity of other genes including the collagenase gene are unchanged in nuclear run on assays. These results clearly indicate that α1(I) gene activity in human primary fibroblasts is strongly depending on GC-rich regulatory elements, while the activity of e.g. the collagenase gene is not. Thus it seems feasible to differentially change the balance of ECM synthesis/degradation in human fibroblasts by a DNA binding drug.

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IMMUNOPHENOTYPING OF ACUTE LEUKEMIAS: CONCORDANCE OF DIAGNOSIS IN TWO DIFFERENT LABORATORIES.

C. Nerl, D. Adorf, J. Thaller, W. Kaboth, E. Thiel and W.-D. Ludwig.

Routine immunophenotyping of acute leukemias has been established as a standard procedure in many laboratories. Although monoclonal antibodies (MAbs) of defined clusters and standard technical procedures are widely used, little is known about the concordance rate for the diagnosis of acute leukemia between different laboratories. In a consecutive series of 37 patients with acute leukemia (ALL, N= 30; AML, N= 7) immunophenotypic profiles, using a battery of up to 20 MAbs, were established at 2 different places (Munich = M; Berlin = B). Unseparated leukemic cells (bone marrow or peripheral blood) were shipped as heparinized samples from M to B usually arriving within 24-48 hrs later. An average panel consisting of 8 MAbs (identical in respect to the cluster designation) was used in all 37 patients in both labs.Only 2/8 MAbs (CD13, CD33) were purchased in both labs from the same manufacturer. Using flow cytometry (FACScan) 293 pairs of markers were investigated in the 37 patients. In the AML patients only one discordant result was obtained out of 64 marker pairs tested. In the ALL patients of 229 markers tested for in both labs 17 differing results were found. 10/17 were based on MAbs coming from different sources (CD20 = 5, CD10 = 3, CD19 = 2). On the contrary,7/17 different results were obtained using identical antibodies (CD33= 5, CD13= 2). Since the expression of these myeloid antigens was not detected in the second laboratory in 6/7 cases this effect might have been due to shedding along the way. On the contrary, testing for various T-cell antigens did not reveal any major discrepancies.Inspite of the discordant marker results described (18/293 = 6%) the final diagnosis made in the two laboratories was identical in 35/37 (95%) cases investigated. In the remaining 2/37 cases a different stage of differentiation of the same lineage was seen.We conclude that immunophenotyping, using a well selected panel of monoclonal antibodies, standard techniques and flow cytometry for analysis is a reliable method for the diagnosis of acute leukemias and can be performed with cells transported at room temperature even after an interval of more than 24 hours.

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axl, a novel receptor tyrosine kinase, is expressed preferentially in myeloid leukemias, interferon treated mononuclear cells, and normal human bone marrow

A.Neubauer, A.Fiebeler, C.A.Schmidt, J.P.O'Bryan, D.Huhn, E.Liu

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We previously described the isolation and molecular characterization of a novel receptor tyrosine kinase, *axl*, from two patients with chronic myelogeneous leukemia (Mol Cell Biol 11:5016). We now report on the expression pattern in hematopoietic tissues and in hematological malignancies. We used a sensitive reverse-transcriptase polymerase chain reaction (RT-PCR) based approach to detect *axl* expression. A 304 base pair (bp) fragment of the tyrosine kinase domain was amplified and its intensity compared with a 204 bp fragment of the same cDNA reaction using primers specific for α-actin. Expression was found in 6/7 myelodysplastic syndromes, 14/26 acute myeloid leukemias, 6/16 chronic myelogeneous leukemias (CML), 5/7 hairy cell leukemias, and 3/39 chronic lymphocytic leukemias. Furthermore, expression was found in normal human bone marrow (N=1), especially in bone marrow stromal cells, whereas normal peripheral blood did not express *axl* (0/11). One patient with CML showed *axl* expression only while he was treated with interferon (IFN). Additional *in-vitro* experiments showed that *axl* is induced in peripheral normal mononuclear cells upon incubation with α-IFN, but not with other cytokines tested. In conclusion, *axl* is expressed preferentially in myeloid cells, and its expression is induced upon incubation with α-IFN. The role *axl* plays in malignant transformation remains to be determined.
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Determination of calcitonin-gene hypermethylation using the polymerase chain reaction (PCR).

A.Neubauer, M.Ritter, E. de Kant, D.Huhn

Methylation of CG residues plays a major role in the regulation of gene expression in the mammalian genome. It has previously been reported that hypermethylation of the calcitonin gene on chromosome 11p, as determined with Southern blotting, can be observed in acute lymphocytic leukemias and Non-Hodgkin's lymphomas, but also in the progression of chronic myelogeneous leukemia CML) from chronic phase to blast crisis (Blood 77:2431 and 2435). In order to work with minimal cell numbers, eg from flow sorted cells, it would be helpful to develop a PCR-based approach to detect hypermethylation of the calcitonin gene. Genomic DNA was completely digested in the presence of an unmethylated PCR modified competitor. PCR was then performed using primers flanking four restriction sites in the 5'-region of the calcitonin gene. To exclude false negative results, primers specific for the ß-interferon gene without restriction sites for HpaII were included in the PCR reaction. Several human cell lines (Jurkat,Molt-4,CEM,HL60,SCC) showed evidence for hypermethylation, whereas, interestingly, the CML blastic phase line, K562, was completely unmethylated. These data show that PCR can be used to detect methylation in the human calcitonin gene and will probably help to better understand the basis of hymermethylation in the progression of human leukemias.

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p53 gene mutations in preleukemic states.

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The p53 gene is frequently mutated in solid tumors and hematological malignancies and these mutations may be important in tumor progression (Nature 348: 681, PNAS 88:5413). In addition, p53 mutations have been detected in the germ line in certain cancer prone families indicating its role in the initiation of malignant transformation (Science 250:1233; Nature 348:747; NEJM 326:1301;1309). We asked if p53 gene mutations can be detected in the myelodysplastic syndromes (MDS), a heterogeneous group of myeloid disorders, which can be used as a model system for leukemic transformation. Single-strand-conformation polymorphism (SSCP) and direct polymerase-chain-reaction based sequencing were performed to detect point mutations in 16 patients with MDS in exons 4-8 in p53. None of these 16 patients harbored a p53 gene mutation. In order to examine the role of p53 gene mutations in late steps of leukemic transformation, we asked if p53 mutations can be observed in the blastic phase of chronic myelogeneous leukemia (CML). However, none of 19 patients carried a p53 gene mutation. These results are in contrast to those published by others (JCI 87:2042), who investigated a group of CML patients mainly from India and found mutations in about 30%. These differences may point to the etiologic role of certain carcinogens and do not support the view that p53 gene mutations play an important role in the initiation and the progression of myeloid leukemias.

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CLINICAL IMPORTANCE OF MUTATIONS IN THE *RAS* PROTOONCOGENES IN *de novo* ACUTE MYELOID LEUKEMIA (AML).

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Mutations in the *ras* protooncogenes are the most frequent genetic alteration observed in AML occuring in approx. 20-25% of the cases. The significance of such mutations was studied prospectively in a well characterized CALGB cohort (median follow-up time: 3 years). Using the polymerase chain reaction (PCR) and mutational specific radioactively labeled oligonucleotides, mutations were found in 18/99 AML patients: N-ras in 10 cases, K-ras in 5, and concurrent N- and K-ras in three cases. The presence of mutant *ras* genes was found to be associated with a low percentage of blasts in the bone marrow (p=0.007). No other association between the presence of a *ras* mutation and clinical parameters (e.g. FAB) was detected. When the outcome of the *ras*-positive was compared to the *ras*-negative patients, a trend towards a better survival in the *ras*-positive group was seen (median survival *ras*-positive 1.55 years vs. 1.07 in the *ras*-negative group (p=0.088)). Age adjusted multivariate analysis revealed cytogenetics and the presence of a *ras* mutation as the two most important predictors for improved survival after age (p=0.01 for cytogenetics; p=0.02 for ras). This improved survival was associated with a trend towards higher CR rate in the *ras* positive group (83% vs 62%, p=0.1), indicating that ras positive AML may be more likely to respond to chemotherapy. Taken together the data show that *ras* mutations may define a subgroup of AML patients with a better prognosis.

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HEREDITARY PROTEIN S DEFICIENCY: FIRST DIAGNOSIS IN ELDERLY PATIENTS

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The prevalence of protein S deficiency (which has an autosomal dominant inheritance) in adolescent patients is about 8%. The first manifestation rarely occurs before the 10th year of life, and mostly takes place between the 20th and 35th year of life. An isolated protein S deficiency was first diagnosed in 11 elderly patients from nine families from January 1990 to February 1992. The age of these patients was between 60 and 75 years. The median protein S activity was 37% and the median protein S concentration was 44%. The other clotting parameters were within the reference range. Clinically, six patients showed fresh thrombembolic events and two patients reported pulmonary infarcts in the history. A further three patients had so far remained asymptomatic. The presence of a first manifestation of an inhibitor defect should thus be considered, even in elderly patients.

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HISTOLOGICAL TYPING AND GRADING OF MALIGNANT NON-HODGKIN'S LYMPHOMAS AND PATIENT SURVIVAL: THE KIEL CLASSIFICATION VERSUS THE WORKING FORMULATION

H. Nizze and U. Bühring

In 163 out of 251 histologically classified malignant Non-Hodgkin's lymphomas, a comparison was made of patient survival with respect to both histological type and malignant grade according to the Kiel Classification (KC) and the three prognostic groups of the Working Formulation (WF). As expected, the survival rate of 94 low-grade malignant lymphoma cases (KC) was significantly better than that of 69 high-grade cases ($p < 0,0001$). Similar results were found both for 46 low grade cases (WF) in comparison to 50 high grade cases ($p < 0,001$) and for 67 intermediate grade cases (WF) compared with the 50 high grade cases ($p < 0,05$). The survival rates of low and intermediate grade cases (WF), however, showed no statistically significant difference. The low- and high-grade malignant lymphoma entities of KC, which together constitute the intermediate grade of WF, differed significantly with respect to patient survival ($p < 0,05$). Therefore, it appears that an intermediate grade is not essential in the KC. Additionally, a grading of centroblastic-centrocytic lymphomas ($n = 20$), based on the size of centrocytes, demonstrated a better survival rate ($p < 0,05$) in cases of the small cell variant ($n = 12$) than in those of the large cell variant ($n = 8$). Sorting these same cases, based on the presence ($n = 9$) or absence ($n = 11$) of a mantle zone surrounding the neoplastic follicles, showed no significant difference in terms of patient survival. It is concluded that supplementary histo- or cytological grading of lymphoma entities can be prognostically useful within the framework of existing classification schemes.

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S-PHASE DETECTION OF IMMUNOPHENOTYPED LYMPHOMA CELLS FROM THE BONE MARROW WITH FLOW-CYTOMETRY

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The quantification of the DNA-content from cell suspensions is possible by means of the flow-cytometric cell cycle analysis.
Non-Hodgkin-Lymphoma (NHL) show clear differances within the proliferative activity. If we look at Lymphoma with low malignancy the part of cells in S-phase is most very small. We can often detect greater proliferation rates regarding high malignant NHL's.
If we carry out the cell cycle analysis with unfractionated cell suspensions there are difficulties by measureing the S-phase. Normal cells influence the size of the value in these analyses. This fact is very important if we investigate bone marrow infiltrations by NHL.
We point out a method of cell cycle analysis with immunophenotyped Lymphoma cells.
Comparing our results with the simple cell cycle analysis we can detect a higher exactness by measureing the proliferative activity of additional immunophenotyped cells.

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IMMUNOTYPING OF BLASTS IN REFRACTORY ANAEMIA WITH EXCESS OF BLASTS

J.Oertel and D.Huhn

A study of immunological markers was performed in 16 patients with newly diagnosed refractory anaemia with excess of blasts (RAEB) and RAEB in transformation (RAEB-T) and in 12 other patients with acute myeloid leukaemia evolving from myelodysplasia. Immunocytochemical investigation of bone marrow blasts was done using a modified indirect immunoperoxidase method. This technique permitted accurate morphological identification of blasts and other cells of bone marrow. The monoclonal antibodies used were anti-CD 34, -c-kit,-HLA-DR, -CD 13 and -CD33.
The range of CD34 expression of blasts in RAEB samples was 1 - 14% (mean 7%) and in RAEB-T samples 30 - 48% (mean 37%). CD 34 positivity was detected in 7 - 94% (mean 46%) of the bone marrow blasts in acute myeloid leukaemia evolving from RAEB!T. Expression of c-kit was demonstrated only in a low percentage of blasts in RAEB, RAEB-T and acute myeloid leukaemia following myelodysplasia. A high percentage of blasts (>30%) in RAEB, RAEB-T and acute myeloid leukaemia following myelodysplasia was HLA-DR, CD13 and CD 33 positive.
We observed the transformation from RAEB to acute myeloid leukaemia in three patients. The proportion of CD 34 positive blasts increased from 2 - 5% (mean 3.2%) to 15 - 72% (mean 48%).
These findings indicate that the positivity of blasts increases with the progression of myelodysplasia to RAEB-T and acute myeloid leukaemia demonstrating expression of stem cell immunophenotype of blasts in these disorders.

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DOSE-INTENSITY AND TOXICITY IN POOR RISK NON-SEMINOMATOUS GERM CELL TUMORS

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Patients with germ cell tumors relapsing after chemotherapy induced complete remission or with incompletely responding tumors under primary treatment have a very bad prognosis with a probability of cure below 20%.To improve their prognosis we started to evaluate the efficacy of high-dose treatment (HDT) followed by autologous stem cell rescue (ASCR). Between 8/89 and 2/92 we treated 55 patients with 56 cycles of escalating doses of carboplatin (1500 - 2000 mg/m^2), etoposide (1200 - 2400 mg/m^2) and ifosfamide (0 - 10 g/m^2). All patients had heavily pretreated tumors between 3 - 15 (median 6) cycles of cis-platin containing regimens and were considered incurable with standard therapy. Prior to HDT two cycles of conventionally dosed PEI were applied to test the sensitivity of the tumor and to bridge the time to HDT. The major toxicities were hematologic and renal or concerned the skin and the mucosae. Two patients (4%) died from treatment related toxicities (pneumonia n=1, renal failure + coma + pneumonia n=1). Response rates were CR/NED 12 (21%), PR-marker negative 16 (28%). At the evaluation 21 of 55 patients were alive in continuous CR/NED or marker-negative PR between 3+ and 26+ months. The probability of overall event-free survival is 34% including 8 patients with an event-free survival between one and two years. These results are very promising in the light of the otherwise extremely bad prognosis.

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Polymerase Chain Reaction for Detection of Cytomegalovirus DNA in Peripheral Blood Leukocytes after Transplantation

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Infections with Cytomegalovirus (CMV) are the most frequent viral complication after liver and bone marrow transplantation and are cause of severe morbidity and mortality. Early diagnosis of symptomatic CMV-infection allows early treatment and can therefore significantly reduce frequency and severity of CMV-associated complications. New sensitive diagnostic techniques for detection of CMV-antigen have been described recently.
We analyzed clinical samples (peripheral blood leucocytes) from 60 patients after liver trans-plantation for presence of CMV-DNA using the polymerase chain reaction (PCR). CMV-DNA was detected in 33 cases, 27 patients remained negative. None of these 27 CMV-PCR negative patients developed a symtomatic CMV-infection. Further, none of the 60 patients included in this study died due to CMV associated complications. 13 of the 33 CMV-PCR positive patients developed a symptomatic CMV-infection. Interestingly, 9 of these 13 patients were pretransplant seronegative for CMV (IgG ELISA). All 13 patients became CMV-PCR negative during antiviral therapy.
Monitoring of CMV-DNA in peripheral blood leucocytes with CMV-PCR does not allow discrimination between viremia and symptomatic infection. Yet, this technique allows -in combination with other parameters (i.e. pretransplant serostatus)- identification of patients "at risk" for development of symptomatic CMV-infection.

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MOLECULAR PATHOLOGY OF HEMOPHILIA A

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Mutations at the factor VIII gene locus causing Hemophilia A have now been identified in many patients from many different ethnic groups. Earlier studies used biased methods which detected repetitive mutations at several CG dinucleotides. More recently rapid gene scanning methods have uncovered an extreme diversity of mutations. The detection of mutations has been especially difficult for a genetic disease, such as Hemophilia A, in which the involved gene is very large and almost all affected individuals have different mutations, most of which being point mutations. So far, over 140 different small mutations-point mutations, insertions, small deletions- and 60 large deletions have been characterized. Using computer analysis the melting properties of factor VIII gene sequences to design primer sets for PCR amplification and subsequent denaturing gradient electrophoresis (DGGE) have been determined. With this method more than 80 different muations have been identified. Another promising method to find small mutations in an efficient way is the analysis of factor VIII gene transcripts, which can be recovered in very low amounts from lymphocyte preparations. Recently, on non-CD 4 cells factor VIII protein could be identified by the aid of immunofluorescence microscopy (appr. 1 out of 2000 cells).

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THROMBOEMBOLIC COMPLICATIONS (TEC) IN HIGH-GRADE MALIGNANT NON-HODGKIN LYMPHOMA (hNHL) BEFORE AND DURING COP-BLAM / IMVP-16 (CBL/IMV) POLYCHEMOTHERAPY

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593 patients (pts) with hNHL treated with CBL/IMV polychemotherapy in a prospective multicenter trial (BMFT) were retrospectively evaluated for incidence and localization of clinically manifest TEC.
To clarify the pathogenesis of TEC in hNHL pts the following parameters were analyzed in the TEC and non-TEC group : Age, sex, Ann Arbor stage, sites of hNHL involvement, evidence of bulky disease, histological subtype of hNHL, Karnofsky index, platelet count, hemoglobin and fibrinogen levels, TPZ, aPTT, LDH , response to chemotherapy, overall (OS) and relapse free survival (RFS).
In the TEC group [35 pts (5,9 %)] age (p=0,035) , Ann Arbor stage and LDH level at presentation were found to be higher and response to chemotherapy (p=0,042) , RFS (p=0,008) and OS lower than in the non-TEC group. Local compression of blood vessels by hNHL (18 pts) or a central venous catheter (2 pts) at the site of thrombosis were documented in 20 pts (60 %) of the TEC group.
Conclusions: 1) TEC in pts with hNHL under CBL/ IMV is rare.
2) Age,a high tumor mass and an insufficient response to polychemotherapy are risk factors for TEC. 3) In contrast to other malignancies a paraneoplastic pathogenesis of TEC in hNHL pts may play only a secondary role, since thrombosis preferably develops at sites of blood vessel compression by hNHL and in elderly pts, but not during rapid destruction of bulky tumor masses and irrespective of the histologic subtype of hNHL.

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GROWTH REGULATION OF BLAST CELLS IN ALL WITH A TRANSLOCATION (4;11): INTERACTION BETWEEN KIT-LIGAND (KL), LYMPHO-HEMATOPOIETIC CYTOKINES AND STROMAL CELLS IN SERUM-FREE (SF) CULTURE

O.G. Ottmann, A. Ganser, and D Hoelzer

Coexpression of lymphoid and myeloid surface markers is a characteristic finding in lymphoblasts of ALL with a t(4;11). An analysis of their response to lymphoid and myeloid growth factors and stromal cells was the aim of this study. CD34+ blast cells from 3 patients with a t(4;11) ALL were cultured in SF liquid cultures in the presence of IL1, IL3, IL7, G-CSF, GM-CSF and KL, alone and in combinations, and in the presence or absence of bone marrow fibroblasts (Fb). Thymidine incorporation, immunophenotype, cell counts and morphology were done before and after 1-5 weeks of culture. Assays for leukemic colony forming cells and normal hematopoietic progenitors were performed using clonal cultures. In the absence of Fb, cell numbers decreased rapidly irrespective of the cytokine combination, a modest stimulation of thymidine uptake was seen primarily with IL3 and IL7. In contrast, an up to 12-fold increase of leukemic (CD19+/CD20-) cells was stimulated by IL3+IL7 in 3 week cocultures with Fb. While KL had no significant stimulatory effect on growth of leukemic cells, KL acted synergistically with IL3 and GM-CSF on the expansion of normal progenitors, with an up to several hundred-fold increase of CFU-GM after three and five weeks.
In conclusion, this culture system facilitates an expansion of both lymphoblasts and normal progenitor cells in t(4;11) ALL, and allows the analysis of differential growth requirements of normal and leukemic populations.

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VENOUS THROMBOPHILIA
I.Pabinger

A striking thrombotic tendency can be observed in certain individuals and families. It was in 1965, that Egeberg demonstrated a correlation between biochemical abnormality (antithrombin III-deficiency) and a thrombotic tendency. Further abnormalitites known to cause thrombophilia are protein C (PC)- and protein S (PS)-def. and dysfibrinogenemia. Other abnormalities (heparin cofactor II-def., factor XII-def. and abnormalities of fibrinolysis) are not truely established as risk factors. The presence of a lupus anticoagulant or malignant disease are acquired risk factors for development of venous thromboembolism.
The prevalence of antithrombin III (AT III)-, PC-, PS-def. and dysfibrinogenemia in patients with a history of thrombosis was found to be around 7%. The prevalence is increased about 2-fold in individuals with a positive family history. In 1% of patients with thrombosis a lupus anticoagulant can be detected.
There is a marked inter- and intrafamilial heterogeneity in the expression of clinical manifestations in individuals with a natural inhibitor def. Previous reports have shown that probably the majority of patients with hereditary PC-def. do not have an increased thrombotic tendency, on the other hand in a minority of families the thrombotic risk is very high. Patients with AT III-def. type II with abnormal heparin binding are not at an increased risk of thrombosis. Considering long term oral anticoagulant treatment information on the individual and familial history and on biochemical abnormalities should be available. Long-term treatment should be introduced in patients with recurrent thrombotic events and in patients with biochemical abnormalities known to increase the risk.

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DETAILED ANALYSIS OF GROWTH REGULATORY GENES IN HUMAN ACUTE MYELOID LEUKEMIA

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Several lines of evidence support the view that inactivation of tumor suppressor genes and/ or activation and deregulation of oncogenes play an important role in the development of neoplasia. We have studied blood and bone marrow samples of 20 AML patients and examined the expression of a number of oncogenes involved in growth regulation. In addition, we analysed the transcript and structure of the p53 gene using Northern and Southern blot analysis as well as single stranded conformation polymorphism (SSCP) and polymerase chain reaction.The myc, raf-1, fyn and vav oncogene was differentially expressed in all patient samples and the respective mRNA levels were high when compared to normal peripheral blood cells. Furthermore the p53 gene was strongly overexpressed in the leukemic cells. Using PCR and SSCP analysis, we searched for loss and mutations of the exons 5, 6, 7, 8 and 9 of the p53 gene in all 20 patients. The analysis revealed in 40% of the patients either a loss or mutation of the p53 gene. The most dramatic alteration was found in the exon 7 of the p53 gene, where the exon 7 was lost in 6 out of 20 patients. These results suggest that there may exist several mechanisms for the maintenance and progression of AML in vivo: (1) overexpression of oncogenes and (2) inactivation of p53 by either mutational activation or specific loss.

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ADHESION MOLECULES IN ESTABLISHED CELL LINES FROM MYELOID AND MONOCYTIC LEUKEMIA
G. Pasternak, L. Pasternak, and U. Karsten

Established cell lines from myeloid and monocytic leukemia are characterized by the expression of a variety of CD markers and adhesion molecules. As has been shown by immunocytochemistry the seven cell lines K-562, HL-60, KG-1, RC-2a, CTV-1, THP-1, and U-937 can be distinguished by five monoclonal antibodies. Each one of the cell lines exhibits its individual antigenic pattern which allows the correct identification of the in vitro culture. When tested for 25 different CD markers only 5 (CD 49e, CD 49f, CD 44, and CD 71) were found on all cell lines. In addition, the presence of the Thomsen-Friedenreich antigen, is a common feature of the leukemic cell lines as detected by our own monoclonal antibody A 68-E/E 3. The distribution of ß1 and ß2 integrins shows a broad variation among the cell lines investigated. While CD 29 is present in all cell lines except in RC-2a, CD 18 is expressed only in 5 of 7. Homotypic adhesion which can be seen during propagation of the cell lines is apparently due to the presence of ICAM-1, LFA-1, LFA-2, and LFA-3 on most of the leukemia cell lines. The cells adhere to monolayers of the human epithelial tumor MaTu.

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TUMOR ASSOCIATED ANEMIA IN SQUAMOUSCELL-CARCINOMA: TREATMENT WITH RECOMBINANT HUMAN ERYTHROPOIETIN (EPO).
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13 patients (12 men, 1 female; median age 59 years [range 45 - 85]) with histologically verified sqamouscell carcinoma and tumor associated anemia were treated with recombinant human EPO. Other forms of anemia (iron or vitamin deficiency, renal failure, hemolysis or chronic bloodloss) had been ruled out. 10 patients had previously received chemotherapy (in 8 cases cisplatin containing regimens). 6 patients had underwent irradiation prior to the enrollement in the trial. Epo was administered in a dose of 150 U/kg body weight thrice weekly. Unless the hemoglobin levels did not increase more than 1 g/dl within 6 weeks, an escalation of the EPO dose to 300 U/kg body weight was performed, without changing application intervals.
1 patient acquired pneumonia and died in the third week of EPO treatment. 12 patients are evaluable for the course of hemoglobin levels: in 2 cases, anemia could not be influenced. In the remaining 10 patients (83.3%) an increase of hemoglobin of > 2 g/dl was found. The median time to fullfill this response criterion was 5.5 weeks [range 1.0 - 10.7]. The median hemoglobin concentration increased from 9.9 g/dl [range 7.6 - 10.7] prior to EPO treatment to 13.85 g/dl [range 13.6 to 14.1] at week 12 of EPO therapy. Moreover, a statistically significant decrease of serum ferritin concentrations was observed, indicating mobilisation of iron stores.

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B-CELL LYMPHOMA LOCALIZED PRIMARY TO THE SUBCUTANEOUS ADIPOSE TISSUE

K.Pecze, A.Poloskey and I.Jakab,

Primary lymphoma of the subcutaneous adipose tissue is rare clinicopathological entity. Only a few previously documented cases have been found in a survey of the relevant literature. A case of primary B-cell non-Hodgkin lymphoma of the subcuteneous tissue is presented. In a case of 81-year-old woman the immunohistochemical investigation of subcutaneous adipose tissue pattern showed centrocytic- lymphoma. The patient is currently alive without evidence of lymphoma after chemotherapy /cyclophosphamide, vincristine, doxorubicin and prednisone (CHOP), six courses/.

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JUDICIOUS APPLICATION OF GROWTH FACTORS AND INTERLEUKINS IN MULTIPLE MYELOMA - A NEW APPROACH ?

D. Peest

Tumor cells in multiple myeloma exert a multiplicity of interactions with other cellular components, such as monocytes and macrophages, T-lymphocytes, polyclonal B-cells, hematopoietic progenitors, and cells involved in bone metabolism. A lot of experimental data and several clinical findings raise strong evidence that cytokines and other soluble factors have a predominant pathophysiological impact in this disease; e.g. paracrine produced interleukin-6 turned out to be the major growth factor for multiple myeloma tumor cells. Findings that cytokines can promote or inhibit tumor growth and may influence tumor complications by direct action on the plasma cells or indirect effects via stimulation of regulator cells suggest the use interleukins, hematopoietic growth factors, and other regulating factors for therapeutical approaches. At present several of these factors have been tested in vitro or are under clinical investigation. Among others, substances such as interferon-α, interleukin-2, interleukin-4, erythropoietin, GM-CSF, G-CSF, and monoclonal antibodies (anti-interleukin-6; anti-CD3) are of special interest. Previous and recent results clearly demonstrate potential impact of these factors. However, just as in other tumors, lots of effort is needed to elaborate the appropriate indications, optimal doses, and schedules for a judicious application of growth factors and interleukins. Multiple myeloma can serve as a suitable model for such an approach.

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Antibody response to pretreatment pneumococcal vaccination and posttreatment revaccination in splenectomized patients with non-Hodgkin lymphomas

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Splenectomized patients undergoing multiagent chemotherapy and irradiation are at highest risk for overwhelming sepsis with Streptococcus pneumoniae (S.pn.). In immunocompetent individuals pneumoccocal vaccines are protective against infections with S.pn. In the present series the antibody response in 11 splenectomized patients with non-Hodgkin lymphomas (NHL) vaccinated with a 23-valent pneumococcal polysaccharide prior to chemo- and/or radiotherapy was studied.

With the bacterial vaccine a 2-5 fold rise of the prevaccination titer against S.pn. (as evaluated by ELISA against an antigen from the 23-valent polysaccharide vaccine) was elicited in 4/11 patients with NHL. Prevaccination antibody concentration in patients with NHL was lower ($p = 0{,}001$) as compared to controls who had undergone splenectomy for other reasons. After vaccination no significant difference in antibody levels against S.pn. was evaluated between both groups ($p = 0{,}082$). NHL patients in remission after having completed chemotherapy received a booster dose of the polysaccharide. Revaccination did not increase the pneumoccocal antibody titer significantly ($p = 0{,}7$).

We conclude that vaccination with pneumoccocal polysaccharides in splenectomized patients with NHL elicits an antibody response in 36% of cases and should therefore be administered. However, a second injection of the bacterial vaccine does not contribute to the protection against invasive pneumococcal infections.

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Follicular dendritic cells in non-Hodgkin lymphoma and neoplastic B-cells display a complementary panel of adhesion molecules

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In non-Hodgkin lymphoma (NHL) with nodular growth pattern, follicular dendritic cells (FDC) form a spherical network which contains neoplastic B-cells. In order to dissect the basis of this close FDC/B-cell association, the antigenic profile of adhesion molecules expressed by individual FDC and NHL-B-lymphocytes was evaluated. FDC isolated from NHL were found to express HLA-ABC (MHC class I antigen), C3bi receptors (CD11b), the very late antigen (VLA) alpha-5- and alpha-6-chain (CDw49e, CDw49f) and the intercellular adhesion molecule-1 (ICAM-1; CD54). Only 50% of the FDC population was positive for the VLA beta-1- and alpha-3-chain (CD29, CDw49c), the vitronectin receptor (CD51) and the vascular cell adhesion molecule-1 (VCAM-1). B-cells obtained from the lymph nodes of patients with centroblastic-centrocytic lymphoma expressed ligands complementary to the adhesion receptors on FDC, *i.e.* LFA-1 alpha- and beta-chain (CD11a, CD18), and ICAM-1 (CD54). Interestingly, monoclonal lymphocytes in the peripheral blood of patients with a leukemic course of this lymphoma entity were devoid of these antigens. These data suggest that neoplastic B-cells without CD11a, CD18, and CD54 surface molecules are unable to associate with FDC and now invade other compartments. Thus, adhesive interactions between FDC and NHL-B-cells may account for the peculiar growth pattern and spread of follicular lymphoma.

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LEUKEMIC CELLS PRODUCE AN ATYPICAL MATRIX-METALLOPROTEINASE, WHICH IS NOT INHIBITED BY RECOMBINANT TIMPs

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In addition to the known 94 kDa metalloproteinase HL-60 cells release a hitherto undescribed 55 kDa metalloproteinase into the culture medium, which preferentially cleaves a peptide bond between alanine and glycine in a synthetic substrate. This proteinase, which is inhibited by chemical inhibitors (phenanthroline, EDTA) of metalloproteinases, degrades extracellular matrix constituents such as denatured collagen type I (gelatine), fibronectin, proteoglycans and collagen type IV. In contrast to all other matrix-metalloproteinases known at present the proteolytic activity of the 55 kDa enzyme is not abolished upon incubation with recombinant TIMP 1 or 2. Moreover, after incubation with recombinant TIMP 1 a 13 kDa cleavage product is generated indicating the proteolytic breakdown of the inhibitor. Thus, the 55 kDa enzyme may influence extracellular matrix turnover not only by proteolytic degradation of matrix constituents, but also by disturbing the balance between other metalloproteinases and their inhibitors through enzymatic inactivation of these proteinase inhibitors. Such a mechanism may contribute to the premature egress of leukemic cells from the bone marrow in acute myeloid leukemia.

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2-CHLORO-2'-DEOXYADENOSINE (CDA) AFFECTS MYELOID PROGENITORS BUT NOT STROMAL CELLS IN NORMAL HUMAN LONG-TERM BONE MARROW CULTURES (LTBMCs)

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2-chloro-2'-deoxyadenosine (CdA) is a new promising substance in the treatment of lymphoid malignancies. Neutropenia and bone marrow suppression is a common side effect in patients treated with CdA. It was the aim of this study to investigate the myelosuppressive effect of CdA in Dexter-type human LTBMCs. LTBMCs were analyzed weekly for up to 8 weeks. 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 CdA-dose of 5nM, no additional cell loss compared to untreated controls was found. However, the numbers of CFU-GM and BFU-E were reduced to 50% at week 1 compared to that of normals, 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 (8 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.
It is known that low doses of CdA reduce the release of IL6 from monocytes and that interleukins (IL6, GM-CSF) secreted by the stromal layer stimulate primitive hematopietic progenitor cells in normal LTBMCs. In order to exclude that the strongly reduced progenitor cell growth found in CdA-treated LTBMCs is the result of a reduced cytokine concentration in the supernatant, IL6 and GM-CSF were measured 3, 6, 12, 24, 48, 72 and 96 hours after 1/2 MC. The results show, that the levels of cytokines investigated were similar in normal and CdA-treated cultures. Therefore it can be suggested, that the dose dependent myelosuppressive effect of CdA is mediated by a direct action on progenitor cells and not by a functionally defective stromal layer.

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POINT MUTATIONS IN THE FMS ONCOGENE- A PCR AND CYTOGENETIC STUDY

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The c-fms oncogene has been shown to transform hemopoetic cells if point mutations at position 301 and 969 are involved. These point mutations have been demonstrated to occur in cells of patients with acute myeloid leukemias (AML) or myelodysplastic syndromes (MDS). In this study we evaluated clinical and cytogenetic data of patients with MDS (RA,RAEB or CMML) or AML secondary to MDS and compared them to the results of the molecular biological analysis.
20 bp long fragments of the c-fms oncogene containing codon 301 were specifically amplified from DNA isolated from patients with MDS using the polymerase chain reaction. Dot blots were hybridized with ^{32}P endlabelled wild type and mutant oligonucleotides.
DNA from patients with a 5q deletion hybridized only with the wild type oligo, indicating the absence of mutations in this fms region. Semi-quantitative PCR using the co-amplification of part of the beta globine gene as reference confirmed observations of fms hemizygosity in 5q deleted patients already found by quantitative Southern blotting.
DNA from 2 of 4 patients with translocations involving chromosome 5q showed cohybridization with both wild type and mutant allele, indicating a possible involvement of the fms gene in disease progression in these patients. The presence of point mutations could be used as a marker of the disease for follow up studies. Sequencing of the mutation positive PCR products in order to confirm the nature of the point mutation is in progress.

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BIOCHEMICAL CHARACTERIZATION OF A NOVEL AUTOCRINE TRANSFERRIN LIKE GROWTH FACTOR IN ACUTE MYELOBLASTIC LEUKEMIA.

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The impact of hematopoetic growth factors on proliferation and pathogenesis of myelogenous leukemia is currently the main focus of research. Nearly all leukemic cell lines are influenced by these cytokines in terms of growth stimulation or differentiation. On the other hand there is evidence that other nonhematopoietic growth factors eventually have comparable influences. In our lab human myelogenous leukemic cell lines have been adapted to growth in serum-free media without supplementation of any protein. Serum-free conditional media (CM) of these cell lines induce growth stimulation of other leukemic cell lines. A transferrin (Tf)-like iron binding kD 50 protein was found to be the main growth factor of these CMs. This protein is produced by the leukemic cells and cross-reacts with antibodies raised against human serum Tf. Purification and further characterization of this protein was performed by chromatographic methods, SDS-PAGE, peptide mapping and amino acid analysis. The kD 50 growth factor differs clearly from human serum Tf in terms of molecular weight, isoelectric point, and chromatographic behaviour. Digestion with V 8 protease, elastase, trypsin, BrCN ect. resulted in different digestion peptides as compared to Tf. Additionally with regard to human serum Tf main differences in amino acid composition exist for the content of following amino acids: Asp, Thr, Glu, Val, Ile, His, and Lys. Comparison with melanotransferrin p97 revealed a closer relationship with only the amino acids Thr, Ile, and His varying significantly. Nothernblot analysis of m-RNA of leukemic cell lines using antisense oligonucleotide probes specific for human serum Tf and melanotransferrin p97 show no idendity. These results suggest, that kD 50 growth factor may be an additional tumor associated member of the transferrin superfamily. Work is in progress to reveal amino acid sequence of this novel growth factor.

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INCIDENCE OF TAL-1 INTERSTITIAL DELETIONS IN T-ACUTE LYMPHOBLASTIC LEUKEMIA AND T-LINEAGE LYMPHOMAS

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The tal-1 gene is localized on 1p33 and has been found to be involved in chromosomal translocations in 5% of T-ALL and to carry an interstitial deletion in 25% of T-ALL (Jonson et al., 1989). It encodes a helix-loop-helix protein which is transcribed in hematopoietic precursor cells and may be involved in gene regulation during early hematopoietic differentiation. Tal-1 interstitial rearrangements represent recombination of two 5´ intronic regions separated by 80 kb in the germline configuration. Tal-1 rearrangements were found to be a marker for malignant transformation in T-ALL (leukemia specific marker). Additionally, lymphoid recombinase most likely mediates recombination and the junctional regions are highly diversified by N-nucleotides and exonucleolytic trimming (clone specific marker) of rearranging regions. Thus, tal-1 interstitial rearrangements potentially represent an ideal leukemia- and clone-specific marker for the detection of occult and minimal residual disease in ALL and lymphoma at diagnosis and after therapy, respectively. Therefore, the incidence of tal -1 interstitial rearrangements was studied in diagnostic tissues (>50% lymphoma/ leukemia involvement) of 41 T-lineage malignancies at different stages of differentiation: 10 T-ALL, 7 T-lymphoblastic lymphomas, 7 T-PLL, 2 T-CLL and 15 T-lineage non-HODGKIN´s lymphomas. DNA was extracted out of single cell suspensions or out of paraffin embedded tissue. Amplification of tal-1 interstitial rearrangement in CEM and of intronic sequences of the vWF gene in all samples studied served as positive controls. Tal-1 interstitial rearrangements were amplified by the polymerase chain reaction using intronic tal-1 oligonucleotide primers as described by Borkhardt et al., 1992., VNTR´s of the vWF gene using primers as described by Geiger et al, 1992. Amplification products were size fractionated on 1% agarose gels and visualized by ethidium bromide staining resulting in a sensitivity of detection of 10^{-2}. No tal-1 interstitial deletions were found in the 41 T-lineage malignancies while amplification products for vWF were readily obtained in all tissues. In conclusion, tal-1 interstitial rearrangements respresent an ideal leukemia and clone specific genetic marker, however our preliminary data indicate that the incidence of tal-1 interstitial rearrangements may be lower than previously reported in T-ALL and T-lymphoblastic lymphomas (n=17) and may be similar in T-lineage lymphomas at more mature stages of differentiation (n=24).

