Blut (1985) 51: 145–239

Blut

Annual Congress of the Austrian and German Societies of Hematology and Oncology

Vienna, October 6–9, 1985

Abstracts

Abstracts of the papers accepted for oral and poster presentation

1 Prognostic Factors in Non Hodgkin Lymphomas

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The prognostic value of clinical and biochemical parameters and of histology was investigated in a study of 336 patients with NHL. The analyses were based on a regression model according to COX. Regarding overall survival, grade of malignancy as histologically defined by the Kiel-classification, serum-LDH-activity, and performance status were found to be factors of equal significance. Stage was the best predictive factor for reaching a complete remission. In patients with complete remission, only histology differentiated groups with a continuous relapse rate from those with relapses occurring only in an interval of about three years from start of therapy. In high grade malignant lymphomas, disease-free survival was influenced by serum-LDH-activity and hemoglobin at the time of start of treatment.

2 The Increasing Significance of Sonography in the Recognition of the Manifestation of Malignant Lymphomas in the Organs

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From 1977 to 1985 a total of 373 patients with malignant lymphomas were kept under sonographic observation. Among them were 89 patients with Hodgkin's disease, 215 patients with non-Hodgkin-lymphomas (NHL) of low grade malignancy and 69 patients with NHL of high grade malignancy. Sonography was used regularly in the initial examination as well as during the treatment. As it was to be expected, liver was found to be affected most frequently, in 9,8% of NHL of low grade malignancy, in 34,8% of NHL of high grade malignancy and in 16,8% of the Hodgkin's disease. On the other hand the intestinal tract was found to be affected considerably more frequently in the NHL i. e. 8,8% in those with low grade malignancy and 18,8% in NHL of high grade malignancy. Only 3,4% of patients with Hodgkin's disease had gastrointestinal involvement. A nodular affection of the spleen was found in 2,8% of low grade malignant NHL and in 4,3% of high grade malignant NHL. But 14,6% of patients with Hodgkin's disease had positive findings. Localized lesions of the spleen and the liver can be recognized sonographically upto a size of 1 cm. Sonography is thereby superior to every other method of obtaining pictures giving evidence of the organ infiltration.

3 Cellular Immunity in Malignant Non Hodgkin Lymphomas: In Situ Quantification of Reactive Immunocomponent Cells in Relation to Tumor Growth

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The number and distribution of reactive non-neoplastic cells (T-cell subsets, NK-cells, macrophages) in more than 100 malignant Non Hodgkin lymphomas (NHL) were evaluated *in situ* on cryostat sections. Results were quantified using stereological methods and compared to histological and clinical findings. In NHL tissues a significant correlation was found between a high number of T helper/inducer cells and (1) prognostically favourable histological subtypes ($p < 0.05$) as well as (2) favourable clinical course ($p < 0.04$) completely independent from histological criteria. The number of NK-cells (Leu-7+) was markedly elevated in tissues of patients with generalized compared to localized disease ($p < 0.03$), in low grade malignant lymphomas ($p < 0.03$) and in treated patients ($p < 0.02$). Macrophage infiltration, however, showed a completely different pattern: These cells were particularly frequent in tissues of patients with prognostically unfavourable, high grade maligant – especially immunoblastic – NHL ($p < 0.0001$) and in generalized disease (stage III + IV; $p < 0.05$).

4 Immunocytological Subtyping of Low Malignant B-Cell Non-Hodgkin's Lymphomas (B-NHL) Using B-Specific/-Associated Monoclonal Antibodies (MoAb)

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Using the Avidin-Biotin method, we examined suspensions of mononuclear cells from peripheral blood, lymph node aspirates and spleen from patients with B-CLL ($n=16$), prolymphocytic leukemia (PLL; $n=3$), hairy cell leukemia (HCL; $n=7$) and centroblastic-centrocytic lymphoma (cb-cc; $n=6$) with a panel of 52 B cell MoAb (Boston workshop, 1984) and CD 5 (Leu-1). The following selected MoAb appeared to be useful for subtyping of low malignant B-NHL: CD 19 (B4, HD37, 4G7), CD 20 (B1, 2H7, 1FS), CD 22 (HD6, HD39, SHCL1), CD 5 (Leu-1) and B 7 (μ-chain of IgM). CD 19 and CD 20 (pan-B-specific) indiscriminately reacted with most of the B-NHL. CD 22 reacted more specifically: all HCL cells were HD6/SHCL1-positive and most of them HD39-positive as well, whereas HD39/SHCL1-expression was found on less than half of PLL/cb-cc cells and only few B-CLL cells. CD 5 was expressed on B-CLL cells only, except for one case of cb-cc. B 7 allowed further discrimination by recognizing all PLL cells, however, it reacted with one third of cb-cc cells and with rare cases of B-CLL. This MoAb-panel suggests patterns of immunophenotypic differentiation of B-NHL, but it has to be substituted by more specific MoAb.

5 Detailed Analysis of Prognostic Factors in 146 Cases of Immunocytoma between 1976 and 1982

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Histories of all 146 patients with immunocytoma (lymphoplasmacytoid 71, lymphoplasmacytic 32, polymorphic 34, not classified 9) were evaluated by multivariate analysis (deadline 12/83). Median age was 64 (26–85) years. 91 cases with marrow infiltration were classified according to RAI (stage 0 15, I 16, II 25, III 18, IV 17) in 55 cases the Ann-Arbor classification was applied (stage I 16, II 18, III 12, IV 9). Treatment included chlorambucil/prednisone, different kinds of combination chemotherapy and/or extended or involved field irradiation. The survival probability for all stages was 48% after 8 years. The polymorphic variant declined to 0% after 6 years whereas the other histopathologic entities were plateauing at 60%. The median survival times for the non-polymorphic subtypes were for stages RAI 0II, IA: not reached, IIA/B, IIIA: 62 months and RAI III/IV, IVA/B: 32 months. Older patients (70 y.) had a worse prognosis (med. surv. 40 mo.; younger pat.: not reached).

6 Cytostatic Drug in Combination with Fractionated Whole Body Irradiation in Patients with Non-Hodgkin's Lymphomas of Low Grade Malignancy Stage III and IV

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While the spontaneous course and the response to therapy is usually quite satisfactory in Non-Hodgkin's lymphomas (NHL) of low grade malignancy stage III and IV, cure is extremely rare. We have attempted to improve the results by combining chemotherapy and irradiation.

19 patients received cytostatic drug treatments (LOP, COP, BACOP) with complete remission (CR) followed by fractionated whole body irradiation (upper and lower body irradiation with 3 Gy each, using a Siemens – MeV Mevatron 12 linear accelerator). Complete clinical remissions have been obtained in 84% of patients with six (31%) remissions still lasting (24 months+ to 44 months+). Until now, 6 patients have died, none of them had reached lasting remission and all of them have developed highly malignant leukemic forms of the disease resistant to treatment. We conclude that combined modality treatment is effective in patients with NHL of favorable histology (cb/cc) and stage III.

7 Centrocytic Lymphoma: Clinical Features and Therapeutic Aspects of a Non-Hodgkin Lymphoma of Intermediate-Grade Prognosis

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The majority of 87 patients with centrocytic lymphoma observed by the Kiel Lymphoma Study Group in a prospective observation study showed initial advanced disease (stage III in 8%, stage IV in 81%, bone marrow involvement in 64% of patients). Forty-one per cent of patients demonstrated initial constitutional symptoms and 47% rapid lymph node growth. In patients with the rare stage I/I_E complete remissions could be induced by radiotherapy alone. Survival probability of patients achieving complete (24%) or partial (45%) remission after chemotherapy of moderate intensity was superior to that of patients unresponsive to treatment. Median survival probability of patients with advanced centrocytic lymphoma was lower than that of corresponding patients with other lymphomas of low-grade malignancy. Available data suggest that centrocytic lymphoma is the only lymphoma of the Kiel classification with intermediate-grade prognosis. Preliminary results of a multicenter randomized therapeutic trial, still in progress (78 patients recruited until early 1985), do not show a significant superiority of the anthracycline-containing CHOP regimen over the less intense COP regimen with respect to rate and duration of remission and survival probability.

8 CHOP Versus COPBLAM for Treating Non-Hodgkin-Lymphomas of High Grade Malignancy: a Retrospective Study

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Between 1980 and 1984 44 patients with non-Hodgkin lymphomas of high grade malignancy were treated according to one of two regimen as a primary chemotherapy. 25 patients received CHOP (cyclophosphamide, adriamycine, vincristine and prednisone) whereas 19 patients were treated by COPBLAM (cyclophosphamide, vincristine, procarbazin, bleomycin, adriamycin, prednisone). In respect to age ($x = 45$ vs. 47 yrs.), histologic subtype, stage, B-symptoms and bone marrow involvement no statistically significant differences existed between the two patient groups. Response was significantly better in the COPBLAM-group ($p = 0.002$) with 15 CR and 3 PR as compared to the CHOP-treated patients (6 CR, 13 PR). A higher proportion of patients with CNS-involvement in the CHOP-group, however, has to be taken into consideration, making a larger prospective study mandatory.

9 Monitoring and Treatment of a Human B-Cell Lymphoma with Monoclonal Anti-Idiotype Antibodies

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In human B-cell leukemias and malignant lymphomas the surface immunoglobulin usually is monoclonal which means the identical variable regions (V_H and V_L), are expressed on each tumor cell. The unique idiotype of each individual lymphoma represents an exquisite tumor-specific antigen. Four different anti-idiotypic antibodies have been produced against the tumor cells from a patient suffering from an immunocytoma. Monoclonal antibodies were employed for the detection of tumor cells in peripheral blood, lymphatic organs, and in bone marrow thus allowing a more precise staging and monitoring. Furthermore, the amount of free idiotype-bearing immunoglobulin shed into the patient's serum could be determined quantitatively by a sensitive RIA. When rapid progression of the disease was diagnosed we decided to conduct a clinical trial using anti-idiotype therapy. Considering the high levels of idiotype-immunoglobulin present in the patient's serum (approx. 1 mg/ml) an alternative approach to intravenous application of monoclonal antibody was designed. Large amounts of the patient's peripheral blood mononuclear cells were incubated *in vitro* with purified monoclonal anti-idiotypic antibody and then reinfused. This antibody therapy resulted in a partial remission of the immunocytoma lasting for at least 11 months.

10 Preliminary Results of a New Drug Combination (VIM) in the Treatment of Non-Hodgkin Lymphomas (NHL)

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Twenty patients with advanced NHL (LB:2, IB:5, CB:2, anaplastic CC:3, CB/CC:6, polymorphic IC:1) were treated with a 3 days schedule consisting of Etoposid-Vepesid® 100 mg/d, Ifosphamid 1 g/d and Mitoxantrone 3 mg/m²/d repeated every three weeks. Five patients received Bleomycin additionally between the cycles. All patients were heavily pretreated (CHOP, C-MOPP and various salvage regimens). 16/20 patients had had irradiation therapy too. Age varied between 40–85 years. 11/20 patients are yet evaluable. Despite the negative selection 1 CR, 2 PR and 4 minor responses (< 50%) of short duration were achieved. Two patients had static disease, which lasted 6^+ months in one of them. Subjective tolerance was excellent but in these heavily pretreated patients 3 life threating septic episodes were observed during agranulocytotic phase. In addition one patient died because of massive gastrointestinal bleeding during marrow depression. Though observation time is too short it seems remarkable that we were able to achieve remissions in patients pretreated with anthracycline containing regimens.

11 Chemotherapy of Relapsing or Refractory Malignant Lymphomas with CCNU, VP 16, Vindesine, High Dose Methotrexate and Dexamethasone

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We treated patients with relapsing or refractory NHL with the following regimen: CCNU 80 mg/m² orally d 1, VP16 80 mg/m² i. v. d 1–3, 22–24, Vindesine 3 mg/m² i. v. d 1 + 22, Methotrexate 1,5 gr/m² i. v. d 1 + 22 followed by folinic acid rescue after 24 h 4 × 15 mg/m² for 3 days and Dexamethasone 4,5 mg orally d 1–14, 3 mg d 15–28, 1,5 mg d 29–42. Repeatment of course at day 43. 17 Patients are treated up to now, 9 male, 8 female. Mean age was 49 yrs (21–72 yrs). Histologies were: 1 NHL centroblastic/centrocytic, 2 immunocytic, 5 immunoblastic, 6 centroblastic, 1 unclassified high grade malignant NHL, 1 ALL, 1 malignant histiocytosis. Treatment results were 3 CR (3-6+mo), 1 PR (1 mo), 2 MR, 5 NC and 6 PD. There was mild hepatotoxicity, hematotoxicity was observed in some patients with bone marrov infiltration.

12 High Quality Chromosome Preparations Obtained from Hemopoietic Colonies Early after Bone Marrow Transplantation

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Several transplant centers recently have described persistence or recurrence of host hemopoiesis after bone marrow transplantation (BMT). These observations prompted us to initiate a more detailed analysis of the development of hemopoietic chimerism after BMT. A method has been developed to obtain high quality chromosome preparations from erythroid (BFU-E), myeloid (CFU-C), and lymphatic (T-cell) colonies thus allowing safe identification of recipient- or donor-origin of cells from the different progenitor cell compartments: After removal of single colonies from the culture matrix cells are suspended in 0.0075 M KCL previously dropped onto glass slides. Removal of excess hypotonic solution under definite experimental conditions is critical. After fixation chromosomes are ready for further investigation including a variety of banding procedures. First results show that procedural cell loss is minimal while the rate of suitably prepared metaphases is high. Karyotyping becomes feasible in 30–60% of all colonies removed from the culture dish including the small colonies obtained very early (days +14, +28) after BMT.

13 A New Method for Simultaneous Demonstration of Chromosomes and Membrane Markers

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The interpretation of chromosomal findings in malignant hematological diseases has so far been hampered by the fact that in general it was not possible to identify the cells from which mitoses were derived. A new procedure which allows simultaneous analysis of karyotype and cell cytology has been published recently by Teerenhovi et al (Blood 64/5). We have succeeded in improving this method which in addition, also enables the simultaneous display of membrane markers on mitotic cells. The variety of staining combinations (triple-fluorescence, cytochemical staining of cells with banded chromosomes) are presented, and examples for the practical application of this new technique, e. g. delineation of normal cell populations and the assignment of karyotypes to heterogenous cell population in CML blast crises and double leukemias, are also demonstrated.

14 Ph' Positive T Cell Subclones Derived From Multilineage Colonie (CFU-GEMMT) of a Patient with Chronic Myelogenous Leukemia

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Bone marrow cells from a patient with Ph' positive chronic myelogenous leukemia (CML) in chronic phase were cultured for multilineage hematopoietic colonies (CFU-GEMMT), erythroid bursts (BFU-E), and granulocytic colonies (CFU-C). T-lymphocytes and B cells within primary mixed colonies of this patient were identified by their reaction with the monoclonal antibody Leu-5 and Bl, respectively. Examined primary mixed colonies contained the Ph' chromosome. Recloned secondary colonies consist of T-cells identified by their reaction with Leu 5 or OKT3. Cytogenetic analysis of secondary T cell colonies, as confirmed by Leu 5 or OKT3 positive cells, were Ph' positive. This finding suggests that T-cells can be generated from the pluripotent stem cell clone in this patient. The observation of B-cell associated antigen positive cells (Bl) in multilineage colonies indicates that the B-cell lineage might be also involved in the neoplastic clone.

15 Expression of c-myc in PHA Stimulated Peripheral Blood T Lymphocytes Producing Pluripoietin (S)

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Phytohemagglutinin (PHA) renders human peripheral T-lymphocytes (Leu 5b positive cells) competent to replicate their DNA and divide. The stimulation of peripheral T cells by PHA, which appears to be a transcription dependent event, leads to the production and release of lymphokines supporting proliferation and differentiation of human pluripotent stem cells (CFU-GEMMT). After the addition of PHA to the culture medium an abundance level of c-myc mRNA can be observed. The c-myc gene product might be an intracellular mediator of the growth response and lymphokine production to PHA. While the function of the c-myc gene product is not clear yet, it seems likely that it is involved in the control of cell proliferation. Our observation for induction of c-myc by PHA on peripheral blood T-lymphocytes suggests that the mitogenic action of PHA induces a gene or a gene family including c-myc that might be responsible for the elaboration of lymphokines supporting colony formation of human pluripotent stem cells.

16 Detection of Commitment ot B-Cell Differentiation in Acute Leukemias by Analysis of mRNA and Gene Rearrangement Using Fluorochrom Labeled μDNA

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Cloned gene probes, now availabel for defined gene segments allow insight into the processes of gene activation and transcription as prerequisite for gene expression. Fluorochrome labeling of such a μ-gene probe allows rapid and quantitative tracing of gene rearrangement by Southern blotting and of the transcription product, the immunglobulin mRNA by in situ hybridization: This has been performed in acute leukemias in order to detect the earliest stages of B-cell differentiation.

Microfluorometric analysis of over 50 samples of acute leukemias after in situ hybridization with μ DNA-FITC revealed that the blast cells of the majority of cALL cases and also of a few null ALL cases exhibited varying amounts of IgM-mRNA. A correlation to B-cell markers as defined by the monoclonal antibodies HD 6, HD 28, HD 37, HD 39, B1, BA-1 and cytoplasmic and surface IgM became evident in combined analysis, where blast cells with high IgM-mRNA content expressed most antigens. Surprising by IgM genes were not rearranged in a few cases where IgM-mRNA was detected.

17 Value and Limitations of Predictive Test Systems in Clinic and Research

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The observation that malignancies considered to be identical by conventional criteria as cytology and/or histology, behave differently with regard to prognosis and/or therapy, resulted in the development of test systems for optimization of therapies in hematology and oncology. The single test systems comprise certain tumor biological properties and malignant processes in different ways. The most common are test systems comprising clonogenic growth, nucleotide metabolism, energy metabolism by bioluminiscence, and the nude-mouse model. Primary goal of these developments was the individualization of the cytoxic chemotherapy. Beyond that, the test systems proved partly to be valuable prognostic parameters. In addition to the attempt of individualizing antitumor therapy, the single test systems are of importance for the development and screening of new drugs and for processing tumor biological problems.

18 Quantitation of Viable Cells in Suspensions with Aggregates and with Low Cell Numbers

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Determination of cell count in suspensions containing aggregates of cells and with very low cell members (microplate assays) with conventional methods is difficult. Two methods will be presented, which have been used successfully in solving this problem: (1) Measurement of cellular DNA using fluorescent staining; (2) Measurement of intracellular ATP using bioluminiscence. Both methods can be used with large sample numbers. They are cheap and processing speed is high. Methods can be performed with standard laboratory equipment. No special care is necessary as it is with radiotracer methods. Sensitivity and reproducibility are similar to other determination methods currently in use. The procedure will be explained and the limits of the methods will be discussed.

19 Effect of Nutritents and Cytostatic Drugs on the Colony Development of Human Tumors (CDHT) in Soft Agar (SA)

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Various patterns of CDHT in SA have previously been demonstrated (Rirkels et al., Int. J. Cancer 1983; 32: 399). We studied the effect of nutrients and cytostatic drugs on the CDHT using cell lines or fresh explants. Cultures were set up in quadruplets using the doublelayer SA system (Hamburger & Salomon, Science 1977; 197: 461). The assay system was modified in order to allow feeding on 3 days per week. Improved growth was observed in melanoma and adenocarcinoma of the kidney with feeding of nutrients. The relative increase in colony number compared to control varied when measured at different time points of the 28-day culture period. Cisplatin and analogs as well as adriamycin resulted in a growth inhibition, which, in comparison to the control group, again varied when analyzed at different time points of the culture period. In conclusion, the analysis of the effect of nutrients or cytostatic drugs on CDHT may lead to different results depending on the time point of the colony scoring during the culture period. Supported by SNF 3.833.0.83.

20 Factors Influencing the Colony-Formation of Bone Marrow Cells and Tumor Cells in the Soft-Agar Culture System

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The influence of cytotoxic drugs, hormones and putative growth factors on the in vitro growth of fresh human tumor cells and bone marrow cells, especially granulocyte-macrophage progenitors, can be studied in bilayer soft agar cultures. The growth of many fresh tumor samples remains inadequate despite the description of many potential growth factors. Correlations of results with clinical practice are often difficult, since in vitro toxicity of cytotoxic drugs does not always correlate with the clinically observed myelotoxicity and is furthermore influenced by drug concentration, exposure time, and type of culture conditions.

21 Regulation of Stem Cell Growth in Aplastic Anemia

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Immune-mediated AA may be directed by mononuclear cell (MNC) products, such as Prostaglandin E or gamma-Interferon. A potential release of PGE by MNC was indirectly measured by blocking its release by MNC with 10^{-5} M Indomethacin: MNC from 5 of 11 normals and from 6 to 11 untreated patients with AA activated more colony formation (as a result of diminished PGE release). Bone marrow from 4 patients with AA, cultured in the presence of a MoAb directed against gamma-IF, revealed increased CFU-GM growth to 154% (100 U IF/ml neutra-

lized by MoAb) and 143% (10 U IF/ml neutralized). 4 normal bone marrow samples showed 141% and 96% growth, respectively. Conversely, MNC from 19 untreated AA patients released only 10% CSF (median, range: 0–75%, normals: 100%). After ATG/Pred treatment ($n=5$) or allogeneic BMT ($n=10$), the CSF release was 52% (25–97) and 95% (60–135), respectively. In mixing experiments, patient's adherent/nonadherent MNC were substituted with normal adherent/nonadherent MNC: We found, that the adherent, as well as the non-adherent MNC fraction of patients with AA released impaired amounts of CSF.

22 Evaluation of the Drug Response in Acute Leukemia by a Short Term in Vitro Test

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To improve the predictive value of chemosensitivity test system in acute leukemias – already shown to be clinically applicable (Cancer 53: 390, 1984) – the criteria indicating sensitivity or resistance of blast cells to cytostatics were newly defined. Cells from patients exhibiting different forms of acute leukemia were analyzed for in vitro responsiveness to anticancer drugs in terms of suppression of ^{3}H-uridine, ^{3}H-desoxyuridine or ^{3}H-thymidin incorporation into cellular nucleic acids. The following criteria were established: Cells are sensitive if a marked inhibition of precursor incorporation – strictly dose related – is found and if the maximum inhibition of precursor incorporation is above the threshold level (= mean value of changes of precursor incorporation induced by a drug in more than 30 tests). 45 patients were analyzed using these criteria. Our data show that among the patients with in vitro sensitivity to at least one of the drugs used for treatment about 75% responded to chemotherapy (CR, PR). In vitro resistance correlated in 80% with therapy failure.

23 The Significance of Different in Vitro Assays for the Pretherapeutic Evaluation of Cytostatic Drugs Used in Leukemia Therapy

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Various assays are used to test the in vitro sensitivity of leukemic cells towards cytostatic drugs. We tested 59 samples from the peripheral blood or bone marrow of 44 patients with leukemia with respect to their chemosensitivity towards AraC, VCR, ADM, Amsa and VP16, using 5 different assays: uptake of radiolabelled thymidine (^{3}H-TdR) and uridine (^{3}H-UdR) prior to and post mitogenic stimulation, respectively; further, clonogenic growth on agar-methylcellulose. Three drugs (ADM, Amsa and VP16) showed only a minor activity in vitro (30 to 50% inhibition), independent of the assay that was used. VCR displayed a significantly higher inhibitory effect in the clonogenic than in the other assays ($p < 0.001$); growth of samples from untreated patients was more inhibited by VCR than of those from pretreated patients ($p < 0.05$). AraC was only active in the ^{3}H-TdR-uptake assay. Furthermore, ^{3}H-TdR uptake post stimulation was significantly more inhibited in samples from patients who responded to therapy ($p < 0.005$).

24 Determination of Drug-Induced Cytotoxicity in Acute Leukemia Using Firefly Bioluminescence in Vitro

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In 25 patients suffering from acute leukemia (19 acute myelocytic, 6 acute lymphocytic leukemia) the effect of cytostatic agents on the ATP-content of leukemic cells was studied in vitro and the in vitro test results were correlated to the therapy results in vivo. Intracellular ATP-concentrations were measured using the bioluminescence assay.

Incubation of leukemic cells with cytostatic agents resulted in a decrease of intracellular ATP, which was most pronounced when adriamycin, amsacrin, cytarabin or daunoblastin were

used. Dose-response curves were derived for the single cytostatic agents. A good correlation was observed between the in vitro chemosensitivity and the in vivo response patterns in leukemia patients, in 22 out of 25 patients sensitivity or resistance could correctly be predicted in the in vitro test system.

25 Differentiation and Stimulation of Melanin Synthesis in Cultured Human Melanoma Cells

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Human melanoma cells tend to cease pigment production in the course of serial propagation in vitro, thus loosing their physiological capacity of autoregulation of growth and autodistruction by accumulation of toxic melanin precursors. We have established 19 cell lines derived from melanomas of 17 patients. The cell lines have been characterized by histo-chemical staining as well as by determination of their growth properties under various culture conditions and their chromosomal constitution. We have studied the response of various substrate concentrations on the induction of differentiation and melanin synthesis in these cells between the 15th and 25th passage in vitro. In most of the lines high tyrosine concentrations (3mM) in the culture medium initially stimulated DNA synthesis followed by growth inhibition, melanin or melanin precursor accumulation and subsequent cell death. The time required for this process varied greatly from cell line to cell line. Studies for combining high substrate concentration with differentiation inducers are under way.

26 Clonogenic Tumour Growth as Prognostic Parameter Before and After Therapy

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Since the Human Tumour Clonogenic System (HTCS) is based on selective growth of tumour cells, this culture method was evaluated for its potential in detecting viable tumour cells in monitoring ovarian carcinoma patients. To evaluate the prognostic value of HTCS, 24 cytologic specimens were obtained from 19 patients at debulking. Three cultures, all from patients who attained partial remission, showed growth in the HTCS. To assess HTCS's ability to detect residual disease, peritoneal washings were obtained from 23 patients at second-look operation. Cytologic examination failed to detect tumour cells in 8 out of 10 patients with residual disease. Colony growth occured in only one specimen, in which no tumour cells could be recognized at microscopic examination. Although HTCS results cannot predict prognosis at the time of debulking, combination of cytologic examination and HTCS cultures potentially provides additive information for the assessment of response at second-look operation.

27 Plating Efficiency, Steroid Receptors and Hormonel Sensitivity of Primary and Metastatic Ovarian Carcinoma Cells

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The human tumor colony forming assay was used to compare growth patterns, content of steroid receptors and hormonal sensitivity among simultaneously tested primary tumor, abdominal metastasis and ascitic fluid from ovarian carcinomas. In 12/35 patients a sufficient colony formation (>30 colonies/dish) for drug testing was obtained. The plating efficiency of the metastatic samples (0,120%) was significantly higher ($p<0{,}053$) than those from the primary tumor (0,076%) or those that were derived from the ascitic fluid (0,082%). Colonies from the metastatic tissues could be evaluated 2–4 days earlier than those from primary tumors. No correlation was found between steroid receptors, the tumor site and the plating efficiency. These discre-

pancies may be due to a heterogeneity of primary tumor and metastasis. The antiestrogen Tamoxifen (1 μM) in 9/12 probes showed a significant dose dependent reduction of colony formation (> 70% of the controls) that was independent from the receptor status.

28 Effects of Interferons on Tumor Cells in Culture

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This study about the biochemical and morphological cell response to an interferon treatment should elucidate its mechanism of action in neoplastic cells and furthermore establish a predictive test for the response to an interferon therapy. Established breast cancer cells were treated with human interferon-alpha 2 and -gamma (hu-IFNα_2 and γ) and investigated according to the following criterions: (1) Measurement of dose dependency for the inhibition of proliferation; (2) Detection of effects on the morphology by scanning electron microscopy; (3) Detection of the HLA-DR (Ia)-expression; (4) Histochemical determination of peanut agglutinin binding glycoproteins by flow cytophotometry; (5) Determination of the estrogen receptor content in intact cells; (6) Measurement of the adhesion of cells on tissue culture plates; (7) Measurement of the invasion into collagen-type I-gels. Hu-IFNγ displayed a broader spectrum of anti-proliferative activity than did hu-IFNα_2. Both interferons tested are capable of having a direct effect on tumor cells. Further studies will be necessary to demonstrate which of these cellular effects correlate best with the in vivo response to an interferon therapy.

29 Predictive Value of the in Vitro Short-Term Test to Detect Cytotoxic Drug-Resistance in Tumour Cells of Malignant Effusions

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The purpose of our study is to objectify the clinical value of the in vitro short-term incubation of tumour cells with ^{3}H-labeled DNA- and RNA- precursors and specific cytotoxic agents. Because of methodical advantages the in vitro examinations were performed only in tumour cells of human effusions. To qualify for the predictive evaluation the tested tumour cells had to show homogeneous sensitive or resistant reaction toward the cytotoxic drugs administered in vivo. 108 patients entered the study. 130 tests were performed. In 76 cases we found a correlation between the in vitro resistance and the clinical tumour progression. In 24 cases no resistance was found in vitro but the tumour progressed in vivo; in 30 cases neither in vitro nor in vivo resistance to chemotherapy was found. In no case, tumour resistance was predicted, which was not clinically confirmed. In patients with malignant effusions a reliable prediction of tumour cell resistance seems to be possible.

30 Significance of the Human Tumor-Nude Mouse System for Clinical and Experimental Research

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Most of the human tumors can successfully be grown in athymic nude mice. We transplanted 400 human tumors, which showed a tumor take in 315 cases (79%). A rapid tumor growth was found in 204 tumors (51%), and 193 (48%) could be transplanted of at least 3 passages. In serial passage the xenografts showed a remarkable constancy, which was examined histologically, immunohistologically, with isoenzymes and in terms of responsiveness to drugs. The comparison of tumor response against anticancer drugs in the nude mouse and in the patient yielded similar results. The xenografts produced a right prediction for resistence in 55/57 cases (96%) and for

tumor responsiveness in 19/21 cases (90%). 164 established tumor lines, which are frozen in liquid nitrogen, are continuously available. These tumor lines are suitable models for therapeutic investigations such as a determination of new drugs and new synergistic combinations as well as for studies of tumor biology.

31 Activity of Antineoplastic Agents in Human Testicular Tumor Bearing Nude Mice

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Human testicular tumors are known to be successfully treated with vinblastine, bleomycin and platinum. However, in several cases because of tumor progression or relapse additional agents would be precious. The purpose of this study was to investigate the anticancer activity of various chemotherapeutic agents on human testicular tumor bearing nude mice. Tumors derived from the established human testicular tumor cell line H 12.1 (embryonal carcinoma). 1×10^7 cells were injected subcutaneously into 6–8 week old, male NMRI nude mice. Treatment was started when tumor volume had reached 1–2 cm^3. Equitoxic doses, app. equivalent to the LD_{10} were used. Only bleomycin and platinum reduced the tumor volume significantly. Vinblastine, vincristine, vindesine, ifosfamide and mitoxantrone almost inhibited tumor growth. Mitomycin C, ACNU, 5-fluorouracil, etoposide, doxorubicin and 4-epirubicin showed only moderate retardation of tumor growth. Actinomycin D was without effect. Besides platinum and bleomycin, the vincaalcaloids, ifosfamide and anthracyclines seem to be effective agents in this human testicular carcinoma model. The model is ready for more detailed investigation on the anticancer activity of new substances in human testicular tumors.

32 Individual Chemotherapy Testing for Patients with Metastatic Urogenital Tumors After Transplantation of Tumor Tissue Into the Nude Mouse

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From patients with metastatic renal cell- (RCC), bladder- (BC), prostate- and testicular carcinoma we transplanted tumor tissue into the NMRI nu/nu mouse for an individual chemo- and immunotherapy testing. Our data demonstrate, that tissue from human RCC is accepted in 50 out of 51 cases, show an identity between the primary and transplanted tumor, constant tumor doubling time, a close correlation between the tumor growth in the NMRI nu/nu mouse and the corresponding patients and the same therapeutic effects in the transplanted tumor as in the corresponding patient. The acceptance rate of BC was about 50% (12/22) and depends on the surgical technique to remove the tumor, the grading and staging. After transplantation bladder tumors show different growth rates, which again correlate with the clinical course of the patients. But after several subpassages the growth rate accelerates in all tumors and there were changes in the DNA index and the proliferation rate as well as in the mitoses index. Chemotherapy results were different when tests were done in the early compared to the accelerated passages which means that an individual specific chemotherapy testing in BC is more complicated than in RCC. A low acceptance rate was observed using prostate carcinoma (3/8) and testicular tumors (1/4) which means that chemotherapy testing is not practicable. On the basis of these results 18 patients with metastatic RCC received 9 different chemotherapeutic agents and 7 patients with metastatic BC received 5 different agents for therapy. Response was seen in all these patients.

33 Aneuploid Cells in Human Myeloma – Immunological Characterization and Clinical Implications

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Cellular DNA- and RNA content analysis by flow cytometry was performed in 103 patients with multiple myeloma (MM) and 40 healthy individuals. All patients developed significant numbers

of aneuploid DNA/RNA containing peripheral blood cells during the course of disease. Tumor-progress was close connected with an increase of the aneuploid cells. The aneuploid cell population is mainly composed of myeloma protein idiotype bearing cells as shown by treatment with anti-idiotypic antisera and complement depending lysis. In addition the quantitative determination of aneuploid cells after 3 days cultivation of peripheral blood and bone marrow lymphoid cells in the presence of cytostatic drugs offers information concerning the drug sensitivity of the individual tumor cell clone. These data provide additional knowledge for monitoring the course and for an individual therapeutic approach in MM.

34 Pharmacokinetic Aspects of Melphalan Therapy in Multiple Myeloma

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Melphalan (M) – plasma-concentration-time curves were investigated in patients on intermittent M + Prednisone (P) therapy ($n = 15$). Five patients received a single i. v. dose of 25 mg M equivalent to the oral test dose. Bioavailability ranged from 0,31 to 0,59. The *inter-* ($n = 15$) and *intra*individual variation at course 1, 2, 3, ($n = 7$) in the area under the plasma concentration – time-curve (AUC) of melphalan amounted to 38% and 28% respectively. This large intraindividual variation of AUC points out, that reduced therapeutic efficacy of conventional oral M + P – versus multiagent therapy of multiple myeloma (still a matter of controversy!) may represent a phenomenon of inadequate median dose rather than of inferior antineoplastic potency of melphalan.

35 Combination Chemotherapy of Plasma-cell Myeloma with M-2 Protokol

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In order to improve the duration and quality of remission in patients with plasmacell myeloma, we have investigated the effects of combination chemotherapy according to the M-2 Protokol. Since 1978, 62 out of a total of 98 patients from our two centers were eligible for study and were treated initially with this regimen, which consists of BCNU, cyclophosphamide, melphalan, prednisone, and vincristine. 12 patients were in stage II and 50 in stage III according to the classification of Salmon and Durie. At least four cycles every 35–42 days were administered before evaluation of response. A maintenance therapy with the same regimen at intervals of 8–10 weeks was given to patients who responded to therapy. Up to April 1985, 52 patients were evaluable. The median age of these patients was 64 years. According to the criteria of the Southwest Oncology Group, a remission was achieved in 31 patients (59.6%). The median survival of all patients was 25 months and for 31 patients in remission the median has not yet been reached after 40 months. The median remission duration is 24 months. The results are encouraging with respect to remission and survival duration.

36 Treatment of Advanced Multiple Myeloma (MM) with the M-2 Protocol. A Follow-up Study

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We have previously reported the efficacy of the M-2-protocol for treatment of MM (Blut (1984) 49: 383–388). Since that report the patients have been followed carefully. The minimum observation time is now 28 months. 37 patients (median age 65 years, range 52–77) were entered into the study between May 1979 and January 1983 and were treated with the M-2-protocol as previously reported. The remission rate was 65% (24/37 patients). Median duration of remission is 20 months. From the onset of treatment median survival is 27 months. The probability of survival at three years is 42,3% (confidence limits 26.1 to 58.6%), and at five years 18%.

At three years there was no significant difference in survival between males and females, Stage II and Stage III, IgA and IgG MM patients. Patients with light-chain MM and B-symptoms did significantly worse. The median survival of responders is 47 months and of non-responders 10 months. 22% of the responders are expected to be alive at five years. The cumulative incidence of leukemia at five years is 17%.

37 Treatment of Multiple Myeloma Refractory to Common Chemotherapy

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Therapy of patients with multiple myeloma that is refractory to alkylating agents, vinca alkaloids and corticosteroids is still difficult because only few effective salvage treatments are available. This report summarizes our experience with three different salvage therapy protocols in 14 patients with multiple myeloma. All patients were at high risk and were refractory to common chemotherapy. In some patient more than one of the salvage regimens was applied. Six patients were treated with etoposide monotherapy, seven patients received a combination of vincristine, cyclophosphamide, etoposide and prednisone (VCPVP), and in ten patients a regimen consisting of high dose prednisone, vincristine and a four-day continuous infusion of doxorubicin was employed. Etoposide was of benefit in one patient only. VCPVP reduced serum M-protein and skeletal disease but showed no benefit in one patient with soft tissue involvement. Comparable to this regimen VAD reduced the M-protein level and had influence on bone lesions, but not on soft tissue myeloma. The study is still in progress.