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TRISOMY 12 IN CHRONIC LYMPHOID LEUKEMIAS - A METAPHASE AND INTERPHASE CYTOGENETIC ANALYSIS

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Trisomy 12 is one of the most common chromosome abnormalities in B-cell chronic lymphoid leukemias, such as B-cell chronic lymphocytic leukemia or immunocytoma. It has been associated with early need for treatment and short overall survival. Cytogenetic analysis of chronic lymphoid leukemias has been hampered by the low in vitro mitotic activity of the leukemic cells. With the development of fluorescence in situ hybridization (ISH) techniques studies of chromosome abnormalities are possible not only in mitotic cells but also in interphase nuclei (interphase cytogenetics). We have analyzed 51 patients (pts.) with chronic lymphoid leukemias using fluorescence ISH. Mononuclear cells from blood, lymph node or splenic tissue were cultered in the presence of B-cell growth factor, anti-μ and PMA or calcium-ionophore and harvested according to standard protocols. Part of the chromosome preparations were G-banded with Wright´s stain for karyotype analysis. Fluorescence ISH experiments were performed on slides stored at -70°C using the following probes: D12Z3 (*Oncor Sciences*), biotin-labeled, a chromosome 12 specific centromere probe and pBS12 (kindly provided by Dr. J Gray), digoxigenin-labeled, a chromosome 12 specific DNA library. Hybridizations were performed as previously described (Lichter et al. *Hum Genet* 80:224-234, 1988). By using fluorescence ISH trisomy 12 was detected in 7 of 51 (13.7%) pts. In these cases the number of trisomic cells ranged from 27.7 to 77%. On G-banding analysis 18 of 42 (43%) pts. so far studied had clonal chromosome abnormalities but only 3 pts. had trisomy 12 and 1 pt. had partial trisomy 12. We conclude that fluorescence ISH is a more sensitive method than conventional G-banding analysis for the detection of trisomy 12. Fluorescence ISH will be of great value in more accurately assessing the prognostic impact of trisomy 12 in chronic lymphoid leukemias.

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OTOTXICITY OF CISPLATIN-CONTAINING CHEMOTHERAPY IN TESTICULAR CANCER.

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Between 1978 and 1991 we analysed 244 patients with nonseminomatous germ cell tumor in view of ototoxicity. 191 patients were administrated, containing cisplatin (CDDP) therapy. CDDP-containing treatment consisted of PVB/PEB in 135 patients, ECBC in 30 patients and VIP/EIP in 26 patients. An audiogram and an orientating otolaryngoscopical examination was performed before each cycle of chemotherapy and at least one audiogram after treatment as control in follow up. 69 patients (36%) revealed abnormalities in audiogram, in most cases a hearing impairment in the high frequency range from 4.000 to 12.000 Hz. Hearing loss in speech frequency range or symptoms like tinnitus were rare side effects. The primary treatment protocol seems to be of influence: whereas 38/135 PVB/PEB patients (28%) revealed abnormalities, this was the case in 20/30 ECBC patients (67%) and in 11/26 VIP/EIP patients (42%). Total CDDP doses below 400 mg already showed significant side effects, so that every patient receiving CDDP-containing treatment need a careful audiologic control. The follow up audiogram in some of these 69 patients showed reversibility of the high frequency hearing impairment in about 50%. Conclusion: We were able to confirm the ototoxic side effects of CDDP-containing chemotherapy. Repeated audiogram controls are necessary during treatment and follow up from the beginning of administration. In contrast to other reports, we found that in some cases the audiogram abnormalities tended to be totally reversible and the hearing impairment must not necessarily be symmetrical.

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CD44 AND CD44 SPLICE VARIANTS IN NORMAL DIFFERENTIATION AND IN TUMOR PROGRESSION

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Lymphogenic metastases of rat and human tumors carry one or several specific isoform(s) of the ubiquitously expressed surface glycoprotein CD44. These isoforms represent splice variants. They carry additional exon-encoded sequences in the extracellular portion of the molecule which are recognized by monoclonal and polyclonal antibodies.

Two of the isoforms were individually expressed in several non-metastasizing rat tumor cell lines. Beyond a threshold level these transfectants became fully metastatic in that they spread lymphogenically. The smallest isoform tested carries an 85 amino acid nonglycosylated extra domain in CD44. The critical role of these isoforms is further documented by the effective inhibition of metastasis formation by i.v. injection of antibody.

The genomic locus spans some 50 kb including at least 10 "variant" exons. Different splice variants are expressed in certain normal tissues, especially in the embryo and in selected epithelial cells of the adult. Interestingly, a small variant is transiently expressed on antigen-stimulated T and B lymphocytes and in macrophages. Antibody or F(ab´)2 fragments directed against the CD44 variant domain, block B and T dependent immune responses in vivo. We hypothesize that immune cells need the surface protein after meeting antigen in the periphery and for an interaction within the draining lymphatic tissues that permit activation and expansion of antigen-specific cells. Further we speculate that tumor cells in progression mimic lymphocytes.

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CORRELATION IN CHANGE OF TUMOR LOAD AND PATIENT'S COMPLAINTS AFTER CHEMOTHERAPY IN METASTATIC BREAST CANCER.

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It is assumed that tumor responses induced by palliative therapy are associated with a benefit to the patients. In this study we test the correlation of change in tumor load and change in intensity of somatic and psychological complaints. **Methods:** The study includes 153 assessments of tumor responses, somatic and psychological complaints in 83 patients with metastatic breast cancer. The patients were treated with one to three cycles of the Triple-M regimen. Complaints (patient's self assessment) and toxicity were recorded before treatment and simultaneously with the assessment of tumor responses after each of the Triple-M cycles in 6-weekly intervals; the intensities of complaints were classified from 1 (very mild) through 5 (very severe). The correlations of changes in tumor load and complaints were tested by using the Fisher's exact test. The sensitivity in detecting changes of the tumor load or of complaints was tested by using Bowker's symmetry test. **Results:** The tumor load was decreased in 25%, unchanged in 60%, and increased in 15% of assessments. The corresponding results for intensities of somatic complaints were (24%, 50%, 26%) and were (43%, 32%, 25%) for intensities of psychological complaints. No significant correlations were seen in the changes of tumor load and intensities of somatic or psychological com-plaints (all $2\sigma > 0.05$). The intensities of psychological complaints but not of somatic complaints were more frequently ($p < 0.05$) reduced than the tumor load when excluding those cycles where no psychological complaints were recorded before the cycle. **Conclusions:** Both, tumor load and complaints were reduced by treatment but tumor load and complaints were reduced in different patients (none of the correlations were significant). Psychological complaints (if not absent) were reduced more frequently than the tumor load. The first result raises the question on whether the reduction of the tumor load or the reduction of complaints or both reflect a benefit from palliation. The second result raises the question on whether the reduction of existing psychological complaints is reduced more frequently than the tumor load or patients reported the less psychological complaints the more the disease progressed. Both of these questions will be addressed by introducing an additional questionnaire for assessment of quality of life.

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AUTOANTIBODIES IN RENAL CELL CARCINOMA PATIENTS TREATED WITH RECOMBINANT INTERFERON-α2A

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Interferon (IFN) treatment may stimulate the formation of autoantibodies. We analyzed the occurrence of several types of autoantibodies in renal cell carcinoma patients (pts) treated with recombinant IFN-α2a (rIFN-α2a) in a controlled, adjuvant trial. Patients with resectable tumors ($pT_{3/4}N_{+/-}M_0$) were randomized after curative surgery to rIFN-α2a (9 MU s.c., three times per week for a maximum of 12 months) versus no treatment (control group). Patient sera were analyzed for the presence of antibodies to rIFN-α2a, extractable nuclear antigens (ENA), double-stranded DNA (dsDNA), pancreatic islet cells, adrenocortical cells, parietal cells of gastric mucosa (PCA), thyroid microsomes (MAB), and thyroglobulin (TAB). Prior to treatment, rIFN-α2a-binding antibodies were present in 5 of 203 pts (2.5 %) analyzed. Of 86 pts treated with rIFN-α2a for more than 2 months, 40 (47 %) developed IFN-α-binding antibodies. Among 62 IFN-treated pts analyzed 5 to 15 months after initiation of therapy, 17 (27 %) were positive for MAB, 18 (29 %) for TAB, and 3 (5 %) for PCA. In the untreated control group, the respective antibody frequencies were 3/62 (5 %) for MAB, 4/62 (6 %) for TAB, and 4/62 (6 %) for PCA. In 14 pts, MAB and TAB occurred simultaneously; 6 pts were positive for MAB only, and 8 pts were positive for TAB only. The presence of IFN-α antibodies, which appeared after a median treatment duration of three months, was not correlated to the induction of MAB or TAB. Thus, apart from the elicitation of IFN-α antibodies, prolonged treatment with rIFN-α2a preferentially stimulated the formation of thyroid-specific autoantibodies but had no impact on the frequency of several different autoantibodies in renal cell carcinoma pts. The presence of IFN-α-binding antibodies failed to inhibit the development of antibodies to thyroid antigens.

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IgG SUBCLASS DETERMINATION OF M-PROTEINS IN MONOCLONAL GAMMOPATHIES: TECHNICAL AND CLINICAL ASPECTS

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Immunofixation electrophoresis has become the standard technique for the detection of monoclonal components (M-proteins) in the sera of patients with suspected monoclonal gammopathies. Commercial kits are available for the rapid and convenient recognition of the principle immmunoglobulin (Ig) heavy and light chain types. Subclass characterization of IgG and IgA type M-proteins, however, is not routinely performed and may be difficult and expensive if carried out according to standard immunofixation protocols. We have developed a simple procedure for the subclass determination of IgG type M-proteins. Sera are separated by agarose gel electrophoresis as for immunofixation (Beckman Paragon® system). Separated proteins are transferred to nitrocellulose (NC) membranes by capillary blot within 20 minutes. Free protein binding sites are blocked with nonfat dry milk in PBS (Blotto) and single NC strips are incubated with highly diluted, IgG subclass-specific monoclonal mouse antibodies (MoAb). Moab are detected with peroxidase-conjugated goat anti-mouse Ig antibodies, and the reaction product is visualized with diaminobenzidine-H_2O_2 substrate solution. The procedure is highly sensitive. Detection limits for M-protein bands were 0.2 mg/l for IgG_1, 6.1 mg/l for IgG_2, 0.6 mg/l for IgG_3, and 0.2 mg/l for IgG_4. Sera from 189 pts with IgG type M-proteins were examined. One-hundred and fifty-one M-proteins (80 %) were IgG_1, 17 (9 %) were IgG_2, 9 (5 %) were IgG_3, and 6 (3 %) were IgG_4. In 6 cases (3 %), the IgG subclass remained uncertain owing to recognition of the M-component by more than one MoAb. Among 65 pts with plasmacytoma/multiple myeloma, the M-protein was IgG_1 in 50 (77 %), IgG_2 in 9 (14 %), IgG_3 in 5 (7.5 %), and IgG_4 in 1 (1.5 %). At the time of diagnosis, there were no IgG subclass-related deviations of laboratory parameters such as ESR, blood cell counts, coagulation parameters, serum Ig levels, presence of cryoglobulins, serum viscosity, retention parameters, and calcium levels in these pts. Likewise, frequency and severity of bone lesions and bone pain as well as the incidence of infectious episodes were independent of the IgG subclass of the M-protein. The same was true for the response to treatment and the survival time. Thus, subclass analyses of IgG type M-proteins may be carried out easily with the assay procedure described. The clinical relevance of these distinctions, however, remains to be established.

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RECOMBINANT ERYTHROPOIETIN (EPO) FOR THE TREATMENT OF ANEMIA DUE TO NEOPLASTIC DISEASE

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In this study 14 patients with hemoglobins < 10 g/dl and/or transfusion dependent anemia received subcutaneously human recombinant EPO (Cilag). Four patients had myelofibrosis (MF), three had multiple myeloma (MM), three had refractory anemia (RA), two had refractory anemia with excess of blasts (RAEB), one had chronic myelomonocytic leukemia (CMML) and one had chronic lymphocytic leukemia (CLL), respectively. None of the patients had iron deficiency, renal insufficiency or hemolysis. The patients were treated with doses of EPO ranging from 150 - 300 U/kg s.c. three times per week. Ten patients (2 MM, 3 RA, 1 RAEB, 3 MF, 1 CMML) responded to EPO treatment. Responders had EPO levels < 500 U/l at baseline, normal levels for TNF-α and only two of them were transfusion dependent with a short history of transfusions.

Responses were achieved after 3 - 18 weeks EPO treatment. Three MDS patients had a rise in hemoglobin level but failed to retain this after further 18 weeks-, 20 weeks- and 28 weeks-EPO application. EPO treatment was well tolerated. Bone marrow BFU-E numbers were low in all patients prior to treatment. Four patients did not respond at the end of 18 weeks treatment. This study suggests that a subset of patients who have EPO levels less than 500 U/l, normal TNF-α levels and low requirement for transfusion therapy may benefit from EPO application when given at pharmacological doses. Our findings further suggest that even initially responding MDS patients may become refractory to EPO treatment, in view of the trend of MDS marrow BFU-E to decline with increasing erythropoietin levels.

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DETECTION OF P53 MUTATIONS IN BREAST CANCER PATIENTS BY AUTOMATED SOLID PHASE DNA-SEQUENCING

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Mutations in the nuclear phosphoprotein p53 are the most frequent genetic alterations in human solid tumors detected so far. These mutations are clustered in highly conserved domains spanning from exon four to nine of the gene. Starting from DNA two to three overlapping PCR-amplifications were needed to span the whole mutation-prone region. Furthermore p53 sequencing data are badly needed to determine the specifity of monoclonal antibodies against mutant p53.

Thus we have established a very rapid non-radioactive solid-phase DNA-sequencing method starting from RNA to sequence through the p53 exons 4 to 9 in both directions with only one primer pair. The sequencing procedure outlined above yields sequencing data the same day. Because of the excellent purity of the sequencing template, reading of more than four hundred base pairs in one direction is possible as a routine. Sequencing with T7-DNA-Polymerase also allows detection of the heterozygous state, in which one allele shows the wild-type sequence, the other the mutated one, respectively. Our sequencing data are a prerequisite for the design of mutantspecific p53 oligonucleotides for the PCR-detection of minimal residual disease.

Breast cancer is one of the most common cancers in the female population and characterized by well defined risk factors for the prediction of clinical relapse such as tumor size, nodal status, tumor grading and estrogen receptor positivity and therfore ideally suited to address the impact of p53 mutations concerning the clinical course of the disease. So far we have sequenced the p53 tumor suppressor gene amplified from tumor samples of 38 breast cancer patients. Nine samples (24 %) showed point mutations with a resulting amino acid exchange. The value of p53 mutations as a independent prognostic risk factor in breast cancer is currently analysed.

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A RETROSPECTIVE STUDY ON CYCLOSPORIN A (CSA) TREATMENT IN 334 PATIENTS WITH APLASTIC ANEMIA (AA).

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The role of CSA in the treatment of AA has still to be defined. We report the results of a retrospective study conducted in 15 countries in Europe and abroad. A total of 334 patients resp. 353 treatment cycles has been analysed. Patient characteristics: initial manifestation 25%, relapsed 10%, refractory 65%. Severity: severe AA in 62% and very severe (VSAA) in 25%. CSA monotherapy (n=118) was compared with CSA+ALG (n=88), CSA + androgens (n=80) and CSA +ALG+androgens (n=39). Results: The overall response rate at 3 mo. (CR+PR) was 41%. Responses further increased at 6 mo.: 53% overall, 46% CSA-mono, 55% CSA+ALG, 52% CSA+androgens, and 70% CSA+ALG+ androgens. Obviously there is no major difference between CSA monotherapy and combined treatment modalities. However there was an advantage in survival for patients treated with CSA only. Response rates stratified by severity for CSA-mono were 53% in non-svere AA, 34% in SAA and 25% in VSAA. Increasing the dose of CSA did not result in improved response rates. Mean CSA dosage was higher in non-responders. There was no influence of serum creatinine increase on response. This study is a retrospective one with ascertainment bias. Beyond question, CSA is effective in AA and these data are an encouragement for further controlled trials.

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ANTISENSE OLIGODEOXYNUCLEOTIDES AS PROBES FOR STUDYING HUMAN HEMATOPOIESIS.

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The molecular events which regulate human hematopoiesis remain largely unknown. However, a number of candiate protooncogenes encoding nuclear transcription factors, elements of the signal transduction apparatus, growth factors, or their receptors, have been postulated to play an important role in governing this process. To directly validate the importance of these genes, and to gain an enhanced appreciation of their function, we have engaged in a series of "knockout" experiments using antisense oligodeoxynucleotides (oligos). Such oligos have been synthesized as native DNA (18 bp) or with phosphorothioate modified backbones (24 bp). The oligos are targeted against the mRNA of the protooncogene of interest around the translation start site. Using this strategy we have been able to disrupt expression of a variety of targets including the c-myb, and c-kit protooncogenes in both normal and malignant human hematopoietic cells. We have found for example, that disruption of c-kit gene in normal bone marrow mononuclear cells (MNC) profoundly impairs erythropoiesis. c-kit AS oligomers inhibit [IL-3 + erythropoietin] driven erythroid colony formation ~70%, and [kit ligand+ erythropoietin] driven colony formation 100%. In contrast, no effect on myeloid colony formation was observed indicating that c-kit function was of uncertain importance for development of myeolid progenitor cells. Using a similar model system we have also shown that the c-myb protooncogene is critical for the growth of all normal hematopoietic lineages and for malignant hematopoietic cell growth in vitro, as well. Recently, we treated human myeloid leukemia-SCID mouse chimeras with phosphorothioate modified AS oligodeoxynucleotides to c-myb. Animals receiving the AS oligomers lived three to eight fold longer than untreated controls, or those which received three different control phosphorothioate sequences. These results suggest that antisense DNA can be a powerful research tool with potential therapeutic applications as well.

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LIPOSOMAL AMPHOTERICIN B FOR TREATMENT OF CRYPTOCOCCAL MENINGITIS IN A PATIENT WITH BONE MARROW DISSEMINATED HODGKIN'S DISEASE IV_B

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We report a case of cryptococcal meningitis in an HIV-seronegative 22-year-old man with disseminated Hodgkin's disease stage IVB who was treated with *AmBisome*^{R} and *amphotericin B* in combination with *flucytosine*. Two weeks after the initiation of cytotoxic therapy (COPP/ABVD), the patient developed nausea and vomiting. At the same time he suffered from a constant frontal headache of increasing intensity accompanied by a clouding consciousness and diminishing vigilance. The cerebrospinal fluid taken at that time had a white cell count of 576 cells/μl. India ink capsule stains of the native cerebrospinal fluid showed massive infiltration with Cryptococcus neoformans. Antimycotic therapy was started with *amphotericin B* (1mg/kg/d i.v.) and *flucytosine* (4x2.5g/d i.v.). Seven days after the initiation of this regimen, cerebrospinal fluid white cell count increased to 712 cells/μl and cryptococcus neoformans could still be cultured. The clinical status of the patient deteriorated accordingly and he went into coma (Karnofsky index: 10). *Amphotericin B* trough levels of 0.068 mg/l in cerebrospinal fluid and of 2.05 mg/l in serum were detected 12 h after intravenous application of 60 mg (1mg/kg/d) *amphoteric B*. *Amphotericin B* was discontinued having no therapeutic efficacy after 7 days of treatment. *AmBisome*^{R} (2mg/kg/d i.v.) was applied instead for the next 28 days and *Flucytosine* was continued at a dosage of 4x2.5g/d i.v.. On day 3 of treatment with *AmBisome*^{R} the patient improved dramatically, regaining normal consciousness and vigilance (Karnofsky: 50). *Amphotericin B* trough levels of 0.11 mg/l in cerebrospinal fluid and of 3.12 mg/l in serum were detected 12 h after intravenous application of 120 mg (2mg/kg/d) *AmBisome*^{R}. Drug level measurements indicate that *AmBisome*^{R} is able to yield higher CSF drug levels than free amphotericin B under comparable conditions and could thus be more effective than free amphotericin B in the treatment of cryptococcal meningitis.

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ROLE OF DIPEPTIDYL PEPTIDASE IV (CD26) AND AMINOPEPTIDASE N (CD13) ON PROLIFERATION OF IMMUNE CELLS

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The dipeptidyl peptidase IV (DP IV) and aminopeptidase N (AP N), ectopeptidases of the plasmamembrane of lymphocytes, have been found to be involved in regulation of lymphocyte activation and proliferation in vitro. The aim of the present paper was to study the influence of peptidase inhibitors and antibodies on proliferation of PWM stimulated peripheral blood mononuclear cells (PBMC) and of DP IV or AP N positive monocytic and lymphocytic cell lines. We could show that specific DP IV and AP N inhibitors, monoclonal and polyclonal anti-DP IV and anti- AP N antibodies inhibited the DNA synthesis as well as the IL-2 and IL-6 production of PWM stimulated PBMC and arrested the cells in the G_0/G_1 phase of the cell cycle. The DNA synthesis of the DP IV positive U937, EL4.6.1 and 7TD1 cells was also found to be decreased in presence of DP IV effectors. Using AP N effectors, the proliferation of the strongly AP N expressing U937 cells was found to be suppressed, too. In future experiments the effect of these peptidase inhibitors will be studied with respect of there influence on the proliferation of neoplastic cells of the immune system.

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CLONOGENIC ACTIVITY OF CORD BLOOD CELLS COEXPRESSING CD34 ANTIGEN AND c-kit RECEPTOR

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Human cord blood or bone marrow cells expressing the CD34 surface antigen include a population of pluripotent progenitors. We identified and isolated a subpopulation of cells coexpressing CD34 and c-kit, a transmembrane receptor with tyrosine kinase activity. Novel monoclonal antibodies (16A6, 14A3, 3D6) directed against the extracellular domain of human c-kit were used for immunofluorescence labeling and sorting of low-density monuclear cells (MNCs) from umbilical cord blood and bone marrow. The frequency of c-kit labeled MNCs from cord blood (mean, 5.0% ± 2.1%, n=16) was similar to that from adult bone marrow (mean, 3.7% ± 1.3%, n= 4). On the average, 1.4% of CD34 positive cells were recorded in cord blood and 2.1% in bone marrow MNCs. Roughly 60% of CD34 positive cells coexpressed c-kit. The ability of $CD34^+$/c-kit^+ cells to form multilineage colonies (CFU-GEMM) was assayed after sorting with an antibody that did not show any significant effect on c-kit ligand or GM-CSF induced colony formation. For $CD34^+$/c-kit^+ cells we found a 20- to 50-fold enrichment as against total MNCs, and a twofold enrichment if compared to the $CD34^+$/c-kit^- population. Furthermore frequencies of BFU-e if compared to GM-CFC were enriched preferentially within the c-kit^+/$CD34^-$ population. To study expression of c-kit in lymphocytic precursors, monoclonal anti-CD7, anti-CD10, anti-CD19 or anti-CD38 antibodies were used simultaneously. In contrast to CD34 expressing cells, no consistent double-labeled subpopulation of lymphocytic cells expressing CD7 or CD10 was detected. The CD38 antigen, which has been used to discriminate the more mature progenitors within the CD34 positive population, was coexpressed on 73% ± 14% (n=3) of c-kit positive cells. Whereas the myeloic differentiation antigen CD33 was found on 30% ± 12% (n=5) of c-kit cells.

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HIGH GRAD MALIGNANT B-CELL NON-HODGKIN-LYMPHOMAS (B-NHL) AND B-CELL-ACUTE LYMPHOBLASTIC LEUKEMIA (B-ALL) IN CHILDHOOD: THERAPY STRATEGY AND RESULTS IN TRIAL ALL/NHL-BFM 86

A. Reiter, M. Schrappe, G. Henze, G. Mann, W.D. Ludwig, R. Parwaresch, ST. Müller-Weihrich, J. Ritter, Th. Klingebiel, E. Odenwald, H. Riehm

In trial ALL/NHL-BFM 86, 206 pts (9 mo - 18 y of age) with B-NHL (165 pts) and B-ALL (41 pts) were treated in 54 clinics in West Germany and Austria. The therapy strategy consisted of a cytoreductive prephase followed by two kinds of therapy courses: dexamethason 10 mg/m² days (d) 1-5, ifosfamide 800 mg/m² d 1-5, methotrexate 500 mg/m² (MD-MTX) 24-h-infusion, and MTX/Ara-C/prednisolon (triple drug) i.th. at d 1, Ara-C 150 mg/m²/12 h at d 4 and 5, teniposide 100 mg/m² at d 4 and 5. In every second course ifosfamide was replaced by cyclophosphamide 200 mg/m² d 1-5, and Ara-C/teniposide were replaced by adriamycin 25 mg/m² at d 4 and 5. Treatment intensity was stratified according to the St. Jude staging system (Murphy, SB.: Semin Oncol 7:332-339, 1980). Pts with stages I and II-R (completely resected) received 3 courses, pts with stages II-NR (incomplete resected), III, IV and B-ALL received 6 courses of therapy. For stage IV and B-ALL pts MD-MTX was replaced by high-dose-MTX 5 g/m², triple drug i.th. therapy was splitted (d 1 and 5), and vincristin 1.5 mg/m² was added at d 1. For CNS pos pts cranial irradiation (24 Gy) was optional. Local treatment was restricted to individual cases with incomplete regression after 2 therapy courses. The following table summarizes the results (probability of 5-year duration of event free survival (pEFS), median observation time 3 years, 6 months).

Stage	I	II-R	II-NR	III	IV	B-ALL
pts	28	13	28	73	23 (7 CNS+)	41
pEFS	1.00	1.00	0.96	0.73	0.63	0.78

Of 25 relapses 23 occurred within 12 months after diagnosis. The majority of failures were due to initial incomplete response to treatment or local relapse, preferably in pts with extensive abdominal tumors (LDH ≥ 500 U/L). Those pts of increased risk of failure are the target group of an intensified approach in the study NHL-BFM 90.

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ARE THE PRE - B LYMPHOID CELLS WITH STRONG CD38 EXPRESSION IN MYELOMA MULTIDRUG RESISTANT ?

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The different cell subpopulations (CSP) in the bone marrow obtained by needle aspiration from 30 myeloma patients were determined by flow cytometry. In double flourescence technique the expression of CD38, CD56, CD9, CD10, CD19, CD20, CD24, and CD34 was measured. For the determination of multidrug resistance we examined the inhibition of the rhodamine 123 efflux (I-R123-E) of the different CSP's. Rhodamine123 is a vital dye which is effectively pumped out from the cytoplasm by the gp - 170 protein.The I-R123-E was determined by flow cytometry in the lympoid, the myeloid and the myeloma area. Further we tried to detect the gp170 expression applying the mrk16 antibody.

We found that the I-R123-E is low in myeloma and myeloid cells and does not change during therapy. In the lymphoid cells the I-R123-E increased significantly during the cytostatic therapy. After several weeks without therapy the I-R123-E decreased spontaneously.

Within the lymphoid region we found a CD38 strong positive cell population . We sorted these cells and confirmed that only lymphoid but not plasma cells showed I-R123-E. In order to proof the hypothesis that the CD38 strong positive cells with I-R123-E are myeloma - associated cells we sorted the lymphoid cells with the highest R123 efflux for gene rearrangement analyses.

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EFFECTIVE IMMUNOTHERAPY OF ESTABLISHED HODGKIN'S TUMORS IN SCID MICE USING A CD16/CD30 BISPECIFIC MONOCLONAL ANTIBODY AND HUMAN PERIPHERAL BLOOD LYMPHOCYTES

C. Renner, C. Pohl, A. Hombach, W. Jung, U. Sahin, U. Kapp, J. Wolf, V. Diehl, R. Schmits, and M. Pfreundschuh.

In order to target NK cells against the relatively NK resistant Hodgkin's derived cell line L540, we developed bispecific monoclonal antibodies (Bi-MAb) by somatic hybridization of the two mouse hybridoma cell lines HRS-3 and A9 which produce monoclonal antibodies (MAb) with reactivity against the Hodgkin's and Reed-Sternberg cell-associated CD30 antigen and the CD16 antigen (FcγIII receptor), respectively. Crude supernatant of the hybrid hybridoma cell line HRS-3/A9 and purified HRS-3/A9 Bi-MAb triggered specific lysis of the CD30$^+$ Hodgkin's derived cell line L540 in vitro, but not of the CD30$^-$ negative cell line HPB-ALL by unstimulated peripheral blood lymphocytes and NK cell-enriched populations. Moreover, the Bi-MAb induced specific regression of established L540 Hodgkin's tumors in SCID mice after injection of human peripheral blood lymphocytes. HRS-3/A9 Bi-MAb hold promise as a novel approach of immunotherapy in Hodgkin's disease.

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ENZYMATIC OXIDATION OF THE ANTHRAPYRAZOLE DuP 937

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The anthrapyrazole DuP 937 has shown exceptional in vivo anticancer activity and reduced cardiotoxic potential in preclinical models and phase 1 and phase 2 studies. The antitumour effect of DuP 937 seems to be associated to intercalative binding to DNA and interaction with DNA-Topoisomerase.

In analogy to mitoxantrone we found that the phenylenediamine-analogous moiety of DuP 937 can be oxidized by activated horseradish peroxidase. The oxidation produces an quinonediimine-analogous intermediate with electrophilic properties. In the presence of glutathione two monoglutathione conjugates and one diglutathione conjugate have been identified. After chromatographic separation the chemical structures of these conjugates were elucidated by means of on-line coupling of high performance liquid chromatography and mass spectrometry. Collisional induced dissociation mass spectra and nuclear magnetic resonance spectroscopy provided unequivocal evidence that conjugation took place at the amino-substituted aromatic ring of the anthrapyrazole.

Our results suggest that alkylation of cellular targets may be a further mode of action of the anthrapyrazole DuP 937.

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G-CSF THERAPY LEADS TO THE EXPRESSION OF HIGH AFFINITY FC-RECEPTORS FOR IGG (FCγRI; CD64) ON NEUTROPHILS AND TO ENHANCED CYTOTOXICITY

R.Repp, Th.Valerius, M.Gramatzki, A.Sendler, J.R.Kalden, and E.Platzer

Neutrophils from patients receiving rhG-CSF in doses of 5 µg/kg/day strongly express the high affinity receptor for IgG (FcγRI). In contrast, FcγRI is absent on neutrophils from the blood of healthy donors and cannot be induced in vitro with G-CSF. In order to test whether the action of G-CSF in vivo is directly on precursor cells, myeloid cells were isolated from the bone marrow and seperated into various fractions by density centrifugation. During G-CSF therapy, polymorphonuclear neutrophils isolated from bone marrow samples expressed FcγRI comparable to blood neutrophils. Nevertheless FcγRI was also found marrow neutrophils of a control group of untreated patients, not bearing FcγRI on blood neutrophils. Granulocytic precursors at different maturation stages ranging from promyelocytes to juvenile neutrophils only weakly expressed FcγRI, but this expression could be increased after 24 h incubation with G-CSF. G-CSF also enhanced the cytotoxicity mediated by neutrophils as could be demonstrated both with Daudi lymphoma cells opsonized with polyclonal rabbit antisera as well as with cell lines from glioblastoma (A1207) or epidermoid carcinoma (A431) coated with monoclonal anti-EGF-receptor antibody 425. Direct involvement of FcγRI could be demonstrated by the use of blocking antibodies to FcγRI and FcγRII. The antibody to FcγRI inhibited only neutrophils from patients during G-CSF therapy but not those of healthy donors, whose neutrophils were completely blocked by anti-FcγRII antibodies. Thus G-CSF therapy may lead to FcγRI expression on blood neutrophils by mobilization of mature cells from the bone marrow expressing FcγRI as well as by direct upregulation on precursors. Those neutrophils show enhanced cytotoxicity what may contribute to the clinical benefit of the drug.

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ADHESION MOLECULES ON CD34+ HEMATOPOIETIC CELLS IN NORMAL HUMAN BONE MARROW AND LEUKEMIA

M.A. Reuss-Borst, H.J. Bühring, G. Klein, C.A. Müller

Expression of selected adhesion molecules of the integrin and immunoglobulin family was investigated on CD34+ precursor cells in normal bone marrow and leukemia to evaluate possible defective homing qualities of malignant hematopoietic precursor cells. Of the ß2-integrin family CD11a was expressed on most CD34+ cells in normal bone marrow and almost all leukemias, whereas CD11b and CD11c were not expressed on CD34+ cells in normal bone marrow, but were found on CD34+ blasts in some leukemias. Of the ß1-family CDw49d (VLA-4) was strongly expressed on normal CD34+ bone marrow cells and on the blasts of all 30 cases of CD34+ leukemic samples, whereas CDw49b (VLA-2) was absent on CD34+ cells in normal bone marrow, but detected on CD34+ cells in a few leukemias which did not constitute a clinical or phenotypic entity according to the FAB classification. The lymphocyte-homing-associated adhesion molecule CD44 (HCAM) and CD58 (LFA-3) were expressed on CD34+ cells in all investigated cases of normal and leukemic bone marrow. ICAM-1 (CD54), the inducible receptor ligand for CD11a/CD18 was not present on CD34+ cells in normal bone marrow, but expressed in some leukemias. So far distinct patterns of expression for some ß2- as well as for ß1-integrins and for ICAM-1 were observed in various subtypes of leukemia. This may point to specific adhesion defects in some leukemias or be related to rare precursor phenotypes of normal bone marrow exaggerated in leukemia.

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DIFFERENTIAL MODULATION OF INTRACELLULAR ARA-C METABOLISM IN NORMAL BONE MARROW CELLS VERSUS AML BLASTS BY GM-CSF.

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Cytosine arabinoside (Ara-C) is one of the most active single agents in the treatment of acute myeloid leukemia (AML). Its cytotoxicity is believed to result from an inhibition of DNA synthesis and from incorporation of Ara-C into the DNA. Recent in vitro studies of our group and others indicate that pretreatment of AML blasts by hematopoietic growth factors like GM-CSF or IL-3 enhances the Ara-C mediated cytotoxicity against clonogenic cells. Furthermore, GM-CSF and IL-3 have been reported to possibly increase the cytotoxicity against leukemic blasts, preferentially. In order to elucidate this phenomenon we investigated the effect of GM-CSF pretreatment on ^{3}H-TdR and ^{3}H-Ara-C incorporation into the DNA of AML blasts (n=31) and normal bone marrow (NBM) cells (n=8) in vitro. Additionally, we determined overall DNA polymerase, DNA polymerase α, TK and dCK activity as well as intracellular Ara-CTP levels. AML blasts and normal bone marrow cells were incubated for 48 hrs with GM-CSF (100 U/ml) followed by 12 hrs incubation in the presence of increasing Ara-C concentrations (0.1-100 μM). In contrast to AML blasts, NBM cells always responded to GM-CSF with increases of TdR and Ara-C incorporation into the DNA and with increases in overall DNA polymerase as well as DNA polymerase α. Rate and amount of the enhancements of TK and dCK activity after GM-CSF were similar for both NBM cells and AML blasts. Compared to the increase of TdR incorporation after GM-CSF (1.5-4.0, median 2.7 vs 1.5-8.5, median 2.3) NBM cells showed a significantly lower increase in the Ara-C incorporation into the DNA than AML blasts (1.5-2.4 fold, median 1.7 vs 1.5-8.4 fold, median 2.3; Wilcoxon test $p<0.05$). Furthermore, the Ara-C mediated inhibition of TdR incorporation into the DNA was significantly lower in NBM cells compared to AML blasts (55% vs 76% at 0.05 μM Ara-C; Wilcoxon test $p<0.05$). In addition, Ara-C induced inhibition of TdR incorporation into the DNA was enhanced by GM-CSF in NBM cells (55% to 64 %, Wilcoxon test $p<0.05$) while GM-CSF had no effect on the inhibition of TdR incorporation in leukemic blasts (76% to 78%). Furthermore, intracellular Ara-CTP levels were 2-40 fold lower in NBM cells compared to AML blasts. In summary, our data suggest differences in the intracellular metabolism of Ara-C between NBM cells and leukemic blasts which might translate into a selective cytotoxicity. This effect can possibly be increased by GM-CSF pretreatment.

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AN OLIGOMER COMPLEMENTARY TO c-raf-1 mRNA INHIBITS IL-2-INDUCED T-LYMPHOCYTE PROLIFERATION.

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Mitogenic signals leading to cell proliferation may engage Raf-1 as it has been shown for a variety of other polypeptide factors including GM-CSF, M-CSF, IL-3, insulin, bFGF, EGF and PDGF. In addition, the viral homolog v-raf, cooperates with c-myc in abrogating factor dependence of murine FDC-P1 cells and also in inducing leukemias and lymphomas. Elimination of Raf-1 may thus favor growth arrest, at least when the growth promoting event ist generated upstream of c-raf-1. In this regard previous studies are of note demonstrating that phorbol 12-myristate13-acetate-mediated proliferation of NIH/3T3 cells is abrogated upon transfection of an antisense c-raf expression vector. Work showing activation of Raf-1 by specific growth factors does not settle the question whether Raf is necessary for transduction of a growth factor signal. However, Raf ~~inhibition does directly address that issue.~~ In these sudies therefore an octadecamer oligodeoxyribonucleotide corresponding to codons 1-6 of human c-raf-1 has been employed to analyze growth promotion of T lymphocytes directed by recombinant human IL-2. In these experiments the proliferative response of IL-2- receptive T-cells (3H-thymidine incorporation) to exogenous IL-2 was substantially reduced when c-raf antisense oligodeoxyribonucleotides which specifically decreased intracellular Raf-1 levels were added to cultures, while in the presence of sense or nonsense oligo- deoxyribonucleotides the proliferative response to IL-2 remained unaffected. Moreover, intracellular Raf translocation induced by IL-2 was prevented when c-raf-1 antisense oligomers were present in the cultures. Taken together, our results demonstrate that RAF-1 is a necessary component of IL-2 signaling in T-lymphocytes.

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EXPERIENCES IN REGIONAL CHEMOTHERAPY OF PRIMARY AND SECONDARY LIVER TUMORS

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Local chemotherapy for isolated, non resectable malignancies confined to the liver was carried out on 62 patients (40 with colon primaries and 22 with other carcinomas). 13 patients were in Wanebo stage I, 38 patients in stage II, and 6 patients in stage III. The cytotoxic agents were given using a totally implantable catheter system. The following treatment protocol was administered; 5-fluorouracil 900 mg/m²/3 hrs/day x 5 every 21 days. Treatment extended over a mean duration of 10 month. Cytotoxic side-effects such as chemical hepatitis, sclerosing cholangitis were observed. The high plasma clearance of epirubicin might be also useful after intrahepatic administration in the attempt to obtain an effective locoregional therapy with a reduced systemic toxicity. The results of intraindividual pharmacokinetic stadies on epirubicin after intrahepatic arterial administration (30 mg/m² over 1,5 to 150 min) are reported. A tumor reduction of 50 % ore more was determined by computer tomography in 21 cases (33,9 %). Objective response rate observed in patients with colon primaries was 37,5 %. Mean time to progression was 12,3 month. 1-year-survival-rate is 76,7 %.

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PLATELET MEMBRANE GLYCOPROTEINS DURING STORAGE OF PLATELET CONCENTRATES: THE INFLUENCE OF LEUKOCYTE-DEPLETION

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A reduction in HLA alloimmunization is obtained when platelet concentrates (PCs) are depleted of leukocytes by filtration. We studied the effect of leukocyte-depletion (polyester filter PL-100, Pall, Dreieich) on the platelet membrane glycoproteins gpIIb/IIIa (CD41a), gpIb (CD42b), gpIV (CD36), gmp-140 (CD62) and gp53 (CD63) during storage using monoclonal antibodies (dianova, Hamburg) and flow-cytometry. Nine "oversized" single-donor PCs were prepared by cytapheresis. After 2 h storage the first sample [A] was taken and the PC was equally divided in two parts. One part was filtered immedeately (sample [B]) and stored for 5 days (sample [C]). The other part was stored for 5 days (sample [D]) and then filtered (sample [E]). The mean values for platelet loss and leukocyte depletion after filtration were 9.1% and 96.4% ([A] vs [B]) and 9.4% and 97.2% ([D] vs [E]), respectively. The effect of filtration on the expression of platelet membrane glycoproteins was not significant. During storage there was a highly significant rise in the mean proportion of platelets which expressed the activation dependent antigens (CD62/CD63) of 13.0/11.4% [B] vs 32.0/30.3% [C] ($p=0.001/0.002$) and 12.2/10.6% [A] vs 32.9/31.0% [D] ($p=0.001/0.001$). Expression of CD42b was significantly lower after storage of the platelets: mean fluorescence intensity 134.8 [B] vs 113.1 [C] ($p=0.008$) and 136.7 [A] vs 114.5 [D] ($p=0.009$). There were no significant differences between samples [C] vs [D] and [C] vs [E].
We conclude that leukocyte-depletion cannot prevent the progressive platelet activation during storage of platelet concentrates.