38 Chimerism and Graft-versus-Host Disease (GVHD) in Dogs Following Hyperfractionated Total Body Irradiation (TBI) and Marrow Transplantation from DLA-Haploidentical Littermates

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In dogs chimerism and GVHD was studied following TBI in various fractionation schedules and transplantation of marrow from DLA-haploidentical littermates. A total dose of 13.5 Gy in 5 days was given either hyperfractionated with 3 fractions of 0.9 Gy per day (14 dogs) or less fractionated with fractions of 4.5 Gy every other day (12 dogs). Dogs given 4.5 Gy fractions became complete chimeras as shown by cytogenetic analysis. 7 dogs died with GVHD at 19, 19, 25, 31 and 151 days, 7 dogs survive as chimeras between 3 and 23 months. With the exception of one dog who showed transiently mixed chimerism all dogs were complete chimeras from day 20 on. Following hyperfractionated TBI 5 of 14 dogs rejected the graft of whom 2 died with marrow aplasia and 3 recovered host type hemopoiesis. One dog died early of sepsis, 2 died of GVHD and 6 dogs are surviving as complete chimeras between 6 and 21 months postgrafting. In conclusion fractionated TBI induces persistent graft-host tolerance across DLA-incompatibility in half of the cases, but hyperfractionation can not prevent rejection in all cases.

39 23 Genetic Polymorphisms of Blood in Patients Before and After Bone Marrow Transplantation – a Proof for Complete or Partial Allogeneic Reconstitution

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In 29 of 45 viennese bone marrow donors and recipients and their families, 23 genetic polymorphisms of blood have been investigated before and after bone marrow transplantation. The antigens of 9 blood group systems, 7 erythrocyte enzyme markers, the Lewis system, the Immunoglobulin allotypes Gm and Km have been tested as well as 4 serum polymorphisms. Whereas

blood groups and enzyme markers of red blood cells of all patients available at least 6 months after bone marrow transplantation had changed to the donor's type, all groups of serumproteins, produced in the liver, remained recipient's type. Immunoglobulin allotypes, however, in some cases kept the recipient's allotype and sometimes changed to the donor's allotype. These results might indicate, that, as described mainly in patients with Severe Combined Immunodeficiency, some of our patients might have retained at least some of their own B lymphocytes producing the recipient's own, Gm and Km bearing Immunoglobulins.

40 Application of Bone Marrow Transplantation in Hereditary Disease of the Lympho-Hemopoetic System

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In a series of patients with various hereditary disease of marrow and marrow dependant disorders, correction by bone marrow (BM) transplantation was attempted. (1) Fifteen infants with syndromes of severe combined immunodeficiency, lacking HLA identical donors, were given haploidentical marrow from parents. Of these, 11 have partial or complete immunoreconstitution and are at home. Graft versus host disease was effectively prevented by T cell depletion of the grafts. (2) Correction of Wiskott Aldrich Syndrome was attempted in 3 boys. One is a long term survivor in excellent health after HLA-matched BM-transplantation. (3) In single cases of Osteopetrosis, Fanconie Anemia, Thalassemia and Chediak Higashi Syndrome, all given HLA matched BM, correction was observed in the first two. Our current experience is encouraging enough to continue BM transplantation in selected cases, where other therapies are lacking or failing.

41 Bone Marrow Transplantation – Results at the West German Tumor Center Essen

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During 1975–1985 105 marrow transplantations were performed for relapsing acute leukaemia ($n = 20$), acute leukaemia in first or consecutive remission ($n = 37$), chronic granulocytic leukaemia ($n = 32$ including 1 syngeneic retransplantation) and panmyelopathy ($n = 16$). In AL in relapse only one patient survived (5%). In first remission AML the survival rate is 50% (11/22) (median follow-up 3.2 years). Patients grafted in a second or consecutive remission of AL had a survival rate of 50% (8/16) (maximum followed-up 2 years). Survival of patients grafted in the chronic phase (22/28) or accelerated phase (6/28) of CGL is 43% (12/18) (median follow-up 1.3 years). Transplantation in a second chronic phase after acute transformation was unsuccessfull due to transplantation related complications in 3 patients. 70% of the patients grafted for panmyelopathy are alive (median disease free interval of 2.1 years). The overall incidence of GVHD in patients at risk is 38% with a significantly higher incidence of GVHD in patients with CGL (total 65%, grade III–IV 31%) in opposition to AL (total 21%, grade III–IV 8%). 29% of patients with AL and 37% of patients with CGL developed fatal interstitial pneumonitis.

42 Allogeneic Marrow Transplantation in Patients with Leukaemia: Comparison of two Different Methods for GvHD Prophylaxis

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1980–1984 40 adults underwent allogeneic bone marrow transplantation for treatment of haematological malignancies (AML, 1. CR $n = 13$, 2. CR $n = 4$, ALL, 1. CR $n = 8$, 2. CR $n = 5$, relapse $n = 2$, CML, chron. phase $n = 7$, blast crisis $n = 1$). 24 patients received Metho-

trexate (MTX) as gvhd prophylaxis (1980–8/1983), 16 patients a T-cell depleted transplant (9/1983–1984). Ex vivo depletion of T-cells was obtained by a monoclonal cytolytic antibody (CAMPATH 1). Results: The survival rate according to Kaplan and Meier was 43% for the MTX-group and 66% for the CAMPATH-group. The difference was statistically not significant. During the first 100 days after bmt CMV pneumonitits was the main cause of death in both groups. Death due to chronic gvhd occurred in the MTX group (5/24 patients) but not in the CAMPATH group (0/16). Relapse rate was low in both groups (2 vs. 1), probably due to intensive chemotherapy before bmt.

43 Allogeneic Bone Marrow Transplantation (BMT) in Patients with Chronic Myelocytic Leukemia (CML)

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Fourteen patients with CML (12 pts. in chronic phase, 1 pt. in accelerated phase, 1 pt. in 3rd chronic phase) were transplanted from HLA-identical siblings. The age range was 5–46 years (median 16,5 yrs.). As conditioning procedure 2×60 mg/kg cyclophosphamide and total body irradiation in 2 pts. with 10 Gy, in 12 pts. with 5×2,5 Gy were used. As GvHD-prophylaxis MTX+ATG-incubation was administered in 3 pts., cyclosporin A in 4 pts. and cyclosporin A+ATG-incubation in 7 pts. CMV-hyperimmunoglobulin and ayclovir were administered in 12 pts. 12 pts. are alive day 42 to 698 after BMT. 2 pts. died from pulmonary complications (interstitial and varicella pneumonia). Both did not receive fractionation of TBI, acyclovir and hyperimmunoglobulin. Acute GvHD grade 2+ was observed in 1 pt., extensive chronic GvHD never occurred. No patient relapsed.

44 Bone Marrow Transplantation in Patients 45 Years and Older

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To evaluate the types and frequencies of transplant related complications, survival rates, and causes of death, we reviewed the courses of all Seattle patients 45 years and older with hematologic malignancies given allogeneic ($n = 39$) or syngeneic ($n = 23$) marrow transplants. Among the twins, 30% are alive. Leukemia relapse and interstitial pneumonitis (IP) were the most frequent causes of death. Nine (23%) of the allogeneic patients are currently alive. CMV pneumonia and septicemia were the most frequent causes of death. The incidence of lethal idiopathic IP in allogeneic patients $>$ 50 years was 33%. Acute graft-versus-host disease of grades II–IV occurred in 27% of the recipients, which is not different from the incidence in younger patients. Seven of 15 allogeneic recipients (47%) transplanted while in remission of AML, in chronic phase of CML, or in preleukemia are alive and well 114–1160 days after marrow transplantation. We conclude that it is justified to offer marrow grafting to patients who are older than 45 years, especially if they are in an early stage of the disease.

45 Reconstitution of Hematopoiesis After Bone Marrow Purging with Ricin-A-Chain Immunotoxin

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Bone marrow cells from healthy individuals were treated with an anti human T-cell immunotoxin (IT_{101}). The treated marrow cells were cultured for erythroid colonies (BFU-E), granulocytic colonies (CFU-C), and multilineage hematopoietic colonies (CFU-GEMMT) containing various myeloid cell lineages and T-lymphocytes. Optimal conditions were defined for the elimination of clonogenic human T-leukemic cells artificially admixed with bone marrow cells. The marrow purging with IT_{101} led to the restoration of hemapoietic colony formation which was abolished in the presence of T-leukemic cells.

The examination of mixed colonies obtained after IT_{101} treatment revealed cells that react with monoclonal Anti T-cell antibodies. This suggests that pluripotent stem cells are not affected by marrow IT_{101} purging and may be able to regenerate lymphoid as well as myeloid lineages.

46 Campath I-Associated Bone Marrow Failure in HLA-Identical Bone Marrow Transplantation: Diagnostic and Therapeutic Problems

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We have observed two cases of graft rejection, one incipient rejection and one presumptive rejection in 23 consecutive HLA-identical transplants for acute and chronic leukemia. All 4 cases had two things in common:
1. They had received a Campath I-purged marrow,
2. graft failure was preceded by a relative lymphocytosis.
In three cases there was an additional major ABO-incompatibility. In all cases $\geq 90\%$ of lymphocytes were activated killer/suppressor cells ($OKT8^+$, DR^+), and in two cases these were demonstrated to be of host origin. This population inhibited CFU-e and CFU-c growth in coculture with autologous T-depleted marrow and this inhibition was mainly due to inhibitory factors in culture supernatants (spontaneous). Interferon, isoagglutinins and I1–2 were excluded as inhibitory factors. In two cases early therapy with cyclosporin A and prednisone appears to have reversed the rejection process. The diagnostic and therapeutic problems of bone marrow failure will be discussed in detail.

47 High Dose Consolidation with Autologous Transplantation of a Purged Marrow Graft in Patients with Acute Leukemia: Heidelberg Data

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Autologous bone marrow transplantation (ABMT) with a purged marrow graft represents a new approach to the treatment of patients with malignant lympho-hemopoietic disorders. 9 patients with acute leukemia (AL) without an HLA-identical sibling have been treated with high dose chemo- and radiotherapy followed by ABMT. Marrow was harvested in complete remission (CR) and treated with an active Cyclophosphamide derivative ASTA-Z-7557. The myeloablative conditioning regimen consisted of super fractionated total body irradiation (1320 rad) followed by Cyclophosphamide (200 mg/kg b. w.). Six patients transplanted in CR are alive in continuous CR for 6.5 months (median time). The longest CR time is 19 months after ABMT. This patient with AML was transplanted in 2nd CR. The longest cryopreservation time for a marrow graft was 3 years 8 months without a significant impairment of hemopoietic reconstitution ability. The upper age limit for patients to be transplanted was 50 years. The mean number of MNC transfused per kg b. w. was 10^8 ($n = 5$). The median number of days after ABMT to reach a blood granulocyte concentration of 500/µl was 43.6, and a blood platelet concentration of 50.000/µl was 100 days.

48 Total Body Irradiation (TBI) and High Dose Cyclophosphamid in Minimal Systemic Tumor Disease: Experience with Liver and Lung Metastases

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Surgery for treatment of metastatic disease due to progression of systemic metastases has proven to be unsuccessful. We have designed an experimental two step protocol for treatment of chemotherapy – resistant hepatic or lung metastases. After surgical removal of clinical detectable

tumor burden by means of liver transplantation or partial lung resection patients are treated for systemic residual micrometastasis with TBI and high dose cyclophosphamide followed by autologous bone marrow transplantation. This treatment modality was associated with no mortality and low morbidity and was applied so far in 5 patients: 4 suffering from breast cancer and one from colorectal cancer. Whereas the patient with the less sensitive primary tumor developed pulmonary metastases within 6 months, the 4 patients with breast cancer are still well and free of relapse from one until 30 months after therapy.

49 High Dose Chemotherapy and Non-Cryopreserved Autologous Bone Marrow Treatment in Advanced Malignant Melanoma

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Only low response rates have been reported in the treatment of advanced malignant melanoma by either mono- or combination chemotherapy. In this study we report the treatment of 20 patients with high dose chemotherapy and autologous bone-marrow support. Five patients were treated with nitrogen mustard the remainder with escalating doses of intravenous melphalan and BCNU. Four patients had complete remissions, seven a partial response, nine no response, one patient was considered not evaluable. Side effects included nausea, vomiting, alopecia, stomatitis and in three patients reversible cerebral dysfunction. The results of this study demonstrate a high response rate of cancer refractory to standard chemotherapy.

50 Histological Classification of CMPD: Observer Disagreement

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200 patients with chronic myeloproliferative diseases (CMPD) were recruited from our files and their slides coded from 1 to 100. Without any further information they underwent a careful reconsideration and classification by 4 independent haematopathologist. A working-classification encompassing 10 entities of CMPD based on histopathologic findings evaluated in respect to cytogenetic and clinical data was used. There was a concordance-rate of 76% of individual independent classification, which was increased to 91% after a second trial and discussion among the panelists. The rate of reproducibility for the individual observer was about 80%. Only 9% of cases proved to be unclassifiable by means of histopathology alone and needed an additional evaluation of the clinical files as well. Therefore it can be stated that this new classification system, which is a considerable extension of so far applied classifications according to WHO or text books, is reproducible with results comparable to those of the observer disagreement studies in Hodgkin's-and non-Hodgkin's-lymphomas.

51 The Diagnostic and Prognostic Relevance of Megacaryocyte Size in Chronic Myeloproliferative Diseases (CMD)

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Morphometric analysis of megacaryocytopoiesis was performed in cytological and histological bone marrow preparation of 104 patients with CMD. The computer-evaluation showed, compared with controls, two separate groups: 32 patients with dwarf megacaryocytes (100–600 μm) and 72 patients with enlarged cells (300–5000 μm).

The comparison with the clinical diagnosis showed a complete coincidence between the dwarf-megacaryocyte-group and chronic granulocytic leukemia, while enlarged megacaryocytes were found in all other CMD, without significant differences between the subgroups.

These results separate CGL from all other CMD and reflect profound differences in DNA-reduplication and maturation. It is suggested to classify CGL as preleukemic disorder rather than as CMD.

52 Essential Thrombocythemia: Symptoms and Clinical Course in 52 Cases

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From 1975 to 1985 52 patients with essential thrombocythemia (ET) have been diagnosed and followed in the Medizinische Poliklinik. 80% of the patients presented with thromboembolic, 10% with hemorrhagic problems. In 10% the diagnosis ET was accidental. 50% of the patients were male, the average age in male patients was 59 years, in female patients 61 years. The maximal number of platelets ranged from 500,000 to 3 million with an average of 1,2 million per µl. In 68% a moderate to marked leukocytosis was present, in 54% splenomegaly. Bone marrow analysis was done in 90% of cases. Megakaryopoiesis was always increased, granulopoiesis was increased in 60%, decreased in 16% and normal in 24% of cases. Erythropoiesis was increased in 20%, decreased in 42%, normal in 38%. The Philadelphia chromosome was negative, whenever determined (11 cases). Therapy included treatment with inhibitors of platelet aggregation and busulfan. At present 40 patients are alive (77%). 10 of the 12 decreased patients most probably died of vascular causes. Two developed acute leukemia. It appears that ET is a more frequent risk factor of vascular diseases than presently recognized.

53 Risk of Transformation, Survival Rates, and Prognostic Factors in 338 Patients with Myelodysplastic Syndromes

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338 patients of the period 1970–84 with sideroblastic anemias, anemias with atypical cells, preleukemic syndromes and subacute leukemias were reclassified according to the FAB-classification (1982) and evaluated by multivariate statistical analysis. Important differences were found between the particular FAB-entities for the survival probability (median survival: RA 65 months, RA with ring sideroblasts 168 mo., RAEB 13 mo., RAEB in transform. 8 mo. CMML 39 mo.) and for the risk of transformation (median time until transformation: RA 46 mo., RA with ring sbl. 202 mo., RAEB 17 mo., RAEB in transf. 3 mo., CMML 64 mo.). Prognostically less favorable were chromosomal anomalies, pathological thrombocyte counts, leukocytes less than 2,0, high need for transfusions and such forms that developed secondary to irradiation, to cytotoxic therapy or to other chemicals.

54 Low-Dose ARA-C in Myelodysplastic Syndroms and CML in Transformation

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33 patients with malignant transformation of myelodysplastic syndrome (MDS) or chronic myeloid leucaemia (CML) (MDS: 20 pat., CML: 13 pat.) received 73 cycles of a low-dose ARA-C treatment. The treatment was administered subcutaneously every 12 hours or as an 12-hour-iv-infusion (12–14 mg/m^2/day) from 5 to 36 days. 20 MDS-patients got 26 treatment-cycles, in 18 cycles a reduction of leucaemic blastcells ($< 0{,}5 \times 10^9$/l) was achieved, 8 times (in 6 patients) a complete remission was seen. In 9/13 CML-patients the ARA-C treatment had to be repeated every 3 or 4 weeks, with this treatment schedule a stable phase till 14 months could be obtained. There was nearly no influence on spleen enlargement or extramedullar involvement. Thrombopenia was a severe side effect of treatment and required treatment termination in several cases.

55 Low Dose ARA-C as an Alternative Therapy for Older Patients with AML

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According to the results of recent studies, about 60% of patients with AML achieve complete remission after aggressive chemotherapy. The rate of CR for older patients ($\geq$ 60 years), however, is only about 35% in studies with a large number of patients. In the last 12 months we treated 10 patients in higher ages (median 68 years) with low dose Ara-C (2×20 mg s. c./day) for 2 to 21 days. Regardless of the result of the initial course of therapy, therapy was continued at monthly intervals with a course of Ara-C in the same dosage for 7 days. With this protocol, PR was achieved in 1 patient and CR in 3 patients (duration of CR: 5, 5+ and 7+ months). The only serious side effect noted was a dangerous fall in platelet count, requiring interruption of therapy in 5 cases. Our results indicate that rates of CR comparable to those achieved with currently used aggressive induction chemotherapy can be obtained with low dose Ara-C. Larger patient groups should be studied to determine whether low dose Ara-C can be recommended as an alternative for older patients with AML.

56 Continuous Infusion Therapy with Low Doses of Cytarabine

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Cytarabine was given as continuous intravenous or subcutaneous infusion 20 mg/m^2 daily for 10 to 21 days. 9 patients were treated up to now, 6 with dysmyelopoetic syndrome (DMS), 1 with CMML and 2 with AML in relapse. In DMS there was 1 PR with rise in peripheral granulocyte counts from 98 to 1400/mm^3 and platelet counts from 16,000/mm^3 to 112,000/mm^3 for 3+ months. There was one minor response with a reduction of blast cells in marrow for 1+ month but without reconstitution of thrombopoeses. 3 patients died within 1 month without effect, another is alive at 6+ months without effect. 1 patient with CMML hat reduction of splenomegaly and transient reduction of WBC for 2 months. In 1 patient with AML in relapse there was stable disease for 1 month. Side effects were considerable hematotoxicity with granulopenia in 6/9 patients and thrombopenia in 1/3 patients (6 already had initial counts below 20,000 mm^3). Severe infection occurred in 4/9, mild in 1/9 patients.

57 Low Dose Ara-C in Preleukemia and Leukemia of the Elderly

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From 24 patients with myelodysplastic syndrome 6 were treated with low dose Ara-C because of refractory anemia with excess of blasts in transformation or acute leukemia. One patient achieved a partial remission lasting three months. Two patients came in a complete remission and are still in remission 15+ and 18+ months. The other three patients developed acute leukemia under treatment. All patients with remissions had severe cytopenia and life threatening complications under remission induction. In our series we could confirm, that complete remissions are possible with low dose Ara-C in preleukemia and leukemia. These remissions were achieved after bone marrow aplasia and not as a differentiation effect of low dose Ara-C as Casteigne et al. initially reported.

58 Acute Lymphoblastic Leukaemia with Prominent Granulation

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The absence of granules or Auer rods in the cytoplasm of blasts is used to separate ALL from AML. There are exceptions of this rule. Since 1977 we observed 10 patients (6 children, 4 adults) with prominent granulation in the cytoplasm of leukemic blasts among the patients with acute

leukemias of two university hospitals and of cooperative therapeutic trials in adults and children (> 1,000 patients). Clearcut differentiation between these cases and acute granulocytic or acute monocytic leukemia was achieved by cytochemical, immunological and electronmicroscopic investigations. Immunologic studies showed the c-ALL phenotype in 2, pre-pre-B type in 1, the absence of all markers in 1 and E-rosetting of part of the blasts in 1 case. Electron microscopic and cytochemical characteristics of the granules are in accordance with lysosomal nature. They contain acid phosphatase, acid esterase, no peroxidase and chloroacetate esterase. Until now there is no difference of the clinical course and the age in comparison to ALL in general, the incidence rate is about 1%.

59 Ecto-5′-Nucleotidase as Marker for Differential Diagnosis in Acute Leukemias

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In earlier studies we could demonstrate a close correlation of the presence of the common-ALL-antigen on leukemic blasts with high, or even extremely high activities of ecto-5′-nucleotidase (5′-N) on these cells. We have recently extended these investigations mainly by including patients from the BMFT-ALL/AUL-study. The trends which had shown up earlier were fully confirmed. Thus, in its 5′-N activity the group of cALL differs highly significantly from other groups of acute leukemias, such as T-ALL, Non-ALL and Null-ALL. Especially the latter observation, the distinction of cALL and Null-ALL seems new and significant. We could also confirm that in the case of high 5′-N activities in cALL we are always dealing with an immunologically normal enzyme. Within the group of cALL 5′-N values vary in a wide range. Such differences were tested for their possible prognostic significance.

60 Ectopic Production of Human and Salmon Calcitonin in Acute Leukemia; Correlation to Clinical Parameters

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The incidence and the correlation to clinical course of elevated human calcitonin (h-CT) and salmon calcitonin (s-CT) serum levels in patients with acute leukemia were investigated. The incidence of elevated h-CT serum levels at diagnosis was 46.7%. More immature forms (AUL and M 1) showed significantly more often elevated levels of h-CT than other subtypes of acute leukemia. Patients with elevated h-CT levels at diagnosis had a significantly shorter time of survival. This was caused by a higher incidence of early death in the group with high h-CT levels. Survival and rate of remission were not different when patients survived longer than 4 weeks after diagnosis. Human CT levels were compared to clinical parameters such as age, LDH, WBC, temperature, serum calcitonin, creatinine, SGOT and serum amylase. Only LDH was significantly correlated to elevated h-CT serum levels. S-CT was found elevated in 19% of patients with acute leukemia, mainly in patients with the subtype M 1. No correlation to clinical parameters could be found for salmon calcitonin.

61 Incidence and Clinical Significance of DNA Aneuploidies in Acute Myeloid Leukemia within the AML Studies 78 and 81

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In 148 patients with previously untreated acute myeloid leukemia (AML) analyses of the cellular DNA-content were carried out by flow-cytometry in order to assess the incidence of DNA-aneuploidies and its relation to morphologic subtype, age, bone-marrow cell count per mm^3 bone-marrow, S-phase index, WBC, remission rate and remission duration. DNA-aneuploidies were identified in 54 of 131 (41%) patients with de novo AML and in 4 of 17 patients with AML

after preleukemic syndromes. The incidence and degree of DNA-aneuploidies was lower for FAB M1 and M2 leukemias as compared to the subtypes M4 and M5. Within the group of M4 and M5 leukemias, patients over 40 years of age had a lower DNA-aneuploidy rate than younger patients. For the other cellular determinants, no differences were found between patients with and without DNA-aneuploides. However, a tendency to longer remissions was observed for patients with DNA-aneuploidy.

62 Pretherapeutic Chromosomal Analysis of Leukemic Cells in Children with Acute Lymphoblastic Leukemia

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Specific chromosomal anomalies in leukemic cells from the bone marrow of children with acute lymphoblastic leukemia (ALL) which can be detected in 60% of cases, are useful for diagnosis respectively classification, and even might be of prognostic significance if all the examined patients are treated according to the same theapy protocol.

We examined the leukemic bone marrow at diagnosis of 50 children with ALL entering the BFM-83 therapy protocol and of 20 patients on the ALL-relapse 83 protocol. Five different groups of chromosomal aberrations could be distinguished (hypodiploid, hyperdiploid > 50 chr., hyperdiploid 47–50 chr., normal karyotype, pseudodiploid) and were compared with immunophenotype, initial leukocyte count and risk factor (of BFM group).

After a clinical observation period of 20 months a probable correlation between frequency of relapse (i. e. resistance to chemotherapy) and karyotype of the leukemic cell clone is becoming more and more obvious.

63 Establishment of two Permanent Human Leukemia-Cell Lines Producing Immunoreactive Calcitonin

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Two permanent human leukemia cell lines designated LG 3 and MS 6 were established from bone marrow aspirates of a patient with acute monocytic leukemia and a patient with acute myeloblastic leukemia respectively. In vitro both lines exhibited a monocytoid marker profile. Cells were positive in staining reactions for unspecific esterase, PAS and acid phosphatase. Cell-surface studies revealed strong reactions with monoclonal antibodies reacting with monocytes. Both lines showed also a strong binding of monoclonal antibodies detecting transferrin receptor. In supernatants and extracts of MS 6 cells immunoreactive human calcitonin was found in raised levels. No significant levels of immunoreactive human calcitonin were found in supernatants of LG 3 cells, while extracts of LG 3 cells contained high levels suggesting the in vitro selection of a non secreting clone of leukemic cells. These data support the concept of ectopic immunoreactive human calcitonin production by leukemic cells.

64 Establishment of Lymphoblastoid Cell Lines Suitable for Investigation of Inherited Metabolic Deficiencies

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Biopsy material for investigational purpose is often limited and available only for a short period often terminated by the course of the disease. Healthy members of the affected family may be willing or not to offer material. Some tissue culture technics have been described to overcome these obstacles. For the investigation of some enzyme deficiencies lymphoblastoid cell lines may

offer a new access. It is known since short that (neutral) maltase is a marker of the stage of maturation of a B-lymphocyte that will lead directly to the plasmacell (Philipp 1983). Acid maltase deficiency is altering plasmacell morphology by inclusions mimicking vacuoles (Pralle 1975). To test whether there are neutral and acid maltase (AM) present in parallel, we established a lymphoblastoid cell line from patients with POMPE's disease (AM-Deficiency) of the iuvenile type. In the cell line of the affected patient the AM-activity was zero, whereas the total leukocyte population showed only reduced enzyme activity. This and the plasma cell deficiency fit into the assumption, that AM is formed as well as the neutral enzyme at this stage of B-lymphocyte differentiation.

65 Cell Cycle Dependent Modulation of Insulin and IGF 1 Receptor Binding to Human Burkitt Type ALL Cells

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We recently demonstrated human Burkitt type ALL cells in longterm culture displaying high affinity binding for insulin and IGF 1. This rapidly proliferating cell line expresses large numbers of receptor binding sites both for insulin and IGF 1. Cell cycle specific separation was performed by counterflow centrifugation. Cells could be enriched to 60–80% purity for G 1-S-G 2. The insulin receptor displayed 10,000–15,000 receptor sites/cell in G 1-, 1,000–5,000 in S- and 40,000–50,000 in G 2-phase. The affinity of insulin binding decreased continuously during cell cycle. The IGF 1 receptor displayed 2,000 receptor sites/cell in G 1, 5,000 in S and 15,000 in G 2. The affinity of the IGF 1 receptor was high in G 1 showing a dramatic decrease towards S phase and a slight increase towards G 2 phase again. IGF 1 shows high affinity binding during G 1 Burkitt type ALL cells. This suggests that IGF 1 may be important for initiation of proliferation. The outstanding reduction in insulin receptor binding sites during S phase indicates refractoriness of the cell to the metabolic action of insulin during DNA replication.

66 Biochemical and Immunological Characterization of Epstein-Barr Virus-Associated Early Antigens with Rabbit and Human Antisera

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Epstein-Barr virus-associated early antigens (EA) consist of a large number of polypeptides. These polypeptides are thought to be involved in the negative control of virus latency as well as the complex regulation of shutdown of host cell macromolecular synthesis, which occurs in all EA+ cells and leads to cell death. An EA-associated polypeptide complex (p 52) EA has been purified from Burkitt's lymphoma cells and used to immunize rabbits. The antisera obtained detect EA as could be shown by immunofluorescence, ELISA and immunoblotting. Two phosphorylated polypeptides (pp 50/58) could be identified by immunoprecipitation with the rabbit sera. 2-D immunoblot analyses showed that the rabbit sera detect series of polypeptides between 50 and 58 kD (IP 8.5 to 4.0). Six polypeptides of 52 kD appear as early as six hrs after induction of EA. All components of the p 50/58 complex detected by the rabbit sera seem to be derived from posttranslational modifications of one polypeptide (p 52).

67 Evidence for a Myelo-Monocytic Differentiation Potential of Common Acute Lymphoblastic Leukemia Cells in Diffusion Chambers

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Mononuclear blood cells from patients with common acute lymphoblastic leukemia (CALL) were cultured in diffusion chambers (DC). After 20 days in culture, the chamber content mainly consisted of granulocytic cells and macrophages making up 60–80%. The granulocytic diffe-

rentiation was independent of the number of granulopoietic progenitors (CFU_C) seeded into the chambers. This was also true in case of the "colony forming units in DC (CFU_D)". In order to test whether leukemic CALL positive blasts themselves are able to differentiate along the granulocytic lineage, cells of the leukemic line Nalm 6M1 were cultured in DC. During culture, a positive reaction of the Nalm 6M1 cells with monoclonal antibodies directed against granulocytic and monocytic antigens could be observed. At the same time, the cells developed a positive naphthyl-AS-D-chloroacetate esterase reaction. We therefore conclude that CALL-positive leukemic blasts in some cases display the capacity to develop myelo-monocytic cell characteristics.

68 Induction of Myeloid and T-Cell Markers in two Cases of Unclassified Acute Leukemia (Null-AL)

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The cellular phenotype remains unclassified in a minority of patients with acute leukemia. It therefore was the aim of the present study to reveal the cellular phenotype in two patients with null-AL by analyzing their proliferation and differentiation in culture after exposure to phorbolester, dbcAMP and 5367-conditioned media. Both cases were cytochemically undifferentiated and reacted both with BA-2 and OKIa. Furthermore a minority of blast cells of the first case was reactive with VIM 2, MY-7 and WT 1. Upon culture both cases newly expressed or considerably increased their reactivities with myeloid markers (MY-7, VIM 2) stem cell (RFB-1) and pan T-markers (WT 1) and – in the second case – thymic markers (OKT 6). In addition both cases expressed the interleukin-2-receptor (Tac). Cytogenetic analysis of the second case prior to and after the various cultures revealed a translocation (4; 17) in all metaphases, proving the leukemic origin of the cells carrying both myeloid and T-cell markers (MY-7, VIM 2, WT 1, OKT 6, OKT 3, OKT 4, Tac). It is concluded that unclassified acute leukemia blast cells might have a bipotential differentiation capacity and that the translocation t (4; 17) might be responsible for this capacity in the second case.

69 Familial Hodgkin's Disease

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The incidence of Hodgkin's disease in consanguineous family members is increased. According to Gruffermann et al, there is a sevenfold increased risk for developing Hodgkin's disease in siblings for the healthy partner. We present a family, im whom three sons have got lymphogranulomatosis. Two sons have died at the age of 12 and 20 years from Hodgkin's disease stage IV B. The third son, 19 years old, has recently developed Hodgkin's disease stage III A. The final diagnosis could only be confirmed after a longer observation period and three biopsies. EBV-titers are strikingly and persistently elevated in this patient IgG 1: 512, EA 1: 32). Chromosomal studies, immunological investigations as well as measurements of EBV-titers have been performed in the parents, the patient and his healthy brother. The results will be discussed.

70 Late Thrombotic Complications After Splenectomy in Patients with Hodgkin's Disease

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According to a material of splenectomized patients with Hodgkin's disease the occurrence of late thrombotic complications has been investigated by epicrisis. Out of a total of 67 patients in 7 patients during the first and the seventh postoperative year the following thrombotic complications have been observed: 2 cerebral apoplexies, 1 thrombosis of the cava inferior vein, 1 thrombosis of the pelvic vein, 1 thrombosis of the femoralis vein, 1 myokardial infarction and 1 pulmonary embolism. The male to female ratio amounts to 4:3. Estimating a frequency of approximately 10% there is no correlation to the usual parameters (age, pretreatment, platelet number) so that multiple factors may play a causal role in these complications.

71 Immuno Suppression and Lymphoretikular Neoplasms: Preceding Disorders of Kaposi's Sarcoma

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Epidemic Kaposi's sarcoma (Ks) is always combined with AIDS. Also the classic variant of Ks is not rarely correlated with other diseases, including hematologic and lymphoreticular neoplasms, autoimmune diseases and preceding iatrogenic immunosuppression. Two cases are presented exemplarily. A 30-year-old female patient had undergone renal transplantation because of chronic renal insufficiency. Five months later, during the following immunosuppressive therapy cutaneous Ks arose. A 67-year-old male patient showed classic form of Ks, typically localised in the lower leg. By routine laboratory examinations and bone marrow biopsy chronic lymphocytic leukemia could be detected, which likely had favoured the opportunistic appearance of Ks.

72 Non Hodgkin Lymphoma (NHL) of the Skin in Infancy and Childhood

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The primary isolated skin manifestation of malignant NHL in childhood is unusual and occures in less than 1% of cases. In our 4 patients (aged from 6 months to 9 years, 2 boys, 2 girls) the diagnosis of lymphoblastic lymphoma was based on biopsy studies of the skin lesions (primary tumor site: scalp 2×, praeauricular and groin 1×). The immunologic typing with monoclonal antibodies yielded various stages of differentiation of the B cell clone. Before chemotherapy was started 2 children developed bone marrow involvement. All patients received multiple agent chemotherapy (3 according to non B-NHL, 1 to the B-NHL protocol). 3 of them are in complete remission with follow up periods of 3, 12 and 18 months, 1 child died after the second bone marrow relapse. Due to the more specific immunologic diagnostic procedures we draw the conclusions that primary NHL of the skin in childhood derive from the B-cell clone.

73 Pulmonary Manifestations in Non-Hodgkin Lymphomas (NHL)

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Pulmonary manifestations with morphologic changes of chest X-rays were found in 28 of 336 patients with NHL, 13 men and 15 women. 10 of them had infiltrations of the lungs at the time of diagnosis of lymphoma. They had a lymphoma of low grade malignancy in 8 and of high grade malignancy in 2 cases. On the X-rays, there were nodules in 4, diffuse infiltrates in 4 and

local infiltrations in 2 cases. Chemotherapy led to a complete remission in 4 of these patients. In 18 patients, the manifestations in the lungs were found secundarily together with a rapid progressive course of disease. 11 of them died within 3 month after onset of lung changes. Histologically, there were 9 patients with a lymphoma of low and 9 with a lymphoma of high grade malignancy in this group. Morphologically the infiltrations were diffuse in 7, localy restricted in 6 patients, 5 patients had pulmonary nodules. In conclusion, pulmonary infiltrates in NHL are mostly secundarily and indicate a rapid progressive course of disease.

74 Differentiation Between Chronic Lymphocytic Leukaemia (B-CLL) and Non-Hodgkin Lymphomas in Leukaemic Phase by the Mouse Red Blood Cell Rosette Assay

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Distinction between B-CLL and other malignant B-cell lymphomas in the leukaemic phase may be difficult. Mouse red blood cell rosette formation of lymphocytes from 100 patients with B-CLL and 20 patients suffering from other B-cell lymphoproliferative disorders, independently of histological subtypes, was examined together with lymphocyte rosette formation of 30 healthy controls. Lymphocytes of B-CLL patients formed rosettes with mouse red cells in a significantly greater number (57.6%) than leukaemic cells of other non-Hodgkin lymphomas (2.14%) and lymphocytes from healthy persons (4.27%). There was no significant difference in the rosette forming cell proportion in different clinical stages of CLL. The relative simple mouse red blood cell rosette assay proved to be of value in differentiation of otherwise near related conditions.

75 Sezary Cell Leukemia with T8+ Suppressor Phenotype

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A 43 yrs old patient presented with fever, hepatosplenomegaly, lymphadenopathy, and a leucocytosis of 99,000/mm^3 with 86% lymphoid cells. There was no evidence of cutaneous involvement. Morphologically the pathologic cells were middle-sized with deep nuclear indentations. By electron microscopy they were Sezary cells with a cerebriform nucleus. The cells were pos. for acid phosph. and PAS, but neg. for POX and ANAE. Immunological phenotyping of peripheral blood cells, bone marrow cells, and lymph node cells revealed a T8+, T4−, T3+, HLA-DR+, TAC− membrane phenotype. However, no significant suppressor activity could be found by functional analysis (NK-activity, MLR, PHA stimulation, ADCC, influence on allogenic CFU-C). The disease had a rapidly progressive course with CNS involvement and lactic acidosis. Despite aggressive chemotherapy the patient died 3 months after diagnosis. In conclusion we believe that this represents the first example of a T8+ suppressor cell Sezary leukemia with lack of cutaneous manifestations but with an aggressive clinical course.

76 T-Cell Prolymphocytic Leukemia (T-PLL) with Unusual Surface Phenotype

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Malignant proliferations of cells committed to the T-cell lineage, although rare, attract great interest because the analysis of these disorders has helped to identify distinct stages of normal T-cell differentiation. Here we report on a 21 year old male, who was referred to us with excessive hepatosplenomegaly, ubiquitous peripheral lymphomas and a highly leukemic white blood cell

count of 233,000/μl more than 90% of which showed typical prolymphocytic morphology. Surface marker analysis by a wide panel of monoclonal antibodies revealed a unique T-cell phenotype not yet reported in the literature: T1–, T3+, T4–, T6–, T8–, T11+, T12+, 9–3+, Leu8–, TQ1+, TAC–; B1–, B4–, CALLA–; VEP13+, 3G8+, Leu11a+, Leu11b+, Leu11c+; Leu7–, NKH2–, NKH1A+, OKM1+; OKT9–, OKT10–, HLA-DR–, My9–. The surface marker profile remained unchanged after 48 h in vitro culture except for the loss of some distinct epitopes of the Fc-receptor molecule (Leu11c, VEP13). The T-PLL cells were inactive in K- and NK- in vitro assays. The significance of these findings for T-cell differentiation will be discussed.

77 Lymphocytic Lymphoma with a Three-Band Gammopathy: Reactivity of One of these Paraproteins with Cytomegalovirus (CMV)

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We report the case of a 70-year-old woman with lymphocytic lymphoma of the lung, abdominal lymph nodes and the bone marrow. Serum electrophoresis showed an M-component and immunofixation revealed the presence of three monoclonal bands in the IgG, IgA and the IgM lane with lambda light chains. Because of the patient's high antibody titre against CMV (1:5120), the possible reactivity of the paraproteins with CMV was investigated. The immunoglobulins were transferred to nitrocellulose sheets by a contact diffusion blotting technique. CMV was applied to these sheets and the IgG-paraprotein was shown to bind CMV using peroxidase-stained antibodies against CMV. The reactivity of only one of the paraproteins with CMV suggests an oligoclonal origin for this gammopathy although only lambda-chains were present. Thus, in addition to the lymphoma the cause of this gammopathy could have been an abnormal immune response to an infection with CMV.

78 Frequency and Prognostic Value of Mitoses in Non-Hodgkin's Lymphomas

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We correlated mitotic activities histological diagnoses and survivals of 300 cases of malignant lymphomas. With the exception of lymphoblastic and T-cell lymphomas, the numbers of mitoses increased in parallel with the nuclear sizes. There was no correlation between a plasmacytoid differentiation and the number of mitoses. Despite the low grade of malignancy of follicular lymphomas, these exhibited greater mitotic counts than did diffusely growing lymphomas with the same grade of malignancy. Large cell lymphomas with round nuclei had only slightly more mitoses than had cases with cleaved nuclei. A subdivision of the 300 cases into three groups on the basis of mitotic frequencies alone resulted in survival curves that were significantly different from each other. We conclude that the mitotic activity is a useful prognostic parameter.

79 Chop-Firstline Treatment in NHL with Unfavorable Prognosis – A Study of the I.G.C.I.

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78 newly diagnosed patients were treated immediately after clinical staging with an age adjusted CHOP-schedule. Median age of the patient population was 56 years (range 22–84). Histologic diagnosis according to Kiel classification was CC large cell type: 11 pat., CB: 9 pat., IB: 18 pat., LB: 9 pat. (patients with bone marrow involvement > 30% blasts, LB of convoluted type and Burkitt-type were excluded), high grade NHL unclassified: 11 pat. Overall response rate was 85%. CR was achieved in 42 patients. No drug related death occurred. 54 pat. are alive (7^+–38^+ months). Extranodal involvement was seen in 33/78 patients. Localized disease (stage I and II E) was seen in 13/33 patients (GI: 7, Testes: 3, Epiduralspace: 1, Lung: 1, Thyroid: 1). It seems remarkable that patients with CC showed a poor outcome despite early aggressive treatment. A risk factor score proved its usefulness in predicting therapeutic response.

80 M-BACOD Chemotherapy in Advanced Stages of High Grade Malignant Lymphomas

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Nine consecutive patients with high grade malignant lymphoma (Kiel classification) at stage III and IV (Ann Arbor) received M-BACOD chemotherapy (3 g methotrexate/m^2). The patients (3 females, 6 males, mean age 47,9 years, range 17–77 years, 5 stage III, 4 stage IV, 4 centroblastic, 4 immunoblastic, 2 lymphoblastic) had no prior treatment and were treated with 6 cycles at 3 weeks intervals. 6 patients had a complete remission, 2 patients are not yet evaluable, 1 patient died while on treatment. 1 patient relapsed 2 month after cessation of therapy, 5 patients are still surviving relapse free. Dose adjustments due to bone marrow toxicity were necessary in 3 patients only. Mucositis and nausea were frequent complications. 1 early death probably was related to therapy. Because of the short duration of the study (1 year) no conclusions as to remission duration can be drawn.

81 T-Cell Lymphomatoid Papulosis Treated with Acyclovir Associated with M. Hodgkin

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We are reporting on a case of M. Hodgkin who developed during chemotherapy according to the MOPP-scheme a T-cell lymphomatoid papulosis. The patient received six times a 5-day treatment with Acyclovir. This treatment was not administered at the same time as the cytostatic therapy. Each time remission, several times even complete remission of the lymphomatoid papulosis could be achieved. Possible connections between M. Hodgkin, T-cell related diseases and virus infections are discussed.

82 Therapy of Burkitt's Lymphoma

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From 6/79 till 2/85 we treated 9 patients (average age 38) with Burkitt's Lymphoma. 7 presented with stage D disease, 1 with stage C and one with stage A. The main presenting symptom was abdominal pain or a rapidly progressing abdominal tumor. 3 patients had bone marrow involvement and 2 had a Burkitt's leukemia. 3 had typical chromosomal aberrations. Therapy consisted of a variety of chemotherapy regimens plus additional radiotherapy and/or surgery. 3 patients achieved complete remissions (median duration 5 month), and 3 partial remissions were obtained. The remaining patients had either progressive, drug-resistant-disease or died early. Only 1 patient is currently alive and in complete remission. The main causes of death were tumor lysis syndrome (4 patients) and therapy related sepsis with progressive tumor (4 patients). This poor outcome is probably due to a high proportion of high-risk patients and suboptimal therapy for this rapidly proliferating tumor.

83 Lymphocyte-Subpopulations in Patients with Malignant Non-Hodgkin-Lymphoma (NHL)

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In thirty patients with NHL of different grades of malignancy the lymphocyte-subpopulations were measured by indirect immunofluorescence microscopy. A slightly decreased number of T-helper cells and a normal range of T-suppressor cells were found in most cases, this could not be correlated to the clinical stage or grade of malignancy. High proportions of maturating and mature B-cells covered by IgG on cell surface were found in patients with low-grade NHL, re-

vealing a possible leukemic dissemination even if the morphology of pheripheral blood cells seemed to be normal. The same antigen-pattern was found in bone-marrow (BM) of patients with low-grade NHL and BM-infiltration whereas in patients with high-grade NHL no specific antigen-pattern could be demonstrated.

84 Vinblastine Induced Thrombocytopenia During Polychemotherapy of Disseminated Testicular Cancer

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In 67 patients with testicular cancer treated with 187 courses of a combination chemotherapy (VBL, BLEO, CDDP) a transient, early onset thrombocytopenia has been observed. At the third day the platelets decreased from 270 ± 90/nl to 100 ± 50/nl ($p < 10^{-10}$). In 10 patients platelet survival studies were performed showing a reduced platelet survival during chemotherapy of 50 ± 20 hours ($p < 0.001$) compared to 100–115 hours of controls. A shift of the proportional accumulation in liver, spleen or lung could not be demonstrated. The electron microscopic examination of the platelets showed a deformation with a loss of the intracellular microtubules within a few hours. These findings in combination with other clinical and experimental data indicate that mainly vinblastine is responsible for the deformation of the platelets and the increased intravascular degradation.

85 Colony Formation and Antioestrogen Sensitivity of Human Ovarian Carcinoma Cells Tested in an Agar Double Layer and in a Methylcellulose Monolayer Assay

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Human ovarian cancer cells from 10 patients were cultured in the agar double layer assay and a methylcellulose monolayer system. Plating efficiency and growth rate in the methylcellulose assay and in the agar double layer proved to be similar. Cytologic and cytochemical staining from plucked cells out of colonies from both test-systems and from the tumor cells prior to plating, revealed the same morphology. In addition the effect of Tamoxifen was tested in both systems. At a concentration of 10^{-6} Mol/l an inhibition of colony formation of more than 70% of controls was observed in the agar and in the methylcellulose system. Compared to the agar double layer system the methylcellulose monolayer assay needs less additives. Furthermore, it is easier to handle with respect to the plating procedure and less time consuming.

86 In Vitro Activity of Triglyzidylurazol and Tiazofurin

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Triglyzidylurazol (TGU) and tiazofurin (TF) are new cytostatic drugs, which at present are being evaluated in clinical trials. We examined the in vitro activity of TGU and TF on human bone marrow (BM), 5 experimental murine tumors, and on human tumors propagated as xenografts in nude mice. The evaluation was done by the clonogenic assay in a modification of the method of Hamburger & Salmon. Effectivity was defined as a > 70% reduction of colonies as compared to control. TGU at the concentration of 0.3 μg/ml in continuous exposure, which was supposed realistic for predictive purposes, was effective in 2/3, BM, 5/5 murine tumors, and in 7/37 tumors of human origin, namely in 3/12 bronchogenic carcinoma, 1/8 colorectal tumors, 1/4 melanoma, 1/1 mesothelioma and 1/1 ovarian cancer. TF gave efficacy at the supposed realistic concentration of 1 μg/ml in 3/3 BM, 3/5 murine tumors, and 2/30 xenografts; 1/4 melanoma and 1/1 ovarian cancer. If a reliable drug concentration is determined in-vitro signal tumors for clinical phase I and II studies may be identified by the clonogenic assay.

87 In-vitro Activity of Five Clinically Used Cytostatic Agents on Human Tumor Xenografts in the Colony Assay

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The cytostatic activity of Adriamycin, Etoposide, Mitomycin-C, Cis-Platin, and Vindesine has been studied on a number of different tumors over a wide dose range. Tests were performed with the modified Tumor-Colony-Assay of Hamburger Salmon under continuous drug exposure. Human xenografts growing in athymic mice were used as tumor material.

There was a clear relationship between frequency of responsive tumors and drug dosage. Comparisons with clinically achievable response rates allowed the determination of the "relevant dose" in-vitro. The following "relevant doses" have been determined: Adriamycin 0.03 μg/ml, Etoposide 0.3 μg/ml, Mitomycin-C 0.015 μg/ml, Cis-Platin 0.1 μg/ml, Vindesine 0.01 μg/ml. The reproducibility of the in-vitro results could be confirmed. If obtained data under continuous drug exposure will agree better with clinical response rates than data under 1 h exposure is still to be demonstrated.

88 Detection of a New Tumor-Associated Carbohydrate Antigen (CA 50) in the Serum of Carcinoma Patients

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A solid phase radioimmunoassay was devised for measuring the value of the carcinoma associated carbohydrate antigen CA 50 in serum based on the use of a specific monoclonal antibody (C 50). Samples of serum from patients with carcinoma, patients with other malignancies or inflammatory diseases, and healthy controls were examined. Serum values of CA 50 exceeding the mean plus three standard deviations for control samples from blood donors were found in a high proportion of patients with colorectal adenocarcinomas, other gastrointestinal carcinomas, lung cancer, gastric carcinoma as well as pancreatic carcinoma. The CA 50 values in samples from patients with inflammatory diseases, including ulcerative colitis, pneumonia and acute pancreatitis with some exceptions were within the normal as were those in patients with various sarcomas and malignant melanomas.

89 Comparing Investigations on the Diagnostic Validity of Several Tumor Markers: CEA, CA 19–9 and CA 50

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The diagnostic validity of several tumor markers (CEA, CA 19–9 and CA 50) is clinically established for epithelial tumors, especially in colon, pancreatic, stomach carcinoma and metostotic lung tumors. The diagnostic validity of the new tumor marker CA 50 (carbohydrate antigen) is analysed in comparing investigations. Data on specificity and sensitivity of several markers are presented. A very good correlation exists between CA 19–9 and CA 50 ($r = 0.8$) especially in epithelial tumors. The correlation is significantly smaller in malignant lymphomas (0.5) and in inflammations (0.6). The correlation coefficient in carcinomas of the stomach and of the colon is 0.8, and in lung carcinomas it is 0.7.

90 Thoracoscopy: Comparison of the Diagnostic Value of Histology and Immuncytology

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The value of immuncytochemical methods in clinical diagnosis of effusions is not proven up to date, especially because the true diagnosis was not ascertained by histology. Therefore, we

investigated effusions by immuncytochemical methods in patients examined by thoracoscopy. About 40 patients suffered from pleural effusions caused by malignancy or by non-malignant disorders. In each case thoracoscopy and cytological and histological investigation of exsudate cells and histology of biopsy material from the pleura were done. Using an indirect immunoperoxydase method, we examined the Ficoll-separated cells with monoclonal antibodies reactive with cytokeratin, vimentin, EMA and different leucocyte- and melanoma antigens; in addition polyclonal antibodies against CEA and transferrin were applied. The obtained results were compared with conventional cytology and histology of biopsy from the pleura.

91 Quantitative Evaluation of X-Ray Films with a Stereological Point Grid for Therapeutic Control of Lung Metastases

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In certain circumstances quantitative evaluation of X-ray and other images can be useful for assessing the efficacy of new antitumoral agents. In this study the simple method of stereological point counting has been used to estimate the total projection of lung metastases. A circular plexiglas plate (diameter 55 cm) was inscribed excentrically with an isometric point grid of equilateral triangles of side 3 cm. The area equivalent to one point is 7.8 cm^2. X-ray films of single lungs were laid upon a light box and the plate randomly placed upon the film. All points coinciding with lung or metastases were counted. The grid was tested on tracings of lungs or metastases as well as on the films themselves. The results were compared with those obtained with a semiautomatic measuring device.

92 New Protocol for Echocardiographic Diagnosis of Adriamycin-Induced Cardiomyopathy

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We investigated, whether echocardiography (E) during increased afterload stress allows earlier detection of Adriamycin (AD)-induced cardiomyopathy than E at rest. We compared 25 normals (N) and 24 cancer patients (P), who had received at least 400 mg/m^2 of AD. During infusion of Angiotensin II (A II) (3000 ng/min) rise of blood pressure was monitored by cuff method, while left ventricular (LV) diameters were recorded by E (M-mode). LV function was judged by the slope K of the linear relation between systolic blood pressure and simultaneous endsystolic LV diameter. Individual coefficients of correlation for K were at least $r = 0.83$. In N values of K were 6.9 ± 1.7, range 4.9–11.9 mmHg/mm. In 6 P LV function was impaired at rest and K was clearly abnormal (3.3 ± 1.1, range 2.2–4.6 mmHg/mm, $p < 0.05$ vs. N). In the remaining P LV function was normal at reset, but K was significantly lower than in N (5.2 ± 2.3, range 2.4–8.9 mmHg/mm, $p < 0.05$). 7 of these P had values of K below the normal range. In one such P AD was continued and clinical, scintigraphic and echocardiographic signs of LV failure ensued. We conclude, that AD-induced cardiomyopathy can be detected earlier when E is combined with afterload stress by A II.

93 Is There an Increased Risk of Thromboembolic Complications in Patients with Malignancy Following Intravenous Application of Radiographic Contrast Medium?

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To assess whether contrast media influence platelet activity or thrombin generation in vivo, we studied 50 pts. (among them 30 with neoplasia) by evaluating plasma levels of β-thromboglobulin (βTG), platelet factor 4 (PF 4) and fibrinopeptide A (FPA) immediately before and at 5 and 30 min after a bolus injection of meglumine glicinate (Rayvist®, 1.5 ml/kg body weight).

In the *20 pts. without malignancy* mean basal levels (± SD; ng/ml) of βTG (29.1 ± 7.3), PF4 (3.5 ± 1.7) and FPA (3.3 ± 2.4) did not differ significantly from those at 5 min. (βTG 27.8 ± 6.5; PF4 3.3 ± 1.6; FPA 2.7 ± 1.4), or those at 30 min. (βTG 32.1 ± 6.7; PF4 3.2 ± 2.0; FPA 3.2 ± 1.1). Among the *30 pts. with neoplasia,* 7 revealed an increased platelet activity prior application of Rayvist (βTG 48 ± 9; PF4 10 ± 7 ng/ml; vs. normals $p < 0.001$). Following the injection of contrast medium, neither in these 7 pts., nor in the 23 other subjects with malignancy a significant change in platelet secretion or thrombin generation occured. We conclude that intravenous application of contrast medium does not lead to an additional risk of thrombotic or thromboembolic complications in pts. with malignancy.

94 Diagnostic – Therapy – Long Term Observation of Lymphedema Following Cancer Treatment

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Lymphedema following surgery and/or radiotherapy of malignant tumors often complicates cancer treatment. About 50% of the women treated for breast cancer develop lymphedema of the corresponding arm. Radical dissection of inguinal lymph nodes almost always results in lymphedema of the leg. Even less aggressive surgery and a more sophisticated irradiation technique can only reduce the extent, not the incidence of lymphedema. 614 (100%) patients with lymphedema caused by tumor therapy were treated with a complex decongestive physiotherapy (manual lymph drainage, compressif bandaging, remedial exercises). Lymphedema developed after treatment of the following tumors: breast cancer 518 (84%), gynecological tumors 48 (8%), malignant melanoma 18 (3%), other tumors 30 (5%). In 131 cases lymphedema was produced by progressive tumor growth. Lymphedema was the first sign of tumor recurrence in 63 patients. A reduction of the edema by more than 50% was achieved in 325 (53%) patients. 178 (29%) patients had size reduction of edema from 50% to 25%, less than 25% reduction was seen in 73 patients. In 36 (6%) patients treatment was ineffective. Long term observation (> 3 years) was done on 221 patients. The effect of the treatment was maintained or improved in 121 patients, 98, however, showed deterioration, 25 of whom reached or surpassed the extent of edema before primary therapy.

95 Advantages and Disadvantages of a Totally Implantable Infusion System (PORT-A-CATH) for Cancer Patients

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26 Port-A-Cath (PAC) infusion systems were implanted in 24 patients (20 solid tumors, 2 lymphomas, 2 acute leukemias) requiring repeated or long-term chemotherapy with the catheter tip in the superior vena cava. PAC was implanted because of poor venous status (14) or to facilitate chemotherapy on an outpatient basis (10). Mean catheter duration was 172 days (range: 16–461 d; total observation period: 4454 patient days). 12 PAC's are still in use. PAC was used for chemotherapy, intravenous nutrition, transfusions and blood samples. Mean number of punctures was 27 (range: 7–112). The following complications were observed: 3 PAC occlusions, which required surgical revision (SR); 2 skin lesions in the region of PAC (1 SR); 2 periods of sepsis in patients with myelosuppression with 1 SR and 1 PAC-related death; 2 periods of thrombosis (2× urokinase treatment and 1 SR of a pseudoaneurysm); 1 dislocation of the catheter tip (SR since arrhythmia occurred); 1 paravasation. Only 2 of these 11 complications could be primarily attributed to the PAC (2× thrombosis). All other complications were at least partially due to difficulties during implantation or to treatment related problems. Acceptance and judgement was favorable by most patients (20 + , 4−) and physicians (8 + , 1−). The PAC provides major progress, since it allows a greater availability of chemotherapy to patients. However, careful training of those involved in the use of PAC is necessary.

96 Tunnelled Central Venous Catheters (Performed Without a Venotomy) in the Oncologic Patient

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Supportive measures in the aggressively treated oncologic patient need a reliable and uncomplicated vascular access. Provided that careful catheter insertion and sterile catheter handling is used, we have recently shown (Pädiat. & Pädol. 19, 418, 1984) that central venous catheters do not bear an increased risk of infection in the neutropenic patient. Twenty patients had a percutaneous subclavian vein puncture from the infraclavicular area using a leader cath system with a metal spring guide (Vygon®) according to the Seldinger-method. The metal wire was then guided through a semicircled subcutaneous tunnel performed from a second needle insertion at the upper parasternal region. The catheter was then placed via the wire into the right atrium and completely buried into the subcutaneous tunnel. Catheters were used for blood drawing as well as for infusion of therapeutic agents and blood products over a median duration of 22 days (3–40). Outpatient care was maintained in most of the patients. With this new technique material-related complications as catheter leaks were not observed anymore and the central catheters could be effectively used on a long-term basis in the majority of the patients.

97 Effects of Aminoglutethimide in a Human Testicular Tumors Model

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Aminoglutetimide (AG), a aromatase inhibitor, has successfully been used in the treatment of estrogenproducing adrenal carcinomas. Purpose of this investigation was to examine the effects of AG in a steroidhormone producing non-seminomatous human testicular tumor model. 4 human testicular tumor cell lines, established in tissue culture, were injected subcutaneously into nude mice or growing tumors were serially transplanted. Growing tumors ($n = 64$) with at least 0.5–1 cm^3 volume were treated with AG (0.42 mg/d/mouse). AG was delivered through intraperitoneal implanted mini osmotic pumps (Alzet). 43% (28 of 64 tumors) showed a significant growth delay compared with untreated tumor bearing control animals ($n = 35$). 15 of these 28 (53%) tumors showed a significant decrease of the tumor volume, good comparable to results obtained with active cytostatic drugs like Ifosfamide in this model. This antitumor effect is dose dependent.

98 Benefit and Costs of Follow-Up Programs in Non-Seminomatous Germ Cell Tumors (NSGCT) of the Stages IIb–IV

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We have evaluated 185/197 patients with NSGCT (stages IIb–IV). The majority was treated with PVB. 138/185 (75%) achieved a 1st CR (131/185 = 71% currently with NED). All patients in CR have been followed by physical examinations, blood chemistry including tumor markers, and chest x-ray (year 1 + 2: 8 ×, year 3: 2 ×, year 4 + 5: 2 ×) and by abdominal CT (year 1 + 2: 6 ×, year 3: 2 ×, year 4 + 5: 2 ×) to a total of 326 patient years (approx. DM 650,000). 18/138 patients (13%) were found in the 1st relapse. 13/18 achieved a 2nd CR, 2/13 were found in a 2nd relapse. 1/2 was brought into 3rd CR. 1 patient died in CR from reasons unrelated to NSGCT. The 20 1st and 2nd relapses were detected with clinical symptoms (6 ×), chest x-ray (6 ×), abdominal CT (5 ×) and tumor marker elevation (3 ×). Only 1/6 patients complaining of clinical symptoms as first sign of the 1st relapse achieved a 2nd CR, and 5/6 died from progressive disease, whereas all 12 patients with 1st relapses detected without clinical symptoms during follow up could be brought into 2nd CR. These data tend to argue in favour of close follow-up programs. 15/18 1st relapses (83%) were found within the first two years and 3/18 later. Since all 3 patients detected with a late recurrence achieved a 2nd CR 5 years of follow-up seem justified.

99 Intraperitoneal Immunotherapy in Patients with Therapy Resistant Ovarian Cancer Ascites

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In a pilot study, patients with therapy-resistant ovarian cancer ascites received intraperitoneal injections of the immunomodulator OK-432 after centhesis of ascites fluid. After weekly injections of OK-432 a complete remission (CR) of ascites fluid was achieved in 4 out of 7 patients (duration 3 months in 2 pts, 6 months in 1 and 12 months in 1 pt). It was attempted to maintain the remissions by intradermal injection. In another 2 pts ascites volume was reduced for more than 50% for 4 and 1 months, respectively. In 1 pt no response was achieved. Patients with CR of ascites are still alive after 13, 12, and 7 months (2 of them with NED). The results of our investigations point to an involvement of immune reactions in these therapy responses.

100 Stimulation of Natural Killer (NK) Activity of Lymphocytes from Peripheral Blood (PB) and Lymph Nodes (LNS) by Interferon (IFN) in Patients with Cancer of the Oral Cavity

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NK activity plays an important role in tumor defense mechanisms. We have determined NK activity in PB and tumor draining LNs of patients (pts) with cancer of the oral cavity. Pts with tumor stage T_{1-2} had NK activity in PB comparable to that of normal donors, whereas those in T_{3-4} had significantly lower levels of activity. In LNs, reactivity was always lower in comparison to PB of identical donors. Preincubation with IFN resulted in an augmentation of NK activity of PB- and LN-derived effectors, whereas in the latter the amount and frequency of augmentation were much lower. The clinical significance of spontaneous and IFN-induced cytotoxicity has to be determined by further follow up of the patients.

101 First Experience with DDP in Combination with 5-FU/VP 16 as Continuous Venous Infusion

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Continuous venous infusion offers a method of achieving a prolonged plasma concentration of antineoplastic agents with relatively short half lives. Cis-platin has become a widely used antineoplastic agent (t/2 32 minutes). The drug administered with a bolus shows typical associated toxicities of acute nausea, vomiting and renal dysfunction.

In animal studies synergistic effects of DDP with 5-FU and VP 16 are known. Since Oct. 1984, 35 patients with various forms of cancer were given a five-day continuous venous infusion containing DDP, 5-FU, as well as, VP 16. Acute nausea and vomiting were drastically reduced. Renal toxicity seems to be reduced also, with retention of neoplastic activity in several kinds of solide tumors. Loss of electrolytes was also seen. An analysis of our extensive pretreated patients, including toxicity and antineoplastic activity of the chosen regimes, will be presented. More than 50% of the patients benefited from the therapy.

102 Results of Chemotherapy in Advanced Gastric Carcinoma

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The combination of 5-fluorouracil, doxorubicin and mitomycin (FAM) designed by Macdonald et al. (1980) has shown some activity in advanced gastric cancer according to the results from several institutions. Treatment with the FAM protocol was started in our institution in 1981. 31

patients treated with this protocol were evaluable. 9 of the patients (29%) responded to the treatment, 2 of them obtained a complete remission, 7 of them a partial remission. The median survival of this group was 51 (24–120) weeks. 7 patients (23%) were considered as non progressive, their median survival was 34 (8–47) weeks. 15 patients (48%) had progressive disease. Their median survival was 16 (2–34) weeks. Patients with a higher performance status (Karnofsky index) were more likely to respond or show no change as compared with those with a lower performance status. Gastrointestinal and hematological toxicity of the cytostatic drugs was tolerable. Our results are in agreement with the initial report of Macdonald and demonstrate a clear survival advantage for patients responding to treatment.

103 High Efficacy of Etoposide (VP-16) and Cisplatin (CDDP) in SCLC – A Phase II Trial

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After a dosage finding study we have conducted a phase II trial with Etoposide (170 mg/m^2, i. v., days 3, 4, 5) and CDDP (50 mg/m^2, i. v., days 1, 7) in pts with SCLC. Since Jan. 1984, 35 pts have entered the study. At present 32 pts are evaluable for response and toxicity. Eligibility criteria included age $\leq$ 70 yrs., Karnofsky PS $\geq$ 40%, no pretreatment, normal renal-, liver- and bone marrow function. Mean age was 57 yrs. (40–70), mean PS 70% (40%–90%). 25/32 pts had ext. dis., 7/32 lim. dis. *Results:* the overall response rate (CR + PR) was 97% (31/32) (96% in ext. dis., 100% in lim. dis.). CR could be achieved in 66% (21/32) pts, (60% [15/25] in ext. dis., 86% [6/7] in lim. dis.). Toxicity was primarily hematologic. A leukocytopenia of WHO grade 3 and 4 was seen in 69% and 25% of the pts. Severe but manageable infections occurred in 3 pts. A thrombocytopenia of WHO grade 3 and 4 was seen in 9% and 6% of the pts., respectively. So far there was no toxic death. 7 pts have died. The others are alive from 3+ to 17+ months. Updated survival data and detailed response and toxicity data will be presented.

104 Treatment of Aplastic Anemia with Antilymphocyte-Serum (ALS)

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We treated 6 patients with aplastic anemia. All patients were pretreated with steroids and anabolics, 5 of them without, another only with transient effect. 40 mg/kg ALS were given for 4 days combined with prednisone in declining dosage and oxymetholone 5 mg/kg daily. Prophylactic platelet transfusions were given at the days with ALS treatment. 4/6 patients responded up to now, one of them transient however. Following pattern of regeneration was observed: rise of peripheral granulocytes occurred first, then regeneration of erythropoesis and finally increase of platelet-counts. Side effects were mostly due to prednisone and oxymetholone with increasing serum transaminases, steroid acne and Cushing-facies. 1 patient was agitated due to high doses of prednisone and 1 patient had life threatening gastrointestinal bleeding. There were only minor side effects due to the ALS: fever, exanthema or serum sickness.

105 Idiopathic Sideroblastic Anemia – Preleukemia or Benign Disorder of Erythropoiesis?

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The incidence of leukemia in patients with idiopathic sideroblastic anemia considerably varies in reported series. Using morphological criteria, the disorder can be devided into 2 groups: (1) 'true' sideroblastic anemia (SA), (2) sideroblastic anemia with dysplastic changes of the granulopoietic and megacaryocytic cells (RARS). In a retrospective study including 42 patients (SA 18, RARS 24), hematological findings, natural course of disease and incidence of leukemia were examined in both groups. Percentage of ringed sideroblasts and pattern of iron deposits were

not different. PAS-positive erythroblasts were found only in patients with RARS (70%). In vitro marrow culture studies showed decreased growth of CFU-GM in patients with RARS ($n=4$), but results were not significantly different from patients with SA ($n=4$). The cumulative 5-year survival rates were: SA 62 ± 15, RARS 24 ± 12% ($p < 0.01$). Main complication in patients with SA was congestive heart failure, whereas patients with RARS often died of infections or leukemia. The cumulative rates of leukemia after 5 years were: SA 14 ± 4, RARS 62 ± 16% ($p=0.038$). Our results show that SA and RARS are heterogeneous disorders with regard to prognosis and pathogenesis. The incidence of leukemia in 'true' sideroblastic anemia is low.

106 Myelodysplasia Preceding CML

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Out of 93 patients with chronic myeloid leukemia (CML) which have been observed in this department in the last 10 years in two cases evolution of CML from a myelodysplastic syndrome could be demonstrated. The myelodysplastic phase in the two patients lasted 25 and 16 month resp. In both cases transfusion requiring anemia and leukopenia were the prominent features of myelodysplasia whereas platelet counts showed normal values. With rising granulocyte numbers and development of CML resp. an increasing thrombocytopenia was found. Transition of myelodysplasia into CML is proved in both cases by multiple cytologic and histologic specimens. Cytogenetic investigations revealed in one of two cases during CML a Philadelphia chromosome. In comparison with transition into acute leukemia the evolution of myelodysplasia into CML is a rare event. The courses of disease and hematological data of both cases are discussed in detail.

107 Low Peripheral Levels of CFU-GM in Patients with CML and Excessive Thrombocytosis

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In 14 patients with Philadelphia-chromosome positive chronic myelogenic leukemia (CML), the peripheral concentrations of CFU-GM (colony forming units – granulocytic, monocytic) were evaluated before, during and after therapy with Au^{198} and Busulfan. Nine patients revealed the expected high CFU-GM levels in the peripheral blood, which decreased during therapy, paralleling the peripheral leucocyte count. However, 5 patients with excessive high platelet count showed unexpected low CFU-GM concentrations within or below the normal range. These findings may be due to a direct inhibitory effect of the platelets on the myeloid stem cell pool or to a competitive reduction of the myeloid stem cell pool in favour of the CFU-Meg (colony forming units – megakaryocytic).

108 Circulating Progenitor Cells in Hairy Cell Leukaemia

K. Geißler, W. Hinterberger, P. Bettelheim, E. Neumann, I. Schwarzinger, K. Lechner (1st Department of Medicine, Division of Haematology and Blood Coagulation, University of Vienna, A-1090 Vienna, Austria)

In order to get insight into the mechanism of pancytopenia in hairy-cell leukaemia (HCL), we assayed granulocyte macrophage committed progenitor cells (CFU-GM), erythroid committed progenitor cells (BFU-E) and pluripotent haemopoietic progenitor cells (CFU-MIX) in the peripheral blood of 14 patients with hairy cell leukaemia. In 8 HCL patients retaining their spleens numbers of circulating CFU-GM, BFU-E and CFU-MIX were under the lower limit of normal controls in 6, 6 and 5 cases, respectively. 6 splenectomized HCL patients had generally more circulating progenitor cells than their nonsplenectomized counterparts. In none of 3 patients treated with recombinant α_2-Interferon (α_2-IFN), therapy induced a persistent normalization of circulating progenitor cells despite normal peripheral blood count. Our results suggest, that a

reduction of the committed and pluripotent progenitor cell compartment might be at least in part responsible for the pancytopenia in the majority of patients with HCL and that a compensated haemopoietic deficiency remains after α_2-IFN treatment even in patients with clinical remission.

109 Circulating Progenitor Cell Levels in Patients with AML and ALL During and After Chemotherapy

K. Geißler, W. Hinterberger, K. Lechner (1st Department of Medicine, Division of Haematology and Blood Coagulation, University of Vienna, A-1090 Vienna, Austria)

In order to assess the reconstitutive capacity of peripheral blood from AL-patients for autografting, we determined CFU-GM, BFU-E and CFU-MIX in the peripheral blood of 16 AML-patients and 11 ALL-patients at various times during and after chemotherapy. In 2 AML-patients being in very early complete remission after induction chemotherapy circulation progenitor cells of all types rose markedly above the mean value in normal subjects. In 1 AML-patient, who did not enter complete remission, such a peak could not be observed. In 8 AML-patients on maintenance chemotherapy circulating CFU-GM were significantly ($p < 0.01$) decreased compared with normal subjects. 5 AML-patients being off chemotherapy had likewise reduced CFU-GM, BFU-E and CFU-MIX ($p < 0.05$), respectively. In 9 ALL-patients on maintenance chemotherapy numbers of circulating CFU-GM, BFU-E and CFU-MIX were decreased ($p < 0.01$). Cycles of vincristine and prednisolone caused a tenfold expansion of circulating CFU-GM, whereas no increment of BFU-E and CFU-MIX could be observed. In 2 ALL-patients being off chemotherapy, all progenitor cell classes were found to be in the range of normal controls. Our results suggest, that PB from AML-patients in very early complete remission seems to be a good stem cell source for autografting.

110 Rapid Separation of Megakaryocytes from Human Bone Marrow

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The analysis of megakaryocytes is so far hampered by the rarity of these cells in bone marrow. Using gradient centrifugation and fluorescence activated cell sorting, we have isolated highly purified (> 98%) human megakaryocytes: Marrow cells ($\leq$ 1.05 g/ml in Percoll) were labeled with monoclonal antibodies against platelet-associated antigens. Positive staining cells (0.4–1.7%) were sorted and identified as megakaryocytes, revealing all stages of cellular maturation. Poly-L-lysine coated wells on glass slides allowed sorting on a single cell basis directly onto the small wells. A requirement for successful sorting is the use of a medium that inhibits platelet aggregation (CATCH-medium). No increase in recovery could be achieved by the addition of prostaglandins or antibodies to Mo 1 antigen and fibrinogen receptor.

In addition to morphological analysis, the ability to separate megakaryocytes with defined antigenic characteristics or distinct ploidy classes (when labeled simultaneously with DNA stains) might provide a useful tool for biochemical or multiparameter studies. Moreover, the possibility to sort cells directly onto small wells might permit molecular biology analysis at the single cell level.

111 Influences of Target Cell Growth Conditions on Conjugate Formation and Chemoluminescence in Human Spontaneous Monocyte Cytotoxicity

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Natural killer cell activity has recently been shown to depend upon culture conditions of tumor target cells. Similar observations have so far not been reported in human spontaneous monocyte cytotoxicity. Using a modified single cell cytotoxicity assay in agarose we analyzed lytic and non-lytic conjugate formation between freshly isolated monocytes and human tumor cell lines

(K 562, Molt 4, HL 60) which had been cultivated for different periods of time at varying density. In addition, chemoluminescence following effector-target cell interaction was monitored on a LKB liquid scintillation counter. It was shown that the target cell growth rate considerably influenced the pattern of conjugate formation with effector monocytes in agarose. These results corresponded with differences in intensity and kinetics of chemoluminescence. Possible relations between target antigens of natural killer and spontaneous monocyte cytotoxicity will be discussed.