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EPIDERMAL GROWTH FACTOR-LIKE ACTIVITY IN URINE OF CERVICAL CARCINOMA PATIENTS

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Elevated urinary excretion of epidermal growth factor (EGF) and/or transforming growth factor-alpha (TGF-alpha) in patients suffering from cervical cancer and other malignancies has been reported (Ucchihasi et al.1982, Stromberg et al. 1987). As TGF-alpha and EGF bind to the same receptor, they are subsumed under "EGF-like activity". The aim of this study was to reexamine the findings of an elevated of EGF-like activity in urine in cervical carcinoma patients and to evaluate the clinical relevance concerning early diagnosis, prognosis, therapy control and early diagnosis of relapse.

A human placenta binding assay was used to measure EGF-like activity in 124 urine specimens of 83 patients with histologically proven gynecologic malignancies.67 of them were cervical carcinomas. 23 urine specimens from 18 healthy women were used as controls. High-molecular weight (HMW) and low-molecular weight (LMW) EGF-like activity were measured separately.

Patients under 50 years showed elevated excretion of LMW and HMW-EGF-like activity in comparison to patients over 50 years ($p < 0,05$).

In the younger patients excretion of HMW-EGF-like activity was statistically significantly higher than in the control group ($p < 0,05$).

Urine of patients with cervical carcinoma under 50 years preoperatively showed higher EGF-like activity than postoperatively. This was not found in the control group with hysterectomy for other reasons than malignancy.

HMW-EGF-like activity correlated with known prognostic factors in cervical carcinoma. Excretion of EGF-like activity in patients under 50 years after curative operation was lower than in patients of the same age suffering from relapse.

These results indicate a possible suitability of EGF-like activity in urine for screening cervical carcinoma in women under 50 years, therapy control, early diagnoses of relapse and as prognostic factor in patients suffering from cervical carcinoma under 50 years.

Further and larger studies are necessary, however, to confirm the evidence.

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REGIONAL HYPERTHERMIA COMBINED WITH CHEMOTHERAPY: EXPERIENCES IN PEDIATRIC ONCOLOGY

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Solid extracranial malignant tumors in childhood bear a high risk of systemic failure and need intensive systemic chemotherapy. For local control surgery and ionizing irradiation are inevitable. In case that such three modality treatment fails or is likely to fail, additional local treatment is desirable. Regional enhancement of drug cytotoxicity by heat is a promising approach to increase therapy intensity but not systemic toxicity. In contrast to the biological efficacy there are some clinical problems, especially the invasiveness of thermometry and the risk of thermal damage of normal tissue.

In the pilot phase of a cooperative GPOH-study regional hyperthermia treatment was restricted to high risk patients in the majority suffering from either locally relapsed soft tissue and Ewing's sarcoma or osteosarcoma and soft tissue sarcoma which were not resectable even with mutilation.

36 patients not older than 16 years were treated in Munich and Essen by externally electromagnetically induced hyperthermia combined mainly with intravenous etoposide, carboplatin, ifosfamide.

Results: 1. Traumatic and local infectious complications of invasive thermometry are very rare. 2. There is no evidence of an increased risk for local or distant metastases until now. 3. Thermal injury of normal tissue occured in about 10% of the patients. It did not severely impair cancer therapy. 4. The temperatures achieved are high enough to increase local drug cytotoxicity significantly (90% of each tumor above 40°C, 20% > 41.5°C).

Meanwhile regional hyperthermia treatment was integrated into a non randomized cooperative phase-II-study for locally relapsed soft tissue and Ewing's sarcoma. If hyperthermia contributs to local tumor control it could probably be utilized to reduce mutilating procedures.

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Retroperitoneal Fibrosis With High-Grade Non-Hodgkin Lymphoma

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A 51-year-old woman complained of the sudden onset of lower backache 3 1/2 years ago. By sonography and computer tomography, a large paravertebral mass encasing the aorta and vena cava as well as obstructing the left ureter leading to renal enlargment, was diagnosed. Histology showed granulomatous tissue with steatosis and fibrosis mass, likely a xanthofibrogranuloma constituting an early stage of retroperitoneal fibrosis. However a lipogranuloma could not be excluded. Antiphlogistic treatment was slated, and for the following 3 1/2 years, no enlargment of the retroperitoneal tumor was noticable.

One month before admission to our hospital, the patient presented with severe abdominal pain, nausea, vomiting and dyspnoe. On the day of entry, bilateral pleural effusions as well as lymphomas along the aorta and diaphragm were diagnosed. The left psoas muscle was infiltrated by tumor. Centroblasts and immunoblasts discovered by cytologic examination in the pleural effusion led to the diagnosis of a high-grade centroblastic-immunoblastic lymphoma. Fiberoptic examination of the upper gastrointestinal tract showed 6 deep ulcera. Mucosal biopsy revealed lymphoma infiltration in between gastric glands. The proliferating blasts contained either one large nucleolus or multiple, medium-sized nucleoli located chiefly at the nuclear membrane. This indicated a high-grade NHL, most likely a centroblastic-immunoblastic lymphoma. By positron-emission tomography with fluorine-18-fluoro-deoxyglucose ([^{18}F] FDG) activity was accumulated along the pleura, stomach and mesenterium. In the retroperitoneal tumor histologically diagnosed as Ormond 3 1/2 years ago, no significant accumulation of activity was diagnosed. A laparatomy showed extensive involvement of the stomach, duodenum, omentum majus, colon and retroperitoneal space. In the meantime a successful chemotherapy has been slated with our patient.

Concerning the differential diagnosis of this patient, we have to consider a primary retroperitoneal fibrosis (Ormond disease) with secondary infiltration by a high-grade lymphoma or as described by M.H. Bennet (1975) "Sclerosis in non-Hodgkin's lymphomata", since retroperitoneal fibrosis is rare disorder, multiple generous biopsies are necessary to ensure a lymphoma is not being overlooked and therapeutic intervention delayed.

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Detection of wild and mutant type p53 in human germ cell tumors by histochemistry staining

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P53, first detected in a complex with SV 40 large T-antigen, was subsequently found to complex to the E1B 58 kd product of adenovirus 5 and 12, and to mammalian heat shock protein HSP 70. The gene is located on the short arm of chromosome 17. It is made up of 11 exons covering 16–20 kb of DNA and encodes a 2.2–2.5 kb mRNA producing a 53 kd nuclear protein. This protein is found in most cells of the body. A consistent deletion of the short arm of chromosome 17 has been seen in many tumors. A study of brain, breast, lung and colon tumors showed, that in the majority of the cases, where p53 was deleted, there was a detectable mutation in the remaining p53 allele, which causes tumor progression by loss of growth control by functional inactivation of p53 gene.

Frozen testicular specimens from 20 patients with testicular cancer were analyzed. The following monoclonal antibodies were used:

1. Clone PAb 1801, derived by fusion of BALB/c splenocytes with NS-1 mouse myeloma cells.
2. Clone PAb 240 was derived by immunization of BALB/c mice with p53-β-galactosidase fusion protein and fusion of splenocytes with SP2 mouse myeloma cells.
3. Clone 1620 derived by immunizing BALB/c mice with VLM tumor cells and fusion of splenocytes with SP2/0-Ag 14 mouse myeloma cells.

The p53 protein in the mutant conformation was localized in the cell cytoplasm, whereas the wild type in the cell nucleus was found in numerous but not in all tumor cells. The wild and the mutant type could be found in the same tumors (e.g. in 3 embryonal carcinomas, 8 seminomas, in the benign Leydig cell tumor, in one immature teratoma, in 3 embryonal carcinomas with seminomas, in one teratoma with choriocarcinoma, in one immature teratoma with seminoma, in one embryonal carcinoma with teratoma). Neither the wild nor the mutant type could be detected in the mature teratoma. In the same of these tumors we also found EBV-DNA. In some of these tumors we could detect the oncogenes: c-myc, N-myc, c-Ha-ras 1, c-fos, and c-jun. The discovery of wild and mutant p53 in human testicular cancer is consistent with the view, that alterations of tumor-suppressor genes play a role in the pathogenesis of this tumor type in cooperation with the Epstein-Barr virus and other oncogenes.

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TOTAL PARENTERAL NUTRITION (TPN) IN BONE MARROW TRANSPLANTATION: USE OF A LIPID CONTAINING MEDIUM CHAIN TRIGLYCERIDES (MCT) - A RANDOMIZED PROSPECTIVE TRIAL

V. Runde, C. Dumont, A. Heyll, G. Meckenstock, and W. Schneider

The positive effect of prophylactic TPN on the long-term outcome of bone marrow transplantation (BMT) is well documented, even in well-nourished individuals. Intravenous fat emulsions are an integral part of a TPN system in which 30-60% of the nonprotein calories are supplied as fat. As particularly stressed and infected patients can not utilize long chain triglycerides (LCT) sufficiently, MCT/LCT emulsions have been introduced into TPN programs. We have investigated the use of a lipid emulsion containing both LCT and MCT in a randomized prospective trial. Thirty patients are evaluable. The regimen was designed to deliver 150 % of the basal energy expenditure (BEE) for the first two weeks of the study, 130 % for the next two weeks and 100 % BEE for the last study week. Two thirds of the the nonprotein calories were supplied as glucose, one third as fat emulsion. Group I received MCT/LCT (Lipofundin) and group II received LCT (Intralipid). Additionally, amino acid solutions were provided in a dosage of 1.5 g/kg/day. Daily body weight, weekly triceps skinfold, plasma triglycerides, cholesterol, free fatty acids, prealbumine as well as routine hematology, biochemistry and liver function tests were measured from BMT day -8 to 70. Metabolic parameters, antropometric measures and patient outcome showed no significant differences between the two groups of patients. In conclusion, MCT/LCT emulsions were found to be as safe and as effective a source of calories as LCT, but there were no significant differences in the metabolic parameters in the two groups.

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TREATMENT WITH ALL-TRANS RETINOIC ACID IS NOT EFFECTIVE IN PATIENTS WITH MYELODYSPLASTIC SYNDROMES

V. Runde, C. Aul, C. Afflerbach, and W. Schneider

Retinoic acid has a well known differentiating activity on leukemic cells in vitro. In acute promyelocytic leukemia, in vivo treatment with all-trans retinoic acid (ATRA) induces complete remission in most of the patients. The current study was performed to evaluate the efficacy of ATRA in the myelodysplastic syndrome (MDS). From July 1991 to April 1992, 15 MDS patients were treated with increasing doses of ATRA. FAB-subtypes were: RA n=4, RARS n=2, RAEB n=6, RAEB-T n=3. All patients were transfusion dependent. Median (range) pretreatment WBC and platelet counts were 2.1 (0.7-10.3) x 10^9/l and 43 (6-319) x 10^9/l, respectively. During the first month ATRA was given at a dosage of 30 mg/m^2. If the treatment was well tolerateted, the dosage was increased to 60 mg/m^2 during the second and to 90 mg/m^2 during the last month of treatment. 10 patients were treated over the complete study period of 3 months. Neither changes in transfusion requirements nor improvements in peripheral leukocyte and platelet counts were observed. Apart from dryness of the skin and mucosa and hypertriglyceridemia in nearly all patients, treatment was well tolerated. In contrast to patients with acute promyelocytic leukemia no thromboembolic events were seen. We conclude that ATRA has no beneficial effect in patients with MDS.

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MULTIDRUG RESISTANCE IN MYELODYSPLASTIC SYNDROMES AND ACUTE LEUKEMIA

V. Runde, C. Aul, G. Meckenstock, A. Höller, and W. Schneider

Expression of multidrug resistance (MDR) in 111 adult patients with myelodysplastic syndromes (MDS) or acute leukemia was studied by means of the alkaline phosphatase anti-alkaline phosphatase technique (APAAP), using the monoclonal murine antibody C219. Reactivity for the P-glycoprotein was defined by the presence of more than 5% positively staining blast cells in the bone marrow. In each patient, at least 100 blasts were examined. Among MDS patients, MDR expression increased with progression of the disease (RA: 0/5 (positive/all samples), RARS: 0/2, RAEB: 6/19, RAEB/T: 4/9, CMML: 6/10, AML evolving from MDS: 10/19). Only MDS patients in whom the bone marrow blast cells showed the stem cell phenotype (CD34) expressed the P-glycoprotein. Forty percent of newly diagnosed AML and ALL patients stained positively for MDR. In the AML group, the percentage of MDR positive samples increased from 70% in patients with first relapse to 100% in patients with second or subsequent relapse. Patients with de novo AML or advanced MDS not expressing the P-glycoprotein had a higher chance of entering complete remission after aggressive chemotherapy than patients with C219-positive blast cells (CR-rates: de novo AML 71% vs. 44%; MDS 86% vs. 40%).

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BACILLUS BACTEREMIA AS A CAUSE OF CEREBRAL HEMORRHAGE IN THE PRESENCE OF SUFFICIENT PLATELET COUNTS

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A 20-year old female with c-ALL in complete remission undergoing maintenance treatment with low-dose cytosine arabinoside, thioguanin and prednisone developed cerbral symptoms with headache, nausea, followed by desorientation the following day. Peripheral blood leukocytes were 1800 /mm^3, platelets 48 000. There was no meningism, CCT showed no abnormal findings, but cerebrospinal fluid examination revealed 160/3 cells (95% neutrophils). Empiric antibiotic therapy with high-dose penicillin, cephalosporin and aminoglycoside was started. On the third day the patient developed massive intracerebral hemorrhage despite of normal blood pressure and minimal platelet counts of 39 000/mm^3, followed by fever >39°C. Bacillus was grown in repeated blood cultures and the antibiotic regimen was changed according to the resistance profile to clindamycin, vancomycin and chloramphenicol, together with anti-edematous therapy. Nevertheless, the patient became comatous und control CCTs on days 4 and 5 showed multiple brain abscesses with diameters up to 15 mm for the first time. The patient expired on day 5 due to central respiratory failure. Autopsy confirmed multiple brain abscesses at the cortex/white matter border with no other septic metastases in other organs. The CNS-tropy of bacillus has been repeatedly demonstrated and exotoxins of bacillus typically cause a necrotizing inflammation. The latter was the most likely reason for the cerebral hemorrhage in our patient. We conclude that bacillus bacteremia should be considered in the differential diagnosis of cerebral hemorrhage in the presence of sufficient platelet counts in immunosuppressed patients.

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VON-WILLEBRAND-FACTOR (vWF) AND THROMBOMODULIN (TM): MARKERS OF ENDOTHELIAL CELL DAMAGE AFTER BONE MARROW TRANSPLANTATION?

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Endothelial cell damage is supposed to be a central pathogenetic mechanism in the development of graft versus host disease (GVHD), a major complication after bone marrow transplantation (BMT). Laboratory methods for the detection of endothelial cell lesions are missing. A correlation between the degree of microangiopathy and elevation of vWF was described earlier. On the other hand TM is expressed on the endothelial cell surface, can be cleaved and measured as soluble TM in in plasma. Trying to find a marker of endothelial cell damage we studied vWF and TM levels in patients after bone marrow transplantation (n=15).
Materials and methods: Citrated blood samples were collected on day -8, -5, -1, 0, 7, 14, 21, 28, and 35 respectively. VWF levels were determined by a commercially available assay (ELISA vWF, Boehringer Mannheim, FRG). TM was also measured by an immunoassay based on monoclonal antibodies (Asserachrom Thrombomodulin, kind gift of J. Amiral, Serbio, Genneville, France).
Results: TM levels before BMT (57,9 +/- 21,7 ng/ml) reached a first peak after conditioning (73,2 +/-27,2 ng/ml) on day 0 and a second peak on day 21 (76,9 +/-46,5 ng/ml). The highest levels were found in a patient with severe complications reaching a summit of 223,7 ng one day before he died. VWF was slightly elevated before BMT (141,6 +/- 41,3%) and rose continously until day 28 (313,3 +/-133,7%).
We conclude that the observed elevation TM and vWF in patients after BMT could be contributed to endothelial cell alterations in these patients.

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PHARMACOKINETICS OF THE STEREOISOMERS OF N-5-METHYLTETRAHYDROFOLIC ACID

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Methyltetrahydrofolic acid (CH_3-THF) is the major serum metabolite of folinic acid (CHO-THF) and possibly contributes to the modulation and enhancement of 5-FU effects by CHO-THF. As a prerequisite for clinical studies with this reduced folate we determined the pharmacokinetics after application of 200 mg/m² d,l-CH_3-THF in ten healthy volunteers. Total d,l-CH_3-THF was quantified by reversed-phase HPLC. The biologically active l- and the inactive d-form was further separated by means of chiral HPLC. After the analysis of 9 probands was completed, the following results for elimination half-life ($t_{1/2\beta}$), AUC, and total body clearance ($Clear_{tot}$) were obtained:

	$t_{1/2\beta}$ (min)	AUC (µM·min)	$Clear_{tot}$ (ml/min)
l-CH_3-THF	187 ± 37	7659 ± 1361	100 ± 25
d-CH_3-THF	516 ± 96	36602 ± 6668	21 ± 4

In accordance with the kinetics of d- and l-CHO-THF, the d-form of CH_3-THF was cleared more slowly than the l-form. Elimination half-life and total clearance of l-CH_3-THF were nearly identical after application of d,l-CHO-THF and after infusion of d,l-CH_3-THF. In comparison to l-CHO-THF ($Clear_{tot}$ about 200 ml/min), l-CH_3-THF is cleared more slowly. This results in higher AUC values of reduced l-folates when identical doses of d,l-CH_3- THF are given instead of d,l-CHO-THF. Provided CH_3-THF/5-FU proves equally effective in human colorectal carcinoma compared to CHO-THF/5-FU, the dosage of CH_3-THF could be reduced by about 50 % when used instead of CHO-THF as 5-FU modulator.

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THE INHIBITORY EFFECT OF 2-CHLORODEOXYADENOSINE (2-CDA) ALONE AND IN COMBINATION WITH CYTARABINE (ARA-C) AND ADRIBLASTIN (ADR) ON THE CLONAL GROWTH OF ACUTE MYELOID LEUKEMIA PRECURSORS (L-CFU) IN VITRO

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The effect of 2-CDA alone and in combination with ARA-C and ADR on the proliferation of acute myeloid leukemia precursor cells (L-CFU) was assayed. Bone marrow blast cells (10^6/ml) were incubated for 24 hours with 2-CDA (20, 50, 100, 150 and 300 nM/L), ARA-C (10^{-7}M/L) and ADR (1µg/ml) separately and in different combinations. After incubation, the cells were cultured in the liqiud phase of the two layer (agar/liquid) culture system (Löwenberg). The number of blast colonies observed on day 9 was inversely correlated with the 2-CDA concentration reaching 68% of control at 300 nM/L. The colony formation after incubation with the three drugs together (5% of control) was signifficantly lower than that after ARA-C and ADR (35%). The results suggest that 2-CDA may be considered as a useful drug in the treatment of acute myeloid leukemia.

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Modulation of ICAM-1 on Pre-B cell lines by cytokines: role of ICAM-1 mediated adherence to endothelial stroma cells

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To analyse the importance of cell-cell interactions for the proliferation of acute lymphoblastic leukemic cells we have determined the baseline expression of ICAM-1 and its modulation by TPA and different cytokines (TNFα and IFNgamma) in a number of human pre-B cell lines (Nalm-6, BV-173, MN-60, REH) by FACS analyses and immunoblots. All pre-B cell lines displayed ICAM-1 in variable degrees with different MG's, ranging from 85 KD to 92 KD. After 12 hrs. of incubation with TPA, IFNgamma or TNFα upregulation of ICAM-1 could be detected by FACS and immunoblot analyses. IFNgamma was generally the most effective cytokine in modulating ICAM-1 expression on pre-B cell lines, since the extent of ICAM-1 upregulation was much higher and the temporal kinetics were different from that found with TNFα stimulation. To determine the function of ICAM-1 mediated cell adhesion we used HUEV in an adhesion assay. Neither the MoAbs to LFA-1 nor MoAbs to ICAM-1 affected adherence of the pre-B cell lines to endothelial cells. MoAb 4B9 (anti-VCAM-1) and MoAb HP2/1 (anti-CD49d) inhibited the adherence of pre-B cell lines to endothelial cells in variable degrees suggesting a role for the interaction with stroma cells. The results of the present study indicate that variable expression of ICAM-1 is a property of pre-B cell lines and can differentially be upregulated by IFNgamma and TNFα but is not involved in the adhesion to HUEV. We conclude that VLA-A but not ICAM-1 mediated adhesion may be important in the interaction of pre-B ALL and bone marrow stroma cells.

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FLOW CYTOMETRIC ANALYSIS OF DEFECTIVE PLATELET MEMBRANE GLYCOPROTEIN (GP) IIb-IIIa IN MYELOPROLIFERATIVE DISORDERS (MPD) OR MYELODYSPLASTIC SYNDROME (MDS)

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We have previously shown that platelet aggregation defects in MPD or MDS can be associated with dysfunction or reduced expression of the platelet membrane GP IIb-IIIa complex. To analyze molecular mechanisms of abnormal GP IIb-IIIa receptor function, we have studied 13 patients (8 with MPD, 5 with MDS) using conformation-specific monoclonal antibodies (MoAbs) and platelet flow cytometry. Ten patients showed defective platelet aggregation in response to epinephrine and/or adenosine diphosphate and 8 concomitantly had a prolonged bleeding time (mean±SEM: 16±4 min; normal 4±2.5 min). Upon platelet stimulation with epinephrine or adenosine diphosphate, binding of PAC1, an MoAb directed to the activated GP IIb-IIIa complex (supplied by Dr. Shattil, Univ. of Pennsylvania, Philadelphia), was intact, as compared to heathy volunteers (n=12), in the 3 patients with normal bleeding time and normal aggregation responses, whereas 8 of the 10 patients with defective platelet aggregation failed to bind PAC1. However, binding of PAC1 increased in response to phorbol myristate acetate, which circumvents receptor-mediated pathways by directly activating platelet protein kinase C. Furthermore, binding of anti-LIBS1, an MoAb that recognizes a ligand-induced binding site on GP IIIa (supplied by Dr. Ginsberg, The Scripps Research Institute, La Jolla), was concomitantly reduced or absent following platelet stimulation with epinephrine or adenosine diphosphate, or incubation with the fibrinogen-mimetic peptide GRGDSP (which binds to normal GP IIb-IIIa without prior platelet activation). These results indicate that platelet dysfunction in MPD or MDS is related, at least in part, to an agonist-specific defect either in activation of the GP IIb-IIIa receptor, or its ligand binding function, or a combination of both.

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TNFα plays an important role in immunotherapy with IL-2 by increasing susceptibility of melanoma cells.

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The role of TNFα in immunotherapy with IL-2 is controversial. TNFα is induced by IL-2 and thought to be responsible for most of the side effects. It is unclear, whether it is of any importance for response to treatment. The data we present here, however, suggest that TNFα indeed is crucial for immunologically mediated regression of melanoma.

Two new melanoma cell lines were established from metastases of patients prior to immunotherapy. Both patients received immunotherapy with IL-2 and showed good response. Using the standard chromium release assay, the cytotoxic activity of peripheral blood mononuclear cells (MNC) against autologous melanoma targets was assessed prior to immunotherapy and after IL-2 treatment, and for comparison also after in vitro activation of MNC with various concentrations of IL2. To increase expression of MHC molecules and the adhesion antigens LFA3 and ICAM1, melanoma cells were preincubated with IFNα or TNFα. These two cytokines are of special interest, since IFNα is frequently used in combination with IL-2 for immunotherapy, and TNFα is induced by high dose IL-2 in vivo. The modulation of cell surface molecules was determined by FACS analysis.

Unstimulated MNC had almost no detectable lytic activity. After immunotherapy with IL-2, MNC exhibited cytotoxic activity against autologous melanoma cells, that could be increased by preincubation of the target cells with TNFα, which effectively augments the expression of ICAM1, and to a lesser extent by IFNα, which mainly upregulates MHC Class I molecules. Autologous LAK cells (cultered in the presence of 1000U/ml of IL-2) had high lytic activity against pretreated as well as unmodified melanoma cells. The results demonstrate that TNFα increases the susceptibility of melanoma cells to lysis by autologous effector cells activated by IL-2 in vivo.

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R-metHuG-CSF COMBINED WITH CHEMOTHERAPY FOR TREATMENT OF ADULT ALL - A PILOT STUDY

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14 consecutive patients with ALL (12 de novo, 2 relapsed)- median age 42 years (18 to 69), 10 c-ALL (2 bcr-abl positive), 2 pre-B ALL (1 bcr-abl positive), 1 early B-precursor ALL and one T-ALL were treated according to the chemotherapy protocol described by Hoelzer et al. In addition r-metHuG-CSF (Neupogen R) was given at a dose of 200 ug/m2 iv. in phase 1 from day 2 to day 21, and thereafter until neutrophil counts were >1000/ul on two consecutive days. In phase 2, G-CSF 200 ug/m2 sc. was started on day 2 (after CP) and continued until neutrophils recovered to more than 1000/ul on two consecutive days. These patients were compared to a historical control group treated with the same chemotherapy protocol but without G-CSF. It included only patients, who achieved CR within 4 weeks (median age 27 years, 22 c-ALL, 3 pre-B ALL, 3 early B-precusor ALL and 10 T-ALL). - 13/14 patients achieved complete hematologic remission (10 within 4 weeks and three at the end of induction treatment). One patient died because of fungal septicemia. G-CSF treated patients had a shorter duration of granulocytopenia (14.5 days vs 21.5 days) in phase 1. In phase 2 the median granulocyte count during weeks 3 and 4 was significantly higher compared to the historical control group. The number of days with fever in phase 2 was lower in the G-CSF group (1,5 days vs 0,8 days). The full dose of chemotherapy (anthracyclins, cyclophosphamide, ARA-C and purinethol) could be given in 11/13 G-CSF treated patients, while in the control group only 23 % of the patients received the protocol without dose reduction or delay. These data indicate, that, (1) G-CSF can be given along with chemotherapy in induction treatment without compromising efficacy, (2) the time of granulocytopenia in phase 1 is shortened by 7 days and the degree of granulocytopenia is amelioreated in phase 2 and (3) dose intensity (dose per time) could be increased.

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CELLULAR PHARMACOKINETICS OF ANTHRACYCLINES AND DRUG-RESISTANCE IN AML

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Besides cytosine arabinoside the anthracyclines (AC), such as daunorubicin (DNR) and idarubicin (IDA), belong to the most active drugs in the treatment of patients with AML. Multidrug resistance (MDR) may be one of the major obstacles to an effective chemotherapy of AML. It is associated with the overexpression of a membrane glycoprotein (Gp-170) acting as an energy-dependent efflux pump for AC and other xenobiotics. MDR1 gene expression has been shown to be an independent prognostic factor in AML. Several drugs including calcium antagonists have been proven to inhibit Gp-170 in vitro. R-Verapamil, the dihydropyridine derivative dexniguldipine (B8509-035) and cyclosporine A are investigated in clinical trials to overcome MDR in AML in relapse. The determination of the cellular pharmacokinetics (CP) of AC in myeloid blasts (MB) from peripheral blood or bone marrow ex vivo either directly by FACS-analysis or by extraction of AC and determination by HPLC with fluorescence detection is a functional test for the efflux capacity. Furthermore, subpopulations (SP) of MB can be characterized in vitro by the efflux of rhodamine 123 (R123), a MDR-dependent dye which does not interact with DNA. The correlation with MDR1 gene expression or the determination of Gp-170 by MRK 16 immunofluorescence may help to understand the different sensitivity of MB to AC on a molecular level. The comparison of the CP of DNR and IDA after simultaneous administration in equimolar amounts led to a significantly longer half life for efflux and a higher area under the curve for IDA. These differences can be explained by a lower MDR-dependent efflux of IDA or by its higher affinity to cellular macromolecules such as DNA. Interestingly, there is a strong correlation between high R123-efflux capacity and the expression of CD34. Thus, MDR may be a physiological detoxification mechanism in bone marrow stem cells. Accordingly, further clinical investigations of MDR-modifiers must be performed with great caution.

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AUTOMATED DIFFERENTIATION OF PERIPHERAL BLOOD COUNTS IN LEUKOPENIC PATIENTS AFTER HIGH DOSE TREATMENT WITH BONE MARROW TRANSPLANTATION BY THE COULTER VCS-TECHNOLOGY

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Due to the application of more intensive chemotherapeutic regimens with support either by colony stimulating factors (CSF) or bone marrow transplantation (BMT) in the treatment of malignant diseases, an increasing number of patients is temporary leukopenic. Conventional morphological examination (CME) of May-Grünwald-Giemsa-stained peripheral blood smears of these patients in the daily clinical routine is time consuming or unreliable for statistical reasons. Thus, we have investigated the automated differentiation of peripheral blood counts in leukopenic patients after high dose treatment with BMT by the Coulter VCS-technology (VCS), which utilizes the simultaneous determination of volume, conductometry and light scatter of at least 1.500 cells in EDTA-collected blood samples in a continuous flow mode, in comparison to CME. Altogether, 150 blood samples with leukocyte counts between 300 and 2.900/µl have been analysed by VCS within 4 hours after withdrawal of blood. In addition, a comparison between CME and VCS was performed in 69 blood samples with normal distribution of cells. There was a good correlation for neutrophils ($y = 0.95x + 26$, $r = 0.93$), lymphocytes ($y = 0.86x + 146$, $r = 0.95$) and eosinophils ($y = 0.82x + 15$, $r = 0.93$), respectively. The number of monocytes was generally smaller with VCS although correlation was good ($y = 0.68x + 74$, $r = 0.91$). There was only a weak correlation for basophils ($y = 0.22x + 14$, $r = 0.35$), due to the small number of cells. During follow-up of patients undergoing high dose treatment with BMT altogether there was a good agreement between the numbers of neutrophils and lymphocytes as determined by VCS in comparison to CME. Thus, VCS allows an earlier diagnosis of bone marrow take. In conclusion, the automated differentiation by VCS is an essential tool in the assessment of blood counts of leukopenic patients. Thus, blood samples with leukocyte counts of down to 300/µl can be evaluated in the daily clinical routine.

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Pharmacokinetics and protein binding of leucovorin diastereomers

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Leucovorin is a racemic mixture of S-R-formyltetrahydrofolicacid (S-fTHF, R-fTHF). Its main clinical application is in methotrexat rescue therapy and for modulation of 5-fluoruracil, predominantly in the treatment of metastatic colo-rectal cancer. In spite of its broad clinical use little is known about the differential pharmacokinetics of the diastereomers and the main metabolite, S-methyltetrahydrofolicacid (S-mTHF). For the appropriate design of new therapies with the recently available pure S-fTHF a detailed knowledge of its pharmacokinetics and protein binding of both diastereomers seems deeply warranted. We recently developed a highly sensitive HPLC assay with detection limits of 2ng/ml for S- and R-fTHF, and 5ng/ml for S-mTHF. On this basis the plasma kinetics and protein binding of S-fTHF, R-fTHF and S-mTHF were measured in 7 patients during a 24 hour period following the administration of leucovorin 300mg/m^2 i.v. over 15 minutes. Results were: **S-fTHF:** $t_{1/2\beta} = 0,76$ h with a coefficient of variation (CV) of 13%, AUC = 15,2 ugxh/ml CV 35%, peak concentration (PC) 26,1 ug/ml CV 62%. **R-fTHF:** $t_{1/2\beta} = 6,65$ h CV 24%, AUC = 251 ugxh/ml CV 46%, PC = 50,8 ug/ml CV 47%. **S-mTHF:** $t_{1/2\beta} = 3,29$ h CV 32%, AUC = 30,6 ugxh/ml CV 48%, peak concentration were reached between 2 - 4 hour = 2,45 ug/ml CV 19%. Protein binding was analysed in spiked plasma samples and patient plasma after leucovorin application. In the presence of the two diastereomers and the metabolite the average S-fTHF binding is 32% (CV 8%). For R-fTHF and S-mTHF the results were 88% (CV 6%) and 59% (CV 11%), respectively. In the absence of R-fTHF, protein binding of S-fTHF and S-mTHF increased significantly to 54% (CV 3%) and 74% (CV 4%). These data indicate that the free plasma concentrations of S-fTHF and S-mTHF, the active metabolite, are only roughly half as high after application of the pure S-fTHF as compared to the administration of the racemic mixture at comparable doses. These findings therefore explain the described lower distribution volume of S-fTHF and the decreased metabolic rate to S-mTHF after application of the pure S-form in comparable dosage to the racemic mixture. Hence, these data question the currently proposed dosing of S-fTHF at equimolar concentrations with the racemic mixture.

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SUCCESSFUL TREATMENT OF NEUTROPENIA IN Tγ-LYMPHOCYTOSIS WITH GRANULOCYTE-COLONY STIMULATING FACTOR (G-CSF)

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Recombinant human granulocyte-colony stimulating factor (G-CSF) has been reported to increase circulating neutrophil numbers in congenital idiopathic, familial cyclic, and acquired chronic neutropenia. Here we report on repeated G-CSF treatment of a 26-year old woman with clonal Tγ-lymphocytosis (constant expansion (5-7x10^9/l) of atypical CD3$^+$CD8$^+$CD16$^+$CD57$^+$ lymphocytes with clonal rearrangement of the T-cell receptor ß-chain detected by Southern Blot analysis of genomic DNA) associated with severe chronic neutropenia (neutrophils < 0.3x10^9/l) and recurrent skin and mucosal infections. Bone marrow biopsy showed a 40% lymphoid infiltration and a maturation arrest of granulopoiesis. In August 1991 treatment with G-CSF at a dose of 480 µg Filgrastim/d subcutaneously was commenced because of a perforated sigmadiverticulitis complicated by diffuse peritonitis and rapid deterioration despite immediate surgical resection with colostoma and therapy with broad-spectrum antibiotics. Together with rapid clinical improvement the peripheral neutrophil count rose to values of more than 20x10^9/l within 7 days and rapidly declined to less than 0.5x10^9/l after cessation of G-CSF. The rapid neutrophil recovery induced by G-CSF was reproducible with reduced G-CSF doses of 300 µg Filgrastim/d (for treatment of a panaritium with incipient hand phlegmona) and 150 µg Filgrastim/d (for prevention of postsurgical insufficiency of descendorectostomy anastomosis). However, on both occasions the elevation of peripheral neutrophil-counts to 11.1x10^9/l and 5.8x10^9/l respectively within 6 days coincided with the development of a painful purpuric vasculitic rash on both legs complicated by renal involvement with hematuria, proteinuria and a serum creatinine rise. After G-CSF was discontinued on day 6, the rash disappeared within 7 days in parallel with neutrophil counts falling to pretreatment levels, the acute renal failure was completely reversible within 3 weeks. The findings in this case suggest that G-CSF can induce a rapid neutrophil recovery at least in selected cases of chronic Tγ-lymphoproiferative disease despite persistence of clonal lymphocytosis. However, because of the risk of severe vasculitic reactions of the skin and, more importantly, of the kindey G-CSF application in Tγ-lymphocytosis should be carefully monitored and primarily restricted to life threatening infections.

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DIFFERENTIAL CELL COUNT AND LYMPHOCYTE SUBPOPULATIONS OF BRONCHOALVEOLAR LAVAGE IN IMMUNOCOMPROMISED PATIENTS WITH PNEUMONIA

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Because of early empirical antibiotic and/or antimycotic treatment broncholaveolar lavage often can not clarify the etiology of pneumonia in acute leukemia and severe granulocytopenia. Looking for characteristic BAL cell profile for infective pulmonary diseases we determined BAL differential cell count, CD4, CD8, CD19 and CD57 positive lymphocytes in 100 immunocompromised patients with fever and pulmonary infiltrates and 12 controls [C], respectively. Patients with acute leukemia and severe peripheral granulocytopenia [A, n=30] had normal differential cell count (46%) or showed a lymphocytic alveolitis (54%), whereas patients with normal peripheral cell count [B, n=70] had increased percentage of lymphocytes and polymorphic neutrophils in BAL: lymphocytes A 25±23%; B 28±22%; C 9±2%, polymorphonuclear cells A4±9%; B 20±23%; C 3±2%. A predominance of polymorphic neutrophils (17±22%) was seen in specimens from patients with bacterial infections, whereas in specimens from patients with pneumocystis infections a predominance of lymphocytes (56±25%) was seen. Patients with and without peripheral granulocytopenia had similar findings in BAL lymphocyte subsets, but CD4- and CD8-positive lymphocytes differed from controls: CD4 A 34±13%; B 35±12%; C45±10%, CD8 A 49±13%; B 49±14%; C 35±6%, CD4/CD8 A 0,7±0,5; B 0,8±0,5; C 1,3±0,4. The lowest CD4/CD8 ratio in BAL was measured in patients with pneumocystis pneumonia (pneumocytis 0,6±0.3; fungi 0,9±0,5; bacteria 0,7±0,3). Pneumonias with more than one infectious agents showed a lower ratio than patients with only one infectious agents.

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INFLUENCE OF MINOR BCR POLYMORPHISMS ON SURVIVAL IN MAJOR BCR POSITIVE CML PATIENTS

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From a group of 131 Philadelphia positive chronic myelogenous leukemia patients we are currently observing, we investigated restriction polymorphisms of 50 patients in the minor breakpoint cluster region (mBCR). All of them had a breakpoint in the major bcr (Mbcr). In these patients we found polymorphisms in mbcr in 11 cases (called group I). In 39 cases we could not detect any aberrant restriction fragments (groupII). Survival analysis showed group I and II to differ significantly. The mean survival duration 37.1 vs. 72.2 months, $p<0.01$, in comparison to a mean survival of 62.1 months in the whole group of CML patients currently under observation. The mean duration of chronic phase is significantly different in those groups (29.1 vs. 55.4 months, $p<0.05$). At the time of investigation 9 patients in group I were in blast crisis (82%) vs. 12 patients in group II (31%) at a comparable mean observation time for both groups. Groups I and II show a difference in the proportion of myeloid and lymphoid blast crises, whereas in group II there was a ratio of 7 to 3, similar to the proportion in our total patient population (19 to 8), group I had a ratio of 4 to 3 with one case of coexpression of myeloid and lymphoid markers on the blasts. This difference is not statistically significant. Furthermore in none of these patients aberrations in mbcr did arise in the course of disease and there was no change in breakpoints in an individual patient over time. From this we conclude that an aberration in mbcr additional to a Mbcr rearrangement may be an indicator of poorer prognosis in CML patients resulting in a faster progression to blast crisis.

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COMPARATIVE STUDY ON ONCE AND THRICE DAILY NETILMICIN IN THE EMPIRICAL THERAPY OF FEVER IN THE NEUTROPENIC PATIENT.