112 Effects of Dexamethasone (DXM) on Natural Killing and Growth Inhibition of K 562 Cells

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Recently dexamethasone (DXM) has been shown to enhance colony growth in human tumor stem cell assays. A direct stimulating effect on tumor cells as well as effects on immunologic effector cells with growth modulating capacity which are present in the testing systems have been discussed. To evaluate the effect of DXM on these immunologic bystander cells we preincubated monocyte-depleted PBMC in DXM ($10^{-6}-10^{-3}$ M) containing medium or in medium alone (controls) and cocultivated them with the erythroleukemic cell line K 562 in a semisolid 0.5% agarose medium. It was shown that DXM treatment of effector cells resulted in a dose dependent reduction of lytic effector-target cell conjugates (natural killing). Furthermore, growth inhibition of conjugated K 562 cells by DXM treated effector cells was significantly diminished resulting in enhanced tumor growth rate during a 24 to 48 h observation period. Possible clinical implications may concern the use of DXM as an antiemetic drug in certain chemotherapy regimens.

113 Effects of Mitoxantrone on Human Hemopoietic Precursor Cells

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Belonging to the synthetic class of anthraquinones, Mitoxantrone binds to DNA thus inhibiting nucleic acid synthesis. When applied in patients with breast carcinoma this drug seems to be as effective but less toxic as Doxorubicin. The major dose-limiting toxicity, however, is myelosuppression, especially leukopenia. Therefore, the effects of this drug on human haemopoietic precursor cells have been investigated. Survival of human granulocyte-macrophage colony-forming cells was determined either after Mitoxantrone preincubation or after permanent incubation of human bone marrow and peripheral blood cells, respectively. For both incubation schedules, our results so far indicate toxic effects of even less than 1 ng/ml Mitoxantrone.

114 Do Cytostatics Destroy Hemopoietic Regulation? – Suggestions for an Experimental Evaluation

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It is known that cytostatics destroy immature hemopoietic cells (CFU-S, CFU-GM, CFU-E etc.). Various drugs can be applied in such a dose that they destroy an equal amount of these cells. Depending on the drug used hemopoietic recovery may, however, differ considerably. Anything from an exponential growth after Hydroxyurea to a very prolonged recovery after busulphan was seen. These observations can consistently be interpreted if one postulates a damage of the homeostatic stem cell regulation. Attempts to quantify the effects by virtue of a mathematical model reveal the incompletness of the present findings. We suggest new experiments which in addition allow a more careful examination of stem cell substructure (eg clonal succession).

115 Wittekind's Method for the Hematological Smear Stains Primary Granules and Accentuates the Secondary Granulation in Neutrophils – Study on Clinical Smears

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By a multivariate analysis Wittekind's new method with pure Azure B and Eosine (WI) was shown to stain more primary granules in neutrophils than the established one of Pappenheim (PA); Blut *48*, 49 (1984). Now, WI and PA were compared on clinical smears from 24 patients with leucopenia ($L \leq 6.0$ G/l), leucocytosis ($L \geq 10.5$ G/l) and normal leucocyte count (L 6.0–10.5 G/l). Parameter of automatic image analysis: Density step with optimum detection of granules. In WI the distribution of the parameter in the leucopenic and normal cases was bimodal. Some patients showed a lot of coarse granules, others lacked them almost completely. In leucocytosis WI gave an unimodal broad distribution shifted to higher densities. Only patients with coarse granules were found. In PA the density distribution always was sharp and unimodal and only somewhat broader in leucocytosis, thus obscuring the real variability of the granulation in neutrophils. In comparison to PA, WI gave optimum detection at higher densities even in cases showing only secondary granules.

116 Effect of Cytostatic Agents on Granulocyte Function (PMA-Induced Production of Superoxides)

R. Kuzmits, G. Steurer, W. Linkesch, H. Ludwig (II. Department of Medicine, A-1090 Vienna, Austria)

Phagocytosis leads to the increased production of superoxide anion (SA) in polymorphonuclear leucocytes, microbicidal agents, which react with the ingested material under emission of photons. The influence of various cytostatic agents on the SA-production of granulocytes was investigated in vitro. Granulocytes were separated from venous blood, activation of the oxidative metabolism of the granulocytes was performed using phorbol myristate acetate (PMA), the luminol dependent chemiluminescence was recorded with a luminometer. No effects on luminol dependent chemiluminescence compared to the cytostatic-free control incubation were observed when bleomycin, cis-platinum, dacarbazine or methotrexate were added to the granulocyte cell suspension. A significant decrease in SA-production was found after incubation with: amsacrine (0.8% after 2 hours), adriamycine (47% after 3 hours), daunoblastine (45% after 8 hours) and vinblastine (38% compared to the control incubation after 8 hours incubation time).

117 Effect of Anti-Inflammatory Drugs on Chemiluminescence

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To investigate the mechanism of anti-flammatory drugs on the oxidative metabolism of polymorphonuclear leukocytes (PMNs) we measured the luminol dependent chemiluminescence (CL), the peak CL (T-max) and the timepoint of T-max. Prednisolone, Chloroquine, Azathioprine and D-penicillamine did not change the total CL, T-max and the time-T-max. Following the incubation of PMNs with Auranofin and Natrium-aurothiomalat total CL and T-max impaired to 45% and 80% of controlls, respectively. The reduction of CL by gold-compounds is due to an alteration of the oxidative metabolism of PMNs and is not caused by scavanging superoxide radicals, since in the absence of PMNs, all substances were unable to quench superoxide radicals generated by Xanthine and Xanthine-Oxidase.

118 A Nonrandomized Prospectivly Controled Study of Nosocomial Infections in Neutropenic Patients with and without Isolation

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The influence of rooming on infections of patients with neutropenia has been studied in single bed (SB) vs multiple bed rooms (MB) and in a laminar air flow unit (LAF). 21 adult patients were included in this prospective, non randomized study. The neutrophils had been at least less than 500/μl and observation time had been two weeks. The days with neutrophils less than 500/μl were days at risk. Fever of more than 38.5 had been counted as days with infection. A study of contaminant microorganisms had been included. 14 men and 7 women with 187 days of neutropenia were observed. The patients in single bed rooms developed more often fever than the patients in multiple bed rooms, although the number of contaminating microorganisms was significantly lower in the SBs. The species of microorganisms were equally distributed. Nosocomial infections seemed to be unaffected by the isolation. Only LAF conditions lead to significant reduction of gram negative bacteria whereas staph aur., streptoc. fec., and candida specs. remained unaffected. A possible role of environmental contaminations by the reduced number of visiting staff and family members must be taken into account.

119 Efficacy of Timentin-Tobramycin as Empiric Therapy in 51 Neutropenic Patients

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Timentin (5.2 g×3/day) and tobramycin (40 mg×3/day) were administered to 51 patients (22 male, 29 female, age range 17–72, mean age 39, 7 years) – mainly leucemias – with neutropenia (< 1,000 PM/mm^3) and fever (> 38°C). Febrile episodes consisted of 22 documented (S. aureus 8, S. epidermidis 2, Enterococcus 1, E. coli 4, K. pneumoniae 1, polymicrobial 1) and 29 clinically apparent infections. Timentin and tobramycin were administered 4 to 20 days (mean 10, 9); 87% of evaluable febrile episodes improved. Among 11 infections due to gram-positive bacilli, 8 were resolved (72%) among 10 cases due to gram-negative bacilli, in 9 of them a success was obtained (90%), also in 1 polymicrobial infection (Ps. aeruginosa + S. aureus + S. haemolyt.) was resolved. Only in 5 patients (9.8%) mild side effects e. g. exanthema, pruritus, phlebitis, were seen; renal toxicity was not observed. These data suggest that the combination timentin + tobramycin is an effective and safe empiric antibiotic regimen in febrile neutropenic patients.

120 Acyclovir Therapy in Herpes Virus Infections

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46 patients suffering from various malignancies (17 NHL, 12 M. Hodgkin, 11 acute leukemias, 4 myelomas, 2 carcinomas), 6 patients with hematological disorders like ITP, SAA, myeloproliferative disease, LAS and 3 patients without preexisting disease were treated with Acyclovir because of herpes virus infection diagnosed by clinical means. All but 7 patients were heavily pretreated with various cytostatic agents and/or irradiation. Most patients were treated with 1500 mg Acyclovir daily for 5 to 13 days. Dosage adjustment was done due to renal function and clinical response. 11 patients received intravenous immunoglobulins in addition. Side effects were neglectable (local irritation, minimal rise in serum creatinine levels was seen in 5 patients). All patients responded to treatment, 6 patients complained of severe neuralgia lasting for more than one month, 3 patients relapsed.

121 Fulminant (Coomb'S-Negative) Hemolytic Anemia Associated with Cytomegalovirus Infection

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This report concerns a 40-year-old woman with a severe hemolytic anemia (hemoglobin values < 5 g/dl; total bilirubin > 90 mg/dl) following few days after cholecystectomy. Hematological and immunological studies, inclusive direct and indirect Coomb's-test were negative. The serological pattern of cytomegalovirus (CMV)-specific IgM- and IgG-antibodies, however, confirmed an acute CMV-infection. Repeated *plasmapheresis* and treatment with *CMV-hyperimmune globulin* was followed by improvement of hemoglobin level and IgM-specific antibody titers fall slowly during reconvalescence. Hemolytic episodes during CMV-infection are uncommonly in adults. The fulminant course of hemolysis and successful treatment with plasmapheresis and CMV-hyperimmune globulin are the prominent features of this case report.

122 Acute Hemolytic Episode as Diagnostic Pitfall in Paroxysmal Nocturnal Hemoglobinuria

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This report is related to a 54-year-old female with first incidence of hemolytic crises. Recent history revealed viral infection, taking of chinin, therapeutic trial with vitamine B_{12} and folic acid and cholecystectomy 4 years ago. Constellation of hematological parameters (hemoglobin 6.5 g% – erythrocytes $1.59 \times 10^6/mm^3$ – MCV 131 fl – Hb_E 40.0 pg) – measured by an electronic counter – in combination with reticulocytosis up to 900‰ pointed to a case of pernicious anemia subjected to therapy.

Differential diagnosis further comprised hereditary spherocytosis, toxic hemolytic anemia and erythroleukemia until diagnosis of PNH was established.

False increase in MCV-values entailing a misdiagnosis of "macrocytic" anemia may be caused by marked anisocytosis with substantial numbers of spherocytes as revealed in this case since electronic counters do not record these cells because of their reduced diameter.

123 Hemolytic Anemia in Alcohol-Induced Liver Damage

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The symptome complex of hemolytic anaemia, high blood lipid level and alcoholic hepatopathy with jaundice is commonly referred to as Zieve syndrome.

In a group of 269 subjects with histologically verified liver disease, the authors have evaluated the incidence of anemia. Hyperlipidaemic type of hemolysis, compatible with the diagnosis of Zieve syndrome was found in 12 subjects (i. e. 16.8% of cases with anemia). The haematological and biochemical findings in the examined group are pointed out and the particularities of the clinical course are discussed along with possible mechanisms involved in the pathogenesis of the disorder.

124 Plasma and Red Cell Lipids in Alcoholics with Macrocytosis

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The cholesterol and phospholipid content and the fatty acid composition in plasma and red cell membranes were determined in 10 alcoholics without liver cirrhosis or liver dysfunction. Cholesterol and phospholipid contents in plasma as well as in red cells were not altered in alcoholics. An abnormal high ratio saturated/unsaturated fatty acids was found in plasma as well as in red cell phospholipids from alcoholics. Linoleic acid was substantially decreased in plasma of alcoholics. This fatty acid abnormality was reflected by a decrease of linoleic acid in red cell

phosphatidylcholine. These results suggest that fatty acid changes taking place in the red cell membranes were secondary to changes in the plasma and reflect plasma/membrane exchanges rather than direct effects of ethanol on red cell membranes.

125 Is Lower Ploidy of Megakaryocytes another Reason for Uremic Thrombocytopathy?

M. Winkelmann, U. Dorr, P. Pfitzer, W. Schneider (Department of Internal Medicine and Department of Cytopathology, University of Düsseldorf, D-4000 Düsseldorf, FRG)

Though it has been generally accepted that serum contains substances which impair platelet function and inhibit erythropoiesis uremic thrombocytopathy is still not clearly understood. Measuring megakaryocyte ploidy patterns of 20 patients with endstage renal failure we could demonstrate that ploidy levels correlate with parameters of uremia. The higher creatinine ($r = 0.5877$; $p < 0.005$) and BUN ($r = 0.6482$; $p < 0.001$) and the lower hemoglobin ($r = 0.4643$; $p < 0.02$) values were the lower was the average ploidy of megakaryocytes. In explaining this phenomenon uremic inhibition of DNA reduplication seems to be more important than dialysis or the underlying renal disease. As megakaryocytes of higher ploidy are thought to produce more reactive platelets than megakaryocytes of lower DNA-content this could be another reason for uremic thrombocytopathy.

126 Ploidy Patterns of Megakaryocytes in Patients with Metastatic Tumors, with and without Paraneoplastic Thrombosis, in Patients with Limited Cancer Disease an in Controls

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An altered platelet heterogeneity could be one of the reasons for thrombotic events in malignancy. Although there is evidence that this heterogeneity depends on the different ploidy of the megakaryocytes, very little is known about megakaryocytes in patients with malignant tumors. We studied the DNA-content of 30 patients with metastatic tumors – 15 with and 15 without paraneoplastic thrombosis, 20 patients with localized cancer and 12 healthy controls. Patients with metastatic tumors had a highly significant increase in ploidy of the megakaryocytes, compared to controls (ploidy index 3.04), independently of whether they suffered from thrombosis or not (ploidy index 3.56; 3.58). This shift was less pronounced or even lacking in patients with localized cancer implying a possible correlation between ploidisation of megakaryocytes and tumor mass. A thus altered platelet population could be another cause for thrombotic events in malignancy.

127 Immunological Investigations in Traumatically Splenectomized Patients with Autologous Spleen Implantation

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In patients with autologous spleen implantation reduced T4/T8-ratios are described. To reconsider these observations, in 9 traumatically splenectomized patients with autologous spleen implantation the lymphocytes and lymphocyte subsets were determined. Additionally the proliferative response to mitogens and the PWM induced in vitro immunoglobulin synthesis was investigated. Patients with autologous spleen implantation showed a lymphocytosis compared to healthy controls (3,502/yl vs. 1,933/yl). The percentage of B-cells, DR^+-lymphocytes, T-cells and T-cell subsets were within normal range, whereas the absolute counts were increased. The T4/T8-ratios differed not essentially from those of the healthy controls. The mitogenic response to PHA, ConA, PWM and OKT3 corresponded to that of the controls as did the serum Ig levels and the in vitro immunoglobulin production. In our collective impairments of the T4/T8-ratios and of the additionally performed immunological parameters could not be confirmed.

128 Increased Suppressor Cell Activities (SCA) in Patients with Common Variable Hypogammaglobulinemia (CVH) and the in Vitro Effect of IL-2 on Ig Synthesis

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Five patients with CVH were investigated for the SCA on immunoglobulin (Ig) production by lymphocytes from normal donors. Mononuclear cells (MNCs) from normal donor or patient were cultivated separately and in coculture and the culture supernatant were measured for Ig isotypes by the ELISA method. 4 patients showed increased SCA on Ig production (SCA: 77–99%, normal value < 60%). In another patient, T-cells from the patient suppressed Ig production by normal MNCs, whereas the irradiated patient T-cells did not affect the Ig production. Recombinant IL-2, when added to patient's lymphocyte culture, brought an increase of Ig production by 3- and 13-fold in 2 patients. Thus, some patients with CVH show increased CA and some of them can produce more Ig in vitro in response to IL-2.

129 Gamma-Globulin-Therapy and Immune Complexes: Some Side Effects are Due to Immune Complexes and Can be Influenced by 5 S Prior to 7 S

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We have treated 71 patients with various forms of humoral immunodeficiences with 7 S immunoglobulins. 9 patients suffered from side effects. In all patients the Ig-concentration was determined before and after substitution of 7 S immunoglobulin by laser nephelometry and immune complex-determination carried out as follows: 4.8 ml of 0.1 buffer and 0.2 ml of serum were mixed, 2 ml of this mixture were incubated with 2 ml PEG (MW 6,000), 7% in borate buffer. The mixture was incubated overnight at 4°C centrifuged at 10,000 g and the precipitate dissolved in 0.1 m NaOH. The determinations were carried out in a spectrophotometer at 280 nm. An optical density of over 0,120 was considered to be pathological according to our standards and controls. In all patients an increase of the optical density after administration of 7 S immunoglobulins could be observed, all this patients with complications were in a significant antigen overload. We started to give these patients 10 g of a pepsin treated 5 S immunoglobulin prior to 7 S immunoglobulins and reduced clearly the severity and incidence of adverse reactions.

130 Immunofunctional Different Groups in Immune Thrombocytopenic Purpura (ITP)

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We studied lymphocyte stimulation by 14 different mito- and antigens, suppressor cell activity and using monoclonal antibodies T-cell subpopulations in 50 patients with ITP and 88 healthy controls. According to platelet kinetics obtained by Cr 51-labelled thrombocytes the ITP collective could be divided into 2 groups, 1 ($n = 40$) with considerably increased platelet destruction within 48 h, 2 ($n = 10$) with platelet destruction within 3–7 days. Cluster and discrimination analyses (F-test; T-test) were done according to programs of the University of California. Controls could be clustered into 3 groups with significantly different mito- and antigen responses ($F > 4-20$; $p < 0.001$), group 1 ($n = 18$) showing medium, 2 ($n = 49$) low and 3 ($n = 21$) high reactivities in all 14 systems, but normal suppressor activity and T-cell subpopulations. Suppressor activity, T-cells and T 4/8-ratio were significantly reduced in both ITP groups ($p < 0.001$). Subsequently, disturbed control of B-cell antibody synthesis may lead to increased platelet destruction by excessive production of thrombocytotoxic antibodies. ITP group 1 showed a lymphocyte reactivity as control group 1, group 2 as control group 2 a significantly lower reactivity. Those control clusters well could be reservoirs for different forms of ITP and slower platelet destruction in ITP group 2 may be due to its lower lymphocyte reactivity and smaller amounts of thrombocytotoxic antibodies, therefore.

131 Storage of Blood and Isolated Lymphocytes Before Analysis with Monoclonal Antibodies

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Transport and storage of blood samples before lymphocyte testing may lead to unexpected differences of results. Weiblen et al. (1983) reported loss od pan-T + and T4 + lymphocytes after overnight storage of whole blood; Dzik et al. (1983) showed a decrease of T4 + lymphocytes when counting was performed 24 h after blood sampling and lymphocyte isolating and marking. We performed lymphocyte subset analysis in 10 normal volunteers:
1. immediately after blood drawing,
2. 24 h later after storing whole blood at 4°C, 20°C and 37°C;
3. we isolated lymphocytes and stored these cells in RPMI-medium for 24 h at temperatures shown above. We used 5 different monoclonal antibodies. Our data indicate that only lymphocyte suspension can be stored at 4°C or 20°C, whereas suspension storage at 37°C as well as whole blood storage at each temperature tested may lead to false and misleading results. The mechanism involved remains unknown.

132 HD 55 – A New B-Lineage Specific Monoclonal Antibody Which Identifies a Mature Subpopulation of B-Lymphocytes

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We have raised in our laboratory a monoclonal antibody designated HD 55 which appears to identify a new B-cell antigen. This marker could not be detected in normal and malignant T cells, monocytes, granulocytes, platelets, and myeloid or erythroid cells in bone marrow even by highly sensitive immunoenzymatic staining techniques. Among SIg^+ cells only a subpopulation of mature B lymphocytes is identified in peripheral blood; no reacting cells were found in normal bone marrow specimens. Immunohistology indicates that the antibody reacts only with germinal centers. HD 55 appears to be restricted to mature stages of B-cell differentiation: This antibody does not react with acute lymphoblastic leukemia, whereas 60% of chronic lymphocytic leukemias and the majority of more mature B-type leukemias were positive. On the basis of its reactivity pattern HD 55 seems not to be related to established B cell specific antigens: B 4, HD 37 (CD 19), B 1 (CD 20), B 2 (CD 21), HD 6, HD 39 (CD 22), and Blast-2 (CD 23). The biochemistry of the HD 55 antigen is currently being investigated.

133 Endothelial Cell Specific Monoclonal Antibody BW 200

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In the past, immunocytochemical studies of normal and neoplastic human endothelial cells were mostly performed using anti-F VIII-RAG antiserum and ulex europeus agglutinin I. Monoclonal antibodies against endothelial cells, described hitherto, only worked on fresh frozen tissue specimen and their specifity was restricted to human tissue. In contrast monoclonal antibody BW 200 recognizes an epitope detectable in the cell membrane and cytoplasm of human endothelial cells of normal and neoplastic tissues. The epitope recognized by the antibody formaline resistant and immunoprecipitation analysis demonstrated that it is, located on an antigen of a molecular weight of 200 kDa, different from F VIII-RAG.

134 Multinucleated Cells in Cultures of Mononuclear Blood Cells Stimulated by Epstein-Barr Virus

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The origin of the Reed-Sternberg cells in Hodgkin's disease is still obscure. It is known that in cultures of lymphocytes, stimulated repeatedly by pokeweed mitogen, Reed-Sternberg-like cells can be observed. These cells are phenotypically mostly T-cells. We have stimulated mononuclear blood cells of normal donors by Epstein-Barr virus, as a T-cell independent mitogen for B-lymphocytes. In these cultures we have rarely seen large multinucleated, sometimes Reed-Sternberg-like cells. These cells were investigated cytochemically, biochemically and by monoclonal antibodies and seem to have shown the phenotype of B-cells. Some of them, however, have markers for monocytes/macrophages. It is conceivable that an in vitro fusion to multinucleated culture cells induced by Epstein-Barr virus has been occurred.

135 Biochemical Purification of a Polypeptide Complex (p 52) Belonging to the Epstein-Barr Virus-Induced Early Antigens (EA)

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The Epstein-Barr virus-induced early antigen (EA) complex is of particular interest since antibodies to this complex are found at an increased frequency in patients with EBV-associated diseases. The determination of IgG and IgA antibody titers to EA has diagnostic and prognostic significance in patients with infectious mononucleosis, Burkitt's lymphoma, nasopharyngeal carcinoma and for EBV-reactivation. We report the first biochemical purification of an EA-associated polypeptide complex. The protein was isolated by chromatography on Blue-, DEAE-, CM- and Phenyl-Sepharose. One- and two-dimensional immunoblots show a major polypeptide of 52 kD with EA activity and some minor components with smaller size up to 40 kD, the later seem to be generated by limited proteolysis of p 52 polypeptides. The isolated protein reacts with IgG and IgA antibodies to EA as could be shown by ELISA. Tests carried out with anti-EA-R+/D-sera suggest, that p 52 belongs to the D components of EA.

136 Comparison of DNA-Binding Proteins from Different Lymphoid Cells

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Normal lymphocytes and lymphocytes from patients with chronic lymphocytic leukemia (CLL) as well as Burkitt's lymphoma cells (Raji) were homogenized; the soluble proteins were subjected to DNA-cellulose chromatography. By two-dimensional gel electrophoresis characteristic patterns could be assigned to the DNA-binding proteins. The binding proteins from normal lymphocytes and from CLL cells caused a greater inhibition of cell-free translation of poly $(A)^+$ RNA than did the non-binding proteins. This difference could not be seen with the corresponding fractions prepared from Raji cells as well as from cells (Raji) induced for the production of Epstein-Barr virus-associated early antigens (EA). Thus, we could not find in these *in vitro* experiments the inhibition of protein synthesis that has been demonstrated in EA-positive Raji cells in culture.

137 Coagulation Abnormalities in Acute Leukemia (AL)

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Coagulation abnormalities in AL are common and pathogenetically heterogeneous. Mechanism which induce coagulation abnormalities are (1) intravascular activation of coagulation (DIC)

(2) decreased synthesis of clotting factors due to impaired liver function (3) infection-related and (4) treatment related clotting changes. In a study 108 patients (98 AML, 10 ALL) activation of coagulation as indicated by an increase of fibrinopeptid A could be found in almost all patients with AML and in some with ALL. Overt DIC was observed in 14.8% of patients with AML but in none with ALL. Induction of DIC was not related to the tumor cell burden, but is rather a specific property of leukemic clones. A yet recognized syndrome of DIC associated with factor X deficiency is described. Both clotting inhibitors, antithrombin III and protein C are reduced in AML but there seems to be no correlation between the presence and absence of DIC and the level of these inhibitors. A striking discrepancy between protein C antigen and protein C activity was observed.

138 Early Death Caused by Bleeding and Leukostasis in Children with Acute Myelogenous Leukemia (AML)

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Among 250 children of the cooperative AML-BFM-studies 78 and 83, 26 (10%) died before or during the first 12 days of therapy (9 vs 17 pats.). The main cause of death was bleeding and/or leukostasis. Most patients had either monocytic leukemia (FAB M 5) (15 pats.) or leukocyte counts $\geq 100{,}000/mm^3$ (20 pats.). These two features, alone or especially in combination, indicate a high risk of early fatal hemorrhage or leukostasis. Autopsy showed intravascular accumulation of leukemic cells in 7 children with extreme high leukocyte counts (median: $286{,}000/mm^3$). 2 of these patients died of progressive respiratory insufficiency, the others of pulmonary and cerebral bleeding. Aside from rheological disturbances complex hemostasiological mechanisms may play an important role. These could be due to the secretion of hemostasiologically active substances secreted by the monoblasts. Precautionary measures in patients with monocytic leukemia and hyperleukocytosis are recommended to avoid early deaths.

139 Asparaginase-Induced Alterations of the Coagulation System

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Patients under treatment for acute lymphoblastic leukemia (ALL) receiving vincristine, adriamycin and in addition L-Asparaginase (ASP) for induction are at a considerable risk (1–2%) to develop life threatening cerebral hemorrhages. In prospective studies severe changes of various hemostatic factors were seen and explained by hyperfibrolysis, decreased production or increased consumption of several coagulation factors. To diminish the toxicity of such induction regimens in following treatment protocols the administration of ASP was postponed until remission was obtained. The coagulation tests now revealed a different pattern of changes, for this reason it has to be distinguished between disease- and treatment-induced disturbances in patients with ALL during induction. A constant finding, however, was a significant decrease in fibrinogen, antithrombin III, alpha 1-antiplasmin and fibronectin. The preliminary results of the evaluation of different treatment protocols suggest that the postponed use of ASP may diminish the risk for life threatening cerebral hemorrhage.

140 Disturbances of Platelet Function in Myeloproliferative (MPD) and Myelodysplastic (MDS) Syndromes

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MPD and MDS are stem cell diseases with significant morphological abnormalities and changes of polyploidy in megakaryopoiesis as well as disturbances of platelet function. Clinically cases of MDS are characterized by bleeding complications whereas in MPD bleeding and thrombotic events may occur simultaneously. Morphological abnormalities correspond to biochemical and

functional disturbances due to changes in glycoproteins with defects of receptors and variations of cyclooxygenase and lipoxygenase metabolism. Due to the multiplicity of disturbances and of clinical courses studies can at the most reveal overall relationships between phases and course of the disease. Longitudinal studies of the course of the diseases are necessary to establish the relationship between individual defects of platelet function and stage of the disease, and to differentiate functional defects resulting from therapy.

141 Acquired von Willebrand Syndrome in Myeloproliferative Disorders

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Two patients developed a severe hemorrhagic disorder during the course of the myeloproliferative syndrome. In both the platelet count was extremely elevated (6×10^9/ml and 3×10^9/ml). The presence of an acquired von Willebrand's disease was demonstrated by a decrease or absence of the larger von Willebrand factor multimers and decreased ristocetin cofactor activity. Factor VIII activity and von Willebrand factor antigen were within the normal range. These observations could be confirmed in seven other patients with the myeloproliferative syndrome, but without bleeding complications. These findings suggest that acquired von Willebrand's syndrome should be considered when a bleeding diathesis develops during the course of the myeloproliferative syndrome.

142 1-Deamino-8-D-Argininvasopressin (DDAVP) Aggravates Thrombocytopenia in Subtype IIB von Willebrand's Disease (vWD)

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Eight patients (pts.), belonging to 4 unrelated families, with an inherited haemorrhagic diathesis showed a laboratory pattern characteristic for subtyp II B vWD. All pts. had a slight to moderate thrombocytopenia with platelet counts ranging from 135×10^3 to $30\times10^3/\mu$l, a strongly prolonged bleeding time (>20 min.), decreased platelet retention (between 35% and 6%, normal >80%), normal or slightly decreased VIII:C (between 157% and 34%), VIII R:Ag (between 120% and 39%), a more pronounced decrease of VIII R:RCo (between 38% and 5%), and hyperresponsiveness of the platelets to ristocetin. DDAVP (0.4 μg/kg b.w./20 min.) raised VIII:C, VIII R:Ag, VIII R:RCo and improved the platelet retention, whereas bleeding time remained unchanged. However, most remarkably, the platelet count in the patients dropped rapidly, reaching the lowest values 30 to 120 min after DDAVP. The decrease ranged from 13% to 91% of the pretreatment counts, reaching values in two patients as low as 5×10^3 and $6\times10^3/\mu$l platelets, respectively. Since bleeding time remained unchanged, and moreover, an increased bleeding tendency due to the aggravated thrombocytopenia cannot be excluded, DDAVP should be avoided in therapy of type II B vWD.

143 Acquired Thrombasthenia due to Anti-GP II b/III a Autoantibodies

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We report on an otherwise healthy female with a fluctuating course of an acquired haemorrhagic diathesis. In periods of bad clinical condition the laboratory pattern (prolonged bleeding time, decreased platelet retention and clot retraction, total absence of ADP, collagen, and thrombin-induced platelet aggregation, but normal agglutination with ristocetin) was characteristic for thrombasthenia. However, in contrast to classic thrombasthenia, the patient's platelets had normal amounts of membrane glycoproteins GP II b and III a. Also, monoclonal antibodies specific for the GP II b/III a complex or GP III a bound normally to the Pl^{A1} positive platelets from the patient. An IgG_1 antibody, which could be eluted from the patient's platelets, inhibited ADP and collagen-induced aggregation in normal PRP and also ADP-stimulated fibrinogen

binding. Since no reason for an alloimmunization was found, the haemorrhagic diathesis of the patient has to be interpreted as acquired thrombasthenia due to anti-GP II b/III a autoantibodies.

144 Successful Herniotomy in a Haemophiliac with Potent Anti-Factor VIII Antibody by Combined Treatment with Plasmapheresis, Porcine Factor VIII and FEIBA

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In a 45-year-old haemophiliac with a potent F VIII antibody herniotomy was urgently indicated because of high risk of incarceration. At admission the patient had an inhibitor titer of 149 BU/ml. By 5 plasmapheresis runs within 10 days (Plasma volume exchanged, 17,500 ml) the antibody titer against human F VIII was lowered to 37 BU/ml. The antibody had only 10% cross reactivity with porcine F VIII (2 BU/ml). Therefore, the patient was treated with porcine F VIII (Hyate C). The initial dose was 71 U/kg bodyweight (recovery 0.66%/E/kg). Subsequently the patient was treated with 47 U/kg every 8 hours. Recovery was 0.96–1.31%/U/kg during the first 4 days of treatment. The biological half life of F VIII was 7–9 hours. The highest F VIII value achieved was 85%. At the 5th day of treatment recovery decreased to 0.08%/U/kg due to the rise of an anti-porcine antibody. Treatment was therefore continued with activated prothrombin complex preparations (FEIBA). No bleeding occured during or after surgery. Subsequently, both the inhibitor activity against human and porcine F VIII rose to high levels (4,210 BU and 4,081 BU/ml respectively).

145 A Variant of Bernard-Soulier Syndrome (BSS) with a Unique Platelet Membrane Glycoproteine Defect

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With a decreased platelet count, increased mean platelet volume, prolonged bleeding time, decreased platelet retention, absence of ristocetin induced platelet aggregation, but normal ristocetin cofactor activity, laboratory investigations in a 15 years aged male patient with a mild haemorrhagic diathesis revealed a pattern characteristic for BSS. Also binding of the monoclonal antibody AN 51 (specific for platelet membrane glycoprotein GPI b) to the patient's platelets was strikingly decreased, but not completely absent. In accordance with this finding GPI b concentration in the membranes of the patient's platelets was also diminished markedly. However, in contrast to other cases of BSS in the patient's platelets GPI b (about 7%) and GP 17 ($< 1\%$) appeared not to be in balance. Familiy investigations did not reveal any abnormalities in platelet function, platelet membrane glycoprotein studies of the family members are in progress.

146 Binding of Factor VIII Coagulant Moiety to Platelets

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To investigate, whether factor VIII coagulant moiety can bind direktly to the phospholipids of the platelet membrane, or binding of the coagulant moiety to platelets has to be mediated by von Willebrand factor, washed platelets were incubated with factor VIII concentrate or factor VIII coagulant moiety alone prepared by immunoadsorbent chromatography. After incubation from the then washed an lysed platelets more factor VIII coagulant antigen (VIII C:Ag) than factor VIII related antigen (VIII R:Ag) was recovered. More VIII C:Ag was recovered after stimulation of the platelets with thrombin and collagen. Measurable VIII C:Ag significantly increased after incubation of the platelet lysate with phospholipase C. Our results suggest that some of the factor VIII coagulant moiety directly binds to the phospholipids of the platelet membrane.

147 Connection Between Blood Clotting Abnormalities and Stages of CLL

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Blood clotting disturbances in CLL could be manifested only in stages III–IV. The most frequent abnormality is the bleeding tendency caused by thrombocytopenia. The alterations in early stages have not been investigated in details. Examinations of blood clotting and platelet functions were carried out in more than 40 CLL patients. The most characteristic abnormalities were the decrease of prothrombin activity, prolongation of PTT, ethanol test positivity, impairment of platelet functions, besides the elevation of plasma beta-thromboglobulin level. A correlation was found between severity of haemostasis alterations and the progression of CLL.

148 Monocyte Dependant and Independant in Vitro Regulation of CFU-GM by Subpopulations of Peripheral Blood T-Cells

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We used subset specific monoclonal antibodies (mcAbs) to dissect T-cells into the helper inducer (T4 + 2H4–), suppressor inducer- (T4 + 4B4–), suppressor effector- (T8 + MO1 +) and cytotoxic subset (T8 + MO1–) and assayed their production of colony stimulating- (CSA) and -inhibitory activity (CIA) in response to lectin stimulation (PHA, ConA) and mcAb (anti-T 11_2 and -T 11_3)T-cell activation via the 50KD T11 surface protein. CSA was assayed by measuring DNA-synthesis of highly purified normal marrow myeloid progenitors. CIA due to gamma-Interferon (γ-IFN) was assessed similarly by neutralysing CIA with mcAb to γ-IFN. The data show that in vitro release of CSA by both monocyte dependant (lectins) and independant (T11 activation) mechanisms is not subset restricted but associated with T-cell proliferation to a degree which varied directly with the extent to which the respective subpopulation was inducible to proliferate, with T4 + 4B4– and T8 + MO1– cells secreting the highest level. In contrast, CIA-release was subset associated, with maximum CIA-release occuring in lectin stimulated cultures of T8 + MO1 + cells. Further analysis of differences in monocyte dependant and independant T-cell stimulation revealed that CIA (γ-IFN) released by T8 + cells mediates stimulation of CSA-release by monocytes.

149 Prognostic Significance of Erythropoietic and Granulopoietic Stimulators in Patients with Acute Leukemia

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It is well established that hemopoietic cells respond to humoral growth factors in vitro, but are inhibited in acute leukemia by secretion products of leukemia cells. Less is known about the regulatory role of humoral factors in vivo and their possible impact on pathogenesis and prognosis in acute leukemia. We studied the stimulating properties of sera from 42 patients with newly diagnosed acute leukemia by adding them at a final concentration of 20% to agar cultures of normal peripheral blood target cells (MNC) of one single donor. After 14 days numbers of granulocyte-macrophage colonies (GMCFU) and erythropoietic bursts (BFUe) were scored in panoptically stained cultures. Counts were analysed in correlation to clinical course and standard laboratory parameters. All patients were treated intensively by standard protocolls. Patients achieving complete remission (CR, $n = 32$) stimulated significantly higher numbers of BFUe (mean $178/10^5$ MNC) than those not achieving CR ($n = 10$, mean 110 BFUe/10^5, MNC, $p = 0.044$). The numbers of GM-CFU were not statistically different. Patients with a high ratio of BFUe/GM-CFU had a significantly higher chance of achieving CR ($p = 0.007$), a lower incidence of relapse ($p = 0.04$) and a higher probability of survival. We conclude that high serum erythropoietic activity is a valuable prognostic factor in acute leukemia and can help to distinguish prospectively patients with a good chance of achieving CR.

150 Pluripoietin(s) Derived from Purified T_4-Helper Cells Support Growth of Human Stem Cells and Megakaryocytic Progenitors

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Medium derived from peripheral mononuclear cells, stimulated with phytohemagglutinin (PHA-LCM), supports the growth of bone marrow derived stem cells. These precursors can be identified by their ability to form multilineage colonies, containing myeloid cells as well as T- and B-lymphocytes.

To study the effect of lymphokines released from T_4-helper cells on pluripotent stem cells (CFU-GEMML) and megakaryocytic progenitors (CFU-M), T_4-lymphocytes were purified (purity $>99\%$) from peripheral blood cells with the use of a fluorescence activated cell sorter (FACS).

It was found that PHA-stimulated T_4-cells release lymphokine(s) that support hemopoietic colony formation of nonadherent and T-cell depleted bone marrow cells. The plating efficiencies for CFU-GEMML, CFU-M, CFU-C and BFU-E were consistently higher with the use of T_4-helper-cell-conditioned-medium when compared to PHA-LCM. Our data demonstrate an important role for T-lymphocytes of helper phenotype in the regulation of human stem cells. These cells may be useful in the molecular cloning of human pluripoietin(s).

151 Cellular and Humoral Regulation of Megakaryocytopoiesis: Influence of T-Lymphocytes and Monocytes on the in Vitro Growth of Megakaryocytic Precursor Cells (CFU-M)

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Cellular and humoral interactions responsible for regulating in vitro megakaryocytopoiesis were studied using a microagar culture system which permits the simultaneous proliferation of human megakaryocytic progenitor cells (CFU-M) and T-lymphocytic colonies (CFU-TL). The proliferation of these colony types depends mainly on two factors: phytohemagglutinin (PHA) and erythropoietin (EP). The direct addition of increasing PHA concentrations to the liquid overlayer resulted in a parallel increase of CFU-M and CFU-TL. If the T-lymphocytes were removed by an E-rosetting technique a marked deminution of CFU-M and CFU-TL number was observed. However, monocyte depletion resulted in a marked augmentation of CFU-M proliferation compared to unfractionated mononuclear cells. In order to confirm that the reduction of CFU-M proliferation observed after T-depletion was primarily mediated by the absence of T-lymphocytes, we have cocultured different concentrations of previously removed autologous T-lymphocytes with a constant number of T-depleted bone marrow cells. A parallel increase of CFU-TL and CFU-M was found if $0.75-7.5\times10^4$ T-lymphocytes were added to the culture. In conclusion, our results indicate that activated T-lymphocytes augment proliferation of human bone marrow CFU-M and that monocytes are of less importance for the growth of megakaryocytic colonies.

152 Proliferative State and Self-Renewal of Clonogenic Leukemic Cells (CFU-L) in Various Types of Myeloid Leukemia

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The CFU-L culture method described by Minden allows the clonal growth and characterization of blast cell progenitors. Growth pattern and self-renewal of leukemic colonies were examined in 15 patients with various forms of myeloid leukemia (de novo AML $n=7$, blast crisis (BC) in myeloproliferative disease $n=4$, RAEB $n=4$). The proliferative state of colony-forming cells was assessed by short-term incubation in cytosine arabinoside (1×10^{-6}M). In all patients, leukemic colonies could be grown from T-depleted blood cells. Plating efficiency (36 to 888/10^5 plated cells) was not correlated with peripheral blast cell count. Colonies were composed of a

majority (> 80%) of blast cells (in one case with Auer rods) which were predominantly peroxidase negative and did not form E rosettes. The suicide rates were not significantly different in the 3 patient groups: AML 65 ± 14, BC 58 ± 15 and RAEB 58 ± 7%. Pooled primary colonies could be replated successfully in 14 cases. Second plating efficiency was much lower in RAEB (5 ± 3) than in AML (258 ± 240) and BC (41 ± 2). The low capacity for self renewal of CFU-L might explain the relatively indolent pace of this leukemia ('smoldering leukemia').

153 Colony Growth of Normal and Malignant Hematopoietic Precursor Cells After Incubation with Synthetic Alkyl-Lysophospholipids (ALP)

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Alkyl-lysophospholipids are synthetic analogues of lysophosphatidylcholine and represent a new class of anti-tumor agents. They are cytotoxic in vitro with a high selectivity for neoplastic cells which in contrast to normal cells lack an alkyl-cleavage enzyme to degrade the ALP molecules adsorbed. To evaluate the potential value of ALP to eliminate leukemic cells from remission marrow prior to autologous transplantation we tested the effect of various ALP on the clonogenicity of normal human marrow cells and promyelocytic leukemia HL-60. A remarkable difference in the dose response to ALP of normal and leukemic cells was observed. After an incubation period of 24 h the same inhibition of clonogenicity in HL-60 occured at ALP concentrations four times lower than in normal marrow cells. Reducing the exposure time to 6 h enhanced the selectivity further: whereas HL-60 colonies were nearly completely inhibited at 16 μg ALP/ml more than 50% of normal CFU-c and BFU-E were recovered after incubation with 48 μg/ml. No further increase in selectivity was achieved by changing the incubation temperature. Both thioether- and alkyl-analogs were active and there was also no difference between methoxy- and acylamino-substituted ALP. We conclude that this selective cytotoxicity make ALP worth to be studied as purging agents in autologous bone marrow transplantation programs. Supported by Deutsche Forschungsgemeinschaft.

154 Rhabdomyosarcoma (RMS) in Childhood – Experiences of the Austrian Cooperative Rhabdomyosarcoma Study

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The modern treatment of soft tissue sarcomas is based on multiple-agent chemo- and radiotherapy as well as on surgery. A main objective is to avoid primary mutilations due to aggressive surgery.

Since January 1982 33 children (20 males, 13 females; aged 4 months to 17 years) with soft tissue sarcomas have been treated according to the Austrian RMS 82 study. Out of 29 protocoll patients 22 had RMS (15 embryonal, 5 alveolar, 2 undifferentiated), 7 revealed with other soft tissue sarcomas. In the RMS group 17 patients are alive, 9 are in first remission 2 months to 3 years after finishing therapy (3 patients with non-resectable bladder tumors in concern of mutilation received chemo- and radiotherapy solely). 6 patients are still in therapy, deaths (5) occured only with abdominal locations. There are two local relapses so far. The therapeutic concept takes primary tumor location and the various stages of disease into concern. The results seem to confirm the applied concept.

155 Cytotoxic Monochemotherapy: Indication and Treatment Results in Patients with Advanced Breast Cancer

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The lack of established treatment modalities in hormonal or cytotoxic pretreated patients with metastasizing breast cancer justified a prospective study of two single drug treatments a) doxorubicin 10–20 mg i. v., weekly, b) mitoxantrone 12 mg/m^2 every 3 weeks, nadir adapted. The study was performed in patients with a) myelosuppression (n = 11), b) carcinomatous bone marrow infiltration (n = 14), c) age ≥ 70 (n = 7) and d) heavily pretreated patients (n = 20). Patients with carcinomatous bone marrow infiltration or myelosuppression (n = 25) achieved the greatest benefit from this treatment modality (objective remission: 28%, duration m: 55 weeks (w); r: 28–82 w; stable disease 66%, duration m: 23 w, r: 12–105 w). Hematologic and gastrointestinal toxicity and alopecia were very mild under both therapy modalities. Patients who had tumor progression under previous conventional polychemotherapy containing doxorubicin had no benefit from the weekly doxorubicin administration (all had progressive disease). These patients should be treated with mitoxantrone.

156 Mitoxantrone in the Treatment of Advanced Breast Cancer

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Twenty-seven patients, aged 43–76 years with advanced breast cancer were treated with mitoxantrone as a single agent treatment (14 mg/m^2 every 3 to 4 weeks) or as combination chemotherapy with cyclophosphamide and 5-fluorouracil. The dosage of this schedule was mitoxantrone 14 mg/m^2 i. v. day 1, cyclophosphamide 100 mg/m^2 per os day 1 to 14, 5-fluorouracil 500 mg/m^2 inf. day 1 and 8, recycled every 4 weeks. Results: 8 patients achieved a complete or partial response and 15 patients stable disease, 4 patients showed further progression. Side effects consisted of mild nausea (30%), moderate alopecia (8%) and severe myelosuppression (in nearly all patients), but no cardiotoxicity was registrated.

157 Low-Dose Weekly Treatment of Metastatic Breast Cancer with 4′-Epi-Doxorubicin (Epirubicin)

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Assuming that the benefits of reduced cumulative cardiotoxicity of low-dose weekly administration (von Hoff et al., 1979) and of the adriamycin derivative epirubicin (Young et al., 1985) in comparison to conventional anthracycline therapies of metastatic breast cancer can be combined, we have treated 31 patients with metastatic breast cancer with 20 mg epirubicin/m^2/week iv. Out of 26 evaluable patients 15 patients responded to therapy: 5 partial remission (15–29 weeks, median 22 weeks) and 10 minor responses (4–17 weeks, median 8 weeks). Side-effects such as leukopenia, alopecia and nausea were markedly reduced in comparison to conventional anthracycline therapies. No cardiotoxicity was observed at a maximal cumulative dose of 500 mg epirubicin/m^2. Our results demonstrate the efficacy of low-dose weekly epirubicin in metastatic breast cancer. Due to less side-effects observed and reduced cardiotoxicity expected, this treatment regimen may be superior to conventional anthracycline therapies in the course of combination chemotherapy of metastatic breast cancer.

158 Sequential Combination Chemotherapy with Epirubicin/Cyclophosphamide/Vincristine and Etoposide/Cisplatin in Small Cell Lung Cancer

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Thirty patients (pts) – median age 51 years, range 40–71; median performance status 80%, range 60–100 – were randomly assigned to receive initial chemotherapy with either EPICO (Epirubicin 40 mg/m^2 iv on days 1 + 2, Cyclophosphamide 750 mg/m^2 iv on days 1 + 2, Vincristine 1.5 mg iv on days 1, 8 and 15) or ETP/DDP (Etoposide 200 mg/m^2 as 24 h-infusion on days 1 + 3, Cisplatin 80 mg/m^2 as 6 h-infusion on day 5) every three weeks. Crossover to the alternative regimen was performed at maximum response – usually after 3 courses. To date, 23 pts – limited disease (LD) 14, extensive disease (ED) 9 – are evaluable for induction therapy. Employing extensive restaging procedures, 3 complete (CR) and 7 partial (PR) remissions could be induced in LD pts, comparing to 1 CR and 5 PR in ED pts. EPICO and ETP/DDP each as the second regimen increased the number of remissions obtained with the first combination. At present, the median duration of remission is 5 months (range 1–14 +), median survival is 9 + months (range 2–16 + with 13 pts still alive). Toxicity including hematological and gastrointestinal side effects was acceptable.

159 Advanced Gastric Carcinoma: A Pilot Study with Etoposide (E)/Adriamycin (A)/Cisplatin (P) = EAP

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In February 1984 a study was begun to find the dosage of E in the combination EAP and to evaluate the effectiveness of EAP for untreated, measurable advanced gastric carcinoma (I = 120/II = 150/III = 170 mg/m^2 E i. v., on days 4, 5, 6 + 20 mg/m^2 A, on days 1, 7 + 40 mg per m^2 P, on days 2, 8). We included 16 patients with histological proven disease. Patients characteristics: male 12-female 4, age 28–70 (56), performance status WHO 1–2, intestinal type 11-diffuse type 5. The WHO criteria were used to evaluate side effects and response. 4 patients were treated with E I, 4 with E II, 1 with E III. 2 of 4 patients E II and the 1 E III showed myelotoxicity greater than WHO 2. The following 7 patients therefore received E I. 2 of 11 patients E I showed myelotoxicity more than WHO 2. Nausea/vomiting: WHO 2 15 pat., WHO 3 1 pat. Nephrotoxicity: WHO 1 4 pat.-reversible. Response (16 pat. evaluable): 2 CR (6 +, 10 + months, 1 CR histological proven), 8 PR (2, 2 +, 2, 3, 5, 4, 6, 6 +, 10 + months). The dosage for the ongoing phase II study: 120 mg/m^2 E i. v., on days 4, 5, 6 + 20 mg/m^2 A, on days 1, 7 + 40 mg/m^2 P, on days 2, 8: 23 pat. included –15 evaluable: 1 CR + 11 PR.

160 Tolerable Toxicity and High Remission Rates in NSCLC – A Phase II Trial with Etoposide (VP-16) and Cisplatin (CDDP)

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After two dosage finding studies a chemotherapy protocol consisting of VP-16 (130 mg/m^2, i. v., days 3, 4, 5) and CDDP (60 mg/m^2, i. v., days 1, 7) was established. Since Nov. 1983, 43 pts with nonresectable NSCLC have entered the study. At present 37 pts are evaluable for response and toxicity. *Pat. characteristics:* Mean age – 60 years (40–70), mean Karnofsky PS – 80% (60%–100%), ext. dis. 25/37 pts, lim. dis. 12/37 pts, squam. cell ca. 17/37, adenoca. 18/37, large cell ca. 2/37. *Results:* The overall objective response rate was 59% (22/37). In lim. dis.

CR + PR was 67% (8/12) (3 CR, 5 PR), in ext. dis. 56% (14/25) (1 CR, 13 PR). In squam. cell ca. response was 82% (14/17), in adenoca. 39% (7/18). No difference in response rates could be observed concerning age and PS. Toxicity was primarily haematologic. Leukocytopenia of WHO grade 3 and 4 was 70% and 3%. However, only 4 pts with a leukopenia of WHO grade 3 had a WBC count between 1×10^9/l and 1.5×10^9/l. A thrombocytopenia of WHO grade 3 occured in 3% of the pts. Updated survival, response and toxicity data will be presented.

161 Active Specific Immunotherapy in Patients with Resected Squamous Cell Carcinoma (SQCC) of the Lung Stage III

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During recent years, adjuvant therapy of patients with sq. c. c. of the lung has made only limited progress. Since more than 15 years we have been dealing with immunotherapy of sq. c. c. of the lung, having developed a method for active specific immunotherapy using membrane preparations of tumor cells (lines) in combination with adjuvants for treatment. In pilot and randomized studies, altogether 30 patients (pts) with operated sq. c. c. have been treated with this method. 10 of these stage III pts are still living without evidence of relapse (4.0–8.2 years, mean 6.5), i. e. 33.3%, in comparison to 16% of pts in the same stage living 5 years after surgery; these data suggest a positive influence of immunotherapy.

162 Results of a Pilot Study with Hyaluronidase as an Additive Drug to Cytostatic Therapy in Malignant Diseases

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It will be reported on 115 patients available to evaluation who have either been treated with 7,500 i. E. or 200,000 i. E. of hyaluronidase, either systemically or intraperitoneally. The following results have been achieved in patients where in spite of non responding to a certain type of chemotherapy, this very chemotherapy was continued but with the addition of hyaluronidase: Myelomas CR 2/17, subjective improvement 13/17. Non-Hodgkin-Lymphomas CR 2/10, PR 5/10. M. Hodgkin PR 1/2. Mammary carcinoma CR 1/13, PR 7/13 (1 PR of a cerebral metastasis). Squamous cell carcinoma of the ENT-region CR 5/6. Non-small-cell bronchial carcinoma PR 1/2. Hypernephroma CR 1/4. Myelosarcomatosis 1 PR. In the rest of the patients hyaluronidase was either added from the beginning to chemotherapy or chemotherapy was changed at the point when hyaluronidase was started. Intraperitoneal treatment with hyaluronidase (6 patients with carcinosis peritonei of solid tumors) led to good toleration of cytostatic agents like adriamycine, cis-platin, 5-FU administered intraperitoneally and thus led to wider therapeutic possibilities. In all patients removal of ascites lasting different periods of time – with a maximum of 6 months – could be achieved.

163 Pharmacokinetic, Preclinical and Clinical Evaluation of α/β-Triglycidylurazol (TGU; NSC 332488) Using an Intermittend Schedule

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A clinical Phase II-study was initiated with 600 or 800 mg TGU/m^2 iv. once every 4 weeks in pts with or without prior chemotherapy. Nine pts with advanced tumors, a median age of 53 yrs (range, 40–62), and a performance status (Karnofsky) of 70% (range, 40–100%) were entered into the study. Nadir counts observed were WBC 2,100/mm^3 (median; range, 1,300–4,400), platelets 100,000/mm^3 (range, 27,000–253,000), and Hgb 9.9 g% (range, 7.7–12). Side effects included minimal nausea and moderate thrombophlebitis on injection side. In 1 pt (colorectal ca.) a partial remission of liver metastases lasted 10 weeks; however, following 3 courses of TGU

(total dose 4,500 mg) he developed a panmyelophthisis with fatty atrophy of bone marrow. Using a HPLC assay (UV detector, 216 nm, sensitivity limit of 50 ng/ml; C_{18} column 300×4.6 mm) peak plasma conc. was 2.7 mg/ml and $t_{1/2}\beta$ 6.0 min (600 mg TGU/m^2). Diffusion chamber assay in NMRI mice (Boyum et al.) showed 30% growth inhibition in L 1210 leukemia following 36 mg TGU/kg iv. In summary, TGU revealed activity in 1 pt with a traditional resistant tumor. Pattern of organ distribution, cumulative bone marrow toxicity, and its cytoreductive effect on myelopoietic cells needs further investigation.

164 Hormonal Changes After Chemotherapy in Patients with Malignant Germ Cell Tumors

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Chemotheraphy (CT)-induced toxic side effects on gonadal function can be seen in the serum levels of sexual hormones and gonadotropines. We investigated 60 patients (pts) with malignant germ cell tumor to define the peak and course of hormonal changes. At specific intervals 1–60 months after CT we analysed the levels of T_3, T_4, prolactin (hPRL), oestradiol (E_2), testosterone (T), LH, FSH (LRF-test) and tumor markers AFP and hCG. All pts were in complete remission at the time of investigation: 52 after surgery + CT, 8 after surgery alone. T_3, T_4, hPRL and T levels were found to be normal, E_2 was elevated in 30% of pts (E_2/T-quotient up to 7) without correlating evidence of tumor relapse or development of gynecomastia. In the first year after CT the mean value of LH increased up to 13.8 U/l (norm 12) – LRF-induced peak up to 9.3 times –, FSH up to 22 U/l (norm 10). In the 3rd year after CR all mean values returned to normal range. Pts. without CT showed minimal elevation of mean FSH, pts. with primary extragonadal germ cell tumor were characterized by marked elevation of LH, FSH, E_2/T, which can be interpreted as a sign of reduced testicular function. The prognosis of fertility impairment depends more on the remaining testis (testes) than on the reversible toxicity of CT.

165 Acute and Chronic Gonadal Toxicity in Patients (PTS) with Testicular Cancer (TC) After Chemotherapy (CT)

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The grade and duration of drug-induced infertility in PTS with TC is incompletely known. We studied 45 PTS with TC treated with cisplatin, vinblastine, bleomycin ± adriamycin (PVB ± A) to establish the impact of CT on hormonal and reproductive functions. Serum was tested for FSH, LH, testosterone (T), estradiol (E), prolactin (P) and semen analyses were done before, during and 1–6 years after CT. Sexual functions were ascertained by questionnaire. 20% of PTS had elevated FSH levels before CT and 95% in the 1st year after CT. In the 3rd year after CT 100% of the PTS showed normal FSH values. LH, T, E and P were normal before, during and after CT. Semen analyses revealed azoospermia in 100% of PTS 1–2 years after CT. 100% of PTS examined in the 3rd year or later after CT showed recovery of spermatogenesis but with a high degree of immotile sperms. In conclusion, although these data emphasize recovery of spermatogenesis in the 3rd year after PVB ± A regimen the minority of PTS fathered children after CT probably due to reduced sperm motility.

166 Changing Pattern of Lymphocyte Subpopulations During Halothane Anesthesia

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Anesthetic drugs are supposed to affect cellular immune response. We investigated the effects of halothane on lymphocyte subpopulations in 24 female patients undergoing refertilization surgery with a mean duration of an anesthesia of more than 2½ hours.

Before the drug, after extubation and during the following day venous blood had been taken and T 11, T 4, T 8 and Leu 7 lymphocyte subpopulations had been determined. Expression of interleukin 2-receptor (Il 2) had also been investigated.

Halothane anesthesia resulted in a significant decrease of T-cells. T 4-cells were mainly effected, which alters the T 4/T 8-ratio. Circulating cells expressing Il 2– receptor were significantly elevated. Preliminary results in patients with regional anesthesia give hints that these changes are really halothane induced.

167 Recovery of Haemopoietic Capacity in Healthy Individuals After Exposure to Aplasiogenic Doses of Xylol/Benzol and Busulfan

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Two persons with formerly unaffected hemopoiesis received toxic doses of xylol/benzol or busulfan. They both fitted the criteria of severe aplastic anaemia for more than three months. The first patient was an analphabetic painter from spain, who cleaned his body after work with xylol and benzol during 8 months. He was excluded from BMT because of an age of 52. Back at his village he received monthly transfusions. 10 months later he presented with marked megaloblastic alterations, but with a Hgb of 12.8 g/dl, leukocytes of 6,700 and thrombocytes of 60,000/μl. The other patient received erroneously 6 mg busulfan (Myleran R) daily instead of the anticonvulsant primidone (Mylepsin R) during 4 months. He suffered from recurrent infections and severe bleedings. 5 months later his blood improved reaching a Hgb of 10.8 g/dl, leukocytes of 4,700/μl and platelets of 57,000/μl. In spite of the claims that the lymphatic system should not be affected by busulfan, severe infections occured and immunoglobulin had been found to be reduced.

168 Bone Marrow Dysplasia and Myeloid Leukemia After Carbimazole

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Antithyroid drugs can induce reversible agranulocytosis and – rarely – aplastic anemia. We observed a 75-year-old woman who developed bone marrow dysplasia and acute myeloid leukemia, following carbimazole therapy. Pancytopenia (Hb 4,4 g%, WBC 1,500/μl, PC 29,000/μl) occured 10 weeks after the initial dose of carbimazole (30 mg/day) which was given because of hyperthyreoidism. Bone marrow aspiration showed a marked reduction of hemopoiesis with a relative increase of lymphocytes, plasma cells and macrophages. Carbimazole therapy was stopped, but pancytopenia persisted. A repeat bone marrow 3 months later was hypercellular with dysplastic changes in all hemopoietic cell lines and presence of micromegacaryocytes, hypogranular myelocytes and pseudo-pelger-cells. 9 months later, the patient progressed to overt myeloid leukemia (FAB-M 2).

Although in the present case the development of 'spontaneous' leukemia cannot be definitely excluded, the time characteristics and the pattern of bone marrow changes with progressively impaired cell maturation suggest a causal relationship between carbimazole and onset of leukemia. Similar cases of carbimazole-induced marrow dyscrasia have not been reported in the literature.

169 Acute Ascending Motor Paralysis Leading to Death After Intrathecal Methotrexate (MTX) and High Dose Cytosine Arabinoside (Ara-C) at a Short Interval

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The case of a 48-year-old man with progressive T-ALL without CNS-involvement is reported, who received MTX i. th. 15 mg hour 0, and Ara-C 5 g as 3 hour infusion hours 48, 60, and 72, resepectively. 22 hours after the third infusion with Ara-C the patient experienced an acute

ascending motor paralysis combinet with severe sensory disturbances. 4 hours after the onset of symptoms, the rapidly progressing neurologic changes lead to the death of the patient due to cardiac and respiratory arrest. The histologic examination of the spinal cord revealed an acute toxic alteration of the anterior cornu cells. As yet, there is no report in the literature on this type of CNS-toxicity with high dose Ara-C. We presume, that the short interval between intrathecal MTX and high dose Ara-C played a causative role. According to this experience, these two procedures should not be combined at such short intervals, as it is included in some salvage protocols for relapsed ALL.

170 Survival from Massive Melphalan Overdose

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The case history of a patient with plasmocytoma who received an overdose of melphalan is reported. After a 5-day course of melphalan with prednisone, she was treated erroneously elsewhere with 50 mg per day of melphalan for another 16 days. This was not discovered until she had ingested a total dose of 775 mg within 21 days. There were no significant side effects noted other than diarrhea from day 15–20. Bone marrow hypoplasia was proved on day 21. With transfusion therrapy during the period of hypoplasia she had neither severe infectious complications nor signs of hepatic or renal dysfunction. The bone marrow recovered within another 2 weeks and the patient is well 3 months after the overdose. At reevaluation plasmocytoma was in remission as can be judged by cytology and electrophoresis. We postulate that there is incomplete absorption of p. o. melphalan and increased excretion by diarrhea may have taken place, though our patient did not reach lethal blood drug levels.

171 Classification of Acute Non-Myeloid Leukemias as Either T- or B-Lineage Malignancies

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Our monoclonal antibody (MAb) HD 37 defines a B-cell specific antigen which represents the broadest available marker for the B lineage (cluster CD 19). The MAb HD 39 recognizes a B-cell specific glycoprotein antigen (m. w. 130 and 140 KD; CD 22) which is present in the cytoplasm of all B cells but is expressed on the cell surface only at later maturation stages. The MAb HD 49 reacts with a T-cell antigen of 40 KD also detected by the MAb 3 Al. Using these MAbs together with HD 11 (I a), J 5 (CALLA), anti-B 1, HD 28 (B-cell associated) and anti-T 6 we have analysed the cell surface of the leukemic cells in 60 cases of acute lymphoblastic and "unclassified" acute leukemias: 47 cases were HD 37$^+$ HD 49$^-$ thus classified as B-lineage malignancies, 8 were HD 37$^-$ HD 49$^+$ thus classified as T-cell neoplasias, only 5 were HD 37$^-$ HD 49$^-$ thus remaining unclassified. The HD 37 positive cases could be further subclassified according to the following phenotypes: HD 37$^+$, HD 37$^+$ B 1$^+$, HD 37$^+$ B 1$^+$ HD 28$^+$. HD 39 was always negative on the cell surface. Using cytocentrifuge preparations in 36 cases also the cytoplasm of the leukemic cells was analysed: In all HD 37 positive leukemias the HD 39 antigen was strongly expressed in the cytoplasm (cHD 39)! These results suggest that B-committed cells with the HD 37$^+$ phenotype represent the earliest stage of B-cell differentiation. Recent findings that heavy-chain gene rearrangement is not restricted to cells of the B lineage stresses the importance of reliable markers in phenotyping B-lineage committed leukemias. It is now possible to classify more than 90% of the non – myeloid leukemias as either T (HD 49/3 Al$^+$)– or B (HD 37$^+$ cHD 39$^+$)-lineage malignancies.

172 Complement Receptors on B-Lymphoid Cells: Detection and Possible Role in Cell Proliferation

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Receptors for C 3 b and C 3 d (two fragments of the third complement component) are present on normal B-lymphocytes and also (see abstract by R. Greil et al.) on CLLs, immunocytomas, centrocytic lymphomas, centroblastic/centrocytic as well as centroblastic lymphomas, but only rarely on lymphoblastic and immunoblastic lymphomas or hairy cell leukaemias. In order to investigate the role of these receptors in the proliferation of normal B-cells we exposed resting (small) and preactivated (= blasts) murine B-cells to polymerized C 3. The results indicate that (i) polymerized C 3 induces further proliferation in blast but not in resting B-cells, (ii) the C 3 d-receptor has to be cross-linked for C 3 to stimulate a B-cell (iii) C 3 mimics the effect on B-cells of macrophage-derived growth factors but not of B-cell growth factor or antigen.

173 Monocyte/Macrophage Heterogeneity as Shown by Monoclonal Antibodies and Monocyte/Macrophage Hybrids

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Although homogenous in origin the monocyte/macrophage lineage expresses a broad spectrum of different functions ranging from nonspecific phagocytosis to specific immune modulation of certain lymphocyte subpopulations. Using monoclonal antibodies this heterogeneity can be shown by immune staining, molecular heterogeneity of monocyte specific antigens, as well as by the possibility to induce macrophage subsets with divergent functional capabilities. In a further step some of the various monocyte/macrophage potencies could be immortalized in permanent macrophage hybrids using enzyme deficient and thus selectionable U-937 cells. With such hybrids mirroring monocyte functional diversity, work is in progress to establish a survey of genetic library encoding differentional potencies of monocytes/macrophages.

174 Immunological Characterization of Haematological Samples Using a Rapid and Simple Immunoperoxidase Staining Technique

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A fast and simple indirect immunoperoxidase staining technique is described, which can also be used for the characterization of cells with high endogenous peroxidase activity e. g. many haemopoietic cells. It is based on the combination of a newly developed glucose-oxidase and glucose procedure for inhibiting endogenous peroxidase activity with a standard two or three layer immunoperoxidase staining protocoll. Glucose-oxidase plus glucose mixture completely inhibits endogenous peroxidase activity without having a detectable deleterious effect on any of the cellular antigens so far studied. In many instances this allows to use only one layer of horseradish peroxidase (HRP) labelled antibodies. The glucose-oxidase plus glucose mixture can also be added to cells together with the HRP labelled antibody solution without loosing its inhibitory effect for endogenous peroxidase activity and without leading to a visually detectable loss of the activity of the HRP conjugate.

175 Monoclonal Antibodies Against Alkaline Phosphatase, Glucose-Oxidase, Galactosidase and Peroxidase for the Detection of Monoclonal Antibodies by Enzyme-Immuno-Techniques in Hematological Cytology

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We developed monoclonal antibodies against alkaline phosphatase, glucose-oxidase, galactosidase and peroxidase which can be used for the detection of monoclonal antibodies by enzyme-immuno-techniques using polyvalent anti-mouse-immunoglobulin as bridging link between first and second monoclonal antibody. The applicability and sensitivity of these different sandwich-assay systems compared to standard techniques are investigated. The advantages and disadvantages of each of the assaysystems as well as the optimal substrates for developing the enzyme reactions and counterstains are discussed.

176 Purging of Bone Marrow with Monoclonal Antibodies and Complement to Eliminate Tumor Cells

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A major problem of autologous bone marrow transplantation in patients with malignant non Hodgkin's lymphoma or acute lymphoblastic leukemia is a possible contamination of the bone marrow with residual malignant tumor cells. Therefore we have tested the cytolytic efficacy of different monoclonal antibodies, i. e. anti-B cell antibody Y 29/55, anti cALLA antibody VIL-A 1, with or without VIB-C 5 and VIB-E 3, anti T-cell antibody LAU-A 1, together with rabbit complement. The lysis of tumor cells (alone or in a mixture with normal bone marrow) was assessed by dye exclusion, indirect immunofluorescence and FACS-analysis. A combination with ethidiumbromide and bisbenzimide allowed us to detect persisting viable tumor cells after the in vitro manipulation. It is possible to reduce the tumor cell load of a bone marrow harvested in remission by at least 2–3 logs. Problems and limitations of bone marrow purging will be discussed.

177 Monoclonal Antibodies Specific for Carcinoembryonic Antigen (CEA) and Related Antigens as Immunohistochemical Markers

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The specificity of 3 murine monoclonal antibodies (mab 130 a, b, c Behring) detecting different epitopes on CEA and/or on related glycoproteins, like nonspecific crossreacting antigen (NCA), was evaluated on formaldehyde fixed paraffin-embedded human tissues, using the indirect immunoperoxidase method. Mab 130 a, recognizing an epitope located on CEA, NCA 55 and NCA 95, was strongly reactive with the majority of the human adenocarcinomas of the lung (17/21), whereas epithelial or biphasic mesothelioma tissues were essentially unreactive (2/17). Mab 130 b, binding to CEA and NCA 95, strongly stained pancreatic adenocarcinoma tissues (11/12), whereas acinic cell carcinomas, solid cystic and endocrine pancreatic tumors as well as tissues from normal or chronically inflamed pancreas were negative. Mab 130 c, reactive to CEA only, stained the majority of coloncarcinoma metastases in liver and lung (15/15) as well as the corresponding primaries (7/9), but reacted only marginally with the external lining of the normal colon mucosa. These antibodies are valuable aids in immunohistochemistry facilitating differential diagnosis of the above mentioned malignancies.

178 Detection of Bone-Marrow Micrometastases Using Monoclonal Antibodies

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So far it has been very difficult to demonstrate micrometastatic foci in carcinoma patients. Using a panel of monoclonal antibodies (anti-$HMFG_2$,-EMA,-cytokeratin) we investigated bone-marrow aspirates obtained from 140 patients with carcinomas of different origin. The aspirates were stained immunocytochemically according to the APAAP (immuno-alkaline phosphatase) method. Following this procedure we were able to detect micrometastases in bone-marrow whereas conventional staging techniques had failed to show any evidence of distant metastases. The results were discussed in relation to predictive indices such as tumor size or involvement of lymph nodes.

179 Different Immune Function and HLA Gene Distribution in Healthy Persons

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Studying immunofunctional parameters in different diseases lymphocytes of 88 healthy controls were stimulated by 14 different mito- and antigens, responses measured by 3 H-thymidine uptake. T-cell subpopulations were determined by monoclonal antibodies. Suppressor cell assays were done. Cluster analysis according to the program of the University of California and discrimination analyses (F-test; t-test for unpaired data with unequal variance) were performed. Surprisingly, we found 3 clusters with extremely different reactivity upon mito- and antigens in the control group ($F > 4-20$; $p < 0.05-0.00005$), cluster 1 ($n = 18$) showing medium, cluster 2 ($n = 49$) low and cluster 3 ($n = 21$) high responses in all systems tested. As suppressor cell activity and subpopulation distribution were normal in all 3 groups, these differences musts be functional ones. As HLA genes had been determined simultaneously, we analysed the clusters' gene composition and observed a certain trend to an increase of some class-1-genes (group 1: A 28; B 13/53; C 2/6; group 2: A 9; B 14/16/37; group 3: B 5/8/21; C 1/7) (relative risk: 3–7; $p < 0.05-0.01$) at a normal Dr-distribution. Furthermore, reclassification to the immunofunctional defined groups by gene distribution succeeded correctly in 70% of cases. Different lymphocyte reactivity may well be genetically determined and those clusters could be reservoirs for different diseases, therefore.

180 Red Blood Cell Transfusion: HLA B 7 Incompatibility May Cause a Delayed Haemolytic Transfusion Reaction

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In order to investigate the clinical significance of HLA determinants expressed on red blood cells (RBC) for transfusions we monitored ^{51}Cr-RBC survival, the direct radioimmune anti-IgG test (DRIAT) and concentrations of LDH and haptoglobin (hp) of patients immunized against HLA determinants and receiving RBC transfusions. Donors were selected who were compatible in typical RBC antigen systems as assayed by conventional techniques but were mismatched for the HLA antigens in question. We observed a delayed haemolytic transfusion reaction (DHTR) in all 3 pts receiving HLA B 7 incompatible RBC. The ^{51}Cr-RBC survival was markedly shortened, the DRIAT became positive, LDH increased and hp decreased. In the eluate the responsible anti HLA B 7 antibody was detectable. A similar reaction was found in only one further pt where HLA A 2 incompatibility was involved. These DHTR could not be anticipated by conventional crossmatch procedures, nor by the measurement of the ^{51}Cr-RBC survival one

hour after transfusion. We conclude that HLA antibodies can lead to a DHTR, especially when HLA B7 ist involved. However, conventional crossmatch procedures in vitro do not predict a DHTR in HLA incompatible RBC transfusions.

181 Fibronectin and its Degradation Products in Units of Whole Blood

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We investigated the levels of fibronectin (FN) by laser-nephelometry in 20 units of whole blood stored up to 39 days. The levels of FN decreased from 19.5 ± 4.8 md/dl on day 1 to 16.6 ± 5.4 mg/dl on day 25 to 10.9 ± 3.5 mg/dl on day 32. The antibody used in laser- nephelometry was coupled to Sepharose 4 B and FN-material isolated by immunadsorption. SDS-PAGE of the isolated material revealed the parent molecule of FN with MW 440 KD and during storage an increasing level of split products. Their MW was estimated by their migration behavior (410 KD, 380 KD, 260 KD, 230 KD, 200 KD, 180 KD, 130 KD, 84 KD, 70 KD, 56 KD and 16 KD). The split products are of clinical importance, since they are capable of inhibiting the activity of intact FN. The comparison of split products from in vitro tests and increasing levels of PMN-Elastase (ELISA, Merck Co.) during storage of whole blood up to 2972 ± 919 ng/dl on day 32 indicate, that granulocyte derived proteases play a role in the degradation of FN.

182 Elevated Levels of C 3 a-des Arg After Storage of Blood Products Under Normal Blood Bank Conditions

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109 units of whole blood were tested for C 3 a-des Arg by RIA (Upjohn Co.). In 4 units of packed red blood cells (RBC) C 3 a-des Arg was measured over a follow-up period of 36 days. 37 units of whole blood were also tested for C 5 a-des Arg by RIA (Upjohn Co.). 77 units of whole blood were examined for C 3 activator-complex by radial immuno diffusion (C 3 activator Partigen, Behring Co.). In whole blood we found as early as after 10 days of storage a highly significant elevation of C 3 a-des Arg from 125 ± 42 ng/ml (day 0) to 534 ± 299 ng/ml (day 10). There was no parallel elevation of C 5 a-des Arg. In packed RBC there was also an elevation of C 3 a-des Arg from 268 ± 109 ng/ml (day 2) to 489 ± 81 ng/ml (day 22). Because the classical pathway of complement activation is dependent on ionisized Ca and because there was no significant elevation of C 3 activator-complex, there might be an activation of complement system by the alternate pathway during storage of blood products.