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The aim of this prospective randomized study was to compare efficacy and safety of netilmicin (6 mg/kg) given once daily (OD) with the conventional thrice daily divided regimen (TD) for initial empirical combination treatment with ß-lactam antibiotics of febrile neutropenic patients. Of the 116 patients 25% had bacteremia (most of them gram-positive), 35% had nonbacteremic documented infection and 40% had suspected infection. Peak serum concentrations of netilmicin in the OD group were higher (median: 18.3 v. 5.9 mg/l), and trough serum levels lower (median: 0.2 v. 0.9 mg/l) than in the TD group. No differences were seen in response rates and toxicity. The overall response rate was 72% in the OD group compared with 69% in the TD group. Comparable rates in both groups were also seen in the response to the initial unmodified (i.e. no change or addition of ß-lactams or of glycopeptides) treatment (57% v. 51%), and in the number of deaths due to infection (one patient in each group). Multivariate statistics using the Cox model identified the type of infection and the increase in neutrophils but not the allocated treatment group, age, underlying disease or initial neutrophil counts as a significant prognostic factor for the response to treatment. An increase in serum creatinine >50% above baseline was seen in two (OD) and three patients (TD), respectively. In the neutropenic patient once daily dosing of netilmicin appears to be as safe as effective as thrice daily dosing.

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CYCLOSPORIN A (CsA) INHIBITS CYTOKINE-INDUCED PROLIFERATIONIN IN B-CHRONIC LYMPHOCYTIC LEUKEMIA (B-CLL)

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We investigated the effects of the immunosuppressant Cyclosporin A (CsA) on proliferation of neoplastic B-cells from patients with B-chronic lymphocytic leukemia (B-CLL). All patients investigated were in a non-progredient phase of the disease, not undergoing specific chemotherapy for the last 3 months. MNC's were isolated as described in Materials and Methods. We used only cell suspensions with > 99% B-cells, analysed by FACS. Cell growth was induced by Tumor Necrosis Factor α (TNFα) or Interleukin 2 (IL-2). We could demonstrate that CsA is able to inhibit cytokine-induced proliferation, measured by ^{3}H-thymidine incorporation, in all cases responsive to TNFα or IL-2. CsA did increase neither the fraction of trypan blue positive cells nor apoptosis, another form of cell death. Growth-inhibition by CsA occured in a dose-dependant manner: 100 ng/ml CsA was an optimal concentration to block more than 90% of cytokine-induced proliferation, whereas less than 10 ng/ml had no effect. We could also demonstrate that the effect of CsA is reversible and that no blocking effect was observed when CsA was added later than 36 hours after stimulation with TNFα or IL-2. CsA did impaire neither the expresion of TNF-α or IL-2 receptors nor the binding affinity of the relevant cytokines. In contrast, we could show that the TNF-α or IL-2 induced proliferation of other lymphomas (e.g. Burkitt's lymphoma) or hairy cell leukemia (HCL), were not affected by CsA. This observation indicates that the inhibitory activity of CsA seems to be specific for B-CLL.

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Distinct combinations of NF-kB subunits determine the specifity of transcriptional activation.

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The transcription factor NF-kB plays a major role in the regulation of cytokines, MHC I and II antigens and viruses. We recently reported the cloning of a new member of the NF-kB multigene family, NF-kB p49/p100 (Nature 352: 733-736, 1991). To under-stand the role of the distinct NF-kB subunits, p49/p100, p50/105, p65 and c-rel, in vitro and in vivo studies were performed. Homodimeric p49 binds weakly to the HIV kB site (identical to the k-light chain kB site) but strongly to the MHC class I kB element. In association with p65 the complex binds with greater affinity to the HIV kB element but still binds the MHC class I motif stronger. In transfection experiments in a T-cell line we have characterized the transcriptional activation of the HIV, MHC class I, interleukin-2 receptor, and two related kB motifs with only single base pair changes. The combination of p49/p65 is most effective in stimulating transcription dependent on the HIV kB site and slightly stimulates reporter plasmids with one base pair changes in the kB sites. These data indicate that: 1. Binding activity to the kB sites does not correlate with transcriptional activation. 2. kB dependent gene expression is regulated through distinct combinatorial associations of NF-kB subunits. 3. Transcriptional activation is affected by single base pair changes in the kB motif. This complexity of regulation might have been evolved to enable the differential expression of diverse genes containing kB sites.

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Overexpression of C-RAF-1 in Acute Myeloid Leucemia

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The protein product of the c-raf-1 gene, a cytoplasmatic 74 kD serine/threonine kinase, plays an important role in signal transduction. In in vitro experiments, amino terminal deletion of the c-raf-1 gene increased oncogenic activity significantly in transfection experiments. No alteration of the c-raf-1 gene has been described in hematopoietic tissues so far. However, rapid phosphorylation of the c-raf-1 gene in myeloid cell lines following hematopoietic growth factor treatment was described recently, indicating that c-raf-1 may play a role in hematopoietic signal transduction.

We analyzeded transcript size and level of c-raf-1 gene expression in 43 AML cases by Northern blot analysis. No alteration in transcript size was detected in any of these cases. However, overexpression of the c-raf-1 gene was found in two AML cases. Equal RNA load was checked by intensity of the ethidium bromide stained gel under UV illumination and confirmed by rehybridisation of the filters with a control gene probe (ß-actin). Southern blot analysis of genomic DNA and sequencing of the amino terminal portion of the c-raf-1 gene revealed no alteration in these two cases. However, other mechanisms (e.g. activating ras point mutations) could account for the observed phenomena.

We conclude that c-raf-1 overexpression can be found in a subset of AML in adults. The biological significance of the observed findings remains to be determined.

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DIFFERENTIAL INDUCTION OF HLA CLASS I ANTIGEN EXPRESSION BY INTERFERON

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Differences in the regulation of HLA class I antigen expression by interferon (IFN) was demonstrated after transfection of the corresponding genes into mouse L cells. Modification of the 5' ends of the HLA-B7 and HLA-B27 genes before transfection 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. For further analysis of the structural basis causing differential induction of HLA-class I genes by IFN 560 bp fragments containing the ICS, enhancer A and B region of the 5'ends of HLA genes were subcloned into pBL CAT2 plasmids and analysed for IFN sensitivity after transfection in mouse L cells. In these cells we could show that low inducibility of the B27 or B38 gene by IFN could be due to an high constitutive promotor acitvity of their 5'ends with reduced IFN sensitivity in mouse fibroblasts. In contrast, 5'-flanking regions of the B7 and B64 genes appear to operate at constitutively low level expression, but are strongly regulated by IFN type I to potentiate gene transcripition. In comparison in vivo modulation of HLA-antigens on peripheral blood lymphocytes, monocytes and hematopoietic precursors during IFNα therapy was investigated in patients with myeloproliferative syndrome. A strong induction of HLA-class I antigens was found on all eamined cells within the first 36 hours. HLA-class I antigen expression was consistently augmented by IFNα in all patients irrespective of their haematologic response. Differential in vivo regulation of HLA-class I antigens was demonstrated by comparison of HLA-A2 with HLA-B antigen expression. These findings may indicate specific regulatory mechanisms for different HLA class I antigen expression possibly influencing T-cell recognition in immune responses.

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IN VITRO GENERATION OF LAK ACTIVITY AGAINST AUTOLOGOUS LYMPHOMA CELLS FOLLOWING BONE MARROW TRANSPLANTATION

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Conventional methods to improve autologous transplantation results with post transplant therapy are limited by toxicity and drug resistance. Lymphocyte activated killer (LAK) cells may provide a novel method to improve results following bone marrow transplantation (BMT). We investigated the possibility of generating LAK cells following BMT from patients with lymphomas. Moreover, we investigated the sensitivity of autologous malignant cells to the cytolytic effect of LAK cells generated following autologous BMT. The results demonstrate that patients' LAK cells recognize and lyse autologous tumor targets. At an effector to target cell ratio of 20:1, there was a mean % specific Cr release of 30+7% for autologous tumor cells compared to the LAK tumor cell line target (OCI-Ly8) of 48+7%. At an effector to target ratio of 40:1, there was 53+8% specific Cr release of autologous tumor targets. These results suggest that LAK cells generated post-autologous BMT are effective in lysing autologous lymphoma cells in vitro and may provide a useful method to improve disease free survival following autologous BMT.

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DETECTION OF BCR/ABL POSITIVE CLONOGENIC CELLS DERIVED FROM SINGLE COLONIES FROM PERIPHERAL BLOOD FROM PATIENTS WITH CHRONIC MYELOGENOUS LEUKEMIA

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Chronic myelogenous leukemia (CML) is a clonal neoplastic disease that arises in a pluripotent hematopoietic stem cell. However, functionally competent non-neoplastic Ph' negative stem cells are known to persist in many patients. Differentiation between neoplastic and non-neoplastic cells is routinely done via cytogenetic determination of Ph' positive cells. However, no assessment on clonogenicity of the cells can be made with that method. In order to quantitate the number of normal and bcr/abl positive clonogenic progenitor cells, we have examined individual 14-day CFU-GM colonies from CML patients via polymerase chain reaction (PCR). Clusters and colonies with 20-50 cells were isolated, total RNA was isolated and complementary DNA (cDNA) was obtained by reverse transcription. PCR was performed on cDNA from each single cluster or colony using primers specific for bcr/abl chromosomal translocation. Alpha-actin primers were used as control for the amount and integrity of the cDNA. PCR products were reamplified in a second PCR using internal primers ("nested PCR"). Using this method bcr/abl transcript could be detected reliably in cDNA derived from 20-50 cells and the percentage of bcr/abl positive colonies was determined. This simple method should facilitate the assessment of efficacy of therapeutic studies performed either in vitro or in vivo in patients with CML.

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EFFECTS OF INHIBITORS OF TOPOISOMERASES I & II ON DNA METHYLATION AND DNA SYNTHESIS IN HUMAN COLONIC ADENOCARCINOMA CELLS *IN VITRO*

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Exposure of human and animal cells to inhibitors of topoisomerase I or II has recently been shown to alter gene expression and induce differentiation in a number of experimental systems. Since DNA hypomethylation is frequently associated with transcriptional activation, we wished to explore the relationship between inhibition of DNA topoisomerases and enzymatic DNA methylation. When HT-29 human colonic adenocarcinoma cells were exposed to the specific topoisomerase II inhibitor teniposide (VM-26), a dose-dependent hypomethylation of DNA was observed during the window of drug treatment. Exposure to the topoisomerase I inhibitor camptothecin (CPT) produced a small but not statistically significant trend toward DNA hypomethylation. CPT-treated cells were found to have up to 19 fold increased levels of topoisomerase II protein, which may have compensated for decreased levels of non-drug-bound topoisomerase I. Both VM-26 and CPT were found to increase [^{3}H]thymidine incorporation into DNA when administered in low dose. Combination of VM-26 and CPT produced DNA hypomethyltion, a synergistic increase in DNA synthesis, and an increasing number of cells entering a higher DNA ploidy cycle. Topoisomerase inhibitor-induced DNA hypomethylation may offer a possible explanation for the induction of differentiation observed upon exposure to this family of drugs. Altered topoisomerase activity occurring during the process of tumor progression may also provide a link between the induction of polyploidy, DNA hypomethylation and aberrant gene expression, three markers of genetic instablility in tumor cells.

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AUTOMATED LEUKOCYTE DIFFERENTIAL: GOALS AND PERSPECTIVES

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The goal of the automated leukocyte differentiation is both the objective, reliable and statistically valid recognition of all normal leukocyte populations of the peripheral blood and the sensitive detection and further analysis of pathological samples. Following recent apparative and methodological progress the multiparametric flow cytometry is interesting for the automated leukocyte differentiation not only for research, but also for routine laboratories. An objective cellular characterisation is reached by the automated measurement and analysis of five independent parameters (cellular forward and side scatter, three fluorescence emissions) under standardised, machine-independent measurement conditions. The analysis of 10,000 to 50,000 cells per blood sample results in statistically valid information even about small cellular subpopulations. The analysis of the cellular light scatter characteristics and of cytochemical staining reactions allows a white blood cell differentiation similar to the use of cytochemistry in morphological cell differentiation techniques. An objective recognition of the cellular lineage and the precise characterisation of the cellular differentiation level even of morphologically indistinguishable lymphocyte subpopulations are obtained through the simultaneous analysis of up to three molecularly defined surface or cytoplasmic antigens with directly labelled monoclonal antibodies. The analysis of the lineage specific expression of enzymes such as the cysteine proteinases of mononuclear phagocytes is an additional parameter for the differentiation of myeloic cells. Pathophysiologically important information in malignant hematopoietic diseases can be obtained from the analysis of the cellular DNA content and proliferative activity. Functional parameters such as the the intracellular free calcium and pH, the cellular production of reactive oxidants, the cellular glutathione levels, or the cellular production of cytokines upon stimulation in addition reveal information about reactive alterations of leukocytes, e.g. in inflammatory processes and should lead to additional diagnostic applications of the white blood cell differentiation.

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MAGNETIC RESONANCE IMAGING OF BONE MARROW IN HEMATOLOGIC DISORDERS

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The ability of MR imaging to detect bone marrow involvement is currently evaluated for different hematological disorders. Unlike plain radiography and computed tomography which may detect destruction of trabecular or cortical bone *secondary* to cellular infiltrates MR imaging depicts malignant bone marrow infiltrates with signal intensities different from those of normal fatty or hematopoietic marrow. Plain T1-W images were usually employed in the past, but more sophisticated approaches promising higher sensitivity and specificity are currenntly developed. Newer techniques include different methods of suppressing signal from fat. These approaches may be used alone or in combination with the paramagnetic contrast agent Gadolinium-DTPA.
The paper reviews the different techniques and discusses the role of MR imaging in detecting marrow involvement in patients with multiple myeloma, Hodgkins disease and the Non-Hodgkin lymphomas.

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IMPROVED EVENT-FREE-SURVIVAL OF CHILDHOOD T-CELL ACUTE LYMPHOBLASTIC LEUKEMIA (T-ALL) AFTER INTRODUCTION OF HIGH DOSE METHOTREXATE IN MULTICENTER TRIAL ALL-BFM 86.

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202 T-ALL patients were enrolled in two consecutive trials, 79 in ALL-BFM 83 (n=677) and 123 in ALL-BFM 86 (n=998), the median follow-up being 83 and 40 months, respectively. The patient groups were not different with respect to age, sex, white blood count, platelets, hemoglobin, mediastinal mass, hepato- and splenomegaly. In both studies, the therapy strategy was composed of induction, methotrexate (MTX) based extracompartment therapy, reinduction, maintenance and brain irradiation. In trial ALL-BFM 86, high-dose (HD) MTX, 5 g/m² instead of 0.5 g/m² as in trial ALL-BFM 83, was applied four times as 24 h infusion. Pts with initial poor response to prednisone (Pred-PR: ≥ 1000/μl blasts in the peripheral blood at day 8) received protocol E instead of protocol M, composed of HD-MTX (4x), HD-cytarabine (2 g/m² x 8), ifosfamide, mitoxanthrone and prednisone. Event free survival (pEFS) in trial ALL-BFM 83 at 5 years for T-ALL pts is 0.56 and 0.73 in trial ALL-BFM 86 (p=0.013). For pts with Pred-PR pEFS at 5 years was 0.41 in trial ALL-BFM 83 (n=26) and 0.44 in trial 86 (n=34). For pts with prednisone good response (Pred-GR: < 1000/μl blasts in the PB at day 8), pEFS at 5 years was 0.62 in trial ALL-BFM 83 (n=53) but 0.84 in trial 86 (n=89). The difference of pEFS for Pred-GR pts in trial ALL-BFM 86 compared to trial 83 is significant (p=0.006) indicating a beneficial effect of HD-MTX for T-ALL pts with Pred-GR.

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ANTICOAGULANT-INDUCED PSEUDOTHROMBOCYTOPENIA (PTP)

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Recognition of PTP due to anticoagulant-induced platelet clumping is important because a falsely low platelet count may lead to unnecessary diagnostic procedures and unjustified, potentially harmful treatment. For a detailed study we selected 10 patients with EDTA-induced PTP that had been admitted to our hospital with previously misdiagnosed "thrombocytopenia". Blood was collected into K_2EDTA (2.2 mg/ml; final concentration), Na-oxalate (10 mM), Na-citrate (15 mM), acid-citrate dextrose (ACD)(NIH Formula A, 1/10 vol), heparin (100 U/ml), hirudin (50 μg/ml) or PPACK (10 μM) and counted immediately in parallel in a Coulter T540 and a Coulter STKS counter and by phase contrast microscopy. Counts were repeated after 10, 20, 30, 60, 90, 120, 240 and 360 minutes. Results: In phase contrast microscopy of finger-stick blood without anticoagulants the mean platelet count was 285 G/L. With the Coulter T540 model the mean platelet count dropped progressively to a minimum of 30 G/L (EDTA), 94 G/L (oxalate), 108 G/L (citrate), 156 G/L (ACD), 111 G/L (heparin), 112 G/L (PPACK) and 139 G/L (hirudin) at 360 min. In EDTA- and oxalate-blood platelet counts decreased very fast within the first 30 min.. The counts with the other anticoagulants fell slowly over time reaching a plateau after 180 min.. The time course of platelet counts was significantly different between the STKS and the T540 counter. With the STKS model platelet counts declined within 30 minutes to 69 G/L (EDTA), 35 G/L (oxalate), 73 G/L (citrate), 80 G/L (ACD), 60 G/L (heparin), 73 G/L (PPACK) and 61 G/L (hirudin). With exception of counts from EDTA- and heparin-anticoagulated blood, counts increased again reaching a stable plateau at about 90 minutes after blood sampling. In the T540 model, PTP was accompanied by a pseudoleucocytosis only when EDTA was used (significant increase in leucocyte count from 7.1 to 10.6 G/L). In contrast, with the STKS model a concomitant pseudoleucocytosis was present also in oxalate-, heparin- and citrate-anticoagulated samples. In EDTA-anticoagulated blood, an abnormal platelet histogram was detected only in 28% of measurements in the T540 model and in 38% of measurements in the STKS counter. Conclusions: It is important to recognize the laboratory artifact of PTP and pseudoleucocytosis to avoid inappropriate treatment. PTP is most pronounced when EDTA-blood is used. However, there is no anticoagulant that reliably prevents falsely low platelet counts in patients with PTP. The least decline in platelet counts was observed when ACD-anticoagulated samples were used. The efficacy in the detection of platelet clumping by automatic cell counters is low. In platelet counts of patients with PTP the correlation between the two counters used and between the STKS counter and the hemocytometer counts is poor.

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PRELIMINARY RESULTS OF TREATMENT WITH WEEKLY APPLIED NAVELBINE (NVB) IN PATIENTS WITH DIFFERENT SOLID TUMORS

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Vinoelbine, Pierre Fabre Oncologie, is a new Vincaalkaloid with high single agent activity especially in lung cancer (NSCLC and SCLC) and breast cancer.
In a clinical study we treated 16 heavily pretreated females median 58 years with breast cancer and 13 pts with lung cancer (11 m, 2 f, 0/4 pretreated SCLC, 4/9 pretreated NSCLC median age 59 years) and 3 pts all pretreated with esophageal tumors and 1 female with ovarian cancer.
At the begining of treatment all pts showed sign of tumor progression. The attention was to apply NVB weekly in a dose of 30 mg/m² palliatively. Pretreatment for breast cancer for all pts consisted of CMF, VAMe, VTM. Pts with lung and esophageal and ovarian cancer were pretreated with Cis-platin containing schedules and radiotherapy.

Results:

Breast cancer	n = 16	PR : 5, MR : 3, NC : 3 PD:5
SCLC ext. II	n = 4	PR : 2, MR : 2
NSCLC	n = 9	PR : 2, NC : 2, PD : 5
Esophageal cancer	n = 3	MR : 1, PD : 2
Ovarian cancer	n = 1	PD : 1

Toxicity: Dosis limiting toxicity was leucopenia 17/32 pts with WHO III. Side effects of local thrombophlebitis 6/32 were reversible and could be prevented by using a central vein catheter.

Conclusion: The presented data of pts with breast cancer and SCLC are encouraging and let us consider NVB as an effective drug even in pretreated pts. Further trials are warranted.

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CLINICAL AND PHARMACOLOGICAL EVALUATION OF THE EFFECT OF DEXNIGULDIPINE (B8509-035) ON THE TREATMENT OF PATIENTS (PTS) WITH ACUTE MYELOGENOUS LEUKEMIA (AML) IN RELAPSE WITH HIGH-DOSE CYTOSINE ARABINOSIDE (ARA-C) AND DAUNORUBICIN (DNR)

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The occurrence of multidrug resistance (MDR) may be one of the major obstacles to an effective chemotherapy of pts with AML. It may be associated with the overexpression of a membrane glycoprotein (Gp-170) acting as an energy-dependent efflux pump for anthracyclines and other xenobiotics. Several drugs including calcium antagonists have been proven to inhibit Gp-170 in vitro. However, effective concentrations mostly cannot be obtained in plasma without severe side effects. In contrast, the dihydropyridine derivative B8509-035 may be a nontoxic modulator of MDR. Pts with AML in relapse were sequentially treated with hAD (2x1,000 mg/m²/d ARA-C i.v., d 2-5, 60 mg/m²/d DNR i.v., d 1-3) and hAD & B8509-035 (1,250 or 1,750 mg/d p.o., d (-2)-7), respectively. Plasma kinetics of DNR and kinetics of cellular and nuclear uptake of DNR were determined in myeloid blasts (MB) isolated from peripheral blood (PB) dependent on B8509-035 either by FACS-analysis or by HPLC with fluorescence detection. Up to now, 11 pts with AML resistant to hAD are evaluable for response and toxicity (7 pts with 1,250 mg and 5 pts with 1,750 mg B8509-035). In 1 pt of the 1,250 mg-group a complete remission was induced, all other pts had a persistence of MB on subsequent hAD & B8509-035 which was well tolerated. Plasma kinetics of DNR was not significantly influenced by B8509-035. In vivo cellular uptake of DNR in MB was about 25% higher when B8509-035 was added. In vitro investigations of MB from PB or bone marrow concomitantly performed showed that the efflux of rhodamine 123 was significantly higher from CD34-positive subpopulations (SP) and could be more effectively inhibited by B8509-035 than by verapamil. However, MB expressing Gp-170 as determined by MRK 16 immunofluorescence were rare and could be only identified in 6/30 SP. In conclusion, B8509-035 seems to be a well tolerated substance to effectively reverse MDR in MB.

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31P-NMR -SPECTROSCOPY (31P-MRS): THERAPY MONITORING IN PATIENTS WITH SOFT TISSUE AND BONE SARCOMAS

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P31-MRS noninvasively provides information on relative concentrations of high energy phosphates and phospholipids in vivo. Aim of the study was to investigate, whether 31P-MRS canbe used as an early indicator of tumor response to chemotherapy and whether it is possible to distinguish between responders and non-responders using the pretherapeutic metabolic profile as assessed b 31P-MRS. 31P-MRS was performed in 9 patients (5 females, 4 males) (mean age: 24.8 ± 4.3 yrs.) for different soft tissue and bone sarcomas. 7 ptients were investigated prior to, during and after chemotherapy. Changes in the spectra were correlated to clinical course and histological tumor regression assessed after surgery according to Salzer-Kuntschik. 2 patients were finally classified responders(R) and 5 non-responders (NR). P31 - NMR spectra were obtained at 25.85 MHz, using a 1.5 Tesla imaging-spectroscopy system and a 16 cm double tuned surface coil. Mean size of the volume of interest(VOI) was 86.3±33.5 cm^3). The VOI was strictly located within the tumor. Magnetic field homogeneity was optimised to 40± 12 Hz. 512-1024 free induction decays were averaged. Prior to treatment all spectra showed elevated PDE (phosphodiester), PME(phosphomonoester) and Pa(anorganic phosphate) and reduced PCr(phosphocreatine) peaks compared to a normal muscle spectrum. In R and NR the pretreatment PDE/PME ratio was 1.33 ± 0.72 and 1.45 ± 0.54, respectively. Pretherapeutic PCr/Pa and PCr/γ-ATP ratios were significantly different in R 1.48 ± 0.35 and 1.03 ± 0.3 compared to NR 0.43 ± 0.36 and 0 .39 ± 0.38. In NR tumor volume remained unchanged or increased during therapy and no change of spectral profiles could be noted during therapy, In responders metabolic changes turned out to be more heterogeneous. Two of the R showed tumor volume reduction of >50%. In one of these PDE/PME increased under therapy. In the other PDE/PME decreased. In the latter patient posttherapeutic pathology found 70% viable tumor cells, indicating that a resisting subclone had developped under therapy. The R without tumor volume reduction showed a dramatic change of the spectral profile towards a normal muscle spectrum, as confirmed by Tl-201 scintigraphy.**Conclusions:**P31-MRS may be a useful early indicator to tumor response during chemotherapy together with other clinical parameters. The pretherapeutic PDE/PME ratio does not seem to differ significantly b-tween R and NR. Pretherapeutic PCr/Pa and PCr/ATP ratios seem to predict tumor response to chemotherapy while pretherapeutic PDE/PME ratio does not.

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DETECTION OF PERIPHERAL BLOOD CELLS DEFICIENT FOR GPI-ANCHORED SURFACE PROTEINS IN PATIENTS WITH PNH AND SAA.

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Paroxysmal nocturnal hemoglobinuria (PNH) is an aquired disorder in which peripheral blood cells deficient in glycosyl phospholipid (GPI)-anchored surface proteins appear in the circulation. On erythrocytes the deficient surface expression of GPI-anchored complement regulating proteins such as DAF and MIRL (CD55 and CD59) leads to an abnormal susceptibility against homologous Complement resulting in the leading clinical symptom of chronic or sudden intravascular hemolysis. In earlier studies we have shown that phenotyping of peripheral blood cells is a reliable and sensitive method to detect the GPI-anchoring defect and to establish the diagnosis of PNH. Here, data are presented of 40 patients with severe aplastic anemia (SAA) recieving standard immunosuppressive therapy with anti-thymocyte globulin (ATG) and cyclosporin A (CsA). 37 % of these patients exhibit GPI-deficient cells detectable in peripheral blood. Deficient cells arise in the granulocyte and monocyte population before erythrocytes are affected and the conventional diagnostic tests for PNH reveal positive results. Moreover, from these data it is evident that the appearance of GPI-deficient cells in the circulation is associated with a poor response to therapy or with relapses after initial remissions.

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ORAL FLUCONAZOLE (F) VS ORAL POLYENES (P) AS ANTIMYCOTIC PROPHYLAXIS IN NEUTROPENIA: AN EUROPEAN MULTICENTER STUDY

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In an open multicenter study involving 18 centers in 6 european countries 538 patients were randomized to either 50 mg/day fluconazole or ≥4*10^6 U Nystatin or 2g amphotericin B.

Study population :		F	P
Diagnoses:	AML + ALL/lymphomas	195/40	210/30
	other malignancies / SAA	32/ 2	24/ 5
	(BMT-patients)	(60)	(50)
Risk Factors:	Antibiotics	181	165
	Corticosteroids	134	117
	Prophylaxis duration (days)	29.3	31.3
	median minimum neutroph./mm³	128	119
Baseline:	positive cultures	23 %	17%
% of +ive cultures			
	Candida albicans / other C.	71/11	72/10
	Torulopsis glabrata	8	8

Results :	F	P	
Oropharyngeal Candidiasis	4	22	p<.001
- C.albicans	(2)	(20)	
- other Candida	(2)	(2)	
Systemic Mycoses	6	9	n.s.
- C.albicans	-	(2)	
- C.krusei	(3)	(3)	
- Aspergillosis	(2)	-	

F prophylaxis is as efficent as P in preventing mycoses in neutropenic patients, there were significantly less mucosal mycoses in the F group.

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MECHANISMS OF CELL GROWTH INHIBITION AND CELL CYCLE ARREST IN HUMAN COLONIC ADENOCARCINOMA CELLS BY DEHYDROEPIANSDROSTERON: ROLE OF ISOPRENOID BIOSYNTHSIS

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There is epidemiological and experimental evidence suggesting that the adrenal steroid, dehydroepiandrosterone (DHEA), has significant chemopreventive efficacy, although its mechanism of action remains uncertain. We have previously demonstrated that DHEA inhibits the isoprenylation of cellular proteins including $p21^{ras}$ by depletion of endogenous mevalonate. Furthermore we have shown that DHEA blocks the $p21^{ras}$ membrane association which is a precondition for the cell transforming activity of oncogenic *Ras* proteins (*Cancer Res. 51:* 6563-6567, 1991). We now report that treatment of HT-29 SF human colonic adenocarcinoma cells with DHEA at concentrations ranging from 12.5 to 200 μM for up to 72 h inhibited growth and arrested cells in G_1 phase of the cell cycle in a time- and dose-dependent manner. Exposure to 25 or 50 μM DHEA also transiently delayed cells in G_2M phase after 48 h. Addition of mevalonic acid (MVA) partially overcame both the growth and cell cycle effects of 25 μM DHEA in the initial 48 h. During prolonged exposure (72 h), the addition of MVA as well as cholesterol was required to reconstitute cell cycle progression. This suggests that the depletion of endogenous mevalonate and other isoprenoids is involved in DHEA-mediated growth inhibition and cell cycle arrest. Thus, it is possible that the depletion of endogenous mevalonate and the inhibition of protein isoprenylation by DHEA may contribute to its anticancer effects.

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ACTIVITY OF RMU SCF AND RHU GM-CSF IN CANINE T-DEPLETED MARROW

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Depletion of T-cells from bone marrow sucessfully prevents Graft-versus-Host disease but engraftment may be delayed or transient. Recombinant human and murine growth factors were studied for stimulation of canine hemopoietic precursor cells in the presence and absence of CD6-positive T-cells. In undepleted marrow recombinant murine (rmu) SCF stimulated growth of CFU-C in a dose dependent manner and the addition of recombinant human (rhu) GM-CSF had little effect on CFU-C growth. In CD6-depleted marrow rhu GM-CSF in addition to rmu SCF was necessary for adequate canine CFU-C growth. The size of colonies was markedly increased by rmu SCF. The effect of rmu SCF can even be obtained by overnight exposure of T-depleted marrow cells.
Both murine SCF and human GM-CSF effectively stimulate the in vitro growth of canine hemopoietic precursor cells. A brief exposure to rmu SCF is sufficient for stimulation of CFU-C precursor cells. The effect of depletion of CD-6-positive T-cells may be compensated by the addition of rhu GM-CSF.

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METHYLTHIOADENOSINE PHOSPHORYLASE DEFICIENCY IN HUMAN MALIGNANCY

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The abnormal growth of malignant cells is eventually mediated by changes in specific metabolic pathways. Methylthioadenosine (MTA) is a co-product of polyamine biosynthesis and is degraded by MTA phosphorylase (MTAase) to adenine and methylthioribose-1-phosphate (MTR-1-P) in all normal mammalian cells. These products of the phosphorylytic cleavage of MTA are recycled to the nucleotide pool and methionine, respectively. The MTAase activity of 21 glioma cell lines and tumor samples of different stages, and 10 osteosarcoma cell lines was determined either by measuring the conversion of [5'-^{3}H]-MTA to [5'-^{3}H]-MTR-1-P in cell-free extracts or by autoradiography with the substrate [8-^{14}C]-MTA. 9/21 (43%) of the glioma cell lines and tumor samples and 5/10 (50%) of the osteosarcoma cell lines were MTAase-deficient. As we hypothesized that under conditions of methionine deprivation and inhibition of purine synthesis (azaserine) MTA-ase-positive cells (HL-60, U373) could use exogenous MTA for generation of methionine and adenine while MTAase-negative cells (CRF-CEM, TE85) would not have this capability, we cultured the respective cells in methionine-free medium / 10% horse serum with and without MTA and azaserine. When cultures were supplemented with MTA, growth of MTA-positive cells increased to control levels, while MTAase-negative cells remained suppressed. Our results indicate that regimens using MTA might be a means of selectively killing MTA-ase-deficient malignant cells while leaving MTAase-positive (benign) cells unaffected.

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DIFFERENTIAL EXPRESSION OF THE C-KIT PROTEIN PRODUCT IN HEMATOPOIETIC MALIGNANCIES

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The stem cell factor (SCF) has been identified as the ligand of a 145- to 160-Kd transmembrane receptor with tyrosine kinase activity encoded by the c-*kit* proto-oncogene. Recent studies have reported c-*kit* mRNA and/or protein expression in 30-87% of acute myeloid leukemia (AML) specimens and indicated that evidence of the c-*kit* product in AML was associated with a poor response to treatment. At the beginning of 1991 we have started to prospectively analyze c-*kit* expression in hematopoietic malignancies and have yet investigated a large series of patients with malignant myeloid and lymphoid proliferations (N=171) of distinct differentiation stages. Two monoclonal antibodies (MoABS: YB5.B8, 17F11) recognizing an extracellular domain of the c-*kit* receptor were used to detect c-*kit* expression and their reactivity was evaluated by flow cytometry. C-*kit* expression (≥ 10% positive cells) was found in most patients with AML (66/103, 64%), irrespective of their FAB-subtype, myelodysplastic syndrome (2/6), and blast crisis of chronic myelogenous leukemia (CML, 3/4) but not in cells from patients with chronic phase of CML (0/5) or acute lymphoblastic leukemia (0/53) disclosing a B-cell precursor or T-cell-lineage immunophenotype. Although the percentage of c-*kit*-positive blasts varied considerably within the AML patients, we did not observe any significant differences in the reactivity pattern of both MoAbs tested. A strong c-*kit* expression (> 50% positive blasts) was found more often in AML cells with an immature CD34-positive, CD13/CD33 weakly positive or negative immunophenotype thus underscoring the value of c-*kit* as a marker for undifferentiated AML. The antigenic profile of c-*kit*-positive myeloid leukemic cells will be correlated with the expression of other progenitor cell markers, myeloid-lineage-associated differentiation antigens as well as cytoadhesion molecules and the prognostic implications of c-*kit*-positive AML will be presented.

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A CASE OF EXTRAMEDULLARY BLAST CRISIS IN CHRONIC MYELOID LEUKEMIA WITH A MEDIASTINAL MASS AND PLEURAL EFFUSION

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A 46-year-old woman with severe dyspnea was admitted to our hospital. The diagnosis of a chronic myeloid leukemia had been made one year previously and confirmed by detecting a M-bcr/abl mRNA using the polymerase chain reaction (PCR). The patient had been treated with busulfan for one year. At the time of admission, the chest x-ray and a CT-scan showed mediastinal enlargement and pleural effusions bilaterally. Lymphoblastic cells found in pleural-effusion specimens disclosed immunophenotypic features of thymic cells (CD 1a+, CD 2+, CD 3+, CD 4+, CD 5+, CD 7+, CD 8+). The cells were found to be negative for B-cell surface markers such as CD 19 and CD 20. The PCR demonstrated a M-bcr/abl mRNA in these cells, thus confirming their origin from the malignant clone. Morphologically, the bone marrow showed a chronic phase of the disease without immunological evidence of an immature T-cell population. The patient was treated according to the ALL-BMFT protocol with a good response and a reduction of the mediastinal mass. Pancytopenia delayed the continuation of chemotherapy, but dyspnea and regrowth of the mediastinal mass after twelve days of delay required the continuation of the chemotherapy which again resulted in an improvement of symptoms. Concomitant mediastinal and cranial irradiation was performed. Seizures and impairment of consciousness occurred and the cell count in the cerebrospinal fluid was much higher than at the previous examination. The cells showed morphological features of lymphoblastic cells. The patient died three months after the occurrence of the first symptoms. Extramedullary blast crisis with T-cell features is reported only in six cases yet and has a poor outcome as in our case.

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ONDANSETRON AND LEVOMEPROMAZINE FOR EFFICIENT EMETIC CONTROL DURING TOTAL BODY IRRADIATION

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Total body irradiation (TBI) and high dose chemotherapy (HDCT) are commonly used as a pretransplant conditioning regimen for allogeneic and autologous bone marrow transplantation (BMT). Patients (pts) undergoing BMT need efficient antiemetic treatment during these preparative procedures. We investigated the antiemetic efficacy of ondansetron and levomepromazine during TBI. Twenty two pts(median age 15 years, [range 4-34], 10 females and 12 males) were prepared for BMT by fractionated TBI. 17 pts suffered from ALL, 4 from CML and 1 from AML. 10 pts received allogeneic, 2 pts syngeneic transplants and 10 pts were autografted. TBI was delivered prior to HDCT on three consecutive days (d), 2x2 Gy/d for pts with ALL and 1x4 Gy/d for pts with AML/CML. 17 pts received 4-8 mg ondansetron once, 3 pts twice and 2 pts three times/d. Eighteen pts received additionally levomepromazine 2 mg/h for about 8 hours daily during TBI, four pts received ondansetron alone. The following emetic control was achieved: complete response (no emetic episodes) in 11 pts (50%), major response (1-2 emetic episodes) in 8 pts (36%) and minor response (3-5 emetic episodes) in 3 pts (14%). No antiemetic failure (>5 emetic episodes) was observed. Our results indicate efficient emetic control by ondansetron and levomepromazine in patients undergoing TBI. The antiemetic efficacy of ondansetron alone during TBI remains to be investigated in a prospective study.

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ACUTE NON LYMPHOCYTIC LEUKEMIA (ANLL) FOLLOWING CYTOTOXIC TREATMENT FOR OVARIAN CANCER

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Therapy related ANLL (t-ANLL) has become a major long-term complication of cancer chemotherapy (CT). The risk of t-ANLL has been predominantly related to treatment with alkylating agents, more recently also to other cytotoxic drugs. We describe the case of a 53-year-old woman with ovarian carcinoma, who received CT for several years and subsequently developed ANLL.

Case report: In 1/84 ovarian carcinoma (FIGO stage III) was diagnosed by laparotomy. From 1/84 to 10/84 she received CT including cisplatinum (DDP), adriamycin (ADM) and cyclophosphamide (CPM) resulting in complete remission (pCR), followed by maintenance CT with 5-fluorouracil (5-FU) and CPM from 12/84 to 6/86. From 9/87 to 4/88 DDP, CPM and epidoxorubicin (EDR), then until 3/89 melphalan (MPH) were administered because of intraabdominal relapse, resulting in stable disease (SD). However, systemic CT had to be discontinued on account of thrombocytopenia (<20000 platelets/µl). In 5/89 progressive disease (PD) had to be stated and from 6/89 to 8/90 fourteen cycles of intraperitoneal carboplatin were administered resulting in SD again. In 9/90 ANLL occured and the patient expired in 11/90. It is likely that in this patient the ANLL is related to one or more of the administered cytotoxic drugs. This fact stresses the conflicting issues of inducing longstanding remissions in ovarian cancer by intensive CT and the induction of t-ANLL by anticancer therapy, reported to occur in up to 15% of patients treated by CT for ovarian cancer.

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COMPARISON OF THE TOXICITY OF TWO DIFFERENT CONDITIONING REGIMENS (BU/CY VS. TBI/CY) FOR ALLOGENEIC BONE MARROW TRANSPLANTATION IN PATIENTS WITH AML AND CML. A PROSPECTIVE RANDOMIZED STUDY.

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31 patients (pts.)(15 women, 16 men) with acute myeloid leukemia (AML) in complete remission (n=8) and chronic myelogenous leukemia (CML) in chronic phase (n=23) received unmanipulated bone marrow from their HLA-identical, MLC-negative siblings. The conditioning regimen consisted either of busulfan (BU 4 mg/kg/day, day -9,-8,-7,-6) and cyclophosphamide (CY - 50mg/kg/day, day -5,-4,-3,-2) = group A (gr.A) or of total body irradiation (TBI 4 Gy/day, delivered by a linear accelerator on days -8,-7,-6, with lung shielding at 9 Gy) and CY as in group A = group B (gr.B). All pts. received a short course of methotrexate and cyclosporin A until day 150 as prophylaxis for graft versus host disease (GvHD). 18 pts. (13 CML, 5 AML; 9 w, 9 m, median age 34 years, range 10 - 50) were randomized into gr.A and 13 pts. (10 CML, 3 AML; 6 w, 7 m, median age 36 years, range 15 - 50) into gr.B. Median follow up is 16,5 months (range 0,2 - 51) in gr.A and 28 months (range 0,3 - 70) in gr.B.