183 Automated Counting of Reticulocytes by Means of Flow Cytometriy

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Reticulocyte counting by microscopy is a time consuming procedure. Therefore, we have tested whether flow cytometry may be employed to automate reticulocyte counting. Fresh heparinized blood was stained by acridine orange. Flow measurements are displayed as a two parametric cytogram represented by cell volume and RNA-content. This allows a satisfactory separation of reticulocytes from other cell types. Counting of more than 100,000 cells per minute increases the accuracy by a factor of 10 as compared to the microscopic technique. Measurements of 33 blood samples with reticulocyte fractions of 0.1 to 14% showed a close correlation ($r = 0.97$) between both methods. Additionally, a histogram of RNA-content per reticulocyte representing their age distribution is obtained from the flow measurements. Therefore, automated counting of reticulocytes by flow cytometry delivers a significantly more accurate determination of number and degree of maturity of reticulocytes in blood within a short time than the tedious counting by microscopy.

184 The Quantitative Buffy Coat (QBC) System in the Hospital Laboratory

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Using the QBC, five erythrocytic, leukocytic and thrombocytic parameters (hematocrit, leukocrit, granulocrit, lympho/monocrit and thrombocrit) can be obtained quickly and simultaneously, with a moderate workload. In the central laboratories of three Berlin hospitals, the system was tested for its suitability in emergency laboratories in a multicenter study according to ECCLS guidelines. The results were analysed statistically. 1984 the relability of the QBC-I was evaluated and the limitations of the system were tested with "difficult" samples (from leukemic and other tumor patients and from intensive care and dialysis patients). The results were satisfactory. After discussion with the manufactures (Clay-Adams) it was decided to continue the evaluation. 1985 the improved QBC-II is available which provides "crit" values instead of numbers. Monoclonal antibody coated capillaries are used, giving sharp-cut layers and therefore more exact results.

185 Classes of Platelet-Associated Immunoglobulins (PAIg) and Platelet-Associated C3c (PAC3c) in Relation to ^{111}In-Platelet-Survival and Site of Sequestration

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The two-stage radioactive antiglobulin test with unlabeled anti-IgG, -IgM, -IgA, or -C3c followed by binding of ^{125}I-Staph Prot A was applied to determine PAIg or PAC3c in patients (pt) with chronic autoimmune thrombocytopenia (cAITP, $n = 19$, platelets (plt) $< 50 \times 10^9$/l). The results were related to ^{111}In-labeled plt life span (MLS) and the site of sequestration. PAIgG was elevated in 13, PAIgM in 11, PAIgA in 1, and PAC3c in 5 pt, respectively. In two pt with normal PAIgG levels elevated PAIgM were measured. The MLS was markedly shortened in all pt (1.4–45.6 h), and correlated with PAIgG only ($p < 0.05$). In pt with splenic sequestration ($n = 10$) PAIgG was elevated in 8, PAIgM in 5, and PAC3c in 1 pt, respectively. In hepatosplenic sequestration ($n = 7$) both, PAIgG and PAIgM were elevated in 4, PAIgA in 1, and PAC3c in 3 pt. In both splenectomized pt with hepatic sequestration elevated levels of PAIgG and PAIgM, and in 1 also of PAC3c were found. The data show that in cAITP neither the proportion nor the class of immunoglobulins of the PAIg or PAC3c predict the site of plt sequestration. The degree of MLS shortening is best indicated by PAIgG levels. Yet, additional measurement of PAIgM may increase the sensitivity in screening for PAIg.

186 Detection of Drug-Induced Antibodies in 4 Patients with Agranulocytosis by Complement Fixation Test

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In 4 of 29 tested sera of patients with agranulocytosis we found drug-induced antibodies by complement fixation test: in 3 cases caused by Metamizol and 1 by Fluorouracil. In the history of these 29 patients 43 drugs have been incriminated as causing agranulocytosis: 12 antibiotics, 12 analgetics and 15 others. The most important measure is to discover, if possible, the offending agent and so to prohibit its further use. Following measures must be done in the early stage:
1. It is most important to send acute-phase serum in the laboratory (max 1 week after diagnosis)
2. A careful history and repeated questioning of patients and family can often reveal a probable cause.
3. Potentially harmful drugs should be noted and tested too.

187 Hodgkin's Disease, Lymphocyte Predominance

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According to the classification of Lennert and Mohri (1974) Hodgkin's disease, lymphocyte predominance is subdivided into three subtypes: nodular paragranuloma (NP), diffuse paragranuloma (DP), and other than paragranuloma subtype. These three subtypes were investigated by morphological, immunohistochemical and clinical methods. The NP and DP differ from the other than paragranuloma subtype in showing special cytological and immunohistochemical properties in their "atypical Hodgkin's specific cells" and in their differentiation of the surrounding lymphoid tissue. The "atypical Hodgkin's specific cells" in NP and DP are positive for j-chain and negative for x-hapten, and thus are possibly B cell derived. Most lymphoid cells in NP and DP are B cells. By contrast Hodgkin- and Sternberg-Reed-cells of other than paragranuloma subtype are usually negative for j-chain and positive for x-hapten. Most lymphoid cells in other than paragranuloma subtype are T cells. NP and DP usually show a better clinical course than other than paragranuloma subtype. Stage I of NP shows the same probability of survival as the normal population.

188 Radiotherapy of Hodgkin's Disease After Primary Failure of Chemotherapy and Recurrence After Chemotherapy

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Eight patients suffering from Hodgkin's disease with primary failure of chemotherapy or recurrence after chemotherapy were irradiated with curative intention. Stages were 2×II B, 1×III A, 1×III B, 1×IV A, and 3×IV B. Radiation dose was at least 40 Gy; four patients received an involved field irradiation, one patient an extended field irradiation, and in three patients total nodal irradiation was performed. None of the eight patients received adjuvant chemotherapy. Six of the eight patients achieved a complete remission now lasting 1, 2½, 8, 11, 12+ and 18+ months. Toxicity of radiotherapy was acceptable. It was more pronounced especially in regions that had already been irradiated or if ABVD had preceded radiotherapy. The two patiens with ongoing remissions after radiotherapy alone show that radiotherapy can bring about long-term remissions after failure of chemotherapy. Nevertheless, those patients should receive adjuvant chemotherapy whenever possible.

189 The Influence of Splenectomy in Hairy-Cell Leukaemia, Classified into Histologic Subtypes According to Bone Marrow Biopsy

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In contrast with the common experience there are cases of hairy cell leukaemia (HCL) that show no improvement after splenectomy. These cases are not clearly distinguished by the generally accepted prognostic criteria (J. Jansen, 1982), namely the value of haemoglobin and splenic size. The bone marrow biopsies and survival-times of 79 patients, all showing the characteristical manifestation of hairy cell leukaemia were evaluated retrospectively. 32 patients underwent splenectomy (group A), 47 patients had no surgical treatment (group B). In all cases semithin sections of the undecalcified specimen were investigated with the light microscope after embedding in methacrylate, and at least five stains were applied. In some cases electron microscopic sections were studied in addition. The survival times were obtained in all. Three subtypes of hariy cells: ovoid, convoluted, and indented were distinguished according to the nuclear configuration, with significantly different median survival times (56, 12, and 5 months respectively, $p = 0.001$). The median survival times differed considerably among group A and B and the three types: ovoid/A 50.4 months ($n = 8$, alive) and 9.1 months ($n = 10$, dead); ovoid/B 82 months ($n = 2$, alive) and

6.3 months ($n = 16$, dead); convoluted/A 63.7 months ($n = 3$, alive) and 9.9 months ($n = 8$, dead); convoluted/B 11.8 months ($n = 18$, dead); indented/A 24.7 months ($n = 3$, dead); indented/B 4.1 months ($n = 11$, dead). These results show that the above named subtypes do not only indicate different prognosis in hairy cell leukaemia in general, but also a different effect of splenomegaly. This intervention is less effective in patients showing the indented type in the bone marrow biopsy than in those that exhibit changes of the two other subtypes.

190 Subtained Improvement of Aggressive B-Prolymphocytic Leukemia After Splenectomy

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The efficacy of bilogic modifiers or enzyme inhibitors on proliferation of lymphocytes has become a well known fact. We report on a 61-year-old female patient, who presented with a high cell count of prolymphocytic leukemia (PL) of B-cell type. Deterioration of performance status lead to treatment in a community hospital. 2.4 gy irradiation to the spleen, 2 times COP- and 1 time CHOP-chemotherapy failed to alter the leukemic process. After referral to our institution we instituted leukapheresis. After 8 courses the patient's status improved markedly and splenectomy could safely be done. Since that time the patient is off treatment. IgM could be redetected for the first time by the RID method 16 months after splenectomy. At this time an infiltration of the bone marrow has still been visible and the largest lymphnode has had a diameter of 0.4 cm. Even now after 50 months and normalization of all blood cell counts prolymphocytes can be seen on microscopic evaluation. Analysis of surface markers and cytochemistry showed a special maturation arrest of a B-prolymphocyte. The cell type and its "homing" in the spleen seem to present a rare association with an unic behaviour.

191 Systemical Chemotherapy with Etoposide, Methotrexate, Bleomycin, Prednisone and Prednimustine in Advanced Mycosis Fungoides

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After local treatment, photochemotherapy and electron beam irradiation systemical cytostatic therapy is indicated in mycosis fungoides (MF) with marked skin lesions, histopathologically positive lymph nodes, elevated atypical cells in the blood and visceral organ involvement. To improve the palliative therapy we attended 11 pretreated MF pts (stages II–IV) with etoposide (100 mg/m^2 po, d 1–5), methotrexate (5 mg/m^2 po, d 1–3), bleomycin (15 mg iv, d 1) and prednisone (50 mg iv, d 1) every 3 wks; after remission and at least 10 courses maintenance therapy with prednimustine (40 mg po, d 1–14) every 4 wks. Results: 1 complete remission (duration 2 mths), 7 partial remissions (2–16 mths, median 6 mths) and 3 stable diseases (1–3 mths, median 1, 5 mths). 4 pts received maintenance therapy (1–9 mths, median 4 mths). Mild nausea occured in all pts and severe thrombocytopenia in 1 pt. Due to the high response rate, the long remission duration and the minimal side effects this treatment schedule seems to be of good palliative efficiency in pretreated MF pts.

192 Improved Survival in Patients with Acute Nonlymphoblastic Leukemia (ANLL) – (VAATP) A Five Drug Regimen

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Since 1980, 63 pts. with ANLL were treated with Vincristin (1.4 mg/m^2, i. v., day 1), Adriamycin (40 mg/m^2, i. v., days 1, 2, 3), Ara-C (100 mg/m^2, cont. inf., days 1–7), 6-Thioguanine (2×80 mg, p. o., days 1–7), Prednisone (100 mg, p. o., days 1–7) (VAATP). 44/63 pts were untreated (group A). 7/63 pts were in relapse or pretreated with other protocols, 9/63 pts had

ANLL after preleukemia, 3/63 pts had ANLL after pretreatment for other malignancies (19/63 = group B). *Patient characteristics:* male (37/63), female (26/63), mean age 57 years (16–74), M1–M5 (55/63), M6 (8/63). *Results:* CR rate was 69% (42/63), i.e. 76% (33/44) in group A and 52% (10/19) in group B. Median remission- and survival duration in group A was 16 and 22 months, in group B 6 and 10 months, respectively. Pts with erythroleukemia (M6) and pts of (M1–M5) with Pas-positive erythroblasts in their remaining erythropoiesis had a bad prognosis. 11/44 pts (group A) are still alive in ongoing complete remission for more than 3 years, 6/11 for more than 4 years. Updated detailed results will be presented.

193 Intensified Induction Therapy and Limited Maintenance Therapy for Treatment of Acute Myeloid Leukemia (AML)

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From August 1982 – April 1985 43 patients with de novo AML (FAB 1–6, median age 53 years, range 14–86 years) were treated with daunorubicin (DNR) 60 mg/m^2/d (> 54 years 45 mg/m^2) day 1–3 and 200 mg/m^2/day ARA-C (100 mg/m^2/day as continuous infusion + 2×50 mg/m^2/day iv) day 1–7. Consolidation treatment consisted of DNR (45 mg/m^2) day 1, Vincristin (V) 1 mg/m^2 day 2 and ARA-C 200 mg/m^2/day (as in induction therapy) day 1–7 and maintenance therapy of 6 weeks cycles of DNR (45 mg/m^2) day 1, ARA-C (200 mg/m^2/day) day 1–5 s.c. (6×). Complete remission (CR) was achieved in 27 patients (63%), of these 70% reached CR after one cycle. Median observation time of CR patients is 13 months. The median duration of remission is currently 14 months. The probability of remaining in first continuous CR at 31 months is 40%, of disease free survival 37.5% and the probability of survival of CR patients 67%. The probability of survival of all treated patients at 31 months is 30%.

194 COAP Regimen: Successful Treatment Approach in Patients with Acute Myeloid Leukaemia with Partial Remission After TAD Induction Therapy

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In five patients with acute myeloid leukaemia three courses of induction therapy according to the TAD regimen (thioguanine, cytarabine, daunorubicin; Gale et al.) resulted in partial remission only. After administration of two to five subsequent courses of the COAP regimen (cyclophosphamide, vincristine, cytarabine, prednisone; Freireich et al.) complete remission could be induced in all these cases. Duration of remission ranges from 4+ to 10+ months. In one of these patients successful allogeneic bone marrow transplantation could be performed. Thus, COAP which is well tolerated in most patients seems to be a successful treatment approach even in patients not achieving complete remission after administration of a cumulative dose of 550 mg/m^2 of anthracycline.

195 Aclarubicin (Aclacinomycin A, ACM) in Single Agent and Combined Chemotherapy of AML

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ACM was used in single agent and combined chemotherapy of acute myeloid leukemia (AML) in two consecutive phase-II studies. ACM, 25 mg/m^2/d×7 days induced 8 complete (CR) and 1 partial remission (PR) in 29 pts (31%) with relapsing and/or resistant AML. The highest response rate was achieved in patients with the first relapse of the disease (6 CR in 12 pts). In a pilot study ACM was combined with Ara-C: ACM 18 mg/m^2/d×7 days for patients ≤ 50 yrs or 12 mg/m^2/d×7 days for patients > 50 yrs, Ara-C 100 mg/m^2/d CIVI for 7 days. Eleven out of 16 evaluable pts responded with a CR. CRs were achieved in previously untreated (8 out of

12) and in pts in first relapse (3 out of 4). Myelocytic (M 1, M 2) as well as myelomonocytic (M 4, M 5) leukaemias responded to the ACM + Ara-C combination. In conclusion, ACM is a very active agent in the treatment of AML and should be further evaluated in phase-II or III studies.

196 Improvement of Relapse Free Survival by Intensified Maintenance Therapy in Adult-ALL

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Since 1980 24 consecutive patients with previously untreated ALL (mean age 32.7 years, range 15–77 years, 10 females, 14 males; 10 T-ALL, 7 cALL, 2 pre-B-ALL, 1 B-ALL, 1 null ALL, 3 not specifiable) received chemotherapy as follows: Induction therapy in the first 11 patients consisted of high dose prednisone, vincristine and methotrexate followed by Ara-C and 6-MP (Omura et al.), the further 13 patients had induction with daunorubicine, vincristine, prednisone and asparaginase followed by cyclophosphamide, ARA-C, 6-MP and methotrexate (Hoelzer et al.). Maintenance treatment included 6-MP, MTX and cyclophosphamide as a basis therapy and every two months an intensification with prednisone and vincristin for 2 weeks. Maintenance was given for two years. 20/24 patients reached a complete remission (83.3%), 4 relapsed within 1.5 years after successful induction therapy. Overall survival at 5-years (according to the method of Kaplan-Meier) is 55%, the probability of relapse free survival and of being in first continuous remission at 5 years currently is 75%.

197 Treatment of Poor Risk Leukemia in Children with High Dose Ara-C and Asparaginase (HD-ARA-C-ASNase)

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14 children (aged 6 ms–17 yrs) received HD-ARA-C-ASNase (3 g/m^2 ARA-C over 3 hrs every 12 hrs for 4 doses and 6000 U/m^2 ASNase i. m. at hr 42). The diagnoses were: 6 ALL bone-marrow relapses (5 inductions, 1 consolidation), 3 high risk-ALL (consolidation), 2 ALL-Nonresponder (NR), 1 AML (NR), 1 CGL in accelerated phase and 1 myelosarcoma in progression. In patients (pt) with ALL bone marrow relapse 3 complete remissions (CR) and 1 partial remission (PR) were achieved, but 1 pt died. In the group with ALL-NR were 1 CR and 1 NR respectively. All pt with ALL consolidation remained in CR. The pt with AML had a PR and both pt with either CGL or myelosarcoma, respectively, did non respond. In a total of 28 treatment courses we recorded the following side effects: severe myelosuppression ($n = 28$), drug fever ($n = 22$) vomiting ($n = 18$), conjunctivitis ($n = 5$), mucositis ($n = 7$), arthropathy ($n = 2$), exanthema ($n = 6$), moderate hepatotoxicity ($n = 6$) and local ASN-ase hypersensitivity ($n = 2$). Since side effects were tolerable, children with acute leukemia may benefit from HD-ARA-C-ASNase as a contribution to conventional chemotherapy.

198 Prognosis and Therapy in Childhood ALL: Which Way to Go?

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Since 1971 some 800 new cases of childhood ALL have been diagnosed and treated in the country. Multivariate analysis of the prognostic factors that may influence cumulative complete remission revealed that after the modality (intensivity) of therapy the following factors had a significant effect: initial WBC count ($p = 0.001$), sex ($p = 0.001$), CNS involvement ($p = 0.01$), hepatomegaly ($p = 0.01$) and L 2 morphology ($p = 0.05$). Stratification into high and low risk treatment groups and intensification of therapy lead to an improvement in complete remission rates (0.72 and 0.49 at 30 mo) but a number of problems remain unsolved.

According to our data, present stratification to 2 prognostic groups is unsatisfactory. Therapy is thought to be too intensive for low risk patients and inadequate for high risk ones. New strategies are sought to improve on current results.

199 Aspergillus Infections Complicating Induction Chemotherapy of Acute Leukemia

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Infections are a major complication in patients with acute leukemia undergoing intensified induction chemotherapy. These are increasingly caused by fungi, especially aspergillus. The diagnosis of aspergillosis is difficult and deep tissue biopsies obtained by invasive procedures are often necessary.

During the last 12 months, 6 patients with acute leukemia (ALL $n = 4$, AML $n = 2$) were found to have invasive aspergillus infection. 4 of these patients had pulmonary aspergillosis, 1 patient presented with cerebral manifestation and another patient with disseminated disease. In 3 cases diagnosis could be confirmed only by invasive surgical procedures (open lung biopsy, craniotomy). Serologic tests were negative in 2 cases of aspergillosis. As antifungal therapy, intravenous amphotericin B was given, in 2 cases in combination with 5-fluorocytosine. In 4 cases additional surgical intervention was necessary. In total 4 of 6 patients survived, 3 of them still being in complete remission of leukemia.

200 Treatment of Acute Myelogenous Leukemia in Relapse with High Dose Cytosine Arabinoside

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The clinical response to combination chemotherapy in AML usually decreases in relapsing patients. We administered 3 g/m^2 of cytosine arabinoside every 12 h for 4–6 days to 11 patients with relapsing or primarily not responding AML. 3 patients were treated twice because of a subsequent relapse. 2 patients were additionally treated with Mitoxantrone 12 mg/m^2, day 3–5. Pretreatment consisted of 1–7 TAD protocols with a median of 2. Results: 6 CR, 2 PR, 3 resistant. 3 patients died of septic complications during leukopenia. Substantial myelotoxicity was observed with a leukocyte count below 1000/μl for a mean of 23 days. Overall toxicity was acceptable. One patient with multiple leukemic skin infiltrates did not benefit from therapy despite a transient regression of the infiltrates. Another patient with meningeosis leukaemica showed restitution of severe hemiataxia after therapy and is in CR for over 12 months. Ara-C HD therapy in adults with AML in relapse resulted in CR in 6 of 14 cases with a mean duration of 6.3 months.

201 High-Dose Cytosine Arabinoside in the Treatment of Refractory Acute Myelogenous Leukemia (AML)

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Six patients (5 male, 1 female) 15–64 years, median 41.2) with acute myelogenous leukemia in relapse, refractory to conventional chemotherapy (2–3 cycles with Daunorubicine, Cytosine Arabinoside and Thioguanine, DAT) were treated with high-dose Cytosin Arabinoside (HD ARA-C) using the following schedule: 2–3 g/m^2 twice daily for 10 consecutive doses. The received total dose ranged from 27–40.5 g, median 31.4 g. Profound myelosuppression (leucocytes 2.0/nl) lasted from 3–22 days (median 15.0 days). Two out of six patients died during the aplastic phase and in four patients the HD ARA-C regimen failed to achieve any remission of leukemia. One out of four non-responders underwent an autologous bone marrow transplantation after 2 additional chemotherapy treatments (Amsacrine and Etoposide). These results suggest that studies with different schedules and dosages of administration of ARA-C are necessary for the improvement of response rate in refractory acute myelogenous leukemia.

202 Efficacy of High-Dose (HD) and Intermediate-Dose (ID) ARA-C in Patients with Secondary Leukemia (SEC AML), Refractory (REF) AML and Relapsing (REL) AML

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17 patients (11 males, 6 females, median age 48, range 14 to 64 years) with SEC AML ($n = 7$, 12 courses), REF AML ($n = 6$, 7 courses) or REL AML after complete remission (CR) ($n = 4$, 7 courses) were treated with HD-ARA-C (3 g/m^2 twice daily for 6 days) ($n = 9$) or ID-ARA-C (0.5 g/m^2 twice daily for 6 days) ($n = 8$). Of the 7 pts with SEC AML CR was achieved in 3, partial remission in 2, 1 was refractory to treatment and 1 died during aplasia. Duration of CR was short (215, 199, and 13 days resp.) but in 2 pts a second and in 1 pt a third remission was easily obtained by the same treatment. Probability of survival of all patients with SEK AML was 55% at 20 months. No CR was achieved in pts with REF AML and in those who relapsed during maintenance therapy. CR was obtained in a pt relapsing when already off therapy. Duration of bone marrow aplasia was similar in both schedules (median 25 days for HD-ARA-C and 29 days for ID-ARA-C) but toxicity was less with ID-ARA-C. In conclusion HD-ARA-C or ID-ARA-C may be an effective primary treatment of SEC AML but seems to be less effective with REF AML or REL AML.

203 Treatment of Relapsing or Refractory Acute Leukemia with High Dose AraC and VP 16

M. Freund, H. Diedrich, H. Poliwoda (Department Hematology/Oncology, Medical School, D-3000 Hannover 61, FRG)

The treatment of relapsing or refractory leukemia requires the development of new chemotherapy regimens. High dose AraC and VP 16 are known to be active in this group of patients. We evaluate therefore a combination of these substances. Treatment regimen consisted in AraC 3 g/m^2 twice daily as a short infusion (2 hrs) i. v. day 1 to 6 and VP 16 100 mg/m^2 as a short infusion i. v. day 1 to 5. We have treated 7 patients up to now, 6 male, 1 female. Mean age was 41 yrs (18–62 yrs). There were 5 AML M 2 FAB, 1 M 4, 1 ALL L 1 (CALLA positive). 3 patients were in their first relapse, 2 in their second, one in his third, the last one was a primary induction failure with persistent hypercellular blast marrow. We achieved 3 CR, 1 PR, 1 NR. One patient is not evaluable yet but has good peripheral regeneration, another died in treatment induced aplasia. Gastrointestinal toxicity was severe, hematotoxicity was tolerable. In our series there was no CNS toxicity and only one case of dermatitis.

204 Treatment of Relapsing or Refractory Acute Leukemia with M-Amsa and VP 16

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Treating relapsing or refractory acute leukemias means a challenge for new substances and new combinations. On this background we started a trial on M-Amsa and VP 16 in this group of patients. Treatment regimen consisted in M-Amsa 100 mg/m^2 and VP 16 100 mg/m^2 day 1 to 5 as short infusions over 1 hour. Up to now 7 patients (5 male, 2 female) are treated, one of them with 2 cycles. Mean age was 52 years. FAB-classification was as following: 1 M 1, 3 M 2, 3 M 4. Four patients were in the first relapse, three were refractory to induction therapy. We achieved 1/7 CR, 1 PR, 1 NR. In 2 patients remission status is not yet evaluable, 2 more died after therapy with peripheral aplasia. The latter 2 patients had been refractory to preceeding induction chemotherapy. Side effects consisted mainly in hematotoxixity. We conclude, that the combination of M-Amsa and VP 16 may be useful in relapsing and refractory AML.

205 Phase III Study with Amsacrine, Cytosine-Arabinoside, Thioguanine (AAT) vs. Daunorubicin, Cyt-Ara-C, Thioguanine (DAT) in Untreated Acute Non-Lymphoblastic Leukemia

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22 patients with untreated acute leukemia (7 blastic transformation of chronic myelogeneous leukemia, 15 acute myeloblastic leukemia) were recruited for a randomized treatment either with AAT or DAT-chemotherapy. AAT regimen consisted of: Amsa 200 mg/m^2/die×3, ARA-C 200 mg/m^2/die×5, Thioguanine 100 mg/m^2 p.o. q 12h×10; DAT regimen dosage was Daunorubicine 50 mg/m^2/die×3, ARA-C 200 mg/m^2/die×5, Thioguanine 100 mg/m^2 p.o. q 12h×10.

10 patients were randomized to receive AAT (16 cycles) and 12 to DAT (24 cycles). The median age was 62 years (AAT) vs. 60 years (DAT). In the AAT group 5/6 evaluable patients reached a complete remission (CR), whereas 6 out of 10 evaluable patients entered a CR on DAT. Median time to reach a CR on AAT was 26 days (20–35 days), on DAT 33 days (17–54 days). On AAT there was a shorter median duration of aplasia with 16 days compared to patients on DAT with 20 days.

206 Plasma Fibronectin During L-Asparaginase Therapy of Patients with Acute Lymphoblastic Leukaemia

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Plasma fibronectin is a large glycoprotein mainly produced in hepatocytes. In addition to other untowards effects L-asparaginase is known for its hepatotoxicity. Therefore we measured plasma fibronectin in eight children and six adults with ALL in the course of L-asparaginase treatment.

In all patients observed fibronectin decreased rapidly after the first L-asparaginase application. Normal levels were found during polychemotherapy before and after L-asparaginase treatment. From this and from correlations with other parameters of liver function including histological examination of liver biopsies we conclude that the decline of fibronectin reflects its reduced hepatic synthesis specificly related to L-asparaginase hepatotoxicity. As fibronectin acts as a mediator of phagocytosis, the implications of low fibronectin for occurence and prophylaxis of septic complications during L-asparaginase therapy will be discussed.

207 β2 Microglobulin in Cerebrospinal Fluid for Diagnosis of CNS-Metastasis in Leukemia and Lymphoma

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β2 microglobulin (β2 MG), a protein produced by nucleated cells, can be found in serum, urine and in cerebrospinal fluid (CSF). The central nervous system (CNS) can be regarded as an isolated compartment with respect to β2 MG. To detect involvement of CNS in acute leukemia (ALL, ANLL) or malignant lymphoma (NHL) and to monitor early relapses we determined β2 MG in CSF of more than 25 patients. Our analyses, which were repeated up to eight times in individual patients, revealed that elevated β2 MG-levels coincide with or may even precede overt CNS involvement. The presence or absence of neurological dysfunction, CSF protein content and cytological examinations were correlated to β2 MG levels. In those patients with CNS manifestations of AL or NHL mean value of CSF-β2 MG was 3.09 ± 0.83 mg/l as compared to 2.0 mg/l for normal individuals, showing a good correlation between elevated β2 MG levels and other signs of CNS involvement. Serial determinations during the course of the disease showed that β2 MG seems to be also a good parameter to monitor the effectiveness of chemotherapy. With successful elimination of malignant cells β2 MG in CSF returned to normal levels.

208 Neurologic Complications in Adult Myelomonocytic and Monoblastic Leukemia (FAB: M4/M5) Occuring after Haematologic Remission

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In 25 patients (14 female, 11 male; 16–68 years of age) suffering from acute myelomonocytic or monoblastic leukaemia complete hematologic remission could be achieved by induction therapy on the basis of anthracycline-cytosine arabinoside combinations. Within 2 to 10 months in 4 female patients (25 to 53 years old) polyradicular neurologic symptoms combined with cranial nerve palsies occured. The onset of the neurologic symptoms either coincided with hematologic relapse (3 patients) or preceded the systemic relapse (1 patient). CSF examination confirmed meningeosis leukaemica, cell count ranged from 64/3 to 1700/3 cells. Intrathecal chemotherapy was applied 5 to 8 times with single applications of 15 to 20 mg MTX + 80 mg CAR leading to CSF remission by which progress of cranial nerve palsies and polyradicular symptoms could be terminated, however neurologic deficit persisted. Neuropathologic examination of involved structures revealed patchy demyelination of cranial and spinal nerve rootes without infiltration. Prophylactic therapeutic measurements in patients with a high risk for CNS involvement are recommended at the time of achievement of haematologic remission.

209 Transformation of Idiopathic Aplastic Anemia Into Acute Leukemia: Significance of Fragile Chromosome 16

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Idiopathic aplastic anemia (IAA) may be considered as a preleukemic state. Cytogenetic studies in this disorder are therefore of diagnostic and prognostic significance. Recently interest has focused on fragile sites on chromosomes as possible predisposing factors to malignant transformation. We report on a patient who developed an acute leukemia (FAB type M4) 2 years after diagnosis of IAA. A fragile site on chromosome 16 was observed in the patient (and in 4 of 11 relatives examined) at the time of diagnosis of IAA. In connection with development of AML a chromosomal evolution with various additional chromosomal abnormalities appeared. Since the fragile site on chromosome 16 was not involved in the evolution of this abnormal karyotype we conclude that there was no direct connection between the fragile site and the development of leukemia in this patient. The fragile site may however be an expression of an undefined genetic disposition to malignant diseases under certain environmental influence.

210 Leukemia Classification by Flow Cytometry After Supravital Staining with Acridine Orange

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The fundamental principles of an automated blood cell differentiation in flow systems after supravital staining of whole blood samples with acridine orange were first worked out by Adams and Kamentsky (1971). Working on this basis, DNA dependent green and RNA dependent red fluorescent signals show the exact position of each individual blast cell in a bivariate system of coordinates. More than 200 patients with leukemic disorders have been measured. The group centers of the various blast populations are presented in a synoptic graph in accordance with the FAB classification.

211 Chromosome Analysis and Flow Cytometry in Immunologically Defined Subgroups of Childhood Acute Leukemia

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In order to detect qualitative and/or quantitative changes in the cellular DNA content of leukemic cell populations, chromosome analysis (CA) and DNA flow cytometry (FCM) were performed in 50 cases of immunologically phenotyped childhood acute leukemia (42 ALL, 8 AML). As an easy method to measure gross quantitative changes, DNA aneuploidies were detected in 40% of cases by FCM. Although usable mitoses were found in nearly 75% of cases, chromosome analysis was hampered by preparation difficulties – especially in ALL. Furthermore, the presence of only normal methaphases did not exclude genetically abnormal clones, since FCM also revealed aneuploidies in several of such cases. FCM on the other hand, failed to reveal near-diploid deviations and qualitative changes (translocations). Hence, corresponding results, i. e. detection of the same clone, were only obtained in 35%. We therefore conclude that only the combination of both methods may provide reliable information on genetic changes in neoplastic cell population.

212 The Support with Leukocytes and Platelets in the Treatment of Acute Leukemia in Adults

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During the period from 1. 1. 1979 up to 31. 3. 1985, 135 patients suffering from acute leukemia, who underwent chemotherapy, were supported with platelets or leukocytes.

All patients needed plateletsupport with the maximum during the second and third week after the start of treatment. On the average the patients received 8 plateletconcentrates during the remission induction.

Only 23 patients (17.03%) were supported with leukocyteconcentrates. Despite the introduction of a more aggressive treatment since 1983, the need for leukocyteconcentrates showed a distinct decline. Between 1979–1982, 17 out 61 patients (27%) received leukocytesupport in comparison to only 6 out of 74 patients (8%) from 1983–1985.

Thus we conclude that approbiate antibiotic therapy is suffient to control infection in severe myelosuppressed patients.

213 Differentiation of Human Lymphoid and Myeloid Cell Lines During in Vitro Culture Under the Influence of Mouse Postendotoxin Serum.

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Human leukemic cell lines of lymphoid and myeloid origin were investigated with respect to their differentiation capacity under the influence of murine postendotoxin serum. The following cell lines were tested during a 2-week culture: K 562, HL 60, KG 1 A, REH and Nalm 6 M 1. Cells were harvested at various time intervals. At each harvesting day cell counts as well as morphological and immunological typing were performed. *Results:* The Nalm 6 M 1 cells die under the influence of mouse serum without any signs of differentiation, the REH-cell line showed a strong positive reaction with the antimyeloid antibody cultured with 10% murine postendotoxin serum. Morphological changes could not be observed. The most important result in the cell lines of myeloid origin was a morphological as well as immunological differentiation of KG 1 A cells considered to be uninducible in this system. K 562 and HL 60 cells differentiated only poorly in the presence of postendotoxin and normal mouse serum.

214 In Vivo Haematopoietic Differentiation Induced by Prednisolone, Daunoblastin and Vepesid in a Case of Acute Undifferentiated Leukemia (AUL)

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In vivo differentiation induction of AUL blast cells is very uncommon. We report a case in which lymphoid and myeloid cell markers appeared sequentially. A 18-years-old woman presented with a high white blood cell count (WBC) of 50,000/μl (90% blasts). The patient's bone marrow was hypercellular, and a diagnosis was made of AUL by typical morphology (French-American-British (FAB) L 1), negative cytochemistry and phenotyping with a comprehensive panel of monoclonal antibodies. Unexpectedly, within three weeks of starting treatment with prednisolon, daunoblastin and vepesid, the leukemic phenotype underwent complete conversion from undifferentiated (FAB-L 1, TdT +) to myelomonocytic (FAB-M 4, TdT–, 80,000 WBC/μl, 90% blasts). In addition, it is of interest that the initial marrow sample (FAB-L 1) of this patient showed myeloid antigeneic determinants using esterase-isoenzyme markers, whereas hexosaminidase-isoenzymes showed a pattern typical for AUL blast cells. By combination of cell surface markers, enzyme markers, morphology and karyotyping, in vivo differentiation induction in a case of acute leukemia with stem cell origin can be proposed.

215 Childhood Acute Lymphoblastic Leukemia (ALL) Phenotypes Defined by Monoclonal Antibodies (MoAbs)

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Leukemic blasts from 285 children with newly diagnosed ALL have been phenotyped by immunofluorescence with a panel of MoAbs directed against B-cell-associated and restricted antigens (HD 37, B 4, VIB-C 5, B 1, Y 29/55), T-cell-specific surface determinants (Leu-9, Leu-1, MasO 36, OKT 11, OKT 3, OKT 4, OKT 8), as well as other non-lineage-restricted antigens (J 5, OKIa 1, OKT 9, OKT 10). In addition, leukemic cells were examined for cytoplasmic and surface Ig, for TdT, and for receptors of sheep erythrocytes. Marker expression in different subgroups of childhood ALL (null-ALL $n = 14$, common-ALL $n = 216$, B-ALL $n = 7$, T-ALL $n = 43$, acute "unclassifiable" leukemia $n = 5$) will be presented in detail. Phenotypic heterogeneity will be related to the distinct stages of normal B- and T-cell differentiation and correlated with morphology, cytochemistry, and with clinical features.

216 Leukemia Typing with Monoclonal Antibodies

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Peripheral blood from 28 patients with clinically suspected leukemia was analyzed by automated flow cytochemistry (H-6,000), cytomorphology and cytochemical stains. In addition, phenotyping of Ficoll-fractionated leukocyte suspensions was performed using a panel of monoclonal antibody conjugates. Antibody reactivity with leukemic cells yielded in 4 patients a T-CLL, in 14 patients a B-CLL. Combined morphological/cytochemical and immunologic cell typing showed 2 patients with hairy cell leukemia. In 3 patients with chronic myeloid leukemia the reaction pattern of anti-myeloid antibodies confirmed myeloid blast crisis. In 2 patients with acute leukemia the blasts were of myeloid origin. Leukemic cells of 3 patients showed neither positive cytochemistry nor antibody reactivity. The clinical usefulness of the three techniques (flow cytochemistry, cytomorphology/cytochemistry, immunologic cell typing) to differentiate leukemias is discussed.

217 Detection of Glycoprotein IIb – IIIa Complex on Leukemia and Lymphoma Cells by the Monoclonal Antibody Hu P 1-m 1

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Marrow smears were incubated with Hu P 1-m 1 (Serotec) and stained with the immune-alkaline-phosphatase-sandwich-technique. In the following cases malignant cells gave positive reactions with Hu P 1-m 1: 1/4 CML blast crisis, 0/2 DMS (dysmyelopoetic syndrome), 0/10 ALL, 6/29 AML, 0/2 EL (erythroleukemia M 6), 0/4 NHL and 0/2 Sézary syndrome. Of the 17 patients treated with TAD, 5 were positive for Hu P 1-m 1, 12 were negative. Median CR-duration was 7 and 3 mo, median survival was 20 and 7 mo respectively. We conclude, that positive reactions of monoclonal antibody Hu P 1-m 1 can be found in blast cells from patients with CML blast crisis and AML. This is probably due to expression of glycoprotein IIb – IIIa complex on the surface of these cells. Reaction with Hu P 1-m 1 seems to be a positive prognostic factor in AML as far as the yet small number of patients allows this conclusion.

218 Monoclonal Antibodies Developed for Diagnosis of T-Cell and Very Immature Leukemias

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Monoclonal antibodies (TH 69, TH 126, TC 25) were raised by immunization of mice with T-cell acute lymphoblastic leukemia (ALL) cell lines CEM and HSB-2. Analyzing blood cells from normal donors ($n = 10$), TH 69 and TH 126 did react with approx. 30% of T-cells, independently whether of helper or suppressor/cytotoxic phenotype, while the determinant detected by TC 25 was expressed only on platelets and, less intense, on monocytes. A variety of human cell lines ($n > 30$) was employed for characterizing antibody specificity. All three antibodies did react with 4 of 4 T-ALL lines, but little reactivity was seen outside the T-lineage. Fresh Sezary cells as well as Sezary line HUT 78 were stained only by TH 126. Testing leukemias and lymphomas ($n > 25$), only TC 25 showed major reactivity outside the T-cell tumors, namely on chronic myelogeneous leukemia in blast crisis and very immature acute leukemias. Thus, while TH 69 and TH 126 will help to dissect T-cell tumors, TC 25 may be usefull in analysis of CML in blast crisis and immature leukemias.

219 Immunocytochemical Analysis of Acute Myeloid and Monocytic Leukemias

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Recent advances in clonal culture of human hemopoetic cells and the growing number of cell surface antigens recognized by monoclonal antibodies require a reconsideration of acute myeloid and monocytic leukemias.

In analogy to the established classification of lymphoblastic lymphomas/leukemias, in this presentation we report our results on attempts to establish a phenotypic characterization of acute myeloid and monocytic leukemias.

This work includes introduction of a new monoclonal antibody, which allows for a clear cut distinction of all myeloid and monocytic cells including the most undifferentiated blasts lacking all other phenotypic properties.

220 T-Cell Phenotypes in the Murine Thymus During Leukemogenesis

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A single injection of 50 mg/kg methylnitrosourea (MNU) induces T-cell lymphomas in over 90% of adult BDF 1 mice within 12–40 weeks. During the latency period and in leukemic mice the expression of PNA (peanut agglutynin), TdT (terminal deoxynucleotidyl transferase) and the thymus leukemia antigen (TLm 4) in the thymus was studied. Thymocytes of BDF 1 mice are TL-negative. Three weeks after MNU, first thymocytes were positive for TLm 4. Also most leukemias contained a high number of TL-positive cells. During the latency period a continous reduction of PNA-positive ("immature") thymocytes was seen, all leukemias were PNA-negative. In contrast, the expression of TdT, another marker of immature thymocytes, remained unaltered. The discrepancy in the expression of PNA and TdT makes an assignment of the leukemic cells to a more immature or more mature physiological cell type impossible.

221 Acute Leukemia in the Area of Freiburg – an Epidemiologic Analysis

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137 new cases with acute leukemia (AL) were registered during the past 6 years. This corresponds to an average of 1.9 cases per 100,000 inhabitants (I) per year. By subdividing the area into three districts the region of Waldshut-Lörrach (335,000 I) which is bordering Switzerland shows an increasing number of AL in the last two years: 3.0 new cases per 100,000 I were found in 1983 and 4.5 new cases in 1984. The possible influence of industrial fallout in this region must be discussed.

222 Chemotherapy of AML in a Patient with Acute Intermittent Porphyria

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There is little information about the safety of cytotoxic drugs in patients with acute intermittent porphyria (AIP). We administered two courses of polychemotherapy according to the TAD protocol to a 33 year old woman with AML who suffered from AIP for 6 years. The urinary excretion of total porphyrins (total po.), porphobilinogen (PBG) and delta-aminolevulinic acid (ALA) was increased before therapy (total po.: 445 µg/l, PBG: 20 mg/l, ALA: 12 mg/l). Continuous monitoring revealed no further increase in the urinary excretion of porphyrins during chemotherapy and supportive care with neomycin, nystatin, hexetidine and lynestrenol for therapeutic amenorrhea. Excretion of all 3 substances increased when fever occurred during myelosuppression. We conclude that chemotherapy of AML according to the TAD protocol and modified supportive care did not influence porphyrin excretion in acute intermittent porphyria.

223 Influence of Alpha-2- and Gamma-Interferon Alone and in Combination with Cytostatic Drugs on the Growth of Human Tumor Cells in Vitro

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We studied the effect of recombinant alpha-2- and gamma-interferon (IFN) alone and in combination with various cytostatic drugs (adriamycin, 4-hydroperoxy-cyclophosphamid, bleomycin, BCNU, VP-16 and vinblastine) on the growth of melanoma cells (cell line CRL 1424) and hypernephroma cells (cell line C 94). We used a clonogenic assay (soft agar assay), whereby the inhibition of proliferation was determined by judging the colony growth related to the control assays. On the cell line CRL 1424, alpha- and gamma-IFN showed marked inhibition, both together acting additively. The combination with cytostatic drugs resulted in additive or subadditive ef-

fects, only the combination with bleomycin inhibited the colony growth stronger then expected. On the hypernephroma cells we found in a similar manner additive or subadditive effects combining IFN and cytostatic drugs in almost all assays, whereas the combination of alpha- and gamma-IFN exhibited synergistic effects ($p < 0.05$).

224 Studies on the Mechanism of Action of IFNα_2 in Hairy Cell Leukaemia

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In order to get more insight in the mechanism of action of IFNα_2 in hairy cell leukemia the effect of IFNα_2 on the incorporation of nucleic acid precursors in hairy cells (HCs) was investigated and compared to the effect of cytostatic agents. In more than 8 patients with HCL the NK-cell-cytotoxicity was evaluated and compared to the NK-activity of healthy subjects and patients with other malignant diseases. While significant differences could be found before treatment between the groups, no significant increase of NK-cytotoxicity was seen during IFN-treatment. The effect of IFNα_2 was also studied on the incorporation of ^{3}H-Tdr and ^{3}H-Udr in HCs and in cells from other leukaemias over various periods of time. The results indicate that IFNα_2 does not immediately effect the proliferative behavior of HCs.

225 Preliminary Report on Neutrophil Function of Patients Treated with H-IFNα_2 for Hairy Cell Leukemia

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Among the most striking features of hairy cell leukemia are pancytopenia and altered host immunity, both leading to lifethreatening infections. Recently H-IFNα_2 has been shown to be effective in reconstitution of normal hematopoesis. Our particular interest was to elucidate whether H-IFNα_2 might also influence intracellular host defense mechanisms of neutrophil granulocytes. A valuable method to test neutrophils for their phagocytic properties is luminol chemiluminescence and killing of Staphylococcus aureus, respectively. Neutrophil granulocytes from patients with hairy cell leukemia were tested before, during and after treatment with H-IFNα_2 (3 Mio I.U.s.c., 3×weekly) over a period of up to 18 months. Before treatment, chemiluminescence responses were markedly depressed, they increased continuously during interferon application and returned again to low levels shortly after interruption of interferon therapy. However, killing properties seemed to remain unaffected throughout the whole observation period. From a clinical point of view H-IFNα_2 seems not only to improve marrow failure in hairy cell leukemia but also to enhance neutrophil intracellular host defense mechanisms.

226 Recombinant Gamma Interferon in the Treatment of a Patient with Malignant Mastocytosis

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In default of chemotherapeutic responsibility, a 61-year-old male patient with malignant mastocytosis was treated with recombinant gamma-interferon (γ-IFN). The anamnestic data showed an excessive loss in weight of more than 10 kg, a marked hepatosplenomegaly and duodenal ulcera. Histologically, a diffuse bone marrow and periportal liver infiltration by mast cells was diagnosed. The serum histamine levels were elevated. For 4 weeks the patient received 50 µg γ-IFN (Bioferon, Laupheim, FRG) 5×/week by continous intravenous infusions about 6 h, followed by an outside treatment with 50 µg γ-IFN s.c. 3×/week. The patient had a 'minor response' with a slight increase in weight and a moderate reduction of initially highly elevated liver enzymes. During the i.v. application of γ-IFN the well known flu-like symptoms occurred, whereas the s.c. application was very well tolerated. Accompanying immunological investigations demonstrated no changes of lymphocyte subsets but a reduction of the PHA response and an increase of the PWM induced in vitro immunoglobulin synthesis.

227 Haemopoietic Reconstitution After Lethal Total Body Irradiation by Combined Use of Cryopreserved Bone Marrow and Mononuclear Blood Cells

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Five patients with metastatic tumors refractory to conventional chemotherapy were treated by total body irradiation with 10 Gray plus ultra-high doses of cyclophosphamid (60 mg/kg×2). Mononuclear cells were previously harvested and separated by density gradient centrifugation on Ficol-Isopaque (1,077 g/l) from 1.500 ml of bone marrow and from three consecutive buffy coats. Cells were cryopreserved in the presence of 10% DMSO and 50% autologous plasma. Selfsustaining haemopoiesis was achieved in all patients within 14 to 28 days. One of the five patients around day 14 experienced a transitory engraftment which was followed by a permanent engraftment on day 28. Serious complications in particular of the infectious type were not observed and four of five patients are well and disease free one to 30 months after treatment.

228 A Cocktail of Monoclonal Antibodies for the Selective Elimination of ALL Cells with Human Complement

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Autologous remission bone marrow is a potential source of repopulative stem cells after ablative chemoradiotherapy of tumor patients. One major obstacle to the use of autologous bone-marrow support is the danger of reinfusing viable tumor cells. We present a purging protocoll with a cocktail of three monoclonal IgM antibodies and human complement for the elimination of acute lymphatic leukemia (ALL) cells of common ALL type and certain B type lymphomas. The purging efficiency was evaluated with leukemic cell lines of common ALL type (Reh-6 and Nalm-6) and with blast cells from common ALL patients. In dye exclusion tests a 99% and in clonogenic assays which detect elimination of up to 5 logs of clonogenic tumor cells a 99.99% (= 4 logs) purging efficiency was observed.

229 Autologous Blood Stem Cell Transplantation Following Myeloablative Therapy in a Patient with Burkitt's Lymphoma

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A 38-year-old male patient with stage III Burkitt's lymphoma was initially treated with COMP. After achieving complete remission hemopoietic stem cells were harvested by means of 7 consecutive leukaphereses and stored in the liquid phase of liquid nitrogen. The total number of MNC collected so far was 7.7×10^8 per kg. b.w. including 2.1×10^5 CFU-GM or 0.6×10^5 CFU-GEMM. Consolidation was performed using a myeloablative dose of total body irradiation (1,320 rad) and CY (200 mg/kg b.w.) followed by the retransfusion of thawed blood derived hemopoietic stem cells. 9 days after autologous blood stem cell transplantation a peripheral blood level of 1,000 leukocytes per μl was reached, 500 PMN and 50,000 platelets per μl after 10 days, and 100,000 platelets per μl 12 days thereafter. 29 days after transplantation the patient was discharged with normal blood counts.

Compared to bone marrow transplantation, blood stem cell transplantation combines the technical advantage of easier access with possible theoretical advantages such as are: 1. the most physiological way of repopulating a cellular matrix is via circulating blood repeating the prenatal development of hemopoiesis; 2. in malignant lymphohemopoietic disorders blood derived hemopoietic stem cells might be less contamined with residual tumor cells than marrow derived stem cells, a hypothesis that still needs to be proven. In the clinical situation those data confirm many preclinical studies showing that complete hemopoietic reconstitution can be achieved after blood derived stem cell transplantation.

230 Evidence for a Lymphocyte Activating Determinant Coded by the HLA Region but Different from HLA-DR, DQ and DP

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We have investigated a family, referred to us for the selection of a bone marrow donor. The serological HLA-typing revealed three siblings identical for HLA-A, B, C, DR and DQ. Surprisingly repeated MLC-testing showed an anusual high reactivity of one sibling against the two others (RR > 40%).

Glyoxylase-testing was informative and supported the occurence of an intra-HLA-D crossing over. An HLA-DP difference was excluded by intrafamiliar PLT- and DP-typing. Finally we performed an HTC-testing demonstrating an HLA-Dw difference between the siblings due to a maternal crossing over event. To our knowledge this family provides first evidence for a lymphocyte activating determinant coded by a locus centromeric to HLA-DQ defined by HTC and MLC reactivity.

231 Canine Blood Mononuclear Cells Inhibit Granulocyte-Macrophage Colony Formation of Adult Bone Marrow and Fetal Liver Cells

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Non-phagocytic, non-adherent mononuclear cells from canine peripheral blood (PBMC) suppressed colony formation in agar of autologous and allogeneic bone marrow granulocyte-macrophage progenitor cells (CFU-GM). Suppression required previous co-incubation in liquid culture of PBMC and bone marrow cells (BMC), was time- and dose-dependent and resistant to X-irradiation of PBMC with 20 Gy. Small BMC were less susceptible than large BMC, whereas day-7 and day-14 CFU-GM were equally inhibited. A similar degree of colony reduction was noted for unseparated, adult BMC and fetal liver cells obtained between days 39 and 57 of gestation. Control experiments suggested spontaneous, contact-dependent inactivation during liquid culture of CFU-GM or accessory cells to be the effector mechanism. Possible recognition structures were different from dog leukocyte antigens A and B. Thus, natural killer cell-like activities may be involved in the regulation of canine hemopoiesis.

232 Improved Demonstration of Chimerism After Allogeneic Bone Marrow Transplantation Using Ultrathin-Layer Isoelectric Focusing of Phosphoglucomutase 1

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Occurence of aplasia after successful allogeneic bone marrow transplantation requires a reliable distinction between graft rejection with revival of host haematopoiesis and de novo aplasia of the graft without revival of host haematopoiesis. Using phosphoglucomutase 1 (PGM 1) isoenzyme typing donor or host origin of different haematopoietic cell lineages can be demonstrated. However, applying conventional isoelectric focusing (IEF) on polyacrylamide gels typing of lymphocytes or granulocytes requires 100,000 to 500,000 cells, which can mostly not be isolated from aplastic patients. Therefore we attempted to enhance the sensitivity of PGM 1 isoenzyme typing. Using ultrathin-layer IEF (240 μm) with debris containing cell lysates and paper wick free sample application we could phenotype lymphocytes and granulocytes down to an amount of 5,000 cells per sample. The power of this new PGM 1 isoenzyme typing will be demonstrated by several examples.

233 Immunohistological Monitoring of the Skin Following Allogeneic Bone Marrow Transplantation – An Additional Tool in the Diagnosis of Cutaneous Graft-Versus-Host Disease

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One of the major impediments limiting the success of bone marrow transplantation (BMT) is acute graft-versus-host disease (GvH-D). Owing to its accessability the skin as a major target of GvHD offers the unique possibility for rapid diagnostic procedures. Since clinical and histopathological appearance need not be diagnostic, we performed additional immunohistological investigations on frozen sections of sequentially performed skin biopsies using a 3-step immunoperoxidase technique. The immunohistological studies yielded the following results: 1. a decrease of epidermal OKT 6 + Langerhans cells (LC), 2. the appearance of NK-marker (Leu 7) bearing cells within the epidermis, and 3. the expression of class II alloantigens by keratinocytes (KC) – which are uniformly class II alloantigen negative under normal conditions – in GvHD. The contention that immunohistological investigations are occasionally superior to conventional H&E studies can be derived from the observation that within the skin biopsies from 7 out of 8 patients with acute cutaneous GvHD HLA-D region encoded molecules were expressed by KC. Furthermore, in 7 out of 8 patients with clinical symptoms highly suggestive of cutaneous GvHD but without diagnostic histopathology class II molecules were already expressed by KC. It is also important to note that patients who received allogeneic bone marrow but did not develop GvH-D never displayed class II alloantigen reactive KC.

234 Discrepancy Between Karyotype and Y-Chromatin Analysis in a Patient After Allogeneic Bone Marrow Transplantation

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A 22-year-old male patient with undifferentiated lymphoblastic lymphoma in partial remission received an allogeneic bone marrow transplant from his HLA-identical sister. 5 months after successful transplantation a relapse of the malignant lymphoma occurred with 73% lymphoblasts in bone marrow. The morphological and immunological characterization of the blasts showed the previously observed recipient type. Y-staining with Quinacrin-mustard revealed typical Y-body in 96% of the cells (normal range in males 75–100%). The control of the peripheral blood of the donor showed no Y-body. Cytogenetic analysis of the bone marrow at time of relapse by a direct method showed normal female karyotype in all 20 analysed metaphases. We made the same observation in another patient with ALL. The explanation for the discrepancy could be that cytogenetic analysis reveals only healthy hematopoetic cells of donor type. In order to discover the malignant cells of the recipient more metaphases should be analysed and/or different culture methods ought to be employed.

235 Reconstitution of Spontaneous Immunoglobulin Secretion After Canine Allogeneic and Autologous Bone Marrow Transplantation: Evaluation by a Reverse Hemolytic Plaque Assay

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In order to study the marked immunodeficiency reported early after human bone marrow transplantation we evaluated spontaenous immunoglobulin secretion in 14 control dogs, 13 healthy short term and 10 healthy long term survivors after canine autologous and allogeneic bone marrow transplantation by a reverse hemolytic plaque assay. Control dogs and dogs sur-

viving 6 and more months after transplantation showed comparable numbers of plaque forming cells (PFC). In short term survivors immunoglobulin secretion was elevated with a pattern of PFC reconstitution specific for each type of graft. Supported by simultaneous studies on specific immune response against tetanus toxoid and on mitogen stimulation the data suggest polyclonal B cell activation which is mediated by two mechanisms: Imbalance of T cell regulation during repopulation of lymphoid organs and allogeneic stimulation of donor T lymphocytes.

236 Antiplatelet and Antigranulocyte Antibodies after Allogeneic Bone Marrow Transplantation (BMT)

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32 patients (21 AL, 4 CML, 7 AA) received BMT from HLA-identical siblings. Allosensibilisation by platelet and/or granulocyte transfusions before and after BMT was investigated using indirect immunofluorescence tests for detecting antibodies against platelets and granulocytes of five random donors. In 19 patients no antibodies were detectable before and after BMT. Eight patients lost their previously demonstrable antibodies and in one patient the antibodies persisted after BMT. Four patients developed alloantibodies in the posttransplant phase.

The kinetics of alloreactivity will be discussed in view of the underlying disease, transfusion frequency, conditioning regimen, GvHD-status and different GvHD-prophylaxis (Methotrexate, Cyclosporine A, T-cell depletion).

237 Prophylactical Treatment of Cytomegalovirus (CMV)-Infection with CMV-IGG-Hyperimmunoglobulin in Patients with Allogeneic Bone Marrow Transplantation (BMT)

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After successful BMT prolonged combined immunodeficiency occurs, which promotes the occurrence of severe CMV-infections, the most frequent lethal complication after BMT at the moment. The few randomised studies on CMV-prophylaxis have suggested that CMV-hyperimmunoglobulin is effective. The efficacy of CMV-hyperimmunoglobulin was investigated in 25 adult BMT-patients (HLA-identical). We analysed the influence of diagnosis, conditioning regimen, GvHD-status, CMV-status in recipient and donor and studied the influence of hyperimmunoglobulin substitution on CMV-serology (CMV-KBR, CMV-ELISA-IgG, CMV-ELISA-IgM). The diagnosis of CMV-infection was based on serology, CMV-cultures, clinical and histological investigations. The results presented here demonstrate the efficacy of CMV-prophylaxis with CMV-hyperimmunoglobulin. The risk factors promoting the occurrence of interstitial CMV-pneumonia in patients receiving CMV-prophylaxis will be discussed.

238 Allogeneic Bone Marrow Transplantation (BMT) for Acute Myeloid Leukaemia (AML)

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12 patients with AML (10 adults, 2 children) received either HLA-matched ($n = 10$), HLA-1-Antigen mismatched ($n = 1$) or haplodentical ($n = 1$) bone marrow grafts from their healthy siblings. 6 patients were in complete remission (CR), 2 patients were in 1. relapse prior to re-induction, 4 patients in relapse had received re-induction cycles, without success. Conditioning consisted of 120 mg/kg Cyclophosphamide and 1,000 rad TBI (Co^{60}, lung shielding:

800 rad, single fraction). All patients received MTX for GVH-D prophylaxis. Currently 4 of 6 patients grafted in CR survive between 180–1,309 days, while only 2 of 6 patients grafted in relapse are alive 72 and 130 days, respectively. The 2 deaths in the CR group were due to cerebral haemorrhage (day 27) and porencephaly (day 213). The 4 patients grafted in relapse died from CMV pneumonia (days 23 and 75), septicemia (day 31) and GVH-D of liver (day 48). 1 patient grafted in relapse (1 haplotypemismatch) died from acute GVH-D. At a median observation time of 260 days, no patient has relapsed. Our limited experience shows, that patients grafted in relapse do much worse, although a minority may keep the option for obtaining long term survival.

239 Allogeneic Bone Marrow Transplantation (BMT) for Chronic Myelocytic Leukaemia (CML): Persistence of a few Ph^+ Mitoses early after BMT is not Indicative for Relapse

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10 patients with Ph^+ CML (9 adults, 1 child, 6 in chronic phase, 3 in accel. phase, 1 in Tdt^+ blast crisis, age 10–38 years, median: 25) received HLA-matched, MLC non-reactive bone marrow grafts from their siblings. Preconditioning consisted of 120 mg/kg Cyclophosphamide and 1,000 rad TBI (lung: 800 rad), delivered from a Co^{60} source in a single fraction, at a dose rate of 8.5 rad/min (pat. 1–3) or 3–4.5 rad/min (pat. 4–10), respectively. Patients 1 and 3 died from CMV-pneumonia, patient 2 died from veno-occlusive disease of the liver, respectively. Currently (20.05.85), patients 4 to 10 survive in complete clinical and haematological remission 226 to 603 days (med. 438). Cytogenetics, performed within 2 months after BMT, showed that in 3 of 8 patients up to 10% of the evaluable mitoses were still carrying the Philadelphia-Chromosome. Beyond 3 months after BMT, however, some Ph^+ mitoses were only detectable in the bone marrow preparations of 1 out of 7 patients. This patient, however, remains in complete remission (603 days), as judged by clinical and haematological criteria. We thus conclude, that persistence of some Ph^+ cells early after BMT is not indicative for an ongoing relapse but may rather reflect chemo-radioresistance of some long-living, yet undefined bone marrow cells.

240 Treatment of ALL in Second Remission with High Dose Busulfan, Cyclophosphamide and Allogeneic Bone Marrow Transplantation. First Report of Two Patients

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A patient with recurrent T-cell leukemia/lymphoma and a patient with multiple CNS-relapses and 1st bone marrow and testicular relapse of common-ALL were treated according to the BFM-study as induction therapy followed by intermediate methotrexate as maintenance therapy. Late intensification with cyclophosphamide 50 mg/kg×4 days and allogeneic bone marrow transplantation was then given using high dose busulfan (4 mg/kg×4 days) to eradicate residual leukemia instead of total body irradiation (TBI). Survival after bone marrow transplantation is now 17 + and 10 + months. Although only applied in two patients we can already conclude from the survival data, that high dose busulfan is at least as effective in ALL as TBI provided that sanctuary sites are being effectively treated before marrow transplantation. On the other hand the late side effects of high dose busulfan are expected to be less than those of TBI.

241 Treatment of Neuroblastoma, Stage IV with High Dose Melphalan (HDM) Followed by Transplantation of Asta-Z Chemopurified Autologous Bone Marrow

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The case of a 4 year 8 months old boy with neuroblastoma metastatic to the bones and the bone marrow is presented. After achieving a partial remission with six cycles of conventional chemotherapy (vincristine, cyclophosphamide, doxorubicin, nitrogen mustard, cisplatinum and DTIC), the patient was given supraconventional chemotherapy (HDM 220 mg/m^2 bolus i.v.) followed by autologous bone marrow chemopurified with the 4-hydroperoxycyclophosphamide-analogon ASTA Z 7654. At the present time (16 months after diagnosis and 12 months after autologous bone marrow transplantation) there is no evidence for active disease according to the follow up studies including bone scan and bone marrow biopsies.

242 Case Report: Successful Pregnancy and Delivery after Bone Marrow Transplantation (BMT) for Severe Aplastic Anemia (SAA)

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A 23-year-old female with transfusion dependent SAA was transplanted from an HLA-identical, MLC-non reactive, ABO-major mismatched (recipient: O pos, donor: A pos) sister. Transfusion dependency persisted due to complete areticulocytosis for 332 days. Isoagglutinine titers of anti-A were 1 : 16 (anti-B 1 : 8). A rapid increase of reticulocytes and a decrease of isoagglutinine anti-A to 1 : 0 (anti-B remained unchanged) occurred after plasmapheresis on day 332.7 months after BMT regular menses started and 21 months after BMT the patient became pregnant. Pregnancy was complicated by mumps in the 14th week but otherwise uneventful. It was terminated at term by caesarean section. The newborn girl (weight: 3,450 gm) had a patent ductus arteriosus Botalli which was corrected surgically. After operation the newborn first showed impaired development for some months. Now 8 months after birth she shows an age-appropriate development. This is the 5th successful delivery known in female patients transplanted for SAA (3 cases Seattle, 1 case South Africa) and the first description of a malformation of such a newborn.

243 Protein S Deficiency and Thromboembolism in 7 Austrian Families

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Protein S is a vitamin K dependent cofactor of the coagulation inhibitor protein C. Protein S: Ag was determined by an immunoradiometric assay in 152 patients with venous thromboembolic disease, normal protein C: Ag and normal AT III activity. 7 unrelated patients with protein S deficiency were detected: 3 were without oral anticoagulant (QAC) therapy (protein S 38, 50 and 52%) and 4 were under OAC therapy (protein S 13.5, 14.5, 26 and 27%). The protein S concentration of the anticoagulated patients was below the range established in 93 patients under stable OAC treatment and without venous thromboembolic disease (protein S = 33 – 74%). All 7 patients with protein S deficiency had experienced deep venous thrombosis (recurrent in 5 patients), 5 had experienced superficial thrombophlebitis and 4 pulmonary embolism (recurrent in 2). All patients had a positive family history with at least 2 family members with known thromboembolic events. Protein S deficiency is, besides AT III- and Protein C deficiency the third known factor for venous thromboembolic disease due to an isolated deficiency of a coagulation inhibitor.

244 Cerebrovascular Complications in Patients with Congential or Acquired Deficiency of Antithrombin III

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A congenital or acquired deficiency of antithrombin III (AT III) increases the risk of intravascular coagulation. These patients have a tendency to develop deep venous thrombosis, but there are only a few reports on cerebrovascular complications. In a 24-year-old patient with congenital AT III deficiency (AT III = 49%) suffering from an extensive deep venous thrombosis and an associated pneumonia a thrombectomy was performed. After developing recurrent pulmonary emboli and disseminated intravascular coagulation the patient was transferred in our hospital and treated with heparin, fresh frozen plasma and antithrombin III-concentrate. During the course of treatment a right-sided hemiparesis and a combined sensory and motor aphasia occured. A CT-scan revealed cerebral infarction in the region supplied by the median and anterior cerebral artery. In a 33-year-old patient with acute lymphoblastic leukemia (c-ALL) treatment with asparaginase resulted in a plasma fibrinogen level of 1.0 g/l and a AT III-level of 33%. He developed hemorrhagic cerebral infarction and a sinus thrombosis. These findings demonstrate that congenital and acquired AT III deficiency increase the risk of cerebrovascular complications.

245 Clinical Trial of Thrombosis Prophylaxis with LMW Heparin and Sodium Heparin in Patients with Major Orthopedic Surgery

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Efficacy and side effects of the low molecular weight (LMW) heparin (KABI 2165) in thrombosis prophylaxis in patients with hip replacement are compared with conventional sodium heparin. This is a randomized open prospective study in 50 patients. The 125 J-fibrinogen test is done on all patients for 8 days. With a positive leg scan, an ascending phlebography will follow. In all cases both LMW heparin and sodium heparin were well tolerated. There was no severe bleeding event. There was a slight but not statistically significant tendency to lower blood loss in the LMW heparin group. The 125 J-fibrinogen test was positive in 3 patients. In 2 patients receiving LMW heparin it was falsely positive with phlebography. In one patient receiving sodium heparin there was a deep vein thrombosis of the lower leg. The advantage of LMW heparin is simplified dosage (one injection daily). Efficacy of both heparin preparations in thrombosis prophylaxis seems to be similar.

246 Diagnosis and Successful Therapy of a Non-Secretory Plasmacytoma Consisting of Undifferentiated Plasmablastic Cells

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A 25-year-old woman presented with pancytopenia. Laboratory data showed an increased blood sedimentation rate and a markedly elevated LDH, but no paraprotein was detectable in serum and urine. Bone-marrow aspiration revealed 92% blast cells of plasmacytoid appearance and bone-marrow biopsy an extensive infiltration by plasmablasts as well as an extreme suppression of all three hematopoetic cell lines. Monoclonal immunoglobulins of IgG-Kappa-type were demonstrated in the cytoplasm and in weak expression at the membrane of those cells together with Ia-like antigens and T 10 antigens, whereas the B-associated BA-1 antigen was not expressed. This phenotype confirmed the plasmacytic nature of the blast cells. No bone lesions were detected by X-ray and bone-scan. Treatment was initiated with doxorubicine 50 mg/m^2, vincristine 1.5 mg/m^2, cyclophosphamide 750 mg/m^2 and prednisolone 100 mg for 5 days. After 6 cycles of this treatment the patient achieved complete remission. For consolidation 3 addition-

al cycles were given. Maintenance treatment is performed with high-dose melphalan (70 mg/m^2) every three months. Our patient remains in CR and has achieved a relapse-free survival of 14+ months.

247 Central Nervous System Involvement in Plasmablastic Myeloma: Immunocytochemical Analysis of Antigenic Phenotype and Proliferation Activity in Spinal Fluid

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CNS involvement is a rare event in multiple myeloma. A case with meningeal involvement preceding final widespread metastasis will be reported. Cells in the spinal fluid were extensively characterized using light microscopic immunocytochemistry. Morphologically the cells displayed a high degree of polymorphism featuring 'plasmablastic myeloma'. Their surface antigen phenotype was T9$^+$ T10$^+$ Ia$^-$ BA1$^-$ B1$^-$ Ig$^-$, thus corresponding to the phenotype of normal mature plasma cells rather than normal plasmablasts. Cytoplasmic immunoglobulin (cIg) with restriction to one light chain was present in only 55% of the cells. Both cIg$^+$ and cIg$^-$ cells in mitosis were found, indicating that Ig-negativity was not related to cell cycle stage but rather represented a loss of capacity to synthesize Ig. This hypothetically reflects a loss of corresponding genes during evolution of the malignant clone. Proliferation activity was assessed using the monoclonal antibody Ki-67 which reacts with a nuclear antigen expressed in G_1- to M-phases of the cell cycle (Gerdes et al., J Immunol 133: 1710). Over 90% of the cells exhibited this antigen, thus evidencing a conversion to high malignancy which presumably predisposed to the outcome in this patient.

248 PWM Mediated Immunoregulation of the in Vitro Production of Monoclonal Immunoglobulin in Multiple Myeloma

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Mononuclear cells separated from the bone marrow of 23 multiple myeloma patients, 8 patients with MGUS and 1 patient with M. Waldenström were set up in short-term cultures. The cells were stimulated by 1 μg pokeweed mitogen (PWM)/ml of culture medium. 10 days later monoclonal and polyclonal Ig were measured in the cell culture supernatants by an ELISA using anti-idiotype, class-specific, and light-chain-specific antisera. Monoclonal Ig was detected in culture supernatants of all investigated patients. In 17 patients the addition of PWM did not alter the production of monoclonal Ig, whereas in 15 others PWM decreased the synthesis of monoclonal Ig significantly. In some patients the production of polyclonal Ig was increased by PWM. These results show, that at least in some patients the malignant clone still responds to regulating signals.

249 Natural Killer Cell (NK) Activity in Peripheral Blood (PB) and Bone Marrow (BM) of Patients with Multiple Myeloma

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NK cells are derived from BM and are important for the regulation of biological defense mechanisms. In the present study we investigated the NK activity in PB and BM of patients with multiple myeloma (MM, $n = 25$). We have demonstrated that NK activity is significantly higher in BM of patients with MM in comparison of healthy donors ($n = 5$), patients with meloproliferative disorders ($n = 15$) and patients undergoing thoracotomy ($n = 14$). In PB no differences have been detected. NK activity of BM and also of PB could be stimulated by interferon and interleukin 2. These studies suggest a biological significance of NK cells in multiple myeloma.

250 Significance of Postabsorptive Serum Iron Increase for the Valuation of Oral Iron Preparations

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There are conflicting results in the literature about the bioavailability of oral iron preparations. Bioavailability thereby is measured by different methods. Here we are reporting on the results of the intestinal iron absorption as measured by means of a whole body counter (reference-method) which are compared intraindividually with the increase of serum iron after oral iron loading (postabsorption-iron curve). In 30 subjects with iron deficiency anaemia therapeutic doses of ferrous sulfate, labelled with ^{59}Fe was administered orally. Before and during the following 4 hours 6 blood-samples were taken. Two weeks after iron administration ^{59}Fe-retention was measured by whole body counting. Intestinal iron absorption amounted from 13 to 53% and max. serum-iron increase from 90 to 330 $\mu g/dl$ respectively. There was a good correlation ($r = 0.93$) between both methods. The individual data for a particular serum iron increase however varied up to a factor 3. Therefore it is concluded that evaluation of the bioavailability of oral iron preparations by means of postabsorption-iron curves is possible only in large and homogeneous groups of patients.

251 Consideration on Thalassaemia in Romania

N. Gingold, C. Predescu, M. Ardeleanu (Hematological Clinic, Clinical Hospital, Fundeni, Bucharest, Romania)

Much more cases of Thalassaemia are discovered in the autochonous population of our country than it was supposed formerly. The diagnosis of this Hb-abnormality has been facilitated by the organisation of a Central Laboratory for Hemoglobinopathies in the Bucharest Center of Hematology and Blood Transfusion. In patients with the presumptive diagnosis of Th. established by the routine analyse of the blood picture, the Hb-electrophoresis is performed and in more than 85% of cases, the presumptive diagnosis is confirmed. It is a predominance of cases of Th-minor, but also cases of autochtone Th-major (the Cooley-anaemia) had been observed. The familial transmission is proved. The surprising extension of this Hb-abnormality in the autochtone population of our country is discussed.

252 Spurious Thrombocytopenia, White Blood Cell Counts, and Abridged Differentials Caused by Platelet Clumping, Cold Agglutinins, and Normoblasts and Blasts Provided by Coulter Counter

J. Anagnou (Medical School Hannover, Department of Internal Medicine, Division of Haematology-Oncology, D-3000 Hannover, FRG)

The platelet and WBC counts of a woman, who was operated for lung carcinoma without bleeding, were 18,000 and 10,900 at room temperature and 269,000 and 4,900 at 37°C, respectively. The abridged differential was also false. This dual and even triple artifact was due to platelet agglutinates seen in the blood smear and the counting chamber. The agglutinates caused a distortion in the low size range left to the lymphoid peak of the WBC histogram and were recognised as white cells. In two other patients with haemolytic anaemia due to cold agglutinins (titer 1 : 4,096 at 4°C and 20°C) the WBC counts were intermittently and on clinical basis inexplicably elevated. Measurements at 4 different temperatures, on 2 separate days revealed that the WBC counts were temperature dependent. Finally, the automatically generated lymphocyte percentage of an out-patient in remission from AML amounted to 68% – an improbable feature for this patient! The WBC volume distribution curve showed only one lymphoid cell peak instead of the normal bimodal pattern of a lymphoid (45–99 fl) and a myeloid (99–450 fl) cell peak. Leukemia relapse was suggested, that was confirmed by bone marrow aspiration and manual differential. The single "lymphoid" peak was due to circulating normoblasts (23 per 100 white cells) and blasts (40%). Several platelet and WBC counts and the corresponding histogramms will be presented and analysed.

253 Spuriously Elevated MCHC, MCH, and MCV Values Estimated by Coulter Counter: A Common Clue to Cold Agglutinins

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Hemolytic anaemia due to cold agglutinins is relatively common. Cold agglutinins may cause agglutination of erythrocytes and formation of microagglutinates, mainly triplets and doublets which may be counted by the Coulter Counter as single cells. Because the spurious lowering of the erythrocyte count is disproportionately greater than the false elevation of the MCV, the result is false haematocrit and erythrocyte indices. In the last 10 years we have observed in our laboratory, which performs over well 900 blood sample analyses a day, at least 10 patients with hemolytic anaemia due to cold agglutinins usually associated with an underlying lymphoproliferative disease and/or an IgM-Gradient. Measurements at room temperature gave a varying set of erythrocyte indices including high, very high, and even impossibly high, but occasionally also normal MCV values. In contrast, the MCHC values were almost invariably high. Warming of the specimens at 37°C in water bath led to accurate values. Inexplicable "macrocytosis" and high MCH, especially intermittent, and in particular impossible, non sense figures for MCHC should alert to search for cold agglutinins, as it will be demonstrated in one of our patients. Several profiles of the erythrocyte-indices from 4 patients will be presented and the significance of each single erythrocyte index as well as of the hematocrit/hemoglobin ratio as a clue to the presence of cold agglutinins will be discussed.