The actuarial survival is 72% (13/18) in gr.A (median duration 37 months, range 3 - 50) and 69 % (9/13) in gr.B (median duration 40 months, range 3 - 70). Early death (before day +100) occurred in 3 pts. in each group and was not related to the conditioning regimen. Late death occurred in two pts. in gr.A and one in gr.B. Median time to take (polymorph-nuclear cells ≥ 500/ul) was 23 days (range 17 - 34) in gr.A (n=16) and 24 (range 18 - 40) in gr.B (n=12). Two pts. in gr.A and one in gr.B died before engraftment could be documented (day +6, +21 and +9 respectively). One pt. with CML who had received BU/CY relapsed at 27 months. Grade II (Glucksberg) acute GvHD was seen in one pt. in gr.A and in 4 pts. in gr.B, grade III in 2 pts. in gr.A and none in gr.B. No grade IV (WHO) toxicity except for mucositis - 11% in gr.A and 62% in gr.B - was observed in either group. Besides the more severe mucositis, transient bilirubin (grade I-III) elevation was seen more often in gr.B than gr.A: 61% vs. 44%, whereas grade II-III ALT elevation occurred more often in the BU/CY group: 61% vs. 46%. 17% of pts. in gr.A and 38% in gr.B suffered from diarrhea grade II-III. There was no difference as to renal toxicity, hematuria, nausea and vomiting between the two groups. One pt.(with AML) developed veno-occlusive disease (VOD) and in one patient a brief generalized seizure occurred after conditioning with BU/CY. We conclude from the interim analysis of our ongoing prospective study that the BU/CY regimen is considerably less toxic to the mucosa of the entire gastro intestinal tract but may be more toxic to the liver than the TBI/CY regimen. In contrast to TBI/CY treatment it also may affect the central nervous sytem causing generalized seizures. The question if both regimens are equivalent in terms of their antileukemic efficacy awaits further observation.

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T47D RETROVIRUSLIKE PARTICLES - PARTIAL CHARACTERIZATION OF THE VIRAL GENOME

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We are currently investigating the expression of human endogenous retroviruses (HERV) in cell cultures of the human breast cancer cell line T47D which can be induced by steroid hormons to produce retroviruslike particles. The particles show B-type morphology similiar to that of the mouse mammary tumour virus (MMTV). One member of the HERV family has been characterized by Ono and coworkers as a proviral endogenous element of 9.2 kb. HERV-K is expressed as a 8.8 kb mRNA in T47D cells. The possibility that HERV-K or parts of its genome are contained in the retroviral particles is still in discussion.

In this presentation we demonstrate by PCR experiments the expression of *gag* and *pol* specific DNA fragments of HERV-K in T47D cells after hormon induction. By a reverse transcription assay we show that the particles contain reverse transcriptase activity and a mRNA, which can be endogenously reverse transcribed to give a cDNA first strand of about 4.5 kb. Using this cDNA as a template in a PCR assay, however, no amplification of HERV-K specific *gag* and *pol* fragments was observed.

From the existing data it may be concluded that the T47D particles either contain a truncated form of the HERV-K genome missing the *gag* and *pol* regions tested or a novel HERV. In order to establish the nature of the sequences harboured in the T47D particles, screening of a cDNA library from the cloned particle genome with a number of retroviral DNA probes is in progress. Using the same probes we carry out Northern blot experiments on purified particle mRNA.

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COUMARIN NECROSIS: ANALYSIS OF 188 CASES REPORTED IN THE LITERATURE BETWEEN 1943 AND 1990

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In the last years we treated 3 patients with severe coumarin necrosis in our hospital. In order to answer the open questions concerning incidence, risk factors, pathophysiology, clinical course and therapeutic strategies of this disease, we reviewed the literature data since 1943.
Between 1943 and 1990, 188 cases of coumarin-induced necrosis of the skin and underlying subcutaneous tissues were described in 111 publications. The incidence of coumarin necrosis is reported to range from 0.01 to 0.1 % of all patients treated with oral anticoagulation. The exact pathomechanism of coumarin necrosis is still unknown. There is good evidence, however, that primary endothelial vascular damage induced by coumarins and transitory thrombophilic diathesis due to an acquired protein C deficiency is a main cause. Half life of protein C is shorter than that of the vitamin K - dependent clotting factors II, VII, IX and X. The maximum of the imbalance between pro- and anticoagulant activity is reached between the 3rd and 5th day after starting oral coumarin therapy.
The **clinical picture** of coumarin-induced skin necrosis passes four stages: 1. initial flush 2. petechiae 3. ecchymosis 4. hemorrhagic infarction (gangrenous necrosis). **Risk factors** for developing coumarin necrosis are: 1. female patients, mainly after delivery or > 50 years. Relative estrogen deficiency is supposed to be an important factor. 2. obesity 3. infection and antibiotic treatment 4. pre-existing hereditary protein C deficiency. Predisposing areas for the lesion are characterized by marked subcutaneous tissue, e.g. female breasts, buttocks and thighs. Possible **therapeutic strategies** are: 1. fibrinolytic therapy 2. continuation of coumarin therapy 3. anticoagulation with heparin 4. application of protein C concentrate or prothrombin complex preparation containing high levels of protein C. Mortality of coumarin-induced skin necrosis is 15 %. The risk of re-necrosis after repeating oral anticoagulation is 19 %. Therefore we suggest that necessary secondary prophylaxis of thromboembolic complications should be best continued with subcutaneous application of fractionated or unfractionated heparin.

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PLATELET MEMBRANE INTEGRINS AND FUNCTION OF CRYOCONSERVED AUTOLOGES PLATELETS IN PATIENTS WITH ACUTE LEUCEMIA

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Introduction:
Polychemotherapy in patients with acute leucemia requires supportive therapy including platelet transfusions. In alloimmunized patients refractory against unselected and HLA-matched platelet tranfusions we used cryoconserved autologes platelets taken in regeneration of bone marrow after chemotherapy by thrombapheresis as described by us earlier. Platelet membrane glycoproteins and platelet functions were measured before, 1 and 24 hours after 31 transfusions to 7 patients with acute leucemia and in 31 freshly thawed cryoconserved platelet concentrates.
Results
Platelet numbers before 1 and 24 h after transfusion were 20571, 67430 and 41735/μl (min), respectively.

time of therapy	Bleed. time (min)	Adhesion Hellem II (%)	Aggregation ADP (%)	Aggregation Kollagen (%)	Glycoproteins Ib (%)	Glycoproteins IIb/IIIa (%)
before Ch.T	5±2	61±36	29±18	70±21	92±4	96±5
concentrate	nd	nd	20±12	42±29	69±23	89±11
1 h after T	11±3	27±17	14±12	36±23	62±21	80±22
24 h after T	13±4	19±22	nd	nd	51±11	85±5
controls	< 6	92±3	53±10	82±8	94±5	95±10

Ch.T = chemotherapy; T = transfusion MW ± SD

GP IV and Laminin were not altered by the procedure.
Conclusion: The results demonstrate that after freezing and thawing platelet membrane glycoproteins in concentrates were conserved to 74 - 93 % and after transfusion to 67 - 83 % of initial. Aggregability of platelets in concentrates was preserved to 43 - 69 % and after transfusion in patients to 32 - 51 %. Good aggregability and conservation of GP of platelets led to a sufficient hemostatic function and prevented any bleeding complications in patients during polychemotherapy.

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THROMBOGENIC RISK OF LONG-TERM INTRAVENOUS CATHETERS IN PATIENTS WITH MALIGNANT HAEMATOLOGICAL DISEASES

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Between 1986 and 1990, a Hickman-line (n = 415) or a Port-A-Cath (n = 57) was implanted into 472 patients aged from 17 to 68 years in our hospital. All patients had underlying malignant haematological or oncological diseases, and the central vein catheters were used to facilitate polychemotherapy and supportive therapy including blood transfusions. To elucidate the thrombogenic risk of the inserted catheters, we studied the incidence of thrombotic events of the upper central veins or of the catheters retrospectively. 58 of 472 patients developed thrombi, 48 of which were localised in the upper veins and 10 occluded the catheters themselves. This indicated an overall incidence of catheter-related thrombosis of 12.3 %.
No laboratory risk factor could be defined. In 22 patients the thrombotic event was associated with cytostatic therapy and in 16 patients signs of infection were present. 11 of 42 patients with a dislocated catheter developed thrombosis indicating a thrombogenic risk of 26 %. Thrombosis also occurred in patients with decreased platelet counts below 50 giga/l (n = 11). Incidence of thrombosis was lower in patients receiving antithrombotic prophylaxis with low-dose heparin as compared with patients without prophylaxis (8,5 % vs. 12,9 %). Time of diagnosis of thrombosis ranged between 5 to 1138 days after implantation (median 102 days). Therapy of catheter-related thrombosis included administration of heparin either as low-dose or thrombin-time-adapted dose. In cases of intraluminal thrombosis fibrinolytic agents were applied. 22 catheters had to be removed and 2 patients had recurrent thrombosis after removal.
In conclusion implantation of long-term intravenous catheters is associated with a high risk of thrombosis in the upper veins. Antithrombotic prophylaxis with heparin should be instituted unless the haemostatic balance is severely deranged.

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SERUM TNF-α LEVEL AS PROGNOSTIC INDICATOR OF RESPONSE TO RHIL-3 THERAPY IN PATIENTS WITH MYELODYSPLASTIC SYNDROMES (MDS).

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In a phase I/II study 21 patients with MDS were treated with recombinant human Interleukin-3 (IL-3). 9 patients were treated at the dose level from 250 to 500 µg/m² for 15 days s.c. bolus daily, 12 patients at the dose level of 60 and 125 µg/m² three times per week for 12 weeks s.c. bolus. Serum was collected before, during and after IL-3 therapy. TNF-α levels were measured by EIA (Medgenix Diagnostics, Bruxelles) with a minimal detectable concentration of 3 pg/ml. In the high dose treatment group white blood cells increased in all patients, platelet counts in 2/9 patients, reticulocytes increased in 1/9 patients. In the long-term treatment group platelets increased in 6/9 patients who completed 12 weeks of therapy. Leukocyte counts increased in 2 patients and reticulocytes in 3 patients. In the case of platelet increase, serum TNF-α levels measured before IL-3 therapy were lower in patients responding to therapy (13,2 ± 1,15 pg/ml, mean ± sem) and remained unchanged during therapy (12,2 ± 2,0 pg/ml), while during the opposite case, i.e. decrease or no change in platelet counts, serum TNF-α levels were higher before begin of therapy (17,1 ± 3,8 pg/ml) and increased during therapy (32,7 ± 6,7 pg/ml) ($p \leq 0,01$). TNF is a potent inhibitor of megakaryopoiesis. IL-6 and G-CSF measured in the serum remained essentially unaffected. These data suggest that the presence or the induction of inhibitory cytokines could alter the response to IL-3 and could be of predictive value for response to cytokine treatment in MDS patients.

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ACTIVATION OF COAGULATION, FIBRINOLYSIS, AND NEUTROPHILS IN LUNG CANCER PATIENTS

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There is a growing body of evidence that coagulation is activated in lung cancer patients. This may be due to an inflammatory response to the tumor with stimulation of macrophages and neutrophils. Coagulation and fibrinolysis may also be triggered by activators released from the tumor cells. It is thought that the activation of haemostasis may be important for tumor growth and metastasis, but little is known so far about the clinical impact.
In 47 lung cancer patients (15 small cell, 20 squamous cell, 6 adeno, 6 large cell) the course of following parameters was assessed prospectively before and during treatment: Fibrinogen, factor XIII, TAT, prothrombin fragment F1+2, D-dimer, plasmin-antiplasmin complex (PAP), and neutrophil elastase. Standard parameters of coagulation (prothrombin times, aPTT) were also monitored and remained essentially unchanged during the study.
The initial levels of TAT and D-dimer were higher in the patients with distant metastases (TAT 8.2±2.4, D-dimer 1169±137) as compared to patients with limited disease (TAT 3.1±0.4, D-dimer 716±104) and were the higher the worse the response to therapy was (TAT in patients reaching CR 2.2±0.3, PR 2.8±0.3, NC 5.33±1.0, PD 6.3±3.2, deceased patients 7.5±2.1). Marked individual alterations were observed during the course of therapy. There were statistical correlations between TAT/F1+2 and D-dimer, between PAP/D-dimer and fibrinogen, and between elastase and factor XIII.
The results of this study indicate that the plasma levels of parameters of an activation of haemostasis are related to tumor spreading and response to therapy in lung cancer patients.

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GROWTH OF HUMAN MULTIPLE MYELOMA CELL LINES IN A SCID MOUSE MODEL

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Human lymphopoeitic cells and their malignant counterparts require a complex regulative interaction with stromal factors and cytokines for their growth. Due to this there are no reproducible in vitro assay systems available which would allow to perform long term studies on the biological properties of lymphoproliferative diseases. The generation of new types of immunodeficient mice (SCID, bg/nu/xid) has allowed to engraft various hematological malignancies. Our objective is to establish a long term animal model of Multiple Myeloma and Lymphomas in the SCID mouse model to study proliferative and regulative properties in vivo. Four Human Myeloma cell lines that had been generated in our laboratory and tested for their response to IL-6 were transplanted into SCID mice. Cells (10^7) were injected either i.v. or. i.p. after irradiation of the animals with 400 Rad. After six weeks line OCI-My 5 grew locoregionally (peritoneum) when injected i.p. or generalized (liver, bone marrow) when injected i.v. Later (10 to 18 weeks) infiltration by tumor cells of liver, spleen, and bone marrow could be detected after either route of injection. Material recovered from the tissues involved matched the patterns of the maternal line by immuno- phenotype and genotype (J_H -rearrangement). Addtionally we were able to generate secondary cell lines from these tumors. The Myeloma cell line OCI-My 1, which is slowly proliferating in vitro, generated tumors at 26 weeks after injection. No in vivo growth was detectable for the lines OCI-My 3 (IL-6 producing) and OCI-My 4 (IL-6 dependant) at 10 to 26 weeks. Currently we are investigating whether we are able to modify proliferation of Myeloma cells in SCID mice by IL-6 and accessory cells. The SCID mouse system might serve as an exellent model to evaluate novel therapeutic approaches e.g. cytokines or immunotherapy.

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WILD TYP p53 SUPPRESSES THE TUMORIGENIC PHENOTYPE OF HUMAN LEUKEMIA CELL LINES

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The mutated forms of the p53 cellular tumor antigen cause neoplastic transformation in vitro. Recent evidence suggest that the loss or inactivation of the normal p53 is a frequent event in several types of tumors. Furthermore alterations of the p53 gene in human leukemias provide evidence for the involvement of p53 gene aberration in a recessive manner in these diseases. One major goal of studying the status of p53 in leukemias is the suppression of the tumorigenic phenotype by restoration of the expression of the wild-type p53. To examine the feasibility of suppressing the tumorigeneic phenotype two human leukemic cell lines, K562 and HL60, which have lost the normal function of p53 as determined by PCR and Northern blot analysis were used for gene transfer experiments with the wild type p53. Expression of the wild-type p53 reduced the growth rate of the respective cell lines and induces differentiation as determined by FACScan analysis. These results suggest that suppression of the leukemic phenotype of the HL60 and K562 cells occured after introduction of wild-type p53 and support the hypothesis that the inactivation of p53 may play a crucial role in tumorigenesis of leukemias.

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FLOW-CYTOMETRICAL DETERMINATIONS OF BLOOD CD34-CELLS: TIMING AND EVALUATION OF PERIPHERAL BLOOD STEM-AND PROGENITOR CELL HARVEST BY LEUKAPHERESIS

St.Serke, J.Zingsem, Th.Zeiler, A.Kirsch, R.Zimmermann, R.Eckstein and D.Huhn

Blood CD34-cells have been monitored in 25 patients receiving conventional chemotherapy, and subsequently either GM-CSF, Il-3, or G-CSF for mobilization of blood CD34-cells. Leukaphereses were started, when a number of > 1500 CD34-cells/ml blood were reached. CD34-cells were also determined in the cytapheresis-products, and also CFU-GM cultures were performed.
There was no correlation of numbers of CD34-cells and of leukocytes, per ml blood, respectively. A weak correlation was calculated for lymphocytes and CD34-cells in blood. Evaluating the cytapheresis-products, a correlation of 0.94 was calculated for numbers of CFU-GM/ml and for numbers of CD34-cells/ml. Mean clonogenity of CD34-cells was 2 %, with a range from 0.9 % to 13 %. The volume of cytapheresis-product necessary to provide 70x(5x10E4) CFU-GM was calculated for each patient. This volume ranged from 10. ml to 1300. ml.
Our data indicate the usefulness of flow-cytometrical determinations of blood CD34-cells. Given the fact, that in some diseases currently treated by autologous blood stem cell transplantation, there is risk for reinfusion of circulating malignant cells, the assay described should allow for determination of the minimum number of cells to be reinfused.

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FLOW-CYTOMETRICAL DETERMINATIONS OF RETICULOCYTES

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Both numbers and stages of maturation of reticulocytes present in blood are valuable parameters in evaluation of the activity of the erythropoietic system. Classical determinations of reticulocytes, by means of microscopy on blood smears following staining of whole blood, are subject to major uncertainties. Immature erythrocytes, i.e. reticulocytes, can also be determined by multiparameter flow-cytometry. These immature erythrocytes can be discriminated from the mature ones as they show a higher RNA-content, and also a higher expression of the transferrin-receptor (CD71-antigen). With both parameters, distinct subsets, correlating to distinct stages of maturation, can be visualized. Consistently, however, only about 70% of reticulocytes as determined by RNA-analysis, will be detected based on CD71-expression.

Principles of both flow-cytometrical methods, as well as limitations of both methods, will be discussed.

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SERUM PROCOLLAGEN III PEPTIDE, TYPE IV COLLAGEN 7S PROLYLHYDROXYLASE IN MYELOPROLIFERATIVE DISORDERS

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Serum procollagen III peptide(P-III-P), serum type IV collagen(C-IV) and serum prolylhydroxylase(PH) were measured in patients with myeloproliferative disorders to elucidate the relationship between these variables and the presence of myelofibrosis in myeloproliferative disorders. Studied were 10 patients with chronic myelocytic leukemia in chronic phase(CML-CP), 3 with CML in blastic crisis (CML-BC), 7 with idiopathic myelofibrosis(IMF), 2 with myelodysplastic syndrome with myelofibrosis (MDS-MF), 11 with essential thrombocythemia(ET), 13 with polycythemia vera(PV) and 10 with acute non-lymphocytic leukemia(ANNL). Serum P-III-P, C-IV and PH in normal controls were 0.43±0.084 u/ml, 3.95±0.91 ng/ml and 46.5±10.4 ng/ml(mean±S.D.), respectively. Serum P-III-P and C-IV were significantly increased in IMF(0.79±0.20 u/ml and 6.72± 2.07 ng/ml, respectively). While serum P-III-P was higher in all types of MPD we studied than that in normal controls, serum C-IV was increased in IMF, MDS-MF,CML-BC and ANLL, and it was not significantly higher in CML-CP,ET and PV. Serum PH was normal in all types of MPD except for ANLL. Immunological staining of biopsied bone marrow collagen revealed that both type III and IV vollagen were increased in myelofibrosis, but type IV collagen was stained more intensely than type III. These reslts suggest that C-IV was more specific for myelofibrosis than P-III-P.

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PRIMARY PLASMA CELL LEUKEMIA: COMPLETE REMISSION AFTER VAD CHEMOTHERAPY PROTOCOL - A CASE REPORT

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Up to now, according to several reports the prognosis for patients with primary plasma cell leukemia as a de novo-disease has been considered extremely poor (median survival time approx. 5 to 7 months).
The 50 year old patient to be presented here was admited to our hospital in may 1991 after having suffered from a feeling of general weakness and fatigue as well as temperatures up to 38.8° C.
Besides elevated levels of LDH laboratory findings showed a hypercalcemia (3.39 mmol/l) as well as anemia and a thrombozytopenia. Furthermore we found a leukozytosis of 32/nl revealing more than 50 % of dedifferentiated blasts. Bone marrow cytology demonstrated a dense, lawn-like infiltration of atypical plasmoblasts (strongly positive reaction of acid phosphatase). Cytogenetic studies elucidated a reduction of the genome to 40 chromosomes with numerous aberrations. Urine immunoelectrophoresis revealed free light chains of the kappa type. The blood concentration of these light chains was below detection limit. Radiography merely revealed suspicious small spotted osteolyses in the pelvic regions. Whole skeleton scintiscanning was without pathological findings.
After treatment of the hypercalcemia with a biphosphonate (BM 21.0955, still in clinical trial) the first 3 courses of chemotherapy according to the VAD protocol (Vincristine, Adriamycin, Dexamethason) were carried out in a more intensive scheme. Already 4 days after initiation of therapy a complete blast remission was achieved in the peripheral blood. A marrow control puncture 6 weeks later (after 3 courses) revealed a complete remission. Pathological light chains in the urine were no longer detectable, the peripheral blood count was completely normalized. After 3 additional VAD-courses in intervalls of 6 weeks the patient is currently still in complete remission.
Considering the so far documented extremely poor prognosis, an autologous bone marrow transplantation during continous primary remission is planned.

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EFFICACY AND TOXICITY OF LIPOSOMAL AMPHOTERICIN B (AMBISOME) IN PATIENTS WITH SEVERE NEUTROPENIA

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Fungal infections are the major cause of morbidity and mortality in pts with severe neutropenia. Amphotericin B (Amp B) is the drug of choice, but administration is limited because of nephrotoxicity and side effects as fever, chills and hypotonia. In order to reduce toxicity and improve efficacy Amp B was incorporated in liposomes. We used the Amp B preparation AmBisome (Vestar Inc) (AmBi) in 14 pts with neutrophils less than 1000/cmm in 15 episodes. Underlying diseases were hematological malignancies (11 AML, 2 NHL, 1 relapsed HD). All pts were resistant to initial antibiotic therapy and received additional antifungal therapy with conventional Amp B. Change to AmBi was due to toxicity in 8 and/or progression of pulmonary infiltrates in 8 and/or persistent fever in 7 pts. AmBi was given in a dose of 3 mg/kg/day. The median duration was 26,8 days (4-42). Response to AmBi was achieved in 11/15 episodes and 9/12 with pulmonary infiltrates and 6/7 of those with radiological signs of pulmonary aspergillosis improved. Only 2 pts improved with recovery of neutrophils, all other pts were still neutropenic. 4/14 pts died due to infections. Although higher doses of AmBi were given it was well tolerated without any prophylactic administration of drugs to reduce side effects. Nephrotoxicity did not occur with regard to creatinine, loss of potassium was not significantly reduced when Amp B was replaced by AmBi.
We conclude that AmBisome provides a well tolerated drug and suggests to be highly effective in this high risk group even in pulmonary aspergillosis.

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LOOKING FOR HCV-CARRIERS AMONG THE BLOOD DONOR POPULATION OF SOUTHERN GERMANY

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Objective: To estimate the prevalence of HCV carriers in a population of blood donors in Southern Germany, using the polymerase chain reaction (PCR) and recombinant protein immunoblot assay (RIBA).

Methods: Sera from 261 blood donors were screened by the second anti-HCV assay (EIA) from Ortho Diagnostic Systems. Serum alanine-aminotransferase level was determined for each sample/donor. All samples were submitted to the PCR and RIBA (Ortho) assays. For the PCR approach, samples were tested with two different sets of primers, both coding for sequences in the 5' non-coding region of the HCV genome. Samples were considered "reactive" if they showed one positive result out of two amplifications. Positive samples were the ones that resulted positive after amplification with the two primer-sets. Negative samples showed two negative results out of two reactions.

Results: Among EIA anti-HCV negative blood donors, we could find 4.3 and 3.0% reactivity in the PCR assay, related to ALT <35 U/l and ALT >35 U/l, respectively. Investigation by RIBA resulted in 0 and 1.5% for the same two subgroups. Among anti-HCV positive blood donors we were able to detect 21.2% and 59.4% in the ALT <35 U/l and ALT >35 U/l subgroup, respectively. RIBA detected 15.2% and 71.4% positivity for those subgroups. The results suggest that the combined screening of donors for ALT and anti-HCV antibodies is able to exclude the great majority of donors carrying HCV sequences.

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SECONDARY AML IN PATIENTS TREATED WITH ETOPOSIDE FOR HIGH GRADE LYMPHOMA

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From 1/84 to 4/92, 111 patients were treated for AML at our institution: AML was "de novo" in 83 pts, secondary to myelodysplastic syndrome (MDS) in 19 pts, and secondary to another neoplasm in 9 pts. Of these, 3 presented with AML following high grade lymphoma.

Case	1 (I.B.)	2 (G.B.)	3 (S.F.)
Age / Sex	42 / f	39 / f	19 / m
Primary lymphoma	centroblastic	T-lymphoblastic	
Treatment of "	4x CHOP/VP16	T-ALL Protocol (Hölzer)	
Etoposide (mg/m²)	1200	1200	1200
AML, FAB subtype	MDS, then M2	M2	M1
Months after primary lymphoma	41	19	20
Immunophenotype of AML blasts	CD15,Elast.⊕; CD11c,14 ⊖	CD4 30%⊕; CD15 ⊕; CD7,10,11c, 14,19 ⊖	CD7 26%⊕ CD15 ⊕; CD10,11c, 14,19 ⊖
Cytogenetics	n.d.	10p+; 11q-	t(10;11)
Treatment	TAD9/HAM	TAD9	TAD9
Outcome	refractory, bleeding	early death	refractory, septicemia

This observation supports recent reports of an excess of secondary AML in etoposide-treated ALL patients (20/580)(Pui, NEJM, 1991, 325:1682), as opposed to the very low frequency of AML (2/9720) in protocols without etoposide (Neglia, NEJM,1991, 325:1330). Possible pathogenetic modes, including treatment-induced second neoplasm, phenotypic shift of gene expression (clonal evolution) and clonal selection in a bilineal neoplasm, will be discussed. The cytogenetic aberrations are features of lymphoid neoplasia rather than myeloid.

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Separation of Haemoglobin Variants using Immobilized pH Gradients

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Using a number of techniques, approximately 457 haemoglobin variants have already been detected and some have been chemically characterized. Fortunately, most do not exhibit serious haematological deficiencies. We have established a new routine isoelectric focusing technique based on immobilized pH gradients (IPGs), which, we believe, may have the high resolving power to detect new neutral and charged haemoglobin mutations. As opposed to classical ampholyte generated pH gradients, IPGs are created using acrylamido buffers having defined pK values. Two such buffers (pK 7.0 and 3.6) are required to create a pH 7.0 - 8.0 pH gradient, which is covalently coupled to the acrylamide matrix. This makes these gradients extreme stable and unsusceptible to protein load. Since only two defined chemicals are used, reproducibility of gel preparation from batch to batch is extremely high, and should aid international standardization of the separation of haemoglobin variants. The main advantage of this technique lies e.g. in the clean fractionation of HbC from HbA2 (not possible using standard electrophoretic techniques) and even the separation of HbF from HbF-Sardinia (β-75 isoleucine --> threonine substitution). The detection of other haemoglobin variants such as Hb-Okayama will also be presented.

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Occurrence of Secondary Lymphoma under Recombinant Alpha-Interferon-Treatment of Hairy Cell Leukemia.

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Second haematologic and non-haematologic malignancies are known to occur in patients with hairy cell leukemia (HCL). However, to our knowledge no report is presently available regarding the development of a second lymphoproliferative malignancy under alpha-interferon (IFN)-therapy.

Since 1989 we have observed 3 such cases.

The first patient responded adequately to alpha-IFN obtaining a complete response after 12 months of treatment.

During alpha-IFN maintenance-therapy he developed a lymphocytic non-Hodgkin's lymphoma (NHL) with lymph node and bone marrow involvement 7 months later.

Patient 2 presented generalized lymph node enlargement due to centroblastic lymphoma 2 months after start of alpha-IFN therapy because of HCL progress.

Patient 3 has been under IFN-treatment since January 1989. Repeated bone marrow biopsies revealed an increasing dense infiltration by lymphocytes in addition to HCL. Actual immunochemistry did not show a clonal population.

Detailed histology and clinical data will be presented.

It seems striking that despite alpha-IFN treatment second lymphoproliferative neoplasias developed.

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Frequency of bone marrow infiltration by centroblastic-centrocytic and centroblastic lymphoma cells as detected by PCR.

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The detection of the t(14;18) bcl- 2 translocation with PCR was assayed for its applicability as a marker for ist overt or occult bone marrow infiltration by lymphomas, classified as centroblastic- centrocytic (cb- cc) or centroblastic (cb) by the Kiel-Classification. Bone marrow smears or sections from our archive were used as a source of DNA and the results of the PCR compared to cytological and histological findings.

34 bone marrow aspirations of patients with cb- cc and cb lymphomas (20 patients with cb- cc, 14 patients with cb) were investigated for positivity of the major breakpoint translocation of the bcl- 2 oncogene into the immunglobulin heavy chain region. Bone marrow cells from 3 patients (1cb, 2 cb- cc) could be demonstrated to carry the translocation, whereas 31 patients were negativ even upon repeated analysis. The 3 patients, which were positiv in PCR also had a histologically verified bone marrow infiltration by their lymphoma. We could not find PCR positive results without histologically detectable infiltration, whereas in 14 patients no translocation could be detected although bone marrow infiltration was shown histologically. Thus, the assay for mbr bcl- 2 translocation was not able to detect more cases of bone marrow affection than histology.

Even if we assume, that only about 50% of this entity of lymphomas seeds into bone marrow and that the PCR for the mbr- region is not able to detect all bcl- 2 positive lymphomas, the amount of bcl- 2 positive bone marrow findings was relatively low. Wether this is due to the fact, that bcl-2 positive lymphomas less frequently infiltrate the bone marrow or due to a divergent group of lymphomas defined by the working formulation and the Kiel classification is under investigation.

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ACUTE MEGAKARYOBLASTIC LEUKEMIA (AMKL) WITH SIMULTANEOUS EXPRESSION OF B- AND T-LYMPHOID ANTIGENS

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Acute megakaryoblastic leukemia is a relatively rare form of acute myeloid leukemia (AML), which can be suspected on morphological grounds, but definite diagnosis requires either demonstration of platelet peroxidase (PPO) by ultrastructural examination or reactivity of leukemic blasts with monoclonal antibodies (mAb) recognizing platelet glycoproteins. Immunophenotypic studies have shown that AMKL blast cells also express myeloid antigens (i.e. CD 13, CD 33) to varying degrees. An aberrant expression of T-lymphoid markers (CD 7, CD 2), which can be found in a significant proportion AML, has also been reported for AMKL.

We describe a 28-year-old male patient with acute leukemia without anteceding hematological disorder, whose blast cell population showed megakaryoblastic differentiation by reacting with one anti-platelet glycoprotein mAb (CD 61$^+$, CD 41$^-$, CD 42b$^-$), a partial TdT-positivity and a simultaneous expression of a T-lymphoid (CD 7$^+$) and a B-lymphoid (CD 19$^+$) antigen on > 80% of blast cells (confirmed by double immunofluorescence staining) suggesting involvement of a pluripotential stem cell. Other T- (CD 2, CD 3, cyCD 3, CD 4) and B-(CD 20, CD 24) cell markers and CD 10 were negative. Among the myeloid antigens tested only one was positive (CD 33$^+$, CD 13$^-$, CDw65$^-$, CD 15$^-$, CD 14$^-$). The blast cells were HLA-DR$^+$ and several markers of cell immaturity were also expressed (CD 34$^+$, c-*kit* protein product). The megakaryoblastic differentiation of the leukemic cells was confirmed by ultrastructural cytochemistry demonstrating PPO in the endoplasmic reticulum and perinuclear space. This unusual immunophenotype in a case of AMKL have - to our knowledge - not been described before.

The pathophysiological implications will be discussed and additional clinical data will be presented.

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EVALUATION BY REVERSE TRANSCRIPTASE POLYMERASE CHAIN REACTION (RT-PCR) OF IMMUNOMAGNETIC BEAD PURGING IN PHILADELPHIA CHROMOSOME (Ph') POSITIVE ACUTE LYMPHOBLASTIC LEUKEMIA (ALL)

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Detection by Southern blot or PCR of the *bcr/abl*-rearrangement, molecular hallmark of the Philadelphia chromosome (Ph'), is associated with dismal prognosis in chemotherapy treated ALL. In patients without compatible allogeneic donor, at our institution autologous bone marrow transplantation is carried out following *in vitro* purging with immunomagnetic beads bound to monoclonal antibodies (Dynabeads^R CD 19 and AB-4 (HLA-DR)). Apart from the conditioning regimen, outcome will depend on completeness of purging which can be evaluated by the highly sensitive, but difficult to quantitate, PCR. To assess the degree of contamination with *bcr/abl*-positive, malignant cells, decimal dilutions of leukemic cells in normal PMN cells were analysed by PCR (35 cycles at 92°C/56°C/72°C, denaturation/annealing/extension), following a modified guanidine thiocyanate RNA extraction and random primed reverse transcriptase cDNA-synthesis. Using M_{aior}- and m_{inor}-*bcr/abl*-specific primers, transcripts could be demonstrated up to the 10^{-3} - 10^{-5} fraction in frozen bone marrow cells from Ph' positive patients, whereas in both fresh bone marrow cells and the fast growing human Ph' positive pre-B-ALL cell-line BV-173 the limit of detection was less than 10^{-6}. As assessed by this procedure, two rounds of immunomagnetic bead purging removed 4 - 5 logs of, but did not completely clear the 10^{-1}-contaminated fractions from *bcr/abl*-positive cells. These purged, yet still residually contaminated fractions were grown in suspension culture and again subjected to PCR, demonstrating *in vitro* relapse. We conclude that this method is highly sensitive for individual, semiquantitative evaluation of bone marrow purging and early prediction of relapse in *bcr/abl*-positive ALL.

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PROGNOSTIC FACTORS FOR COMBINED THERAPY OF ADVANCED NECK NODES (HYPERTHERMIA, CHEMOTHERAPY, RADIOTHERAPY)

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A phase II study about a trimodal approach for advanced nonresectable H & N tumors has been started at the Universityhospital Rudolf Virchow Berlin in 1990. The therapy regime includes 3 cycles of an induction chemotherapy (d1: Cisplatin 100 mg/m^2, d1-5: 5-FU 600 mg/m^2, d1-5 : Leucovorin enhancement 250 mg/m^2 plus 50 mg bolus d1) overlapping with a definitive radiotherapy up to a tumor dose of 66-70 Gray. Local hyperthermia (LHT) of the N3/N2-nodes has been recommended as a third modality parallel to the cisplatin-infusion on d1 and eventually later on during the radiation course. More than 20 patients with advanced H & N disease (N2/N3) have been treated by 1-12 LHT-sessions (average 6 LHT/patient). The LHT is performed by the wave guide applicator MA-150 at 434 MHz or the spiral applicator SA-115 at 160-180 MHz by use of the BSD-2000 hyperthermia system. Closed end catheters are implanted under CT guidance for thermometry. Therapeutic temperatures (≥ 42 °C) were achieved in >90% of sessions. The toxicity of LHT in the cervical region is very low, no serious complications have been observed. In about 30% some local complaints appeared. For all patients a follow-up is performed by CT and clinical examination. The results implies an overall response rate of >70% for a patientgroup with a poor prognosis.

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COMBINED MODALITY TREATMENT WITH SEQUENTIAL CHEMOTHERAPY, CHEMO-/RADIOTHERAPY AND SURGERY IN LOCALLY ADVANDED ESOPHAGEAL CARCINOMA (EC).

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Chemotherapy (CT) with 5-FU, leukovorin, etoposide and cisplatin (FLEP) has proven to be active in advanced EC (EJC 27, suppl2:427, 1991). Simultaneous chemo-/radiotheray (RT) seems to induce higher pCR-rates, compared to CT alone. Therefore we initiated an ongoing pilot study of preop. CT with FLEP (F 500mg/m^2, L 300mg/m^2, E 100mg/m^2, P 30mg/m^2, d1-3, qd 22-28), 2-3 cycles, followed by RT of 40 Gy (2 Gy daily within 4 weeks) with concurrent CT (P 50mg/m^2 d1+8, E 100 mg/m^2 d3-5, 1-2 cycles), followed by transthoracic esophagectomy (TE) 4-5 weeks after RT. Since 3/91 19 pts. with locally advanced EC (T2-T3 NX M0) have been entered: median age 55y. (44-70); Adeno-CA/SCC 2/16; T2 4, T3 14; upper third 3; tumor length ≥ 5cm 15 pts. Results after CT: Too early 1; major response 10(59%), including 1 CR; NC 5; P1; toxic death 1 pt. Fifteen pts have run through the protocol. Two pts with CR after CT/RT refused surgery and were irradiated up to 67 Gy. 11 pts underwent TE: 6 pCR, 4 R0-resections, 1 R2-res. No pt. died postop. One pt. relapsed from pCR with bone metastases. Toxicity of CT/RT+CT was mainly hematologic (WHO grade, dose of E had to be reduced after the first cycle in 8/17 pts): Leukopenia 3° 39%/33%, 4° 22%/41%; thrombopenia 3° 37%/33%, 4° 6%/8%; infection 3° 10%/5%, 4° 5%/0; mucositis 3° 11%/13%. Conclusions: This intensive preoperative multimodal treatment is toxic but feasible. Especially perioperative mortality was not increased. The pCR (6) and CR/NED (6) rate of 80% (12/15) of those pts. who are off treatment is promising. Further pts accrual is planned.

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MODULATION OF DOXORUBICIN (Dox) CYTOTOXICITY IN TWO GASTRIC CARCINOMA CELL LINES: COMPARISON OF CYCLOSPORIN A (CiA) AND FK 506 IN VITRO.

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Multidrug resistance (mdr) is a major problem in anthracycline treatment of gastric cancer. CiA is a well known modulator of the P-glycoprotein (Pgp) related mdr. FK 506 is a macrolide with a strong immunosuppressive activity in vivo. We studied the effects of CiA and FK 506 on the cytotoxicity (cytotox) of Dox in two human gastric cancer cell lines (HM2 and HM51) with different expression of Pgp. Methods: Expression of Pgp was determined by immunocytochemistry using the specific monoclonal antibodies C-219 and JSB-1. Chemosensitivity was estimated with the MTT colorimetric assay. Exponential cell growth was shown for both cell lines within 96 h. Continuous drug exposure was performed with the IC_{30} of Dox for 24 h, either alone or in combination with 24 h preincubation of 0.1, 1 and 10 μM CiA or FK 506. Cytotox was measured after 96 h. Results: Pgp expression was high in HM2, and low in HM51 cell line. The intrinsic cytotox of FK 506 was lower than CiA: 19% vs 100% inhibition for HM2 and 66% vs 82% for HM51 at 10 μM. 10 μM FK 506 increased the Dox cytotox 2.5 fold in HM2 cell line, but at lower concentrations there was only a moderate effect of FK 506 on Dox cytotox. CiA did also enhance Dox cytotox, but only at concentrations with significant intrinsic cytotox. Conclusions: FK 506 as well as CiA increases the cytotox of Dox in two human gastric cancer cell lines with different expression of Pgp. However, the intrinsic cytotox of FK 506 was lower than that of CiA.

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EXPERIMENTAL EFFICIENCY OF IMMUNOMAGNETIC BONE MARROW PURGING USING THE T-CELL-MODEL.