254 Classification of Anaemias by MCV and RDW*

J. Anagnou (Medical School Hannover, Department of Internal Medicine, Division of Haematology-Oncology, D-3000 Hannover, FRG)

Initial classification of anaemias usually is based on either morphologic criteria or the erythrocyte indices. However, due to the fact that MCH does not provide any additional information to MCV, and that MCHC estimated by Coulter Counter is low nearly only in severe iron deficiency anaemia, classification of anaemias on the erythrocyte indices is practically limited into micro-, normo-, and macrocytic forms.

We have undertaken a classification of anaemic conditions on the basis of the MCV and RDW values now routinely estimated by the Coulter Counter of the latest generation. The Coulter Counter Model S Plus IV measures the heterogeneity of the red cell size as coefficient of variation and prints it out as RDW (in %) in the routine haematology report. RDW has proven to be a good index of anisocytosis. Anaemias can be divided by MCV and RDW into the following six groups (examples in parentheses): 1) Microcytic-isocytic (β-thalassaemia minor) 2) Microcytic-anisocytic (iron deficiency anaemia) 3) Normocytic-isocytic (anaemia of chronic disorders) 4) Normocytic-anisocytic (osteomyelofibrosis) 5) Macrocytic-isocytic (aplastic anaemia) 6) Macrocytic-anisocytic (pernicious anaemia). The hemolytic anaemia due to cold agglutinins was in most cases macrocytic-anisocytic, the set of MCV and RDW was, however, varying. The value of this classification will be discussed on the basis of the above mentioned and additional anaemic disorders.

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* RDW = *R*ed cell volume *D*istribution *W*idth

255 Bone Marrow Evaluation Simulating Multiple Myeloma in Acquired Immune Deficiency Syndrome (AIDS)

M. Paulsen, W.-D. Ludwig, H. Seibt-Jung, M. Bur, H. Rühl (Abteilung Hämatologie/Onkologie, Klinikum Steglitz, FU Berlin)

Recent case reports indicate that the bone marrow is a target organ in AIDS (reviewed by J. L. Spivak, Am. J. Med., 1984, 77, 224). We report here on three 27-, 44-, and 45-year-old homosexual men with AIDS. The diagnosis of AIDS was based on clinical features (chronic generalized lymphadenopathy, opportunistic infections) and immunologic abnormalities (lymphopenia,

reversal of T cell subset ratio, absolute reduction in Leu-3a+ cells). Bone marrow aspiration revelated a marked increase in plasma cells (up to 40% of the nucleated cells) in all three patients. Furthermore, alterations in marrow cellularity, a left shift in the myeloid series and an increase in histiocytes were observed. No monoclonal gammopathy could be detected, serum immunoglobulin levels were elevated in all three patients, consistent with polyclonal B-cell activation. Abnormalities of bone marrow evaluation will be presented and discussed in the context of the clinical and immunologic data of these patients.

256 Studies on the Staging System of Plasmocytomas

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The prognostic relevance of the staging system proposed by Salmon and Durie was analysed in 101 patients suffering from multiple myeloma. All patients had been treated according to the same protocol in order to avoid bias due to different treatment strategies. Prognosis of patients in stage I and II did not differ, whereas stage III was associated with a less favourable course of disease, however of borderline statistical significance only. In contrast classification according to subgroups A and B correlated rather precise with prognosis. Analysis of individual factors showed that values for hemoglobin and monoclonal immunoglobulin as well as degree of osteolytic bone lesions were of only minor prognostic importance. On the other hand serum calcium and creatinine were significantly correlated with prognosis. Further analysis revealed that these two parameters were also correlated. It could be shown that serum calcium levels were of prime importance in distinguishing between subcollectives of different prognosis.

257 Psychological and Social Support of Children with Cancer and their Families

U. Kaufmann (Kinderpoliklinik der Justus-Liebig-Universität Giessen, Abteilung Allgemeine Pädiatrie und Hämatologie-Onkologie, D-6300 Giessen, FRG)

In recent decades, improvements in treatment have changed the prognosis for childhood cancer. Now a chronic disease, it requires intensive therapy during a period of several years. The procedures to which the child is submitted, the effects and side effects of treatment, and the uncertainty of the future place a great stress upon the child and his family. Age of child, previous coping, coping of other family members, a good support system and lack of additional stresses seem to correlate well with healthy coping.

Group therapy with the families on a self help basis serves as an effective method of communication, problem identification and solution. Experiences with this kind of group therapy over a period of 5 years are discussed.

258 Structures of Human Interferons

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In recent years, progress in protein chemistry and molecular biology has allowed elucidation of the structures of human interferons (IFNs) and their genes. Classical, virus-induced (type I) IFN comprises three classes of proteins: IFN-α, a family with 10–15 members, the recently discovered IFN-ω (also referred to as IFN-α subclass II), and IFN-β, both represented by only a single protein. Type I IFNs consist of 165 or 166 amino acids (IFN-α, IFN-β) or 172 amino acids (IFN-ω); all proteins are structurally related, but serological crossreactivity is found only between members of the IFN-α family. Genes for all type I IFNs as well as a number of related pseudogenes are located on the short arm of chromosome 9; they do not contain introns. In contrast, the gene coding for IFN-γ (type II-IFN, formerly immune IFN) is found on chromosome 12 and contains three introns. IFN-γ contains 143 amino acids; unlike type I-IFN, it is acid-labile and forms non-covalently bound dimers. Its sequence shows only very little homology with type I IFNs.

259 Regulation of Viral Replication by Interferons

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IFNs inhibit the replication of most DNA or RNA viruses. A number of potential antiviral mechanisms of IFNs have been described. These may result in inhibition of virus penetration or uncoating, virus RNA synthesis or methylation, protein synthesis and virus maturation or release.

The availability of highly purified, biologically active IFNs produced by recombinant DNA technology opened the way to clinical studies of the effectiveness of IFNs in the prophylaxis or treatment of viral diseases. Controlled trials with IFNs have been performed in patients with infections with Herpes simplex virus, Varicella-Zoster virus, Cytomegalovirus, hepatitits B virus, rhinoviruses, and papillomaviruses. Prophylaxis with IFNs was shown to be effective against cytomegalovirus reactivation in immunocompromised patients and against rhinovirus-induced common colds. Treatment with IFNs was demonstrated to be of benefit for patients with herpes zoster, herpetic keratokonjunctivitis, chronic hepatitis B, and papillomavirus-associated diseases (juvenile laryngeal papillomatosis, condylomata acuminata).

260 Regulation of Cell Growth by Interferon

A. J. Schwarz (Schering Corp., Bloomfield, N. J., USA)

The Interferons (IF) besides the well known anti-viral effect possess, also a variety of other important activities; such as the inhibiting effect on cell growth. This so called anti-proliferatic effect probably plays a significant role in the anti-tumor activity of the IFs. Studies will be described utilizing a variety of human tumors in a xenograft model which clearly demonstrate the growth inhibiting effect of IF-Alpha$_2$, a genetically engineered, highly pure IF-Alpha sub-type. These anti-proliferitic effects will also be clearly demonstrated in tissue culture of normal and malignant cell lines. In addition, data will be presented comparing the anti-proliferatic activity of IF-Alpha$_2$ with the one of IF-Gamma. It will also be shown that the cell growth inhibiting effect of the genetically engineered IF-Gamma is equal to that of the naturally produced IF-Gamma.

261 Interferons in the Regulation of Cellular Immune Responses

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This review deals with the immunoregulatory role interferons exert on cellular immune responses. Non-immune and immune-interferons are released from leukocytes or T cells subsequent to challenge with viruses or antigens. They modulate immune responses primarily by their capacity to control expression of HLA-antigens and synthesis of prostaglandins. Immune-interferon enhances recognition of antigens by T helper cells by its unique capacity to induce expression of HLA-DR. Both types of interferons increase lysis of target cells by T killer cells due to their capacity to increase expression of HLA-A, -B structures. These positive interferon effects are counter regulated by enhanced prostaglandin release.

262 Differences in the Regulation of HLA-A 2 and HLA-B 7 Antigen Expression by Interferon

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It is known that interferons modulate the expression of the major histocompatibility antigens. In order to examine the regulation of single HLA-genes by interferon, the genes for the HLA-A 2 or the HLA-B 7 antigen were integrated into mouse Ltk$^-$ cells by DNA mediated gene transfer. The appropriate HLA proteins were expressed on the cell surface of these cells, as could be shown with monoclonal antibodies recognizing different epitopes of the HLA-A 2 or the HLA-B 7

antigen. The exposure to mouse interferon of cells transfected with the HLA-A 2 or HLA-B 7 gene resulted in an up to 3.5-fold increase of the HLA-A 2 antigen and even in an up to 10-fold increase of the HLA-B 7 antigen. It still remains to be established why interferon enhances the expression of the HLA-B 7 gene more than the expression of the HLA-A 2 gene.

263 Influence of Interferon Treatment in Vitro on the 2–5 A-Synthetase Level of Leukemic Cells

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The formation of 2–5 A-synthetase, an enzyme which produces 2–5 A-oligoadenylates from ATP in the presence of double stranded RNA, is induced by interferon. Thereby an endoribonuclease present in the cells is activated, which can degrade mRNA. It is suggested, that this process is involved at least in some of the antiviral and antiproliferative effects of interferons. We estimated the level of the 2–5 A-synthetase in the mononuclear cell fraction of healthy donors and patients with leukemia before and after in vitro treatment with different interferons. In most cases, basal levels of the enzyme were strongly reduced in leukemic patients. However, following in vitro treatment with interferon, in some forms of leukemia (CML, Ph+) an up to twenty-fold elevation of the enzyme level could be detected, whereas only a two to four-fold elevation was found in healthy persons. The possible connection of these findings with different leucemic transformation mechanisms as well as their therapeutic implications are discussed.

264 Interferon-α: Current Status in the Treatment of Human Leukaemias

J. D. Schwarzmeier (1st Medical Clinic, University Vienna, A-1090 Vienna, Austria)

The observation that IFN-α is a highly effective agent in the treatment of patients with hairy cell leukaemia (HCL) has generated renewed interest in this compound and has stimulated clinical trials in a variety of haematological malignancies. It has been shown that recombinant IFN-α is at least as potent to reduce the leukaemic cell load in HCL as natural IFN-α. The results of a multi-center study using rIFN-α_2 and involving more than 70 patients in USA and 25 patients in Europe with HCL will be presented. In addition a brief overview on published clinical trials with IFN-α in acute leukaemia, chronic lymphocytic and myelogenous leukaemia and in myelodysplastic syndromes will be given.

265 Clinical Results and Mode of Action of Interferon-Alpha in the Treatment of Hairy-Cell Leukemia

G. Gastl, W. Aulitzky, J. Troppmair, R. Flener, Ch. Huber (Abteilung für klinische Immunbiologie, Universitäts-Klinik für Innere Medizin, A-6020 Innsbruck, Austria)

We report on results of the treatment of 14 patients with advanced hairy-cell leukemias with low dose interferon-alpha$_2$. Within three months responses were seen in 13 of 14 cases. In vitro studies further revealed that neither anti-tumor responses mediated by classical T-cells nor by NK-cells were responsible for these responses. They rather indicated a direct action of interferon on the in vivo growth of the malignant cells.

266 Treatment of Hairy-Cell Leukaemia (HCL) with Alpha Interferon: – British Results

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More than 50 patients with HCL have now been entered in the British interferon (IFN) trial started at UHC and continued at other centres under the co-ordination of the Wellcome Research Laboratories. All patients, irrespective of previous treatment, showed evidence of response.

All but one patient showed a good peripheral blood response with reduction of hairy cells to low levels and improvement if cytopenias; the peripheral blood response was complete in 15 patients. The marrow response was less complete and, although HC infiltration was reduced in 22 patients, HCs were reduced to low levels in only 9. The response rate was greater with increasing duration of therapy. Even when a complete response had been initially attained, cessation of IFN was followed by a gradual return of the disease which was responsive to treatment. Repeated immunological marker studies were carried out in 23 patients. There was no evidence of differentiation of the HCs and light-chain-restricted B cells were reduced in parallel with the disappearance of morphologic HCs. Phenotypic NK cells were not increased and there was no evidence of reappearance of normal B Cells, especially OKT 8^+ suppressor cells, were moderately reduced resulting in enhancement of OKT 4/8 ratios.

267 Treatment of Hairy Cell Leukaemia with Recombinant Alpha$_2$-Interferon (Results of a Multicenter Study)

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27 patients (18 males, 9 females) suffering from hairy cell leukaemia were treated with recombinant alpha$_2$-IF produced by Fa. Boehringer Ingelheim. 21/27 patients received induction-therapy by 5×10^6 i. U. i. m. or s. c. daily for 1–7 months, 6/27 patients started with a lower dose ($5\times10^5-20\times10^6$ i. U. per week for 6–7 months). 14/27 patients were set on maintainance therapy by $2\times10^6-4\times10^6$ i. U. for 1–6 months. 13/27 patients had been splenectomized before onset of treatment, and 26/27 patients showed progressive disease when admitted to the study. In 10/ 16 thrombocytopenic patients thrombocytes increased to values above 100,000/μl within the first month, severe granulocytopenia recovered in 14/24 patients within 3 months, Hb-values didn't increase before 3–4 months. Hairy cells were reduced to a various degree in peripheral blood and bone marrow. Dependent on the time of treatment so far there are 3 CR, 16 PR, 4 MR, 3 SD and 1 progression.

268 Investigation of Recombinant Human Interferon Alpha-2 (rIFN-α 2) in Chronic Myelogenous Leukemia (CML)

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The antiproliferative effect of rIFN-α 2 was investigated in patients (pts) with Philadelphia-positive CML. All pts were in the chronic phase of the disease with increasing leucocyte counts before projected bone marrow transplantation. Pts characteristics are: male/female 5/1, age 22–43 years, performance status 0, 9–48 months (median 14) after diagnosis, all pretreated. rIFN-α 2 was given in a dose of 4×10^6 U/m^2 daily by subcutaneous injections. Pretreatment and follow-up evaluations included: complete blood counts; determinations of liver and renal functions, LDH levels, serum electrolytes, clotting factors, vitamin B 12 levels and CFU-C; bone marrow aspiration and biopsy; cytogenetic studies. Hematologic remission of the disease was obtained in all pts after 3–16 weeks (median 6) of treatment. The white blood cell counts decreased from $32-120\times10^3/\mu$l (median 68×10^3) to $3.3-8.1\times10^3/\mu$l (median 4.9×10^3), and the thrombocyte counts from $180-831\times10^3/\mu$l (median 443×10^3) to $110-230\times10^3/\mu$l (median 177×10^3), respectively. Parallel reductions occured in serum LDH and B 12 levels. Enlarged spleens decreased in 3/3 pts. Treatment was continued at lower dosages for 1–9 months (median 4+). During these periods a decrease of Philadelphia-positive analyzable metaphases could not be observed. Adverse reactions including fever up to 39.8°C, chills, headache, and fatigue were present only during the first weeks of treatment hardly interfering with the life-style of these patients.

269 Treatment for Excessive Thrombocytosis in Myeloproliferative Disorders with Recombinant Interferon Alpha-2C

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Recombinant interferon alpha-2C (Boehringer Ingelheim, International) was administered in 11 patients (3 male, 8 female; age 54–80 years, median 67.9) with excessive thrombocytosis and myeloproliferative disorders. Initial dosage was 5 to 10×10^6 U/d 5–7×/week IFN and as a maintainance treatment 5×10^6 4–7×/week. In 7 out of 8 patients eligible for evaluation (duration of therapy is 8+ weeks or complete remission prior to week 8) complete remission (platelet count <440/nl) of pretherapeutically increased platelet values (716–1,510/nl) was achieved. In one case decrease of platelet counts from 1,510/nl to 1,160/nl was observed after 8 weeks of treatment. The platelet half life was already reduced significantly in 9 out of 11 untreated patients (t/2 54–114 h, median 73.5 h) and decreased (t/2 21–28 h) significantly ($p<0.01$) in all cases during IFN therapy. In our opinion IFN seems to offer a biological alternative to cytostatic drug therapy in excessive thrombocytosis of myeloproliferative diseases.

270 Results of Interferon Therapy in Multiple Myeloma

H. Ludwig, W. Linkesch, R. Kuzmits, W. Scheithauer, P. Pötzi, J. Kühböck, R. Flener (for the EMSI study group) (II. Medizinische Universitäts-Klinik und Ernst Boehringer Institut für Arzneimittelforschung, Vienna, Austria)

A prospective randomized trial was conducted to compare the efficacy of recombinant interferon alpha-2C (Boehringer Ingelheim, International) and VMCP polychemotherapy (L-PAM, CTX, VCR, PRED) in 42 patients with multiple myeloma. Out of 21 patients randomized to the interferon group, 18 were evaluable for side effects and in 14 cases the therapeutic effects could be evaluated. 19 of the 21 patients randomized to the chemotherapy group were eligible for both criteria.

Interferon therapy effected responses in 2 (14%) patients and minor responses in 4 (29%) cases. 7 (50%) patients showed stable disease courses and in one case the disease progressed under interferon therapy. The respective results in the chemotherapy group were distributed as following: 11 (57%) responses, 6 (32%) minor responses, and 2 (11%) stable courses of the disease. Interferon was preferentially active in patients with IgA myeloma as well as during the early stages of the disease.

271 Recombinant Alpha A – Interferon (Roferon-A®) in the Treatment of the Therapy Resistant Multiple Myeloma and of the Lymphoplasmocytic Immunocytoma

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The extent to which therapy resistant paraprotein producing diseases can be influenced by rIFNαA-Interferon was the topic of a pilot study. Two different doses were tested. During a first series we treated six patients with progressive multiple myeloma and one female patient with a lymphoplasmocytic immunocytoma-dose: once a month 100×10^6 IE/m^2 rIFNαA as intravenously short infusion and 3 times weekly 10×10^6 IE/m^2 rIFNαA subcutaneously over 3 months. During a second series patients were given daily 3×10^6 IE/m^2 rIFNαA over a week subcutaneously.

We raised the dose during the following weeks dependant on its compatibility up to 10×10^6 IE/m^2. The second series is not yet finished. We will comment on the reactions to the therapy as well as the secondary effects being observed.

272 Intralesional Treatment of Advanced Malignant Melanoma with Natural IFN

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Natural Interferon alpha was investigated for its antitumoral activity in 20 patients with advanced malignant melanoma. 6 Mio U IFN was injected into or at the edge of soft tissue metastases. Patients were treated thrice weekly for at least 28 days. If progress occurred under therapy, treatment was stopped. Patients with stabile or shrinking disease remained on IFN-therapy. Complete response was defined as disappearance of all measurable tumor metastases, partial response as an at least 50% reduction of tumor masses. Of 20 patients treated one complete and 4 partial responses were observed. 50% of all injected tumor nodes shrank in size under IFN therapy. In 5 patients injected and non-injected tumor lesions became smaller during treatment. In none of the patients a reduction of a none-injected metastases occurred without a reduction of the injected tumor lesion.

273 Interferon (IFN)-Treatment (Recombinant IFN-Alpha-2 C or Recombinant IFN-Gamma) in Metastatic Renal Cancer

R. Kuzmits, W. Scheithauer, R. Flener[1], H. Ludwig (II. Department of Medicine, University of Vienna, [1]Ernst-Boehringer Research Center, A-1090 Vienna, Austria)

Eleven patients with metastatic renal cancer received IFN-therapy, 8 patients received rec. IFN-alpha-2 C (10×10^6 U/die, later dosis adaption), 3 patients received rec. IFN-gamma (1 mg/die, later dosis adaption). 8 patients presented initially with pulmonary metastases, 5 patients had bone metastases, 3 patients showed metastases in the liver and 2 in soft tissues. IFN-therapy induced a complete remission in 1 patient with pulmonary metastases, 2 patients showed a mixed response, 3 patients showed stable disease and in 5 patients progression of disease was seen. IFN-therapy seems to be effective in pulmonal disease (reduction of pulmonary metastases in 3 patients), whereas in bone and soft tissue metastases only stabilization of the disease could be observed.

274 Significance of Histological Parameters in Severe Aplastic Anaemia (SAA)

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Bone marrow biopsies of 135 patients with SAA were compared with 118 biopsies showing fatty atrophy of the bone marrow, but no concomitant aplastic anaemia. Follow-up biopsies were obtained of 12 patients with SAA treated with bone marrow transplantation. A total of 40 histological parameters were evaluated and correlated with time of survival which was obtained for 78% of the SAA-group and 100% of the FAM-group. Marked cellular infiltration of inflammatory type was observed in 15% of the patients with atrophic marrow but not suffering from hematopoietic failure, and in 85% of patients with SAA. The histological changes were classified either as atrophic (chronic) or necrotic (acute) type of myelitis. More severe inflammatory infiltration as well as more pronounced exsudative capillary changes were correlated with unfavourable prognosis in SAA but not in the FAM-group. Inflammatory changes of the bone marrow are more pronounced in SAA than in FAM. They indicate unfavourable prognosis especially in the former group, and they are observed to disappear after successful bone marrow transplantation.

275 Hematopoietic Suppressor Activity Generated by Peripheral Blood Cells in Aplastic Anemia: Evidence for a Complex Process

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Peripheral blood buffy coat cells from patients with aplastic anemia prior to therapy were incubated for 1 or 4 days in the absence (i) or presence (ii) of lektins (PWM, PHA), or (iii) preincubated with Cyclosporin A (CyA) and then exposed to mitogens. Supernatants were tested for antiviral activity and their effect on the growth of CFU-GM, CFU-E and BFU-E from normal bone marrow. Results: (1) IFN was produced only in response to mitogens. (2) IFN-containing supernatants suppressed CFU-E/BFU-E, while (3) CFU-GM proved to be more resistant to factor-mediated inhibition. (4) Inhibitors and stimulators acting on different target cells coexist in some supernatants. (5) The inhibitory effect could be exerted with only 1% (v/v) of the supernatants. (6) Inhibitory activity generated by mitogen-priming only partially proved to be IFN-gamma. (7) Washing of the target bone marrow after 60 min. preincubation with supernatants completely abolished the inhibitory effect. (8) T-cell depleted bone marrow target cells were not impaired by IFN-containing probes. (9) Doses of $> 1\,\mu g$/ml CyA inhibited IFN production in vitro.

276 Antithymocyte Globulin (ATG) / 6-Methylprednisolone (MP) Treatment in 8 Adult Patients with Severe Aplastic Anemia (SAA)

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8 adult patients (4 female, 4 male, 22–60 a, median: 44 a) with transfusion dependent SAA were treated with combined immunosuppression (IS): Horse-ATG (ATGAM, Fa. Upjohn) 15 mg per kg×8 days and 6-Methylprednisolone 20 mg/kg×4 days with reduction to the half every 4 days and withdrawal of MP after 1 month (except 2 patients with MP maintenance therapy of 0.5 mg/kg for 156 and 224 days, res.). Oxymetholon was given in all patients except 1 but had to be stopped between 10 and 385 days (median: 28 days) after IS because of deterioration of the liver enzymes in 6 patients. 1 patient, starting with 0 granulocytes failed to regain any granulocytes and died from antibiotic resistant pseudomonas septicemia on day 23. The other patients are alive 38–728 days after treatment (median: 187). 5 patients became transfusion independent (1 after additional treatment with Cyclosporin-A), but complete normalization of all cell lines was not achieved. 2 patients, 38 and 60 days after ATG/MP, are too early for a final evaluation. No relapse occurred up to now (71–728 days, median: 422 d after IS). These results show that ATG/MP is an effective treatment for SAA, although complete remission is rarely obtained and long term prospects are uncertain.

277 Treatment of Aplastic Anemia with Antithymocyte Globulin, Prednisolone and Cyclosporin A

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Twelve patients with aplastic anemia (9/12 with severe aplastic anemia) were treated with an immunosuppressive regimen consisting of antithymocyte globulin, prednisolone and cyclosporin A (CsA) (group I, $n = 5$) or prednisolone and CsA (group II, $n = 7$). 10 patients are alive with a follow-up of 8 to 13 months; 2 patients in group II died of a sepsis 5 resp. 7 weeks after treatment. 7 patients had evidence of hematologic recovery: First signs of a response after 23 to 61 days were followed by 1 complete remission, 2 partial remissions and 1 minimal improvement (group I), resp. 2 complete remissions and 1 minimal improvement (group II). Major side effects could be attributed only to CsA (gum hypertrophy, tremor, minimal nephrotoxicity). Interestingly, the quality of remission proved to be dependent on the continued administration of CsA in 3 patients. Thus CsA seemed to be an effective part of the immunosuppressive regimen employed. This observation is a strong argument for the role of T cells in the pathogenesis of at least some cases of aplastic anemia.

278 Cyclosporine in Severe Autoimmune Thrombocytopenia (ATP) and Aplastic Anaemia (SAA)

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Cyclosporine's (Cy-A) encouraging effects on some experimentally induced immune responses in animals and autoimmune diseases has prompted us to use Cy-A in the treatment of two conditions of proven or suspected autoimmune origin, eg, the ATP and the SAA. Between August 1983 and December 1984 we have treated 8 patients with refractory ATP as well as 2 patients with SAA (without available compatible bone marrow donor) unresponsive to conventional treatments and antilymphocyte serum. All 10 patients had significant bleeding. Cy-A was given orally (10 mg/kg bd wt, divided in two doses daily) for 19 up to 51 days (in most cases 6 weeks). Cy-A levels of 300–800 ng/ml whole blood were aimed for. 3 out of 8 patients with ATP showed a significant improvement of the platelet counts and the haemorrhagic diathesis. 2 patients are maintaining their remissions for over 18 months: one patient with a very low dose of Cy-A and the other one without maintenance therapy. In the third responder Cy-A was discontinuated without relapse. Both patients with SAA did not respond. Toxicity was tolerable. However, in view of the benign natural course of ATP the indication for a trial with Cy-A should be made very cautiously, unless the effectiveness and safety of such a treatment has been proven by a randomized study.

279 Bone Marrow Transplantation (BMT) in Severe Aplastic Anemia (SAA): Results Obtained in 20 Consecutive Patients

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20 pretransfused SAA-patients (11 adults, 9 children, med. age: 17 y, range: 4–37) underwent allogeneic ($n = 18$) or syngeneic ($n = 2$) BMT. The disease duration was 6 months (med., range: 1–38), 11 cases were "idiopathic", 4 had a history of hepatitis, 4 had drug exposure and 1 child suffered from Fanconi-anemia. Allogeneic recipients were conditioned with 200 mg/kg Cyclophosphamide (CPM). Rejection prophylaxis consisted of donor buffy coat cell donation on 4 days post graft (15 pat) or preirradiation of the recipient (4: TBI, 300 rad, 1: total nodal, 400 rad). MTX was given for GVH-D prophylaxis. At present (20. 05. 85), 15 patients survive from 55 to 1220 days post BMT (med. 665). Two patients died from infection (Day 4: Systemic Candidiasis, day 85: gut GVH-D, Septicemia). The patient with Fanconi-anemia died from left ventricular failure (day 14). One patient, accidentally pretransfused with maternal platelets, rejected his graft twice and died in aplasia on day 140. One patient died on day 410 from brain edema after prolonged reactive schizophrenia. Both syngeneic recipients obtained engraftment only after immunosuppression (CPM 200 mg/kg) was given prior to a second bone marrow graft from the same donor. Survival of the whole group is 75% after 3 years.

280 Immunophenotyping of Chronic Lymphoid Malignancies: Reliability of the GP 130/140 Marker in Hairy Cell Leukemia Diagnosis

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The monoclonal antibody HD 39 identifies a B cell specific antigen with a molecular weight of 130/140 KD which is present in the cytoplasm of all normal and malignant B cells but is expressed on the cell surface only in mature B cells (Boston, II Int. Congress on Leukocytes differentiation antigens, Sept. 1984). Among 185 blood samples from patients with chronic lympho-

proliferative disorders only 12 cases showed a reactivity with this antibody. In 11 out of these 12 cases a hairy cell leukemia was confirmed by cytochemistry and histology; on the other hand HD 39 surface positivity and a "hairylike" morphology could be induced in CLL cells upon stimulation with TPA. In 3 of our hairy cell leukemias it had primarily been the HD 39 reactivity that led to further diagnostic procedures indicating that HD 39 may be very helpful in detecting cases with atypical presentation. Moreover, the number of hairy cells can be accurately monitored in single patients: this appears to be a very useful follow-up parameter during interferon therapy.

281 UL-90: A Monoclonal Antibody Specific for Pre-Pre-B Cells

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UL-90 is a cytotoxic mouse monoclonal antibody (subclass IgM) reactive with a subpopulation of Common-ALL antigen (CALLA) positive leukemia blast cells but not reactive with lymphoid cells of earlier (AUL) or later stages of B cell differentiation. Cells of early or late T-cell lineage or myeloid/monocytoid lineage are completely negative. TdT-positive cells from bone marrow do not express the UL-90 antigen. Among the CALLA-positive group the reactivity of UL-90 is exclusively restricted to cells which are negative for cytoplasmic IgM heavy chains, thus making this antibody an attractive candidate for the identification of pre-pre-B ALL. Furthermore, hematopoietic progenitor cells (CFU-GM, BFU-E, CFU-GEMM) are spared by treatment with UL-90 and complement. UL-90 might be useful for in vitro purging of bone marrow autografts in patients with pre-pre-B ALL.

282 Receptors for the Third Component of Complement: Their Association with Maturation Stage in Non-Hodgkin Lymphomas (NHL) and their Possible Implication with the Development of Follicular Structures

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The expression of the various receptors for the third component of complement was studied on cryostat sections of about 170 NHL using a pannel of monoclonal antibodies. Our results conform a significant coexpression of the complement receptors one (CR1) and two (CR2) regarding all histological entities of the Kiel classification ($\chi 2 = 32.2$; $p < 0.0005$). The expression of both, the CR1 and the CR2 was apparently restricted to NHL of "intermediate maturation stage" (i. e. chronic lymphocytic leukemia, immunocytoma, germinal centre cell lymphomas). Within these entities CR positive cases often showed a follicular arrangement of the neoplastic cells. Although these follicular structures were usually abortive, the correlation between the expression of the CRs and the follicular appearance of the neoplastic cells was confirmed by another finding: Using morphometric methods higher numbers of T-helper lymphocytes ($\chi 2 = 5.2$; $p < 0.025$) as well as natural killer cells ($\chi 2 = 7.35$; $p < 0.01$) were observed in CR positive NHL. Both types of reactive cells are usually present within the germinal centres of reactive lymphatic tissues.

283 Expression of Interleukin-2 Receptors and the Ki-1 Antigen on Malignant Lymphoma Cells of Histiocytic Origin

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Diffuse histiocytic lymphoma of the Rappaport classification represent a wide diversity of cell types the majority of which are of B-cell others of T-cell origin and only few originated from the macrophage lineage. Here we report on the surface phenotype of two cases of true histio-

cytic lymphoma which have been studied by immunperoxidase staining of aspirated lymph node cells and compared with the DHL-1 line which was established from a patient with diffuse histiocytic lymphoma. Both the lymphoma cells as well as the DHL-1 cells failed to express markers specific for T-cells, B-cells and monocytes. However, they strongly reacted with monoclonal antibodies (mAbs) to the IL-2 receptor (anti-Tac), the Ki-1 antigen, the transferrin receptor (OKT9) and HLA-DR molecules as well as to the novel MAX. 26 antigen which is shared by macrophages and subsets of B- and T-lymphocytes but absent from granulocytes. Whereas lysozym, alpha-trypsin, chymotrypsin and ferritin were found within the cell no cytoplasmic immunoglobulins could be detected. The staining of a minor portion of the lymphoma cells with EMA (a mAb that recognizes an epithelial cell membrane antigen) is at present not understood. The Tac$^+$ Ki-1$^+$ OKT9$^+$ MAX. 26$^+$ Ia$^+$ phenotype in the absence of any lineage specific antigen may be unique for true histiocytic lymphoma.

284 Receptor for Interleukin 2 on Tumor and Reactive Cells in Non-Hodgkin Lymphomas

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Cryostat sections of 130 tumor tissues (lymph nodes and bone marrow biopsies) of patients with Non-Hodgkin lymphomas were evaluated by the immunoperoxidase method with a panel of monoclonal antibodies, including the anti-Tac antibody, which detects the interleukin-2 receptor. A considerable number of reactive T lymphocytes demonstrated strong staining for this antibody (range: 0 to 20% of tissue cells). In addition, tumor cells of the B cell lineage expressed this antigen in about half of the patients, though in lower density. A high incidence of positive cases was observed in hairy cell leukemia and CLL and in a lower percentage in immunocytomas and centrocytic lymphomas. We conclude, that the receptor for this growth factor is not only a feature of activated T lymphocytes, but also of neoplastic B lymphocytes, particularly of certain histologies.

285 Interferon γ-Stimulates the Expression of IL-2 Receptors on Human Monocytes

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During cultivation in the presence of IFNγ human peripheral blood monocytes acquire reactivity with anti-Tac monoclonal antibody. Twenty-four hours after stimulation 30–70% of monocytes are Tac positive. Reaction with two other anti-IL-2 receptor monoclonal antibodies and the inclusion of subclass specific control antibodies indicate the specificity of binding. Moreover, high doses of recombinant IL-2 bind to IFN stimulated, but not to freshly isolated monocytes, as estimated in a sandwich indirect immunofluorescence staining technique with an anti-IL-2 monoclonal antibody. Further studies to characterise the structure and function of the detected antigen are under progress.

286 Intraclonal Heterogeneity of Antigenic Phenotypes in Acute Lymphoblastic Leukemia: Evidence for Two Independent Mechanisms

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Although monoclonal in evolution, leukemic blast populations are commonly heterogeneous, known by morphology in myelogenous leukemias and evident by immunological multimarker analysis in ALL as well. In order to better understand this heterogeneity, immunological phenotypes in ALL blast populations were compared with the phenotype sequences of T- and B-lymphocyte maturation in thymus and bone marrow. For phenotypic analysis a large panel of monoclonal antibodies, applied singly and in combination, and a sensitive immunocytochemical method were used. The phenotypic mixtures encountered in both T- and c-ALL correspond-

ed to segments of the normal T- and B-lymphocyte maturation sequences, and thus appeared to represent 'skidmarks' of differentiation arrest. In addition to this sequence of normal phenotypes, subsets of blasts with an abnormal antigenic phenotype could be identified in several leukemias, lacking antigens which are found on normal cells of corresponding maturation stage. The latter finding suggests that cells can randomly loose, in the course of their malignant evolution, capacities to elaborate normal cell products, which hypothetically reflects a loss of the corresponding genes.

287 Correlation of Surface Marker Analysis and Morphological Diagnosis in Acute Myeloblastic Leukemia (AML)

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In order to compare the surface antigen structure of AML cells with morphologic diagnosis based on FAB classification bone marrow specimen from 27 adults with AML were characterized by morphological criteria, classical cytochemical techniques, and phenotyped by immunofluorescence with a panel of monoclonal antibodies (MoAbs) directed against myeloid-, monocyte-associated surface determinants (My 7, My 9, VIM-D 5, VIM-13, Leu-M 3, Mo 2), glycophorin A (VIE-G 4), platelet glycoprotein II b–III a (J 15), HLA-DR (OKIa 1). We observed a clear correlation between surface marker profile and FAB classification. Three morphologically/cytochemically undifferentiated leukemias could be affiliated with myeloid lineage by immunologic analysis and in one patient immunological markers revealed involvement of both myeloid and lymphoid cells. Morphological and immunological data will be presented, and the clinical and prognostic significance of surface antigen analysis in AML will be discussed.

288 Low Dose Interferon-α-Therapy of Hairy Cell Leukemia

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Eight patients (3 men and 5 women at the age of 36–53 years) with hairy cell leukemia were treated with a low dose Interferon-α-2 therapy of 1 Mio U per day given s. c. for 1 month followed by 1 Mio U IFN-α given thrice weekly. The diagnosis of hairy cell leukemia was based on the clinical characteristics and the demonstration of typical hairy cells in peripheral blood and bone marrow specimen. The tartrat-resitent acid phosphatase stain of bone marrow was positive in all 8 patients. 2 of the 8 patients were previously splenectomized. None of the patients received chemotherapy prior to IFN-α treatment. 5 of 6 patients showed after more than 3 months of IFN-α treatment a normal blood cell count. In 4 of 5 patients the splenomegaly disappeared. So far a complete response ocured in none of the patients.