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Autologous bone marrow transplantation is performed in poor prognosis T-All patients when no HLA-compatible donor is available. However, the likely presence of contaminating leukemic cells in the autologous bone marrow is a major concern.

To establish a bone marrow purging protocol, we used an experimental model in which peripheral blood MNC from CML-patients or normal bone marrow cells were ficoll-separated and then contaminated with 1% and 10% cells of the human T-Cell-Line Jurkat. The T-Cells were previously marked using the Hoechst 33258 dye method. T-Cell-removal was achieved using monoclonal antibodies (MoAb) and immunomagnetic beads (Dynabeads M-450 coated with Sheep-anti-Maus-IgG). In comparison with the indirect method the direct method (MoAb coupled to the IgG-coated beads before use) led to an increased elimination efficiency of 19,8% (direct: mean 1,36 log, range 0,91-1,63log; indirect: mean 1,09log, range 0,84-1,32log) with increased MNC-recovery (direct: mean 45,1%, range 19-62,3%; indirect: mean 42,6%, range 30,8-45,8%). For the direct method we varied the total beads concentration from 0,9 mg to 7,2 mg beads per 1*10^7 MNC and analysed the effect of two successive treatments of the sample. Four MoAbs were tested alone in comparison to different MoAB-cocktails. Using the direct technique in a two step procedure with an MoAb-cocktail against the CD2-, CD3- and CD5-antigen we obtained a MNC-recovery of 51,35% (range 28,6-74%) and a depletion efficiency of 3 log (range 2,82-3,16 log) which corresponded to a removal ofmore than 99,9% of the T-Cells. This corresponds to the antigen-positive fraction of the Jurkat-cell-line.

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rG-CSF FOR DOSE-INTENSIFICATION OF CHEMOTHERAPY IN HIGH GRADE MALIGNANT NON-HODGKIN'S LYMPHOMAS (HG-NHL)

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In an attempt to increase dose intensity of chemotherapy, patients with HG-NHL stage II-IV were treated with 8 alternating cycles of VIM (VP16, ifosfamide, methotrexate) and CHOP (cyclophosphamide, adriamycin, vincristine, prednisolone) with a stepwise reduction of the time interval between treatment cycles. To overcome hematotoxicity, which is mainly neutropenia with this protocol, rG-CSF (Amgen-Roche) 5 µg/kg sc was given for 9-10 days between the treatment cycles. In a first phase of the study, intended cycle duration was 17 days during the initial 4 treatment cycles. In a second phase the treatment interval was reduced to 14 days for 4 cycles. In the third step patients were treated at 14 day intervals throughout all 8 cycles of chemotherapy. 14 patients entered the first phase, 10 the second and 19 the third phase. During rG-CSF treatment there was a rapid increase in neutrophils beginning at about day 10. Chemotherapy could therefore be continued in the planned interval in >80% of all cycles at both 17 and 14 day intervals. With rG-CSF support, dose intensity could be increased in patients with HG-NHL without increased toxicity. Whether this will lead to better tumor treatment outcome is currently being studied in a randomized phase III trial comparing a regular 3 weekly treatment schedule with a two weekly regimen combined with G-CSF support.

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DELETION OF THE RETINOBLASTOMA TUMOR SUPPRESSOR GENE IN CHRONIC LYMPHOCYTIC LEUKEMIA

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Inactivation of the retinoblastoma tumor suppressor gene (RB-1) is thought to be an important step in the oncogenesis not only of retinoblastoma but also of various other human malignancies. In chronic lymphoid leukemias of B-cell origin an involvement of RB-1 was suggested based on cytogenetic data, but so far molecular studies using Southern blot analysis failed to provide clear evidence for this hypothesis. We examined RB-1 and its chromosomal locus 13q14 in B-cell chronic lymphoid leukemia by dual color fluorescence in situ hybridization (FISH) of interphase lymphocyte nuclei and by G-banding analysis of metaphase chromosomes. In FISH experiments RB-1 was delineated in one color while a differentially labeled cosmid probe mapping to chromosome 21 served as a positive control for hybridization efficiency. Of 32 patients (pts) analyzed so far, four pts had aberrations of chromosomal band 13q14 on G-banding, a frequency which is in agreement with previous reports. In contrast, FISH revealed a single RB-1 hybridization signal in high numbers of interphase nuclei of nine pts (28% of cases). In these pts two hybridization signals of the cohybridized cosmid were present in more than 80% of nuclei with a single RB-1 signal, demonstrating that a deletion of RB-1 and not an insufficient hybridization efficiency was responsible for the large number of nuclei with a single RB-1 signal. Six of the nine pts with RB-1 deletion in interphase cells had two normal chromosome 13 homologs on G-banding analysis and nearly all metaphases analyzed by FISH showed two RB-1 hybridization signals. Additionally FISH applied to blood smears confirmed the RB-1 deletion in lymphocytes, whereas cells morphologically appearing as granulocytes had two signals. Our data demonstrate that the interphase cytogenetic approach by FISH increases the accuracy of the detection of chromosomal aberrations especially in cell populations with low mitotic activity. The frequency of RB-1 deletions in B-cell chronic lymphoid leukemia is significantly higher than previously assumed and is in the same range as in retinoblastoma.

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PENTOXIFYLLINE DID NOT PREVENT TRANSPLANT-RELATED TOXICITY IN 30 ALLOGENEIC BONE MARROW TRANSPLANT RECIPIENTS.

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Recent data (Bianco et al., Blood 1991, 78, 1205-11) have suggested that pentoxifylline (PTX) reduces morbidity and mortality in patients (pts) undergoing bone marrow transplantation (BMT). Based on these encouraging results, we prophylactically administered PTX to 30 consecutive allogeneic BMT recipients (AML 10, CML 10, ALL 6, NHL 2, MM 2; 15 standard-risk, 15 high-risk; donor: HLA-id.-rel. 24, mism.-rel. 5, matched unrel. 1). PTX (12-30 mg/kg/day by continuous infusion) was started one day prior to conditioning, switched to oral PTX when tolerated and discontinued on day 100. Clinical data were compared to a historical control group of 63 pts (AML 21, CML 25, ALL 8, NHL 2, MDS 3, SAA 4; 47 standard-risk, 16 high-risk). PTX was well tolerated at all dose levels administered and no pt experienced significant adverse side effects. Twenty-six of thirty PTX pts engrafted. One pt experienced graft failure and three pts died too early to be evaluable. Currently, 15/30 (50 %) pts survive with a median follow-up of 160 (70-399) days. 63 % of the PTX recipients developed severe hyperbilirubinemia (>3mg%), 10 % renal insufficiency (serum-creatinine >1.5mg%) and 57% acute GvHD Grade II-IV. The respective values for the control group were 28%, 32%, and 51%. Mucositis requiring narcotics occured in 90% of pts receiving PTX. No difference was observed regarding the days of fever, TPN requirements, and the duration of hospitalization. Based on these results, we can not confirm the previously published beneficial effects of PTX on transplant-related complications.

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BCL-2 GENE REARRANGEMENTS AND BCL-2 EXPRESSION IN A LARGE SERIES OF NODAL AND EXTRANODAL B-CELL NON-HODGKIN'S LYMPHOMAS

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We analysed 43 cases of nodal and extranodal B-cell lymphomas for presence of a BCL-2 gene rearrangement by conventional Southern blotting and for expression of the BCL-2 gene by immunohistochemistry. Among nodal lymphomas, a BCL-2 gene rearrangement was found in 10 of 15 cases with cb/cc, in 0 of 3 cases with cb and in 1 of 4 cases with cc. BCL-2 expression was seen in 17 of these 22 cases, being found consistently in cases where a BCL-2 gene rearrangement could be detected. This is in accordance with the concept of BCL-2 gene rearrangements resulting in BCL-2 deregulation. On the other side, the finding of 5 lymphomas, which did not carry a detectable BCL-2 gene rearrangement and also did not express BCL-2, is consistent with their follicular center-cell derivation because follicular center-cells are the only peripheral B-cells which physiologically do not express BCL-2.

BCL-2 expression was also frequent among primary gastrointestinal B-cell lymphomas (11 low-grade malignancies, 10 high-grade malignancies) and was seen in 15 of 21 cases. In contrast to nodal lymphomas, however, only 2 BCL-2 gene rearrangements were detected. When looking closer at 7 nodular growing lymphomas, it turned out that two could be defined as true follicular lymphomas because of presence of BCL-2 gene rearrangements, BCL-2 expression, expression of CD10 and lack of vimentin. Another 3 nodular growing, BCL-2 protein positive lymphomas had no detectable BCL-2 gene rearrangements, and had a reverse immunophenotype in respect to CD10 and vimentin expression. They might represent lymphomas of extrafollicular origin, which invaded pre-existing follicles of MALT. A routine discrimination between these two types of nodular growing, BCL-2 protein positive, gastrointestinal lymphomas - which might be of clinical importance - should be achieved by PCR.

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UNEXPECTED EFFECTS OF GM-CSF AND IL-3 ON LYMPHOBLASTIC B-CELLS IN VITRO

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Hematopoietic growth factors like GM-CSF and IL-3 act on early multipotential progenitors as well as on granulocyte/monocytic progenitor cells. However, GM-CSF and IL-3 are not strictly lineage specific for cells of the myelopoiesis. It has been reported that they can stimulate lymphoid cells, indicating that these cells may express GM-CSF- and IL-3-receptors. We therefore investigated the effects of IL-3 and GM-CSF on 15 human lymphoid precursor B-cell lines derived from a variety of different B-cell neoplasms. Monoclonal lymphoid B-cell lines were used to exclude secondary effects by accompanying non-tumor cells. Thymidine uptake, surface membrane and nuclear antigen expression, immunoglobulin secretion, receptor expression and secretion of cytokines were monitored before and after stimulation with IL-3 and GM-CSF. GM-CSF and IL-3 were used at 10, 100, and 1000 U/ml over a time period of 24 to 72 h. In 14 out of 15 B-cell lines we observed an effect upon IL-3 or GM-CSF treatment. We found a growth inhibition in one cell line and a proliferation in two other cell lines. The majority of cell lines exhibited alterations in their expression of proliferation and activation antigen expression. The surface membrane expression of the IL-3 and GM-CSF receptors as well as secretion of cytokines were correlated with the reaction of the cells to IL-3 and GM-CSF stimulation. We conclude that some B-cell tumors react upon treatment with GM-CSF and IL-3. The effects could be regulated through direct effector-ligand interactions. These in vitro findings will prompt further investigations of lymphoma cells from patients treated with these growth factors.

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PROTO-ONCOGENES AND TUMOR SUPPRESSOR GENES IN MALIGNANT TESTICULAR GERM CELL TUMORS

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Systematic investigations have revealed changes in the expression levels of the c-kit and hst-1 proto-oncogenes, the c-kit ligand SCF as well as the retinoblastoma tumor suppressor gene (RB) in GCTs:

67 primary GCTs were examined to determine the prevalence and nature of RB gene alterations. Decreased expression of RB gene mRNA was found in all testicular GCTs (both seminomas and non-seminomas) examined. The RB protein was not detectable immunohistochemically in the undifferentiated cells of any GCTs whereas the differentiated malignant cells in 14/15 teratocarcinomas expressed the protein. No gross alterations of the RB gene were found at DNA level in any of the examined specimens. This, combined with the presence of the RB protein in the more differentiated tumor cells of teratocarcinomas suggest that changes in transcript levels rather than mutation(s) of the gene may be responsible for the absent or decreased RB-expression in human GCTs. To date studies on the mechanism of RB regulation have demonstrated that it occurs at the protein level by phosphorylation of the p105 gene product. The findings presented here indicate that additional regulation might occur at the transcript level.

Seventy testicular germ cell tumors and normal testicular tissues were analyzed at the DNA, RNA and protein levels for the c-kit and hst1 proto-oncogenes aswell as the c-kit ligand 'stem-cell-factor (SCF)' using Northern and Southern blot analyses and immunohistochemistry, respectively. c-kit and its ligand SCF were expressed in normal testicular tissue. c-kit was expressed in 24/30 (80%) seminomas but in only 3/40 (7%) non-seminomatous tumors, whereas hst1 was expressed in 24/38 (63%) of non-seminomas but only 1/24 (4%) of seminomas, demonstrating an inverse relationship in the expression pattern of these 2 oncogenes in human testicular germ cell tumors. SCF was not expressed in either subtype of GCT as determined by Northern blotting, however, the protein was detected immunhistochemically in the cytoplasm of some tumor cells. No gross alterations in the c-kit, SCF and hst1 locus were found at DNA level. It is concluded that the detection of the c-kit surface receptor in normal human germ cells and its natural ligand SCF in Sertoli cells suggests the presence of a local trophic regulatory system that seems to be active in human spermatogenesis. Furthermore, alterations of oncogene and suppressor gene expression are frequently detectable in testicular GCTs.

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Fibrolamellar Hepatocellular Carcinoma as Coincidental Tumor in Patient with Germ Cell Tumor: A Case Report

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Fibrolamellar hepatocellular carcinoma is a slowly growing tumor arising in normal liver in young adults, comprising 3,5% of all hepatocellular carcinomas with a better prognosis.

We report a case with the diagnosis of coincident fibrolamellar hepatocellular carcinoma in a patient with germ cell tumor. A 24-year-old patient with teratocarcinoma (St.Ib, Lugano classification) had an orchiectomy as treatment. A local recurrence in inguinal lymphnodes was diagnosed four months later and treated with four cycles of PEB. One month after the end of chemotherapy a solitary liver lesion was first noticed by sonography. Because of increasing ß-HCG levels after sixteen months, this patient recieved two cycles PEI followed by high-dosis-chemotherapy with ABMT. With this treatment he achieved a markernegative PR with a remaining solitary lesion in his right liver lobe. Surgical resection revealed a fibrolamellar hepatocellular carcinoma, which was completly excised.

Nearly every fibrolamellar hepatocellular carcinoma has been diagnosed after surgery, tumor marker may be neurotensin levels and serum vitamin B12 binding-capacity. Five year survival is about 60% after resection or transplantation. There is no concept of a possible etiology of this tumor; this case reminds to consider and look for second tumors in the follow-up of patients after chemotherapy.

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Immediate Early Transcription Factors in Renal Cell Tumors

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A class of cellular oncogenes known as immediate early transcription factors function as transcriptional regulators in that they are known to both positively and negatively control cellular growth properties. This class of genes includes *c-fos*, *c-jun* and *EGR-1*. To determine whether these genes are altered in renal cell carcinomas, 47 pairs of renal tumors and adjacent normal tissues were analyzed for these 3 genes at the DNA-, RNA-, and protein levels using Northern- and Southern blots, and immunohistochemistry respectively. The expression of these 3 genes was found to be coordinately regulated at the transcript level in the vast majority of normal and malignant kidney tissue samples. Overall, approx. 50% of the tumors showed high expression, as opposed to 50% of tumors with little or no expression of these genes. Moreover, markedly higher mRNA levels of *c-fos*, *c-jun* and *EGR-1* could be demonstrated by Northern analysis in almost 40% of the tumors when compared to their adjacent normal tissues. However, there were some differences of expression within the adjacent normal kidney samples, thus suggesting that these genes are differentially regulated even within different anatomical regions of the normal kidney. These expression patterns were confirmed by immunohistochemical analysis using polyclonal antisera to the *c-fos*, *c-jun*, and *EGR-1* gene protein products: Tumors with high mRNA levels showed high expression of the transcript factor proteins on the cellular level, whereas in the tumors with decreased mRNA levels little or no nuclear staining was detected in the tumor cells.

Although no correlations of these molecular findings with tumor stage, grade or patients outcome were found so far, the striking differences of the expression of immediate early transcription factors may be of importance in the pathogenesis and clinical course in renal cell tumors.

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INCREASED CIRCULATING ICAM-1 LEVELS ASSOCIATED WITH PULMONARY COMPLICATIONS DURING TREATMENT OF ACUTE LEUKEMIA

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Aggressive chemotherapy of akute leukemia results in neutropenia with prolonged hospital stay and is often associated with pulmonary infections. Intercellular adhesion molecule-1 (ICAM-1, CD 54), a member of the immunoglobulin superfamily, has been shown to be involved in the binding of leukocytes to target cells and endothelium. After induction by cytokines, ICAM-1 is expressed on a variety of hematopoietic and non-hematopoietic cells. We performed serial determinations of circulating ICAM-1 levels (ELISA, Bender MedSystems, Wien) in 5 patients during treatment of acute leukemia. During this period, 3 episodes of pneumonia occurred and etiologic agents could be confirmed as E.coli, Candida, and Aspergillus, respectively. TNF-α, which is known to produce a strong upregulation of ICAM-1 expression, could not be detected in plasma during pulmonary infections. In additon, we observed 2 cases of acute respiratory distress syndrome of unknown etiology. In all events circulating ICAM-1 increased at least 2 fold and maximal levels were found in acute respiratory distress syndrome. Elevated ICAM-1 levels associated with pulmonary complications occured not only during overt leukemia but also in bone marrow aplasia. Thus, we suggested the ICAM-1 molecule to be of endothelial origine. The turnover and the role of these circulating molecules in the pathophysiology of infections in immuno-compromised patients remains to be established. From these first observations, pulmonary complications in leukemia appear to result in increased circulating ICAM-1 levels.

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RECOVERY OF IMMUNOGLOBULIN LEVELS FOLLOWED BY AUTOIMMUNE HEMOLYTIC ANEMIA IN FLUDARABINE-INDUCED COMPLETE CLINICAL REMISSION OF CLL

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In 1989, a 60 year old female patient was admitted to our hospital with rapidly progressive chronic lymphocytic leukemia, classified as Rai IV. A total of 17 treatment cycles of anthracycline based chemotherapy was applied during 20 months. On disease progression, Fludarabine Phosphate at a dose of 25mg/qm for 5 days was administered. After 4 courses complete clinical remission was achieved with normalization of leukocyte count and resolution of lymphadenopathy and bone marrow infiltration. Immunoglobulin levels increased continuously from 540 to 820 mg/dl into the lower normal range and the patient developed a severe hemolytic anemia of autoimmune origin. Direct Coombs test became positive and IgG and C3d molecules could be demonstrated on red blood cell membranes. Hemolysis could be controlled by immunosuppressive therapy with prednisone and azathioprine. Unfortunately, the patient died of unrelated ischemic cerebrovascular complications. Autoimmunity is a well known complication in CLL, but is usually correlated with disease activity. In this rare case of Fludarabine-induced complete clinical remission of CLL with recovery of immunoglobulin levels, autoimmune hemolytic anemia is difficult to explain. Because malignant B-cells generally produce IgM-antibodies of monoclonal origin, the autoimmune phenomenon could be attributed to therapy-induced disturbance of the immunoregulatory network. Even though the T/B-cell ratio was balanced, Fludarabine may have led to a loss of a certain subset of T-lymphocytes (CD45RA), which has recently been implicated in the control of autoimmune mechanisms. Increased succeptibility of T-lymphocytes to Fludarabine has been demonstrated by in vitro studies.

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Sequential analysis of immunoglobulin gene rearrangements in peripheral blood from lymphoma patients receiving cyclic chemotherapy (CHOEP)

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In an ongoing study 41 pts presenting with lymphoma or lymphatic leukemia (CB: n=9; CB-CC: n=17; ALL: n=6; CC: n=4; T-NHL: n=1; NHL unclass: n=2; T-CLL: n=1; immunocytoma: n=1) were screened for immunoglobulin light and heavy chain or T-cell receptor gene rearrangement by Southern blotting. In 9 pts (CB-CC: n=3; ALL: n=3; CB: n=1; NHL unclass: n=1; immunocytoma: n=1) only BM samples were available and rearrangement was detected in all but two pts with CB and c-ALL. In 16 pts (CB-CC: n=7; CB: n=5; CC: n=2; ALL: n=1; T-CLL: n=1) PB samples only were analyzed and rearrangement could be detected in all but two pts with CB or CB-CC, respectively. In the remaining 16 pts samples from BM and PB could be studied simultaneously. While rearrangement was found in all BM samples a rearrangement was present in the PB of 8 pts only. Nine pts (CB II EA: n=1; CB III A: n=1; CB IVA: n=2; CC IV A: n=1; CC IV B: n=1; CB-CC III A n=1; CB-CC IV B: n=2) exhibiting a rearrangement in the PB at diagnosis were followed regularly by analysis of PB samples while treatment with CHOEP progressed. After three cycles of CHOEP 3 pts had returned to germline configuration of the respective genes involved in rearrangement at diagnosis and these pts remain alive and well 20, 20, and 21 months after diagnosis. Five pts showed a persisting or reappearing rearrangement at the end of chemotherapy and 3 of these pts have died 6, 9, and 9 months after diagnosis. Two pts are alive and well 22 and 23 months after diagnosis although the last evaluation of PB cells showed a reappearing rearrangement of immunoglobulin genes. One further pt awaits testing after the last cycle of chemotherapy.
We conclude that up to 50 % of lymphoma/lymphatic leukemia pts exhibit detectable rearrangements of immunoglobulin and/or T-cell receptor genes in the peripheral blood which then offers the opportunity to easily follow the kinetics of the diagnosed rearrangements under chemotherapy. Our results do not allow firm conclusions as to the clinical value of such studies. However, further investigation seems warranted as those pts achieving a molecular remission by the end of chemotherapy remain in clinical remission for extended periods of time while on the contrary, 3/6 pts with persisting or reappearing rearrangements have relapsed and died already whereas 2 pts with rearranged immunglobulin genes at the end of therapy remain alive and well closed two years after diagnosis.

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EFFECTS OF RECOMBINANT HUMAN STEM CELL FACTOR (rhSCF) ON THE GROWTH OF HUMAN TUMOR CELL LINES IN VITRO

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Stem cell factor is a pluripotent cytokine which is believed to play an important role in proliferation and differentiation in various types of cells. We have studied the in vitro growth-modulating effects of rhSCF (0.1 - 10 ng/ml) in 7 tumor cell lines using continous exposure experiments in a capillary human tumor cloning system, a tritiated thymidine uptake assay and by counting cell numbers. Tumor types included: 4 renal cell (A-498, Caki-1, Caki-2, ACHN) and 3 colorectal carcinoma cell lines (HT-29, LS 180, WiDr). No significant stimulation or inhibition of soft agar colony formation was observed in most cell lines. These results were confirmed in the tritiated thymidine uptake assay and by counting cell numbers. However, a borderline increase of tritiated thymidine uptake to 159% ± 18% compared to control was observed in Caki-2 cells after 3 days of incubation with 1 ng/ml rhSCF. Similarly, an increase in cell number after 3 days of incubation with 1 ng/ml rhSCF to 166% ± 36.6% and with 10 ng/ml rhSCF to 155% ± 33% was noted in A-498 cells. No time- or dose-relationship was noted. We conclude that under our experimental conditions rhSCF had no profound growth modulating effect on established human tumor cell lines.
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CYTOKINE EXPRESSION IN HODGKIN'S DISEASE

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The characteristic histology of Hodgkin's Disease (HD), in which low numbers of Hodgkin and Sternberg-Reed cells are surrounded by a large number of reactive cells, suggests a functional relationship between these cells, possibly mediated by cytokines. To study the role of cytokines in the biology of HD, we first analysed the expression of a panel of growth factors in HD derived cell lines in primary biopsy specimen and in serum of patients with HD. Our data indicate that the cells produce a variety of cytokines such as IL-1, IL-5, IL-6, M-CSF, G-CSF, GM-CSF, TNFα, lymphotoxin and TGFß. In addition, the receptors for IL-2 (α and ß chains), IL-6 and M-CSF could be detected in some of the cell lines. In primary tissues we have found the expression of IL-6 and IL-6 receptors and of IL-2 Rα and IL-2 Rß in Hodgkin and Sternberg-Reed cells by in-situ hybridization and immunohisto-logical experiments. ELISA analyses revealed expression of IL-3, IL-6, IL-7, IL-8, GCSF and soluble IL-2 R molecules in the serum of patients with HD. Thus, we conclude from our data that IL-6 and perhaps additional cytokines are involved in Hodgkin's Disease.

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ACTIVATION AND ADHESION ANTIGENS ON CIRCULATING MONONUCLEAR CELLS DURING THERAPY WITH DIFFERENT HEMOPOIETIC GROWTH FACTORS

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Regulation of surface antigens by hemopoietic growth factors was studied by flow cytometry on lymphocytes and monocytes of patients treated with rG-CSF, rGM-CSF and rIL-3 . The effects of these cytokines were observed under three conditions :

rG-CSF in patients with advanced bladder cancer treated with M-VAC. They received a single dose of rG-CSF (5 µg/kg/s.c.) before chemotherapy. Cell surface marker analysis was done before and after cytokine application.

rGM-CSF in patients with small lung cancer treated with different doses of this cytokine (5 µg or 10 µg/kg/s.c.) followed by the ACO-II scheme. Antigens were analysed as described above.

rGM-CSF and rIL-3 treatment of patients with different solid tumors in complete remission for peripherial stem cell harvest. An application of rGM-CSF (5 µg/kg/d on 5 consecutive days) was combined with several doses of rIL-3 (2.5 µg, 5 µg or 10 µg/kg/d) and different application periods (3, 7 or 14 consecutive days).

Methods : Ficoll-separated mononuclear cells were studied by two color FACS-analysis using a broad panel of FITC and PE conjungated monoclonal antibodies. Cells were characterised by lineage specific antibodies (CD 2, CD 14, CD 19 among others); coexpression of activation markers (CD 25, CD 38, CD 71 and MHC II) and adhesion molecules (CD 11b, CD 44 and CD 49wd) was studied.

Results : After rGM-CSF and rG-CSF treatment activation of T-lymphocytes was observed by coexpression of CD 25, CD 71 and MHC II, while rIL-3 had no influence on T-cell activation. rG-CSF downregulated adhesion molecules (CD 11b, CD 44 and CD 49wd) on lymphocytes. On monocytes rGM-CSF upregulated CD 54, CD 71 and MHC II. In contrast MHC II was downregulated during rG-CSF application. Furtheron, rG-CSF reduced the antigen density of CD11b and CD 44 on monocytes.

The studies showed differential regulation of activation and adhesion molecules in vivo during treatment with different hemopoietic growth factors.

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TREATMENT WITH ALKYLLYSOPHOSPHOLIPID AND LONGTERM BONE MARROW CULTURE FOR PURGING OF CHRONIC MYELOID LEUKEMIA

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Chronic myeloid leukemia (CML) is a clonal hematopoietic disease of the pluripotent stem cell. Cure can only be achieved by bone marrow transplantation. Allogenic bone marrow is available only for a minority of patients. Autologous bone marrow may be used, if in vitro treatment is successful for elimination of Ph 1 positive cells.

15 bone marrow samples of patients with newly diagnosed untreated CML in chronic phase were treated with the alkyllysophospholipid ET-18-OCH3 (ALP) followed by long term bone marrow culture (LTBMC). Colony growth assays and cytogenetic analysis were done at day 0,21,28 and 35 of LTBMC. In 14 of 15 cases without ALP treatment the philadelphia chromosome was still detectable after several periods of LTBMC. In one case there was no analysable metaphase.In three out of the 15 cases treated with ALP we found Ph 1 negative metaphases in cytogenetic analysis after three and four weeks of LTBMC.

These preliminary results indicate that treatment with ALP might enhance the rate of conversion of Ph 1 positive CML bone marrow precursor cells during LTBMC.

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SUCCESSFUL ORTHOTOPIC LIVER TRANSPLANTATION FOR A PATIENT WITH VENOOCCLUSIVE LIVER DISEASE AFTER BONE MARROW TRANSPLANTATION

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Hepatic venoocclusive disease (VOD) is a complication of allogeneic bone marrrow transplantation (BMT) occurring in 10-30 % of patients. The mortality of these patients is 30-40 %. We report a 38 year-old patient with an acute myeloid leucemia (M5, FAB) in first remission after conditioning with busulfan 4 mg/kg/day orally for 4 days and cyclophosphamide 60 mg/kg/day for 2 days. On day +8 after BMT (HLA identical sibling donor, ABO- and sex-mismatched, immunosuppression with cyclosporine A and "short-MTX") the patient developed fever and increasing C-reactive protein. Antibiotic therapy was ineffective. 5 days later a progressive hepatomegaly, rise of bilirubin (maximum 200 umol/l), rise of liver transaminases (maximum of GOT/GPT/GLDH > 1000 U/l), ascites and refractoriness to platelet transfusions occurred. A clinical diagnosis of VOD was made. Hepatorenal syndrome developed and daily dialysis was initiated. Because the liver function progressively deteriorated, orthotopic liver transplantation (LTX) was planed and carried out at day +23 after BMT. The transplanted liver was ABO-compatible with the bone marrow graft. Postoperatively the patient received steroides, cyclosporine A and monoclonal murine IL-2-receptor antibody for immunosuppression. Liver function parameters improved quickly, but hemodialysis was necessary for further 4 weeks. No GVHD was observed neither after BMT nor after LTX. 7 month after BMT bone marrow function was slightly impaired due to cytomegalovirus-infection and subsequent gangciclovir-therapy (in complete remission). 6 month after LTX liver function was still completely normal. No signs of rejection or GVHD were seen.. The Karnofsky index of the patient was 70-80 %. We conclude that orthotopic liver transplantation is a feasable approach for rescue in rapidly progressive veno-occlussive liver disease after BMT.

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GENISTEIN, A SPECIFIC TYROSINE KINASE INHIBITOR, PREVENTS CYTOLYTIC T-CELL ACTIVATION

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Modulation of growth and functions of lymphocytic cells is possible by chemically interfering with intracellular signalling events. In recent years the biochemical signals following the activation of T-lymphocytes via the T-cell receptor (TCR) have been intensively studied. The tyrosine kinases have been identified as important mediators of TCR-induced activation of leukemic cell lines (L. E. Samelson, Cell, 1986). In order to analyse the role of tyrosine kinases in murine cytolytic T-cells we used the specific tyrosine kinase inhibitor genistein. Concentration dependently, genistein inhibits IL-2-dependent cellular proliferation and TCR-mediated cytolytic T-cell effector functions. These functions include a) cytolytic activity, b) exocytosis of preformed granules, and c) *de novo* protein synthesis. Biochemical characterisation of intracellular signalling events reveals two steps in TCR-induced signal transduction sensitive to genistein. First, phosphatidyl-inositol turnover is blocked suggesting a tyrosine kinase activity proximal to phospholipase C activation. Second, genistein is able to inhibit phorbolester/calcium ionophore-induced exocytosis of granules and *de novo* protein synthesis. Implications of findings on the regulation of lymphocytic functions are discussed.

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CD 8 DEFICIENT MUTANTMICE CANNOT REJECT MURINE LEUKEMIA AFTER TUMOUR IMMUNIZATION

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Immune control of tumours is thought to be mediated through specific, antigen dependent mechanisms that are T cell dependent and through mainly unspecific, K(?) and NK mediated processes. The existence of antigen specific rejection mechanisms has been shown in early experiments involving immunization with irradiated tumour cells and subsequent challenge with vital tumour cells (Foley 1953, Main and Prehn 1957, Klein 1960). To further dissect the cellular subsets responsible for the observed postimmunization tumour rejection , we have utilized a novel mouse strain that is completely CD 8 deficient and was generated by homologous recombination (Fung-Leung et al, Cell, 1991). These mice lack CD 8 killer T function in CTL assays. CD 8 deficient mice were backcrossed into a B6 background (F5) and immunized with 1 Mio. irradiated ALC cells weekly x 5. ALC is a radiation virus induced murine thymic leukemia (B6 background). CD 8 +/- and wild type mice as well as non-immunized mice of the three phenotypes served as controls. One week after the last immunization, mice were challenged with 1000 live tumour cells after a 400 rad irradiation of the mice. Results (tumour incidence for immunized and non immunized mice) are shown in the table:

WT im	WT non-i	CD 8 +/- im	CD 8 +/- non-i	CD 8 -/- im	CD 8 -/- noni
0/6	8/8	**1/9**	8/8	**4/5**	6/6

These results show that CD 8 killer T cells are necessary for the rejection of ALC leukemia cells in immunized mice.

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SINGLE CELL ANALYSIS OF REED STERNBERG CELLS: PHENOTYPICALLY SIMILAR HODGKIN'S LYMPHOMAS ARE MOLECULARLY DISTINCT

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Although it was the first malignant lymphoma to be recognized as an individual disease entity, Hodgkin's disease [HD] , its origin and cause, remains an enigma to clinicians and researchers alike. Heterogeneity in clinical presentation and biologic behaviour suggests that HD might not represent a single disease. Due to the scarcity of Reed Sternberg [RS] tumour cells in affected tissues, we have examined single RS cells from 11 patients with HD with a global PCR method (polyA-cDNA PCR) and from 4 patients with combined RT cDNA+DNA PCR for p53. PolyA cDNA PCR generates 500bp 3'mRNA cDNA "libraries" with preservation of relative abundance from small samples. To examine specificity, cell line controls were examined with 3' cDNA probes. Jurkat 3' cDNA was cloned into a vector, and the library screened for TCRß sequence. 420 bp of non-mutated TCR sequence were found. Probing of libraries from 6 patients with at least 7 successfully amplified single RS cells showed lack of c-fms expression, very frequent c-myc, c-fes/fps and fyn expression in single cells. Lck (T-cell tyrosine kinase) and lyn were expressed to varying degrees in different cases. Hck (monocytoid/B cell kinase) was found in one NS case and no other case. CD 4 was expressed in most RS cells from LP and NS cases. TNFß was expressed to high levels. GATA-3, a T cell specific transcription factor, was expressed in T lines but not in single RS cells. This molecular footprint shows that RS cells are activated hemopoietic cells and shows consistent differences for kinase proto-oncogene expression. p53was expressed in 3/4 cases examined by RT PCR, genomic sequence was found in 4/4cases.

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GRAFT REJECTION AFTER BONE MARROW TRANSPLANTATION: THE ROLE OF T CELLS, GVHR AND MARROW CELL DOSE.

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Graft rejection is a problem after transplantation of T cell depleted bone marrow. We investigated factors influencing graft rejection using two animal models. I. LEW rats ($RT1^l$) were treated with a lethal dose of busulfan (35 mg/kg) and escalating doses of cyclophosphamide (30 - 240 mg) prior to transplantation of increasing numbers ($1x10^7$ - $4x10^8$) of either GvH-reactive CAP ($RT1^c$) or GvH-nonreactive F1(LEWxCAP) bone marrow (42 experimental groups, n=5-33 per group). Cell dose variations of 50% ($2x10^8$/kg) or differences in the CY dose of 30 mg/kg significantly influenced red cell and platelet recovery, the percentage of donor type ($RT1^{c+}$) lymphohematopoietic cells, and the number of deaths with neutrophils < 500/ul. Virtually no difference, however, was observed between GvH-reactive and GvH-nonreactive grafts. The addition of thymocytes did not improve engraftment. II. Different T-cell depletion (TCD) techniques were tested in lethally irradiated (7.5 Gy) Balb/c mice ($H\text{-}2^d$). After transplantation of 0.1, 1, and 10 x 10^6 MHC-mismatched but GvH-nonreactive (BalbxC57)F1 ($H\text{-}2^{dxb}$) marrow cells, rejection rates were 36%(n=11), 14% (n=14), and 0% (n=15) for unmanipulated BM (NTCD) and 45%(n=11), 8%(n=12), and 0%(n=12) after TCD with anti-Thy-1. There was no influence of TCD on chimerism: a median of 47% (NTCD) vs. 36% (TCD), 79% vs. 78%, and 83% vs. 94% of $H\text{-}2^{b+}$ donor cells were detected at day 50. After injection of 0.1, 1, 10, and 40 x 10^6 unmanipulated GvH-reactive C57BL/6 ($H\text{-}2^b$) cells, the incidence of rejection was 87%(n=8), 67%(n=26), 21%(n=33), and 0%(n=17). Similar rejection rates were observed after TCD with anti-Thy-1: 87%(n=8), 64%(n=14), 27%(n=33), and 0%(n=17). Similarly, TCD with anti-CD4/CD8, or Leu-Leu-OMe successfully prevented GvHR but did not affect rejection rates and long-term chimerism, provided that exactly the same cell numbers were grafted. We conclude that: (1) The marrow cell dose is critical for successful engraftment. (2) The risk of rejection is not reduced by GvHR. (3) T cells are not essential for long-term hematopoietic recovery. (4) Intensified immunosuppression can compensate for reduced BM cell doses.

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PARAMETERS OF UNSPECIFIC IMMUNITY IN PATIENTS WITH HIGH GRADE MALIGNANCY NON-HODGKIN LYMPHOMAS IN THE REMISSION PHASE OF DISEASE, SUBJECTED TO IMMUNOMODULATION WITH CALF THYMIC EXTRACT

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The study comprised 13 patients aged 19-66 years (median 54 years) with high grade malignancy non-Hodgkin lymphomas in the remission phase of disease (remission duration 26-103 mo, median 45 mo). Following parameters of unspecific immunity: absolute numbers of peripheral blood lymphocytes, their subpopulations, neutrophils and monocytes, serum levels of IgG, A, M and skin tests with recall antigens were evaluated prior to and after immunomodulation.
All patients were given Thymus Factor X (Polfa) in dosage of 10mg s.c. every 2nd day for the 1st month, followed by the same dose every 3rd day for 5 months.
Disturbances in parameters studied were observed prior to immunomodulation. Moreover, all of patients suffered from frequent viral or Candida albicans infections of the oral and pharyngeal mucosa. None of these infections ocurred since the 2nd month of immunomodulation. After 6 months of immunomodulation disturbances in parameters of unspecific immunity persistend, but the CD4/CD8 ratio tended to improve.

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RESULTS OF 24^h HEART MONITORING IN LYMPHOMA PATIENTS TREATED WITH EPIRUBICIN.

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39 patients (pts) with malignant lymphoma (30 Hodgkin's disease: 16 women and 14 men, aged 17-70 years, mean 36.7 in clinical stages CS IIA-IVB, and 9 with high grade non-Hodgkin lymphoma: 3 women and 6 men aged 21-64 yrs mean 46.6 in CS IV) were treated acc. to multidrug cytostatic protocols containing epirubicin (epiDX).

Using the 24^h ambulatory ecg acc. to Holter, on the day before, the day of the first drug application as well as after therapy completion heart rate, arrhythmias and conduction abnormalities were estimated. Mean value of heart rate diminished a little in the consecutive examinations, being significantly elevated in comparison with the healthy control, on the day of drug administration and the day before. Supraventricular extrasystoles (SVES) were observed in 10 pts before the treatment, just after EpiDX in 14, and after therapy completion in 5. In 3 patients suffering from ischemic heart disease (IHD) several episodes of atrial tachycardia were noticed on the day of drug injection.

Ventricular extrasystoles (VES) have been found in 9 pts: in two they appeared only on the day of EpiDX inj. and in 3 with IHD their amount was twice as high as it was before.

The authors conclude that EpiDX in a part of pts may decrease the heart rate and in some, especially with IHD, provoke VES generation.

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HIGH ACTIVITY OF IDARUBICIN (IDA) IN COMPARISON TO DOXORUBICIN (DOX) AND 4-EPIDOXORUBICIN (EPI) IN GASTRIC CARCINOMA CELL LINES.

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Multidrug resistance (mdr) is a major problem in anthracycline tratment of gastric cancer. The cytotoxicity of the new anthracycline idarubicin seems to be independent of P-glycoprotein (Pgp) related mdr. Cytotoxicity of DOX, EPI and IDA was studied in two human gastric cancer cell lines (HM2 and HM51) with different expression of Pgp. Methods: Expression of Pgp was determined by immunocytochemistry using the specific monoclonal antibodies C-219 and JSB-1. Chemosensitivity was estimated with the MTT colorimetric assay. Exponential cell growth was shown for both cell lines within 96 h. Continuous drug exposure was performed with concentrations of IDA, EPI and DOX between 0.0625 and 5.0 μM for 24 and 72 h. Cytotoxicity was measured after 96 h. Results: Pgp expression was high in HM2, and low in HM51 cell line. In HM2 IC_{50} of DOX was 5 (24 h) and 11 fold (72 h) higher than in HM51. In contrast to DOX the IC_{50} of IDA was only moderately elevated in HM2. In comparison to DOX the IC_{50} of IDA was 3 (HM51) and 10 fold (HM2) lower [0.18 vs 0.48 μM (24h), and 0.06 vs 0.20 μM (72 h) for HM51: 0.24 vs 2.38 μM (24 h), and 0.22 vs 2.27 μM (72 h) for HM2]. There was no significant difference betweeen IC_{50} of DOX and EPI. Conclusions: IDA is active in both human gastric cancer cell lines with different expression of Pgp. However, in contrast to DOX and EPI the efficacy of IDA was independent of Pgp expression.

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PILOT STUDY OF FOLINIC ACID (L), ETOPOSIDE (E), 5-FU (F) AND SEQUENTIAL CISPLATIN (P) FOR ADVANCED GASTRIC CANCER (GC).

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ELF is one of the newer active regimen for GC which induced approximately 50% of CR/PR. In this ongoing pilot study, cisplatin was added to ELF because of its high single agent activity in GC and lack of overlapping toxicities of E, F, and P. Treatment plan: L 300 mg/m^2 10 min iv; E 120 mg/m^2 50 min iv; F 500 mg/m^2 15 min iv were given on d 1,2,3; and P 50 mg/m^2 iv d 8,15; q d 28. Eligibility criteria: histologically proven locally advanced and irresectable (LAD) or metastasized (M1) GC; no prior chemo-/radiotherapy; measurable and/or evaluable disease; age $\leq$ 70 yrs; WHO performance status (PS) $\leq$ 2; informed consent. Patients (pts) characteristics (n=17): male/female 14/3 ; age 58 yrs (42-69); PS 80% (60-100); tumor extension LAD/M1 6/11. Treatment results: too early to evaluate (< 2 cycles) 1 pt; PR 11/16 (69% [46-92%]), MR/NC 3/16, PD 2/16, PR in LAD 4/6 (3pts NED after second look surgery), PR in M1 7/10; median remission duration 7 months ; median survival time not yet reached. Percent of WHO grade > 2 toxicity in 57 administered cycles: leucocytopenia 12%, anemia 2%, thrombocytopenia 7%, infection 4%, nausea/vomiting 5%, diarrhea 9%, stomatitis 4%; no treatment related death. Due to side effects (especially thrombocytopenia and diarrhea), cisplatin could not be given in full doses in 42% of all courses. Conclusions: These first results indicate a high efficacy of ELF-P for advanced GC. Compared to ELF alone, toxicity is markedly increased with respect to myelosuppression and diarrhea, but manageable.

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THE ROLE OF CHEMOEMBOLIZATION (CE) IN THE TREATMENT OF HEPATOCELLULAR CARCINOMA (HCC).

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CE combines tumor ischemia with locoregional chemotherapy. It may have particular utility in patients with HCC, whose tumors tend to be vascular and refractory to standard treatment. At UC San Francisco, we have treated 115 patients on sequential trials of CE since February, 1986, using gelfoam as the embolizing material, combined with various chemotherapies. Entry criteria include a KPS $\geq$ 60%, liver-predominant tumor, patent portal vein, no gross ascites and bilirubin < 5 mg/dl. Our pilot experience with gelfoam alone (N=14) revealed no evident activity, so patients were then treated with a combination of gelfoam, doxorubicin, mitomycin-c and cisplatin. The mixture was injected under fluoroscopic guidance via a percutaneous catheter into the hepatic artery until stagnation of blood flow was achieved. 84 patients (median liver replacement = 50%) were treated palliatively with this combination. 60% of patients had selective arterial embolization due to underlying cirrhosis and/or hepatitis. By CT scan criteria, there were 21 PR's (25%) and 21 MR's (25%). Liquefaction necrosis occured in 77% of patients, and alpha-fetoprotein declined by > 50% in 43/56 patients. The median survival for all patients was nine months; 25% of patients were alive > two years. Patients are now being treated palliatively on a protocol using Lipiodol in the same CE mixture. Seven patients have been treated, responses are similar. Eight patients have been treated with the original CE mixture as preoperative therapy for orthotopic liver transplantation (OLT). To be eligible, patients have tumors < 5 cm, are HBsAg - and have no metastases. Six patients had OLT; all are alive and free of disease (months 10,10,16,21,24,42); one patient had carcinomatosis at surgery and one awaits OLT. The toxicity of CE includes transient fever, nausea, vomitting and abnormal liver enzymes. Complications have included hepatic abscess (2), bacteremia (2), and tumor lysis syndrome (1). There have been three treatment-related deaths (liver failure, 2; hemorrhage, 1). In summary, CE is reasonably well tolerated and is active against HCC. The best particle/drug combination and the role of CE in treating HCC are uncertain.

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EFFECT OF STEM CELL FACTOR AND ERYTHROPOETIN ON THE GROWTH OF ERYTHROID PROGENITOR CELLS (BFU-E) IN MYELODYSPLASTIC SYNDROMES.

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Peripheral cytopenia is a key symptom of myelodysplasia (MDS). 15 % of cases with anemia can be significantly improved by erythropoetin (EPO) alone. Since in vitro data demonstrated a synergistic effect of stem cell factor (SCF) and other hematopoietic growth factors including erythropoetin on the growth of normal hematopoietic progenitor cells, we investigated the effect of EPO and SCF in MDS. 9 bone marrow apirates were analyzed in a colony forming assay (3 RA, 2 RA-RS, 2 RAEB, 2 RAEB-T cases). In 4 out of 5 cases with RA or RA-RS the number and size of BFU-E colonies were significantly increased by EPO and SCF in comparison to EPO alone. One of these 4 patients showed no BFU-E colonies with EPO. In comparison to normal bone marrow the number of colonies still was reduced. In 2 RAEB and 2 RAEB-T cases no growth of BFU-E colonies was observed even when both factors were added. We conclude that EPO and SCF act synergistically on erythroid progenitor cells in patients with RA and RA-RS. The responsiveness of erythroid progenitors is possibly lost during transformation to AML. The factor combination may be useful in the treatment of refractory anemias.

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IMMUNOLOGIC AND CLINICAL ANALYSIS OF ADULT ACUTE MYELOID LEUKEMIA WITH AND WITHOUT CYTOGENETIC ABNORMALITIES

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A retrospective analysis was made for adult patients with acute myeloid leukemia in order to correlate cytogenetic and immunologic findings as well as the clinical outcome after conventional induction chemotherapy.

From 1985 to 1990, we investigated 184 patients (119 pat. <60, 65 pat. >60 years of age). Cytogenetic analyses were performed in 146 and results obtained in 123 (84%) patients. 56 (46%) of these cases showed no chromosomal aberrations, whereas 67 (54%) presented with numeric and/or structural aberrations.

For 46 patients without chromosomal abnormalities, immunological phenotyping was performed by flow cytometry. We found an aberrant expression of CD7 in only 2 cases (4%), while no other atypical antigens could be detected. The expression of stem cell marker CD34 was analyzed in 25 cases of which 8 (32%) were positive. Within the total 46 cases, 34 (74%) were HLA-DR- and/or CD34-positive.

It can be concluded that the absence of cytogenetic abnormalities is associated with a low incidence of aberrant antigen expression. Data on prognostic factors and clinical outcome are related to analyze the homogeneity of cytogenetically normal acute myeloid leukemias.

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AUTOMATED WHITE BLOOD CELL DIFFERENTIAL COUNT. AN UPDATE

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The manual or visual differential leucocyte count (DLC) is performed when a human observer locates and classifies cells microscopically on a stained blood film. The imprecision and labour-intensive characteristics of this classical haematology procedure have led to the development of automated differential counters which has been of two categories: 1- Pattern-recognition instruments which simulate by computer the human eye and brain and classify cells (100 to 500) on the basis of programmed properties of size, shape and staining on a conventionally stained blood film, and 2- Flow-cytometry methods which classify large numbers of cells (more than 20.000) in suspension employing electromagnetic waves (low and/or high frequency), light scatter, cytochemistry and differential lysis ,either singly or in combination. Pattern-recognition instruments, have never been developed to their full extent due to the comparatively small number of evaluated cells and their low rate of specimen throughput. On the contrary, the flow-cytometry instruments, in addition to its high throughput rate and the classification of a very large number of cells,provide several new red cell and platelet measuring parameters and incorporate "flags" or "alarms" which indicate abnormal blood samples and the need for microscopy review. The most modern and sophisticated haematology analyzers provide, in general;1-eight parameter complete blood count 2- Some new indicators relative to RBCs and PTLs 3-five (NE-8000, STKS, CD 3000, COBAS) or six (H*1) WBC population DLCs, 4-scattergrams relative to leucocyte populations, 5-series of morphologic and distributional flags.

Although some differences have been observed in instrument performance, neutrophil comparisons have been always excellent. Equally, the small mature lymphocyte is also easily recognized and inter-instrument comparisons are fairly satisfactory provided the proportion of large lymphocytes is normal and there are no atypical lymphocytes present. This is also true for eosinophils. On the contrary when cells are present in low proportion (i.e,basophils), the comparability is in general very poor. For monocytes, some disagreement has been observed between the different instruments, but also by visual assessment of these cells. In conclusion, the topic of DLC continues to hold much interest for both the improvement in the identification of circulating cells and the achievement of standardization of the different technologies which could allow a harmonized interpretation of the different "signals" provided by the peripheral WBCs.

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PRECLINICAL ACTIVITY OF TAXOTERE (RP 56976, NSC 628503) AND TAXOL AGAINST HUMAN TUMOR COLONY FORMING UNITS

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Taxotere (TE) and taxol (TA) are new antitumor agents currently undergoing clinical evaluation. We have studied the antineoplastic effects of these agents on in vitro proliferation of clonogenic cells from freshly explanted human tumors using a capillary soft agar cloning system. Final concentrations used for TE and TA were 4.0, 0.4 and 0.04 μM. At present, 45/72 specimens (63%) are evaluable for head-to-head comparisons using a 21-28 day continuous incubation (12 renal cell cancer, 7 colorectal, 7 gastric, 6 melanoma, 13 others) with a median colony formation of 19.7/capillary in controls (range 5.3-604.8). Both agents showed concentration-related antitumor activity. At 0.4 μM median colony survival was 0.57 x control (range 0.04-0.96) for TA and 0.43 (0.04-0.77) for TE. On a concentration basis, TE was more active than TA in 27/45 specimens.- A concentration related increase in cytotoxicity was also observed after a 1-hour short term incubation. At 0.4 μM, median survival was 0.61 x control for TA (range 0.09-0.87) and 0.49 (0.15-0.81) for TE. On a concentration basis, TE was more active than TA in 21/41 specimens. We conclude that Taxotere and taxol are active against in vitro tumor colony formation from freshly explanted human tumors. The spectrum of activity includes tumor types like melanoma, renal cell and colorectal cancer with known intrinsic resistance to antineoplastic agents.

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paroxysmal nocturnal hemoglobinuria (PNH) as late hematological complication in aplastic anemia (AA) after immunosuppressive treatment (IS)

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Development of clonal disorders such as PNH, myelodysplastic syndromes or acute myeloid leukemia is observed in a considerable proportion of patients with AA following after IS.These results imply that long term follow-up investigations in AA after IS are necessary.
In a recently completed study we have investigate 29 patients (15 males, 14 females, range 18-65 years, mean age 43, range after IS: 2-128 months, mean 32 months) with acquired AA for signs of PNH.
The Ham-test and assessment of phosphatidylinositolglycan (PIG)-anchored surface molecules in peripheral blood cells analysed on a fluorescence-activated cell sorter FACScan by using monoclonal antibodys were performed serially.
In four patients indicators of clonal diseases were found:
In one patient a PNH was diagnosed prior to IS with positivity of both the Ham-test and PIG-analyses and clinical evidence of hemolysis. He achieved a complete remission (CR) of the AA.
The second patient showed first evidence of a deficiency of PIG-anchored proteins three months after IS, Ham-test was negativ. There was no clinical response to treatment.
The third patient developed deficiency of PIG-anchored proteins 18 months after IS in association with relapse after CR, Ham-test was negativ.
In the fourth patient was first diagnosed a severe AA in 6/84, treated with antilymphocyte globulin (ALG),methylprednisolone (MP) and androgens followed by a CR. First relapse occurred 2/85, a second course of ALG/MP resulted in a second CR. Second relapse occurred 11/85, monotherapy with ciclosporin A (CSA) was followed by a CR, which was initially CSA-dependent. Despite continuation of CSA-therapy a third relapse was observed 3/89, treatment with ALG, MP and CSA again resulted in a CR.
During the whole observation period PNH-tests were negativ.
In 9/90 she relapsed again and for the first time a PNH was diagnosed by both the Ham-test and demonstration of PIG-deficiency. By following treatment with ALG/MP and CSA in combination with Interleukin-3 a CR was again achieved.
These results demonstrate the importance of long term follow-up investigations in obtaining more information about the frequency of PNH, and most likely clonal disorders in general in AA after immunosuppressive therapy.

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recombinant human Interleukin- 3 (rh IL- 3) in aplastic anemia (AA) complicated by septicemia during immunosuppressive treatment (IS) --- a case-report.

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A 25 year old female patient presenting in 2/88 with a severe AA, was treated with antilymphocyte globulin (ALG) and methylprednisolone (MP) and reached a complete remission (CR) 2/89.
In 7/89 she relapsed and achieved a partial remission (PR) under ciclosporin A (CSA) and prednisolone.
In 3/90 the second relapse was diagnosed with an absolute neutrophil count of 500/µl. In 7/90 she was treated within a phase I/II-study with rh IL-3 500 µg/day given as subcutaneous bolus injection from day 9-90 in combination with ALG/CSA/MP. Fever developed 8 days after start of treatment, blood cultures showed growth of Staph. aureus, ciprofloxacin-therapy was successful.
Within the following two weeks the neutrophils increased to 2800/µl without fever or signs of infection, an unusually rapid increase compared to ALG/CSA/MP-therapy alone.
Five weeks after beginning of therapy the patient developed clinical evidence of infection with fever, chills and septic skin lesions. Again staph. aureus was found in blood cultures.
Abdominal ultrasound examination showed multiple abscesses of the liver, confirmed by computertomography; a bone scan showed foci in the left tibia and in the head.
Antibiotic treatment with imipenem/vancomycin, followed by teikoplanin i.v. and then clindamycin orally for about 6 months resulted in a complete cure. Since 05.09.90 no fever was observed.
On 22.10. 90 CR of the AA was demonstrated and rh IL-3-therapy was terminated according to the treatment plan.
On 19.02.91 CSA was stopped, the patient is still in CR at last follow up 20 months after start of therapy.
The early rise of neutrophils under ongoing IS with CSA/MP suggests, that treatment with rh IL-3 was an important factor for the survival of a severe infectious episode in the second relapse of a severe AA.

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TRANSFORMING GROWTH FACTOR-β1 INTERFERES WITH THE PROLIFERATION-INDUCING ACTIVITY OF STEM CELL FACTOR IN MYELOGENOUS LEUKEMIA BLASTS THROUGH DOWN-REGULATION OF THE C-KIT PROTOONCOGENE PRODUCT.

Sven de Vos, Marion A. Brach, Yoshinobu Asano, Wolf-Dieter Ludwig, Peter Bettelheim, Hans-Jürgen Gruss, and Friedhelm Herrmann

TheTransforming Growth Factor-β family of proteins has potent hematopoietic regulatory properties. The major biological effect of TGF-β on hematopoietic cell growth relies on its reversible inhibition of cell entry into cycle. TGF-β can act as a positive or negative regulator of normaland malignant hematopoiesis dependent on the presence of other factors as well as the responding cell type. Available experimental evidence suggests that TGF-β acst as a selective negative regulator of early hematopoietic progenitor cells while sparing the more committed progenitors.The mechanism by which TGF-β inhibits hematopoiesis is, however, not completely understood. Blast cells obtained from patients with acute myelogenous leukemia (AML) expressing surface binding sites for human Stem Cell Factor (SCF) proliferatively respond following exposure to this molecule. In the presence of human TGF-β1, the capacity of SCF to augment the proliferative state of AML blasts was, however, almost completely abolished. This inhibitory action of TGF-β1 could be reversed by a neutralyzing anti-TGF-β1 antibody. Studies on the mechanism of action of TGF-β1 on SCF-induced proliferation of AML blasts revealed that TGF-β1 treatment of these cells was associated with downregulation of SCF-receptor surface expression (detected with a specific monoclonal antibody) without affecting the transcriptional activity of the c-kit protooncogene encoding the SCF-receptor.

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MOLECULAR CHARACTERSITICS OF THE EPIDERMAL GROWTH FACTOR RECEPTOR SYSTEM IN RENAL CELL CARCINOMA

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Anomalies of the epidermal growth factor receptor (EGF-R) gene, including amplification and overexpression, have been reported in renal cell carcinoma in vivo. Furthermore, all these tumors coexpress at least one of its ligands, transforming growth factor-alpha (TGF-alpha) and EGF, suggesting the existence of an autocrine growth stimulatory loop. We have studied normal kidney and renal cell carcinoma tissue from 50 patients and 4 corresponding tumor cell lines and examined the structure and quantity of the EGF-R gene and its transcripts using Southern and Northern blot analysis as well as single stranded conformation polymorphism (SSCP). In addition, we analysed the genes and transcripts coding for ligands of the EGF-R, TGF-alpha and EGF. EGF-R gene amplification was detected in all 50 tissue specimens whereas no amplification of the EGF-R gene was detected in the corresponding normal kidney. Studies on EGF-R rearrangement and/ or mutation is in progress. All tumors revealed a significant overexpression of EGF-R and TGF-alpha, but underexpression of the EGF when compared to normal kidney tissue. The four tumor cell lines showed a similar expression pattern of the EGF-R system, however overexpression of the TGF-alpha and EGF-R was more pronounced. Interestingly , in 3 out of the 50 renal cell carcinoma and one tumor cell line an aberrant EGF-R transcript of 8.0 Kb was detected.. Our data suggest several mechanisms for the activation of the EGF-R mediated growth stimulatory pathway in renal cell carcinomas in vivo: (1) expression of a structurally altered receptor may have escaped normal control and (2) autocrine/ juxtacrine- and or paracrine stimulating mechanims involving coexpression of receptor and ligands with overexpression of the EGF-R.

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EFFECTS OF TEMPERATURE ON METABOLISM AND ACTION OF OXAZAPHOSPHORINE ALKYLATING AGENTS IN HUMAN TUMOR XENOGRAFTS

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Tumor temperature may vary greatly depending on the topographical situation (37°C from core to 32°C on the skin). We have shown in human tumor xenografts that the effective ness of oxazaphosphorine cytostatic drugs (cyclophosphamide, ifosfamide) is steeply temperature dependent increasing with rising tumor temperatures from 32°C to 43°C. We observed a drug and thermodose dependent increase of the therapeutic efficacy of cyclophosphamide (CP) and ifosfamide (IFO) in sensitive tumors. However, in primary CP and IFO resistant tumors only hyperthermia at 43°C for 1 hr in combination with the same dose of CP and IFO caused significant tumor regressions. To date, the cause of the enhanced therapeutic efficacy of CP and IFO at elevated tumor temperature is poorly understood. In the tumor models studied we observed increased cytotoxicity of thermochemotherapy with CP/IFO without significant changes of nutritive blood flow, tumor-pH or tumor oxygenation. In order to get more information on the in-vivo pharmacokinetics of CP and IFO we determined the blood concentrations of CP and IFO and of activated CP and IFO following iv. application of the drugs in nude mice under different liver temperatures. The temperature dependent change of the concentration of both activated drugs was considerably less steep than the temperature depending change of the therapeutic efficacy. We assume that the increased cytotoxicity of thermochemotherapy with CP and IFO is caused by the temperature dependency of the alkylating reaction.

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IMMUNOPHENOTYPIC FEATURES OF CSF-1 RECEPTOR POSITIVE ACUTE MYELOID LEUKEMIAS (AML)

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Colony-Stimulating Factor 1 (CSF-1/M-CSF) is a 70-to 90 Kd glycoprotein that stimulates proliferation and supports differentiation and survival of the mononuclear phagocyte series in a lineage-specific way. This hematopoietin is the ligand of CSF-1 receptor (CSF-1R), a product of c-fms protooncogene. Analysis of CSF-1R expression in acute leukemia cells using monoclonal antibodies (MoAbs) or Northern blot technique revealed detectable levels of CSF-1R only in AML, suggesting that CSF-1R can be used as an specific marker for leukemias of myeloid origin.Recent studies, however, indicated that CSF-1R was also present in acute leukemias with coexpression of myeloid- and lymphoid-lineage-assiociated antigens. We therefore tested a large series (N=91) of both childhood/adult AML and ALL samples in order to evaluate the incidence of CSF-1R expression and to correlate them to immunophenotypic features. CSF-1R was detected by using the MoAb c-fms/CSF1 receptor (Ab-2; Oncogene Science,Inc.) and expression was evaluated with flow cytometry. Six of 27 children (22%) and 12 of 46 adults(26%) with AML were receptor-positive, whereas all samples(N=18) with a B-cell-precursor or T-cell-lineage immunophenotype were found negative. Immunophenotypic features of monocytic differentiation were detected only in 38% (CD4) and 22%(CD14) of AML with CSF-1R. A considerable number of CSF-1R+ AML(approx. 50%) expressed features suggesting an immature myeloid phenotype, (i.e.,CD34 and/or CD7 and/or TdT+). In conclusion our study indicates CSF-1R+ in about 25% of AML and confirms results that CSF-1R+ AML were not restricted to leukemias with monocytic differentiation.

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DETECTION OF THE IFN-INDUCED HUMAN MX-A PROTEIN BY IMMUNOSTAINING

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The human intracellular Mx-A protein is specifically and dose-dependently induced by type I-interferons in vitro and in vivo (1,2).No other known cytokines including IFN-y are able to directly stimulate the synthesis of this protein. So far, however, it is unknown which cell population is capable of synthetizing MxA in response to IFN-a,ß,w. We investigated this question by studying leukocytes from patients treated with s.c. exogenously administrated rIFN-a2b.

Blood smears were obtained from healthy persons and cancer patients recieving either no or 1 to 10 Mio.IU of rIFN-a2b thrice weekly. In an Mx-A-ELISA employing two specific monoclonal antibodies the blood samples of the control persons were Mx-negative, while all IFN-treated patients were Mx-positiv in this assay. In contrast when one of these two antibodies were used to stain blood cells via the APAAP method, at an antibody-concentration of 56 µg/ml (1:100) all leukocytes showed a weak positive signal in the cytoplasma. These finding suggests that healthy persons contain low amounts of this intracellular protein in their leukocytes. At an antibody-concentration of 11,2 µg/ml (1:500) the monoclonal antibody employed was unable to stain cells from control persons.However, using this concentration leukocytes from patients under IFN-therapy (>2Mio IU/thrice a week) were Mx-positiv by this staining method. Even at 5,6 µg/ml (1:1000) such cells were consistently found to be Mx-positiv. MNC as well as granulocytes showed a granular specific staining in the cytoplasma. More than 95% of the leukocytes studied were Mx-positiv. Employing this staining method neither thrombocytes no erythrocytes were found to be Mx-positiv.

We therefore conclude first that the Mx-A protein is specifically detectable by immunostaining and second that nearly every leukocyte in peripheral blood produces significant amounts of Mx-A upon IFN-a stimulation in vivo. After proving the specifity of this staining procedure it seems likely that this method is helpful in identifying in vivo activation of the IFN-system in different organs and tissues.

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PROGNOSTIC FACTORS FOR THE RISK OF DISEASE PROGRESSION IN LOW AND INTERMEDIATE GRADE NON-HODGKIN'S LYMPHOMA (NHL): A MULTVARIATE ANALYSIS.

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Purpose: The prognosis and clinical course of low and intermediate grade NHL are highly variable, and the staging systems currently available are not fully satisfactory with regard to an accurate prediction of disease progression and survival. Several well established prognostic parameters were therefore compared with some more recent markers of tumor progression in a prospective clinical trial.

Patients and Methods: Between January 1987 and October 1991, 316 patients (pts; 190 male, 126 female; mean age ± S.E.M. 61.4 ± 0.7 yrs, range 22-86 yrs) with the following histopathological entities were included in the study: 152 chronic lymphocytic leukemia (CLL), 56 immunocytoma (IC), 2 prolymphocytic leukemia, 72 centrocytic-centroblastic lymphoma (CCCB), 12 hairy cell leukemia, and 22 other low grade NHL. Pts on radio- or chemotherapy were excluded. Disease progression was defined as progression of the tumor parameters (e.g. thrombocytopenia, tumor size, anemia) by > 25% within 2 months. Univariate and multivariate analyses (Cox's regression model) of progression-free survival were performed in 219 pts evaluable, in whom all of the following prognostic parameters were determined at inclusion in the study: age, sex, platelet and white blood cell counts, peripheral blood lymphocytes and neutrophils, hemoglobin, serum thymidine kinase (TK), serum $ß_2$-microglobulin ($ß_2m$), serum lactate dehydrogenase, presence of B-symptoms, number of lymph node areas involved, and Karnofsky index (KI).

Results: All variables except B-symptoms, age, and sex, showed a significant relationship to progression-free survival (univariate analysis). Using a multivariate analysis, $ß_2m$ was found to be the best prognostic parameter. Only three parameters provided significant additional information ($P < 0.05$): KI, platelet count, and TK. When only CLL and IC pts were analysed, similar rankings were obtained: 1. $ß_2m$, 2. KI, 3. platelet count, 4. TK, 5. age ($P < 0.05$). In CCCB pts, only three parameters provided all significant prognostic information: 1. platelet count, 2. $ß_2m$, 3. sex ($P < 0.05$).

Conclusion: A limited number of clinical and laboratory variables seems to provide sufficient information about progression-free survival of low and intermediate grade NHL. Serum $ß_2m$ and TK are likely to give significant prognostic information and may therefore be helpful to develop improved staging strategies for these malignancies.

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KARYOTYPE IN MULTIPLE MYELOMA AND PLASMA CELL LEUKEMIA

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Between October 1988 and October 1991 one hundred and four patients with multiple myeloma and six with plasma cell leukemia were studied cytognetically. Abnormal karyotypes were found in bone marrow cells of 33 patients (30 %). Most pathological karyotypes were complex with numerous modal and structural anomalies. Numerical anomalies involved most frequently chromosome 11 and structural aberrations occured most often in chromosomes 1, 11 and 14. The most consistent structural aberration was a 14q+ chromosome (10 patients) resulting from a t(11;14)(q13;q32) in 4 patients and a t(8;14)(q24;q32) in one patient. Sequential cytogenetic studies were performed in 15 patients. In five of eight cases with a normal karyotype at diagnosis, chromosomal anomalies were detected when disease progressed. In concomitant cytogenetic/cytologic studies it was found that in the majority of patients with normal karyotype the mitoses originated from contaminating normal bone marrow cells. Pathological karyotypes were detected more frequently in pretreated than in untreated patients, in patients with plasma cell leukemia than in patients with multiple myeloma, in patients with stage III and dense bone marrow infiltration than in patients with stage I. Patients with abnormal karyotype, irrespective if pretreated or not, had a significantly shorter median survival than those with normal karyotype.

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DYSMEGAKARYOPOIESIS IS RELATED TO PLATELET ABNORMALITIES AND FAB SUBTYPE IN MYELODYSPLASTIC SYNDROMES

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Myelodysplastic syndromes (MDS) are stem cell disorders characterised by morphological abnormalities of one or several cell lines. We investigated the frequency of dysplastic megakaryocytopoiesis in MDS subgroups according to the FAB classification and their relation to functional and morphometric platelet abnormalities. Platelet parameters and morphologic megakaryocyte abnormalities were evaluated in 32 untreated MDS patients, and megakaryocyte ploidy could be determined in 22 of these patients. Megakaryocyte ploidy was determined by Feulgen staining. Platelet volume parameters were obtained using an electronic particle counter, and platelet density equilibrium centrifugation was performed with continuous Percoll gradients. Platelet aggregation was induced by ADP and collagen in platelet-rich plasma.
In pure sideroblastic anemia (n=6), there were no signs of megakaryocyte dysplasia, and megakaryocyte ploidy was increased. Platelet count was normal and platelet volume was low. In 7 patients with refractory anemia with or without ringed sideroblasts, megakaryocyte ploidy was almost normal, platelet count normal or slightly decreased, and platelet volume normal or increased. In patients with excess of blasts and in transformation to acute leukemia (n=12), megakaryocyte dysplasia was frequent and megakaryocyte ploidy lower than normal. Mean platelet volume and platelet distribution width were increased, and fibrinopeptide A levels in plasma were elevated. Patients with CMML (n=7) had strong dysplastic features of megakaryocytopoiesis, and megakaryocyte ploidy was slightly increased. Mean platelet volume and distribution width were elevated, platelet aggregation was impaired and platelet density was low. In all other MDS subgroups, platelet aggregation was almost normal and platelet density slightly lower than in control subjects.
In summary, megakaryocyte ploidy is related to platelet volume in MDS. Abnormalities of the megakaryocyte-platelet-system are quite specific for MDS subgroups and range from reactive changes in pure sideroblastic anemia to severe involvement of megakaryocytopoiesis with platelet dysfunction in CMML.

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ABSENCE OF THE RETINOBLASTOMA GENE PRODUCT OCCURS FREQUENTLY IN MONOCYTIC LEUKAEMIAS

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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. Knowledge about the involvement of RB in the pathogenesis of acute myeloid leukaemias is still scarce. In this study we looked at the expression of p105 in 20 myelomonocytic and monoblastic acute leukaemias by Western blotting and immunocytochemistry using the anti-p105-monoclonal antibody PMG3-245. We found absence or nearly undetectable p105 levels in 11 patients (55%). Absence of p105 was correlated with a higher leukocyte count at presentation (133.000/µl vs. 83.000/µl) and with the occurrence of extramedullary leukaemia (8/10 vs. 2/10). We conclude that inactivation of RB with lack of p105 expression occurs frequently in myelomonocytic and monoblastic leukaemias and that this may be correlated with a more malignant course of the disease.

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A PHASE II STUDY OF IODODOXORUBICIN IN ADVANCED NON-SMALL CELL LUNG CANCER (NSCLC).

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4-Iodo-4-deoxydoxorubicin (I-dox) is a doxorubicin analogue that has shown activity in preclinical studies against different cell lines including Lewis lung carcinoma (ca) (Cancer Res 47, 1987). Phase I studies with this compound have already been performed (Contrib Oncology 37, 1989). Therefore we initiated a phase II trial in patients (pts) with advanced, non-operable NSCLC. Inclusion criteria: age < 75 yrs, WHO performance status (PS) < 3, no prior chemotherapy, no prior radiotherapy of indicator lesions. Treatment plan: I-dox 80 mg/m^2 iv, q d 22 until progression. Pts characteristics: male/female 19/4, PS 1 (0-2); age 60 yrs (48-71); squamous cell ca 10, adenoca 5, large cell/undifferentiated ca 8; stage IIIa 2, stage IV 21. Results: evaluable for response 23: PR 3/23 (13% [3-34%]) (remission duration 3.5, 5 and 6.5 months), NC 9/23 (39%), PD 11/23 (48%); evaluable for toxicity (WHO grade) 23: leukocytopenia 3° 7/23 (30%); infection 3° 1/23 (4%), 1 pt died of poststenotic pneumonia after the first cycle (treatment unrelated); thrombocytopenia 2° 1/23 (4%), 3° 2/23 (9%), anemia 3° 2/23 (9%), 4° 1/23 (4%); nausea/vomiting 2° 4/23 (17%), 3° 1/23 (4%); alopecia 3° was observed in only one pt (4%); bone pain 3° 1/23 (4%), ösophagitis 3° 1/23 (4%), no other toxicity occurred. Conclusion: In this dose and schedule, I-dox induced acceptable toxicities but is only marginally active in advanced NSCLC.

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ENLARGEMENT OF PERIPHERAL LYMPH NODES: CORRELATION OF ULTRASONIC AND HISTOLOGICAL FEATURES. A PROSPECTIVE STUDY

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In an ongoing prospective study 21 patients with enlarged peripheral lymph nodes have been examined by a 5 MHz-probe until now.
Characteristic criteria have been worked out for a differentiation between reactive lymph node enlargements, malignant lymphomas and an infiltration by a carcinoma. At the same time the lymph nodes have been examined by a computer-supported colour-coded Doppler-ultrasound to show the intranodal blood supply. The ultrasound examinations had been photo-documented in detail. A well visible lymph node had been marked and exstirpated consecutively. The fresh material has been examined cytologically, histologically and immunohistologically. In the scope of a comparative examination the documentated pattern of lymph nodes have been correlated with histological slices.
In the same period lymph nodes of 20 other patients of well known histology have been examined by the criteria mentioned above.
We conclude that an ultrasonic differential diagnosis between a reactive lymph node enlargement, a malignant lymphoma and an infiltration by a carcinoma seems to be possible.

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THE INFLUENCE OF CLINICAL IMPLICATIONS AND AGE OF PLATELET CONCENTRATES ON THE EFFICACY OF PLATELET TRANSFUSION

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We report data concerning 1016 platelet transfusions in 144 hematologic patients, receiving 1-37 HLA-A,B-matched single donor platelet concentrates (PC). Posttransfusion response was analysed calculating the corrected count increment (CCI). 33 (23%) of 144 patients had to be excluded from the study because of the presence of HLA-antibodies. The age of PC (range from 5 to 24 hours in conventional PVC-bags and from 5 hours to 5 days in special gas-permeable bags) showed no influence on CCI, nor after 1 hour neither after 24 hours. Reliable information about the presence of bleeding, splenomegaly, sepsis, disseminated intravascular coagulation or fever could be obtained in 751 of 1016 transfusions, 237 of these 751 transfusions were performed in absence of one or more of the above mentioned conditions. Patients with one or more of these problems showed a significantly reduced CCI after 24 hours compared to those without any factor impairing platelet increment. CCI after 1 hour was not influenced, as it is in patients with HLA-antibodies, e.g.. These results corroborate the necessity of a strictly monitoring of postransfusion response to PC, especially in patients who seem to be refractory to platelet transfusion.

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RETROPERITONEAL LYMPH NODE DISSECTION (RLND) AS STAGING AND THERAPEUTIC PROCEDURE - STILL UP TO DATE?

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Today, we distinguish three kinds of RLND: diagnostic in stage I, therapeutic primary RLND in stage II A/B and salvage-RLND for residual masses after chemotherapy. The staging of testicular tumors is false negative in 17 - 38%. Reliable risk factors to definitely define a stage I are not yet defined. Therefore, modified ejaculation-protective RLND with rapid tissue sections is the "therapy" of choice, even now. Fertility is sufficiently preserved and the progression rate of 17% will be outweighed by an easy follow up with longer intervals than under surveillance.
RLND alone for stage II A/B results in a progression rate of 49%. Currently we prove the value of RLND + adjuvant chemotherapy vs. primary chemotherapy + RLND in a prospective multicenter trial. Endpoints will be the impairments by therapy. Advantages are the pathological staging with the possibility of a tailored therapy and the reduction of chemotherapy from 3-4 to 2 courses, whereas the application of two invasive therapies is perhaps an overtreatment.
Residual masses after chemotherapy require a surgical intervention. Whereas some authors claim a salvage-RLND for all patients, others try to discriminate patients according to the histology of the primary and the size of residual tumors who don't need this operation. Our own results revealed fibrosis/necrosis in all seminomas < 5 cm and embryonal carcinomas < 3 cm. For primaries with teratoma components all patients with residual disease need RLND. Generally, the preoperative markers have to be negative; only in some special cases without progressive disease and a marker plateau RLND may be beneficial.

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ERYTHROPOIESIS AND AUTOLOGOUS BLOOD DONATION

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We studied autologous blood donation (AD) in 427 adults (42% male, 58% female) aged 19-84 years (55+/-17y) scheduled for elective orthopedic surgery. All patients received adequate oral iron substitution. The mean number of autologous units (U) collected per patient was 2.3 (range 1-4). Men showed significantly ($p<0.001$) higher hb-values prior to first donation (15.2+/-2.4 g/dl), prior to (13.1+/-1.2 g/dl) and post surgery (11.2+/-1.5 g/dl) than women (14.0+/-1.1/11.9+/-1.1/10.4+/-1.4g/dl). Decline of hb during the donation period was independent of sex and correlated with the number of U donated (2.3+/-1.0). Younger donors (<60y) performed slighthly better than older ones (>60y) in this regard. Analysing the influence of time on preoperative hb-regeneration characterized by the point of preoperative donation stop showed that erythropoiesis generally cannot compensate the blood loss caused by AD during the preoperative period. Therefore, the risk of additional homologous blood application especially depends on the predonation hb value apart from the degree of perioperative blood loss. Only 13% of male (n=20) but 25% of female (n=67) ($p<0.01$) patients needed additional homologous blood transfusion. Women are at a substantial disadvantage with regard to AD, therefore. During the postoperative period most patients' hb-values recovered slower than those of a control group receiving neither autologous nor homologous blood, indicating that the often heared postulate of an advantage in postoperative hb-regeneartion caused by preoperative AD is not true. Ferritine, analysed prior to first donation, correlates well with the number of U donated: 1: 88+/-62 ng/ml, 2: 104+/-82, 3: 153+/-153, 4: 157+/-118 and dropped substantially during the donation period despite of iron substitution. As the number of U donated seems to depend on the level of iron stores we will rather have to analyse the effect of long time predonation iron substitution than that of recombinant human erythropoietin.

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CLINICAL PHARMACOKINETICS (CP) OF IDARUBICIN (IDA) AND DAUNORUBICIN (DNR) IN PATIENTS (PTS) WITH ACUTE MYELOGENOUS LEUKEMIA (AML) AFTER SIMULTANEOUS ADMINISTRATION

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The new anthracycline (AC) analog IDA (4-demethoxy-daunorubicin) is used in the induction therapy of pts with AML in combination with cytosine arabinoside in a dose of 12 mg/m²/d, day 1-3. As the comparison of the CP of different ACs is limited by large interindividual variability, we have determined IDA and DNR and their metabolites in plasma during the first 24 h after simultaneous administration of IDA together with an equimolar tracer of DNR. Another advantage of this procedure is that the uptake of both ACs by the same population of myeloid blasts (MB) from peripheral blood and their nuclei can be measured simultaneously. Thus, information about the functional consequences of multidrug resistance (MDR) on the CP of both ACs may become available by correlation with the expression of P-glycoprotein. Up to now, CP has been determined in 9 pts. In plasma pharmacokinetic parameters, such as maximal concentrations c_{max}, half lives for efflux $t_{1/2}$ and areas under the curve AUC_{0-24h}, were about the same for IDA and DNR. In contrast, in MB as well as their nuclei, c_{max} were significantly higher, $t_{1/2}$ were significantly longer and, consequently, AUC_{0-24h} were significantly greater for IDA and idarubicinol (IDAol) than for DNR and the corresponding daunorubicinol (DNRol), respectively. These differences can be explained by a lower MDR-dependent efflux of IDA and IDAol or by their higher affinity to cellular macromolecules such as DNA. The determination of velocity constants for efflux in individual populations of MB in a multicompartment model by the "topfit"-computer program may further elucidate the clinical importance of MDR on the cellular pharmacokinetics of ACs in AML.

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REVERSAL OF PLATELET ALLOIMMUNIZATION BY CYCLOSPORIN A IN A CASE OF APLASTIC ANAEMIA

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Alloimmunization is the major problem in patients with long term substitution of platelets. We report a case of aplastic anaemia with an evident reversal of platelet alloimmunization by cyclosporin A (CSA).

Severe aplastic anaemia (SAA) was diagnosed in a 33 year old woman, who had no HLA-identical bone marrow donor. Treatment with androgens and corticosteroids failed and she was admitted to our hospital. Haemoglobin was 68 g/L, reticulocyte count 8 x 10^9/L, granulocyte count 0.5 x 10^9/L and platelet count 13 x 10^9/L. The patient was completely refractory to platelet transfusions. Alloimmunization due to 22 red blood cell and repeated platelet transfusions was documented by polyspecific antibodies reacting against 88% of the HLA-panel in the lymphocytotoxicity test.

A treatment of SAA was initiated with CSA without antithymocyte globulin (ATG) because of platelet refractoriness. Four months later, haematological values had not improved, but the anti-HLA antibodies had disappeared and the patient responded well to random donor platelet transfusions. Therapy with CSA was continued and in the following year additional treatment with ATG and ATG + IL-3 was performed. There was no substantial effect on SAA. Transfusions were necessary and efficient during the whole time. Three months later, the patient developed acute leukaemia and died of pneumonia.

This case demonstrates that the treatment with CSA reverses alloimmunization and refractoriness to platelet transfusions and also prevents alloimmunization during a long term support of platelets.

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TREATMENT OF DISSEMINATED HUMAN HODGKIN'S LYMPHOMA IN SCID MICE WITH RICIN A-CHAIN IMMUNOTOXINS

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Second generation immunotoxins constructed by linking monoclonal antibodies against CD25, CD30 or IRac via the bifunctional SMPT linker to deglycosylated ricin A-chain have been demonstrated to be highly effective against solid human Hodgkin's lymphoma in nude mice. We established a SCID mouse model of *disseminated* growing human Hodgkin's tumours to further evaluate the anti-tumour effects of these constructs. Injection of 1x10^7 L540 Hodgkin cells i.v. induced progressively growing lymphomas in several organs including liver and lymph nodes in most animals as demonstrated by immunohistochemistry. The effect of immunotoxin treatment was quantified by hybridization of extracted DNA to a human DNA probe via dot blotting. Ricin A-chain immunotoxins used for treatment were RFT5γ1.dgA (CD25) and IRac.dgA which recognizes an unclustered antigen on Hodgkin and Reed-Sternberg cells. A single injection of 8 ug of either immunotoxin administered i.p. one day after challenge with L540 cells abrogated tumour formation in most animals. Combination of the two immunotoxins ("cocktails") were superior to single immunotoxin treatment.

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Antibody Development Against p53 But Not Against H-ras in Small Cell Lung Cancer Patients May Reflect the Degree of a Protein's Involvement in Oncogenesis

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We have previously demonstrated anti-p53 antibodies in lung cancer patient sera and found that they occur only in patients whose tumors have missense mutations of p53. In this report we have examined 61 small cell lung cancer (SCLC) for development of anti-H-ras antibodies. Whereas almost all of the SCLC tumors have p53 mutations, none have H-ras mutations. Thus, we addressed two questions: (1) Do SCLC patients develop anti-H-ras antibodies, even if they lack H-ras mutations? (2) Is the frequency of such anti-H-ras antibodies in SCLC patients lower than anti-p53 antibody frequency?

We probed E.coli produced, purified H-ras protein run on immunoblots with SCLC patient sera. (1) We were not able to show anti-H-ras antibodies in SCLC sera nor in normal controls, including sera of autoimmune patients. The only SCLC serum exhibiting a low titer against *E.coli* produced H-ras protein, in further examination showed a band in the 21kDa region with E.coli lysate alone and failed to give a 21kDa band when tested against a *Baculovirus* produced H-ras. (2) Thus, there is no evidence for anti-H-ras antibodies in SCLC as none one out of 61 patients developed such antibodies. Contrary to this10.3% of the SCLC patients exhibit anti-p53 antibodies. -In conclusion, we demonstrate that in contrast to the observation of anti-p53 antibodies there is no evidence for anti-H-ras antibodies in SCLC cancer patients. This fact is consistent with lack of H-ras mutations in this disease. We hypothesize that antibody development against cancer related genes is not simply related to tumor necrosis but may indicate the degree of a protein's involvement in oncogenesis.

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EXPERIMENTAL TREATMENT OF A REFRACTORY ITP WITH ASCORBATE

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Idiopathic thrombocytopenic purpura (ITP) is an autoimmune disorder caused by autoantibody mediated destruction of platelets in the spleen. Different forms of treatment include administration of glucocorticoids and gammaglobulins followed by splenectomy if treatment fails. In case of refractory vinca alcaloids, immunsuppressive drugs or alternative experimental therapy can be tried. There are some reports in the literature that ascorbate may increase the platelet counts in patients with ITP (1,2).

Here we report one patient with refractory ITP and examine the effects of ascorbate on platelet release.

The 22-year old patient was affected March 91 showing petechial hemorrhages. The platelet count was 11 x 10^9/l and platelet autoantibodies were detected; additionally bone marrow cytology was compatible with acute ITP. No viral or bacterial infection could be investigated before ITP became manifest. The case history included an acute ITP 13 years ago treated successfully with prednisone 1mg/kg for 3 weeks.

This time all common treatments failed: prednisone, gammaglobulins, splenectomy, vincristin and azathioprin had been tried for 8 months. In addition high doses of methylprednisone (1g/d over 5 days) failed success. Before starting the more aggressive therapy with cyclophosphamid we started orally doses of 2g ascorbate given on an empty stomach. Basal platelet levels before ascorbate therapy were below 5 x 10^9/l, two weeks after beginning they elevated to 34 x 10^9/l. One month after individual therapy with ascorbate the platelet count was within the normal range. At day 54 the platelet count reached 1193 x 10^9/l, so we reduced the ascorbate doses to 1.5g/d. Presently, 168 days after the start of ascorbate therapy platelet count is 589 x 10^9/l. The present study demonstrates that ascorbate may increase platelet count in some patients with refractory ITP. The mechanism of action is still unknown. We conclude that treatment with ascorbate in patients with refractory ITP should be tried before starting aggressive therapy because having no side effects.

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DIFFERENT IMMUNOREACTIVITY IN THE OLD AGE

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Stimulation of lymphocytes with mitogens and antigens is established for in vitro testing of immunoreactivity. After defining a model for the overall in vitro immunoreactivity in healthy individuals, we examined blood samples of members of the Berliner Altersstudie(BASE) (n=206, females=110, males=96, x=84,5a, r=76-93a, SD=3,4a). We stimulated peripheral blood lymphocytes with 12 different mitogens and antigens (protein A, con A, PWM, PHA, measles, rubella, tetanus, diphtheria, tuberculin bc, streptolysin, mumps, vaccinia). Simutaneously blood count, flow cytometric measuring of lymphocyte subsets (CD4, CD8, cytotoxic T cells, NK cells), cell activation (IL2-receptor, MHC classII expression), HLA-A, -B, -Cw, -DR antigens and various red cell antigens were determined. Using multivariate statistical analysis (cluster analysis,SPSS) proved by t-test, F-test, discriminant analysis (SPSS) all individuals were classified in clusters of low, intermediate, and high responders, which differed significantly ($p<0.05-0.001$) in all stimulation systems tested. Using t-test and χ^2-test (SPSS) there were no clear correlations between blood count parameters, lymphocyte subsets, cell activation markers, age or sex and in vitro response, but a certain trend towards decreased overall immunoresponsiveness in elder people, and clear correlation between HLA and red cell antigens and the cluster membership. Like in the control group immuno-responsiveness in elder inviduals can be dissected in at least three functionally different clusters not influenced by lymphocyte subsets or in vivo cell activation, but by the patterns of both, red cell and HLA antigens.

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RADIOGENIC MYELOPATHY AFTER IRRADIATION OF A HEAD & NECK CANCER

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Radiation therapy is a common therapeutic approach in squamous cell carcinoma of head & neck (H&NSCC). Until 2 months after irradation in the acute phase main side effects are reversible mucocutaneous changes. Delayed complications with irreversible changes in connective tissues like osteoradionecrosis and perichondritis are rare. We report the case of a 67-years-old woman suffering from H&NSCC treated with radiation followed by myelopathy of the cervical spinal cord within three years.

CASE-REPORT: In 2/88 a H&NSCC was diagnosed in a cervical lymph node without any other tumor manifestation treated with extirpation followed by radiotherapy with a total tumor dose of 69 Gy. In 10/88 tracheostoma was performed due to radiogenic laryngical perichondritis. In 4/91 osteoradionecrosis of the left mandibula was diagnosed. Thereafter gradually neurological changes resembling syringomyelic dissociation of the cervical myelon developed with right sided hemiparesis, left sided hypalgesia and pallhypesthesia, and Lhermitte's sign. Diagnostic investigations in 6/91 revealed normal cerebrospinal liquor, normal serum levels of folate and cobalamin, no signs of metastatic H&NSCC. In computed tomography and three subsequent nuclear magnetic resonance imaging procedures within 6 months constant halo-like GDPA-enhancement of a intramedullary focus consistent with radionecrosis was present. Funicular myelosis, spinal cord infarction, and intramedullary tumor masses were ruled out. A radiogenic myelopathy of the cervical spinal cord was diagnosed. Treatment with dexamethasone (4 mg per os daily for 6 months) and physiotherapy resulted in improvement of physical activity. Although in this case radiotherapy was very successful in inducing a longstanding still lasting complete remission, the appearance of severe side effects negatively interferes with therapeutic attention.

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SCLERODERMA-LIKE LESIONS AFTER A SINGLE DOSE BLEOMYCIN

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Bleomycin (BLM) is a widely used well-known anti-tumor agent against lymphomas and solid tumors like squamous cell carcinomas (SCC). Main side effects are idiosyncratic (fever, chills), gatrointestinal, and vascular toxicity, and dependent on the cumulative dosage lung toxicity and mucocutaneous lesions. We report the case of a 42-years-old man with psoriasis vulgaris suffering from recurrent metastatic squamous cell carcinoma of the right lower extremity, who received anti-cancer chemotherapy (CT) and after a single dose of BLM (15mg i.v.) developed severe mucocutaneous toxicity.

Case report: In 10/89 spinalioma of digit.V tarsi was diagnosed and treated with amputation followed by radiotherapy. In 6/90 and 1/91 amputations of metatarsus and lower leg were performed because of relapsing disease. In 12/91 progressive disease was diagnosed with multiple subcutaneous tumors in the thigh and enlarged inguinal lymph nodes. Three courses of CT (cisplatinum 100mg/m² d1, 5FU 1000mg/m² d1-d5) combined with regional hyperthermia were administered without severe side effects or worsening psoriatic efflorescences. At day 8 of the third course for the first time a single dose of 15mg BLM was given. This caused within 4 days severe mucocutaneous changes: pruritus, Raynaud phenomenon, sausage fingers, colored bumps on fingertips, palms and plants, and skin rash. Skin biopsy showed a skleroderma like pattern without psoriatic lesions. No signs of renal or pulmonary toxicity were present. 100mg prednisolone per os daily resulted in a complete regression of skin lesions within 2 weeks. Although mucocutaneous toxicity after BLM is well-known to develop with increasing cumulative doses, to our knowledge this is the first report of appearance of severe scleroderma-like lesions after a single 15mg-dose of BLM.

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T-Zellerkennung von Nierenzellkarzinome:
Molekulare Charakterisierung antigener Strukturen

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Klinische Studien zeigten einen therapeutischen Effekt des immunmodulatorischen Lymphokins Interferon-γ auf das Nierenzellkarzinom. Diese und andere Hinweise auf ihre potentielle Immunogenität lassen Nierenzellkarzinome als besonders geeignet erscheinen für die Suche nach der Natur von Tumorantigenen, die von *in vitro* induzierten, gegen autologe Tumorzellen gerichteten Effektorlymphozyten erkannt werden.

Bei einer Reihe von Patienten wurden sowohl Nierenzellkarzinomlinien als auch Zellinien aus normalem Nierengewebe in Gewebekultur etabliert. Im nächsten Schritt wurden durch gemischte Lymphozyten-Tumorzellkulturen mit autologen Blutlymphozyten tumorreaktive zytotoxische T-Lymphozyten (CTL) generiert. Im Nierenkarzinommodell MZ-RC-1257 wurde dabei HLA-A2 als Restriktionselement für die Erkennung von Tumorzellantigenen durch autologe CTL identifiziert. Zwei von vier HLA-A2 exprimierende Nierenkarzinomlinien - darunter MZ-RC-1257 - wurden darüber hinaus von allogenen, in dem Melanommodell AV etablierten tumorreaktiven CTL erkannt. Über Transfektions- und nachfolgende Klonierungsschritte planen wir die Identifizierung der Gene, die für die auf der Nierenkarzinomlinie MZ-RC-1257 durch autologe und allogene CTL erkannten Antigene kodieren. Dann wird es möglich sein, zu genauen Aussagen über die Spezifität der Expression dieser Antigene in verschiedenen Geweben zu kommen und ihre Eignung als Zielstrukturen für immunmodulatorische Therapieansätze zu evaluieren.

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IMMUNOPHENOTYPIC IDENTIFICATION OF PERSISTENT LEUKEMIC CELLS IN THE BONE MARROW ASPIRATES OF AML PATIENTS IN COMPLETE REMISSION. B. Wörmann, M. Safford, S. Könemann, K. Zurlutter, K. Schreiber, K. Piechotka, M. Drescher, S. Toepker, Th. Büchner, W. Hiddemann and L. W. M. M. Terstappen

Myeloid leukemic blasts can be distinguished from normal myeloid progenitors based on the different expression of cell surface antigens. The dominant features are the presence of lymphoid - lineage associated antigens on myeloid cells, the asynchronous expression of myeloid - lineage associated antigens, and the overexpression or loss of myeloid - lineage associated antigens. These differences can be assessed using multiparameter flow cytometry with three fluorescent dyes. We have started a prospective study on bone marrow aspirates of patients with newly diagnosed AML to evaluate the potential of this method for the detection of residual leukemic cells in hematologic complete remission. 80 patients entered the study between 4/89 and 2/91, median age 58 years. Treatment was performed according to the protocols of the German Multicenter AML Cooperative Group. Clinical outcome was monitored for at least 15 months. 15 patients died early, 15 were nonresponders. 45 of the 50 CR patients were also analyzed by multiparameter flow cytometry at achievement of hematological complete remission. Residual cells with the leukemic phenotype were detected in 30 / 45 patients (67 %) at this timepoint. The median percentage of leukemic cells was 3 % (range 0.4 - 60 %). The projected rate for continuous CR was 59 % for patients without detectable leukemic cells compared to 21 % for patients with residual leukemic cells ($p < 0.02$). Bone marrow aspirates of 25 patients were again analyzed two to four months later in continuous CR. 13 patients had residual leukemic cells, all have relapsed, compared to 3 of 12 patients without residual leukemic cells. Our data suggest that AML patients with persistent cells carrying the leukemic phenotype in complete remission have a high risk of relapse.

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INTEGRATION OF EBV NEAR THE BREAKPOINT OF A CHROMOSOMAL TRANSLOCATION (11/19) IN A BURKITT'S LYMPHOMA CELL LINE ALTERS LATENT VIRAL GENE EXPRESSION

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Infection with the Epstein Barr virus (EBV) and deregulation of the cellular oncogene c-myc have been postulated as the essential factors in the pathogenesis of EBV-positive Burkitt's lymphoma (BL). We recently questioned the significance of c-myc deregulation for the malignant BL phenotype by demonstrating suppression of tumorigenicity despite continued c-myc deregulation after fusing a BL cell line with autologous EBV-immortalized lymphoblastoid cells (LCL). In order to further elucidate the mechanisms leading to tumor suppression in this hybrid model we analyzed physical state and latent gene expression pattern of EBV in the parental and hybrid cell lines. Non radioactive in situ hybridization of an EBV cosmid clone to metaphase chromosomes revealed that the parental BL cell line BL60 contains exclusively EBV integrated into the host cell chromosomes. Integration takes place near the breakpoint of a chromosomal translocation t (11/19) which is present in BL60 in addition to the BL-specific t(8/22) translocation. A large deletion which affects about 10 % of the EBV genome including the coding sequences for the EBV latent membrane protein (LMP) and the EBV terminal protein (TP) is found in the integrated BL60 EBV. In contrast, only episomal, non deleted EBV molecules can be detected in the autologous LCL IARC 277. The integrated BL60 EBV as well as the episomal IARC 277 EBV are present in the BL/LCL hybrids. Although commonly present within the same hybrid cell background, however, both viruses show a different latent gene expression pattern, i.e. expression of the LCL derived latent proteins EBNA 1, EBNA 2 and LMP versus non expression of the respective BL derived latent viral proteins. While non expression of the BL60 LMP is readily explained by deletion of the whole gene, non expression of the BL60 derived EBNA 1 and EBNA 2 genes might reflect differentiation dependent transcriptional downregulation due to the influence of cellular control elements located near the integration site. These results provide a first example that chromosomal integration of EBV can influence expression of transformation associated latent viral proteins.

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DETECTION OF PLATELET-REACTIVE ANTI-CARDIOLIPIN ANTIBODIES IN IDIOPATHIC THROMBOCYTOPENIC PURPURA

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Elevated levels of serum anti-cardiolipin antibodies (ACLA) have been demonstrated in patients (pts) with idiopathic thrombocytopenic purpura (ITP). However, little is known about the reactivity of these antibodies with cardiolipin (CL) in platelet membrane. Thus, we investigated sera of ITP pts for both elevated values of serum ACLA and the presence of platelet-reactive serum ACLA.
Serum ACLA (IgG, IgM) values were determined by an enzyme-linked immunosorbent assay (Loizou, 1985). Adsorption of serum ACLA to freeze-fractured platelets was used to test for platelet-reactive ACLA.
One of 27 pts with elevated serum IgG and 9 of 26 pts with increased serum IgM ACLA showed a marked adsorption of ACLA to platelet membrane exceeding that found in controls (serum ACLA negative pts). But adsorption was significant only in pts positive for IgM ACLA ($p < 0.001$).
We conclude that ACLA of IgM class bind to platelet membrane CL in some ITP pts and may contribute to enhanced destruction of platelets by the reticuloendothelial system.

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THE EFFECT OF DIFFERENT CELLULAR COMPOSITION OF BONE MARROW ON MAFOSFAMIDE RELATED CFU-GM TOXICITY

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It is well known, that the presence of erythrocytes in cell suspension during marrow purging with mafosfamide ameliorates the toxic effect of this agent. The aim of our study was to clarify if the presence of autologous granulocytes and lymphocytes, and non-autologous malignant cells from the patients with acute myeloid leukemia (AML) and chronic lymphocytic leukemia (CLL) in the bone marrow cell suspension during incubation with mafosfamide (100 ug/ml) had any effect on normal marrow CFU-GM survival. It was shown that the presence of granulocytes, AML-blast cells and CLL-lymphocytes in different concentration had no effect on the mafosfamide related reduction of the normal marrow CFU-GM survival. The effect of autologous lymphocytes was dependent on the functional state of these cells. Phytohaemagglutinin preincubated lymphocytes, in contrast to resting cells, increased CFU-GM derived colony formation. This effect was probably secondary to additional stimulation of CFU-GM by colony stimulating activity released by activated lymphocytes. Similar results were observed in cultures where PHA-leucocyte-conditioned medium was applied.

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RIFN-α2A IS MORE IMMUNOGENIC THAN RIFN-α2B IN PATIENTS WITH CML

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In a multicenter trial 106 newly diagnosed, evaluable patients with chronic myelogenic leukemia were treated with daily subcutaneous injections of either 5 Mill. I.U./m^2 rIFN-α2a or rIFN-α2b. Each center participating in this study chose one of the two rIFNα-subtypes for treating their patients. Inclusion criteria, IFN-dose and -schedule were identical for both rIFN-α2a and -2b treated CML-patients. Also, mean IFN-dose administered and mean duration of treatment in both patient populations were statistically not different. During rIFN-therapy patients were monitored for emergence of IFN-binding (EIA) and neutralizing antibodies in their sera (bioassay). For 93 patients both clinical data and antibody determinations are available for analysis. Of 33 patients receiving rIFN-α2b two (6%) developed low-titered and none high-titered IFN-antibodies. In contrast, of 50 rIFN-α2a treated patients four (6%) had low-titered and 10 (17%) high-titered IFN-antibodies detectable in their sera. Therefore, patients receiving rIFN-α2a developed significant more and higher titers of rIFN-antibodies than rIFN-α2b ($p < 0,035$). Subsequently, the association between IFN- antibodies and secondary resistance to rIFN-α was investigated. Since all patients with an IFN-antibody-titer above 900 IBU/ml relapsed despite a continuous IFN-therapy, a stringent association between high-titered rIFN-α-antibodies and secondary resistance was established. Within 12 months of IFN-therapy 18 patients developed a secondary resistance after initially responding to rIFN- α2 despite continuous therapy. 9 of these patients had high-titered rIFN-α2 antibodies (50 %) ; all patients received rIFN-α2a. Therefore, rIFN-α2a is more immunogenic and induces significantly more secondary resistances than rIFN-α2b in CML.

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MODIFICATION OF THE HUMAN CELL CLONOGENIC ASSAY IN METHYLCELLULOSE BY INCREASING THE AGAR CONCENTRATION FOR THE MIXTURE OF BONE MARROW CELLS WITH HUMAN SOLID TUMOR CELLS IN CULTURE

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The clonogenic methylcellulose assay according to Fauser and Messner (Blood, 54, 1197 (1979)) is usually carried out for cultures of normal human hematopoietic bone marrow cells. It has also proved to be quite successful in cultures of bone marrow cells mixed with leukemic and lymphoblastic cell lines. But there were some problems when the contamination of the normal bone marrow cells was performed with human solid tumor cell lines, since it was hardly possible to differentiate the growth of both cell types. There was a dispersed growth of the human solid tumor cells with patterns similar to the monolayer growth in culture flask so that no well defined colonies could be discriminated. If the human solid tumor cells, however, were cultured without bone marrow cells the human tumor clonogenic assay proved to be very successful in the capillary method which in contrast to the methylcellulose assay contains much more agar (1:5,5 = 0,18 %). Therefore, to improve the growth of both cell types, the cells of human bone marrow progenitors and the cells of human solid tumor cell lines, we modified the human clonogenic tumor assay based on methylcellulose by increasing the agar concentration according to the well tried corresponding capillary test. In this modified assay we were now able to observe the growth of both cell types. The colonies of the solid tumor cells could be easily differentiated from normal hematopoietic colony forming units such as CFU-GEMM, BFU-E, CFU-E and CFU-GM. This new agar modification of the assay allowed us to carry out several in vitro experiments in which we purged bone marrow cells previously contaminated with solid tumor cells by the selectively cytotoxic ether lipid ET-18-OCH_3 in cultures simulating remission bone marrows. This was conducted as support for possible clinical trials for autologous bone marrow transplantation in solid tumor patients. Supported by Wilhelm Sander-Stiftung 88.028.2.

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EFFECTS OF RECOMBINANT HUMAN STEM CELL FACTOR (rhSCF) ON CLONOGENIC PROLIFERATION OF FRESHLY EXPLANTED HUMAN TUMORS IN VITRO

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SCF is a multi-potent cytokine acting on a number of cells including hematopoietic progenitors, mast cells, melanocytes and germ cells. The purpose of our study was to determine wether rhSCF modulates growth of clonogenic cells from freshly explanted human tumors. 73 specimens were studied in a capillary soft agar cloning system. 9 (12%) specimens had to be excluded from further analyses (4 bacterial or fungal contamination, 3 confirmed benign on pathology review, 2 technical failure). 50 of the remaining 64 specimens (78%) showed evaluable growth in controls (11x gastric, 10x kidney, 6x colorectal, 5x melanoma, 4x lung, 14x other tumor types). Median colony formation in controls without rhSCF was 26.4 colonies/capillary (range: 3.7 - 611.8). Final concentrations of rhSCF were 0.1 - 10 ng/ml. A significant concentration-dependent stimulation of tumor forming units (colony formation ≥1.5 x control) was observed in only 2/50 evaluable specimens (4%) - one gastric cancer: 1.25 x control at 0.1 ng/ml, 1.91 x control at 1 ng/ml, 1.99 x control at 10 ng/ml; one melanoma: 1.37 x control at 0.1 ng/ml, 1.65 x control at 1 ng/ml, 1.77 x control at 10 ng/ml. Inhibition of tumor colony forming units (colony formation ≤ 0.5 x control) was not observed. Our data indicate, that rhSCF is not a major growth modulator of freshly obtained clonogenic human cancer cells in vitro. - Supported by the Wilhelm Sander Stiftung (grant 90.055.1).

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EFFECTS OF INTERLEUKIN-8 ON TUMOR COLONY FORMING UNITS FROM FRESHLY EXPLANTED HUMAN MALIGNANCIES

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Interleukin-8 (IL-8) is a 8 kD cytokine involved in the functional regulation of granulocytes. Because of its chemoattractant and degranulating activities it is thought to act as pro-inflammatory agent. We have studied the effects of recombinant human IL-8 (rhIL-8) on in vitro soft agar colony formation of 83 freshly explanted human tumors using a capillary cloning system. 9 (11%) specimens had to be excluded from further analyses (4 bacterial/fungal contamination, 3 confirmed benign on pathology review, 2 technical failures). 57 of the remaining 74 specimens (77%) showed evaluable growth in control capillaries without IL-8 (13 x kidney, 12 x gastric, 7 x colorectal, 6 x melanoma, 4 x lung, 4 x breast, 11 x other tumor types). Median colony formation in controls was 27.8 colonies/capillary (range: 3.7 - 611.8). Final concentrations of rhIL-8 were 1 - 100 ng/ml. A significant stimulation of tumor colony forming units (≥ 1.5 x control) was observed in only 1/57 evaluable specimens (2%) at 100 ng/ml (melanoma: 1.53 x control). Inhibition of tumor colony forming units (≤ 0.5 x control) was not observed. We conclude that rhIL-8 does not modulate in vitro growth of freshly explanted human tumor colony forming units.

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THE INFLUENCE OF HYDROXYETHYL STARCH IN BONE MARROW PROCESSING

T. Zeiler, V. Weisbach, J. Zingsem, G. Wittmann, R. Zimmermann, H. G. Heuft, R. Eckstein

Hydroxyethyl starch (HES), a synthetic analog of naturally occuring starch is used in leukapheresis as a sedimentation agent. HES induces rouleaux formation of red cells and hence produces a sharply defined interface between red cells and buffy coat, resulting in increased leukocyte yields and less erythrocyte contamination of the separation product e.g. granulocyte concentrates. HES has been recommended for the processing of bone marrow (BM) in ABO incompatible transplantations. Here we present a study on the influence of HES in BM processing with cell separators (cobe spectra, Fresenius AS 104). In a series of 116 routine BM separations prior to cryoconservation 9 separations were performed using HES as sedimentation agent in a concentration of 1:10 v:v. We monitored the influence of HES on the recoveries of white blood cells (WBC), mononuclear cells (MNC), colony forming units (CFU-GM) and red blood cells (RBC). The mean values are given below.

	WBC	MNC	CFU-GM	RBC
with HES (n=9)	102,7%	56,1%	74,2%	6,8%
SD	±183,3	±21,0	±24,8	±5,1
without HES (n=107)	43,5%	60,9%	76,2%	4,9%
SD	±22,5	±32,0	±69,7	±4,2

Cell counts were performed on a sysmex counter and microscopically, differentation of MNC by microscope and CFU in a semiliquid medium. There was a significant difference in recovery of leukocytes (p=0,002) but no significances in MNC, CFU or RBC recoveries.
We conclude from this data that the use of HES in automated BMT processing merelly increases the recovery of granulocytes and does not improve the results of separation, therefore.

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QUALITY OF SINGLE DONOR PLATELET CYTAPHERESIS CONCENTRATES

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Here we report a quality control on a series of 3201 single donor cytapheresis platelet concentrates (SDPC) with regard to the requirements for the preparation of SDPC given in the "Richtlinien zur Blutgruppenbestimmung und Bluttransfusion" (published in 1992). According to these guidelines SDPC should contain more than $2*10^{11}$ platelets total in a plasma volume less than 300 ml and the contaminating leucocytes should not exceede $5*10^{8}$ in total. All concentrates in this study were produced running the standard separation protocols as recommended by the manufacturer. The separators and separation protocols we used were:

A = AS 104 (Fresenius) dual needle program (n = 901)
B = AS 104 (Fresenius) single needle program (n = 59)
C = Cobe Spectra (Cobe) dual needle program (n = 150)
D = V 50 (Haemonetics) time saver platelet program (n = 1937)
E = PCS plus (Haemonetics) platelet program (n = 154)
There are signifiant differences in the quality of the concentrates in respect to the cell separator and the separation protocoll. The percentage of concentrates not conforming the quoted quality requirements are given below:

	A	B	C	D	E
Platelet count:	(11,4%)	(5,1%)	(3,4%)	(8,1%)	(15,7%)
Leukocyte count:	(2%)	(0%)	(2%)	(19%)	(7,3%)
Volume:	(5,7%)	(89,8%)	(8%)	(26,3%)	(9,8%)

Since a remarkable part of all the concentrates does not conform to the quality requirements there is still need for further improvement of cytapheresis procedures.

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MOBILISATION OF PERIPHERAL BLOOD STEM CELLS WITH G-CSF ALONE WITHOUT PRECEDING CHEMOTHERAPY

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Peripheral blood stem cell transplantation (PBSC) offers an alternative to autologous bone marrow transplants, especially in malignant diseases with bone marrow contamination. It has been proven in several animal models and in humans that pluripotent stem cells are circulating in a number sufficient to enable sustained trilineage engraftment after transplantation. Stem cell mobilization can be promoted by the administration of various cytokines or chemotherapeutic substances like cyclophosphamide or both. We explored the administration of G-CSF for peripheral stem cell collection in patients with Hodgkin's disease, Non Hodgkin lymphoma and inflammatory breast cancer, who had fully recovered after chemotherapy. G-CSF was administered subcutaneously at a daily dose of 10 ug/kg BW. Leukapheresis was performed on three consecutive days starting on day 5 of G-CSF administration and $3\text{-}6x10^{10}$ mononuclear cells (MNC) were collected. At present five patients have been transplanted; recovery of absolute neutrophile count (>500) could be observed on day 12 (mean, range 10-12), recovery of platelets (>20.000) on day 15 (mean, range 13-21). The number of CD 34 positive cells in peripheral blood and leukapheresis harvest (HL) was determined by FACS-analysis. Peak values of CD 34+ cells in peripheral blood (max. 10% of MNC) and in HL (max. 14% of MNC) were found between day 4 and day 6 after the start of G-CSF treatment. The colony formation was monitored in peripheral blood and HL. Peak values were reached between day 4 and day 6 of G-CSF treatment. Mobilisation of PBSC with G-CSF is effective, logistically simple and feasible and offers an alternative to autologous bone marrow transplantation.

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MOBILIZATION OF PERIPHERAL BLOOD DERIVED HEMATOPOIETIC PROGENITOR CELLS BY LEUKAPHERESIS.

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High dose chemoradiotherapy followed by the reinfusion of previously collected and cryopreserved autologous peripheral blood derived hematopoietic stem cells (PBSC) has been shown to be successful for the treatment of different malignancies. To harvest PBSC, blood cell separators with leucocyte collection procedures are being used routinely. In previous studies we found the best collection results in PBSC-separations with process volumes higher than 10 L. To investigate a potential stem cell mobilization by the separation itself, WBC, MNC, CFU-GM and CD34 positive cells were analyzed in the patients peripheral blood before, during and after the separation as well as in the corresponding fractions of the PBSC-concentrates.

After an initial decrease of all cells, MNC, CFU-GM and CD34 positive cells increased after 5 - 6 L and, at the end of the separation, attained higher concentrations than before the procedure in some cases.

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Ara-C Metabolism after Priming with rhGM-CSF Evaluated Using an Algorithm for the Predicition of Ara-C Incorporation into DNA

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A model has been developed to predict the incorporation of cytosine arabinoside (ara-C) into DNA given the extracellular drug concentration and the cells' outfit with relevant enzymes. From published as well as our own data, the following steps can be described quantitatively: uptake of ara-C into the cell by facilitated diffusion, phosphorylation, deamination, ara-CTP degradation and ara-CMP incorporation into DNA. Repair mechanisms and dCTP interactions can also be calculated. The algorithm is programmed in a commonly used spreadsh-et application and solves intracellular ara-C levels as well as ara-CTP levels for steady state conditions.

In it's current form, the algorithm can accurately predict dose responses for ara-C-DNA from extracellular ara-C concentrations as compared to experimental measurements of ara-C-DNA formation in the majority of cases (n=31). A quantitative relation of the currently available values towards ara-C mediated cytotoxicity is also under investigation.

The model is used to describe quantitative changes in individual steps of ara-C metabolism after priming of cells with rhGM-CSF. Conventional analysis of single parameters did not reveal any uniform changes in enzyme activity, such as deoxycytidine kinase, thymidine kinase, DNA polymerase, or other parameters such as ara-CTP half life following priming with rhGM-CSF. The majority, but not all, patients had an increase in DNA polymerase α activity and of ara-C cytotoxicity. The new approach allows to identify a variety of individual response patterns: dCK, ara-CTP half life and DNA polymerase can all be altered into different directions, yielding different net effects on ara-C-DNA formation.

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Pentosan polysulfate inactivates heparin binding growth factors and inhibits tumor growth

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We have shown, that the heparinoid pentosan polysulfate inhibits tumor growth and angiogenesis, which are induced by Kaposi's FGF, a member of the heparin binding growth factor family. In the present study we set out to determine the secretion of heparin binding growth factors by different human carcinoma and sarcoma cell lines. All tumor cell lines studied secreted heparin binding growth factors, which stimulated the growth of endothelial cells and fibroblasts in vitro. The non tumorigenic cell lines investigated did not actively release heparin binding growth factors. Pentosan polysufate inactivated heparin binding growth factors in vitro and inhibited the growth of the tumor cell lines in thymusaplastic mice. Our results indicate, that heparin binding growth factors, which stimulate angiogenesis are secreted by tumor cells, but not by normal cells. Inactivation of heparin binding growth factors by polysulfated sugars might provide a more specific tumor therapy, which would avoid the side effects of conventional chemotherapy.

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IMMUNOPHENOTYPIC IDENTIFICATION OF PERSISTENT LEUKEMIC CELLS IN THE BONE MARROW ASPIRATES OF AML PATIENTS IN COMPLETE REMISSION.

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Myeloid leukemic blasts can be distinguished from normal myeloid progenitors based on the different expression of cell surface antigens. The dominant features are the presence of lymphoid - lineage associated antigens on myeloid cells, the asynchronous expression of myeloid - lineage associated antigens, and the overexpression or loss of myeloid - lineage associated antigens. These differences can be assessed using multiparameter flow cytometry with three fluorescent dyes. We have started a prospective study on bone marrow aspirates of patients with newly diagnosed AML to evaluate the potential of this method for the detection of residual leukemic cells in hematologic complete remission. 80 patients entered the study between 4/89 and 2/91, median age 58 years. Treatment was performed according to the protocols of the German Multicenter AML Cooperative Group. Clinical outcome was monitored for at least 15 months. 15 patients died early, 15 were nonresponders. 45 of the 50 CR patients were also analyzed by multiparameter flow cytometry at achievement of hematological complete remission. Residual cells with the leukemic phenotype were detected in 30 / 45 patients (67 %) at this timepoint. The median percentage of leukemic cells was 3 % (range 0.4 - 60 %). The projected rate for continuous CR was 59 % for patients without detectable leukemic cells compared to 21 % for patients with residual leukemic cells ($p < 0.02$). Bone marrow aspirates of 25 patients were again analyzed two to four months later in continuous CR. 13 patients had residual leukemic cells, all have relapsed, compared to 3 of 12 patients without residual leukemic cells. Our data suggest that AML patients with persistent cells carrying the leukemic phenotype in complete remission have a high risk of relapse.

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ALL TRANS RETINOIC ACID : A TARGETTING DRUG FOR THE DIFFERENTIATION OF ACUTE PROMYELOCYTIC LEUKEMIA.

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All-trans retinoic acid (ATRA) is able to specifically differentiate acute promyelocytic leukemic cells (APL) in short term culture (Chomienne et al, Blood 1990). Patients with APL achieved complete remission within 1 to 3 months by a progressive maturation of leukemic cells (Huang et al, Blood 1988, Degos et al, Lancet 1990). The advantages of this differentiation therapy is the rapid disappearance of the bleeding disorder and the absence of aplastic phase avoiding the early deaths occuring in 15 to 30 % of patients with conventional chemotherapy. However relapses occur when ATRA alone is maintained. For this reason, a chemotherapy is added after complete remission obtained by ATRA. A pilot study on 27 patients was proposed with the sequential combination of ATRA and chemotherapy leading to 70 % of actuarial event free survival (83 % actuarial disease free survival) at 18 months. An European trial randomizes conventional therapy to the sequential ATRA-chemotherapy protocol (70 patients included).

Retinoic acid receptor (RARα) is rearranged by the specific translocation t(15;17) of APL (de Thé et al, Nature 1990) ; a PCR technique was developped (Castaigne et al, Blood in press) in order to ensure the diagnosis and to follow the minimal residual disease. Transfection experiments of the chimaeric gene in granulocytic cells (HL60) specifically inhibits the in vitro differentiation induced by retinoic acid (Farzhaneh & al Nature in revision).The arrest of maturation of granulocytic lineage could be one of the major step of the leukemogenesis. ATRA is able to revert the arrest of maturation may be through a modulation of the expression (increased) of the normal allele of RAR (Chomienne et al, J. Clin. Oncol. 1991), which could overpass the impairment induced by the chimaeric protein on target responsive elements. One of the steps of the repair is the modulation of programmed cell death (PCD). Bcl-2, a gene involved in the PCD, is modulated in in vitro studies, arguing for the engagment of the cell in the natural death (Chomienne et al, in preparation). The beneficial effect of "differentiation therapy" probably is due to the induction of the natural death of the malignant cell.

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Presence of macrophages (Mφ) and transforming growth factor beta (TGF-ß) in the hematopoietic microenvironment appear to influence cell cycle kinetics in acute myeloid leukemia (AML).

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Total cell cycle time (Tc) of leukemic myeloblasts was measured following a one hour bromodeoxyuridine (BrdU) infusion administered to 23 APL and 56 AML patients. Tc was significantly slower in APLs (93.6h vs 56.0h, p=0.002). To investigate the biological basis for this peculiarity, the HM was examined for the presence of TGF-ß, a known inhibitor of hematopoietic progenitors. By using a monoclonal anti-TGF-$ß_2/ß_3$ antibody in plastic embedded bone marrow biopsies, a markedly higher concentration of TGF-ß was found in 23 APLs compared to 30 AMLs (p=0.0002). Double-labeling of biopsies for TGF-ß + BrdU revealed that S-phase cells in APL occurred in "Geographically Restricted Islands of Proliferation" (GRIPs). Since TGF-ß is chemotactic for Mφ, a monoclonal antibody (EBM-11) was used to investigate the role of these stromal cells in hematopoiesis. Once again, a dramatic infiltration of APL biopsies by three distinct types of Mφ was observed. Proliferating GRIPs were especially found in intimate contact with Mφ. We conclude that cytokines/stromal cells in the hematopoietic microenvironment directly affect the proliferative characteristics of leukemia cells in vivo.

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