O418

Combined Anti-Inflammatory and Angiostatic Treatment of Hormone Refractory Prostate Cancer

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Purpose: Peroxisome proliferator-activated receptor gamma (PPARγ) and cyclooxygenase-2 (COX-2) are frequently over-expressed in both prostate cancer cells and in the adjacent stroma cells, and could be targets for therapy of hormone refractory prostate cancer (HRPC). Methods: The current study for symptomatic patients with HRPC was designed to evaluate the efficacy and toxicity of repetitively administered low-dose capcitabine (twice daily 1 g/m², day 14 to 28, every 3 weeks) combined with an anti-inflammatory/angiostatic treatment including rofecoxib (selective COX-2 inhibitor) 25 mg po day 1+, pioglitazone PPARγ agonist, 60 mg po day 1+, and dexamethasone 1 mg daily, day 14 to 28, every 3 weeks during continuation of hormone ablation.

Results: Eighteen men (median age 68 years), 10 of whom had received previous radiotherapy and/or chemotherapy (56%), were enrolled onto study protocol. Using clinical response guidelines set forth by the Prostate Specific Antigen Working Group, 33% of patients were found to have a > or = 80% reduction of PSA, 11% of patients were found to have a reduction < 80% and > 50%, 38% were found to have a < 50% decrease in PSA, and 17% experienced disease progression while receiving treatment. Up to date study medication was discontinued due to PSA progression in 4 patients after 3 to 6 months, 14 patients are still on treatment (median 6 months, range 2 to 9 months +). Two of 3 patients with measurable disease achieved partial remission. In 33% of patients Karnofsky performance status improved. 56% of patients could reduce analgetic medication due to positive pain response. Morphin medication could be terminated in two patients. Major toxicity (grade 3) was observed in one patient (edema). Conclusions: In contrast to the recently reported low efficacy of standard dose capcitabine in HRPC, the present all-oral therapy approach combining stroma cell- and tumor cell-targeted therapy including repetitively administered low-dose capcitabine (metronomic chemotherapy) shows for the first time efficacy and low toxicity, which compares to standard therapy with docetaxel in terms of PSA response and improvement of clinical response parameters.

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Thalidomide in Refractory Germ-Cell Tumors

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Purpose: To assess the activity of thalidomide in patients (pts.) with germ-cell tumors (GCT) and progressive inoperable disease after salvage chemotherapy.

Patients and Methods: Between April 2002 and January 2003, 15 pts were included in a phase I/II study and treated with escalating daily doses of 200 to 600 mg of thalidomide. All pts had relapsed or progressed after salvage treatment and were considered inoperable. Pts has had received HD-VIP containing cisplatin, ifosfamide and etoposide and 102 pts were treated with Tax-HD-VIP containing cisplatin, ifosfamide, etoposide plus paclitaxel. All pts had received 3-4 HDCT cycles each supported by autologous PBSC resulting in a median cumulative dose of etoposide of 4.9 g/m² (range, 2.2 to 9.4 g/m²).

Results: The median follow-up of pts alive at least one year after therapy and thus being at risk for the development of s-AML was 50 months (mo) (range, 12 -128 mo). Two hundred thirty eight (74 %) pts were alive at a median follow-up of 46 mo (range, 3 to 118). One patient developed a secondary acute lymphoblastic leukemia (s-ALL) involving a chromosomal translocation t(11;19)(q23;p13.3) 24 mo after the start of chemotherapy and 16 mo after HD salvage chemotherapy (cumulative etoposide dose of 8 g/m²) which he had subsequently received for relapsed disease. This resulted in a cumulative incidence for secondary leukemia of 0.48 % (95% -CL: 0-1.42). Two additional pts with primary mediastinal GCT developed a myelodysplastic syndrome (MDS) 23 and 11 mo after chemotherapy considered to be non-therapy induced but biologically related to the GCT. No secondary solid tumors were observed. Conclusions: HDCT as first-line therapy for advanced testicular cancer was associated with an acceptably low risk of developing secondary malignancies including s-AML.

O420

Low risk of secondary Leukemia following First-Line High-Dose Chemotherapy for Patients With Advanced Germ Cell Cancer

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Purpose: We investigated the incidence of secondary leukemia (s-AML) in patients (pts) treated with first-line high-dose chemotherapy (HDCT) plus autologous peripheral stem cell transplantation (PBSC/T) for advanced germ cell tumor (GCT). Methods: Three hundred twenty-three pts with median age of 30 (range, 15 - 47) from two consecutive prospective Phase-II studies of the German Testicular Cancer Study Group were analyzed. Of these 221 pts had received HD-VIP containing cisplatin, ifosfamide and etoposide and 102 pts were treated with Tax-HD-VIP containing cisplatin, ifosfamide, etoposide plus paclitaxel. All pts had received 3-4 HDCT cycles each supported by autologous PBSC resulting in a median cumulative dose of etoposide of 4.9 g/m² (range, 2.2 to 9.4 g/m²).

Results: The median follow-up of pts alive at least one year after therapy and thus being at risk for the development of s-AML was 50 months (mo) (range, 12 -128 mo). Two hundred thirty eight (74 %) pts were alive at a median follow-up of 46 mo (range, 3 to 118). One patient developed a secondary acute lymphoblastic leukemia (s-ALL) involving a chromosomal translocation t(11;19)(q23;p13.3) 24 mo after the start of chemotherapy and 16 mo after HD salvage chemotherapy (cumulative etoposide dose of 8 g/m²) which he had subsequently received for relapsed disease. This resulted in a cumulative incidence for secondary leukemia of 0.48 % (95% -CL: 0-1.42). Two additional pts with primary mediastinal GCT developed a myelodysplastic syndrome (MDS) 23 and 11 mo after chemotherapy considered to be non-therapy induced but biologically related to the GCT. No secondary solid tumors were observed. Conclusions: HDCT as first-line therapy for advanced testicular cancer was associated with an acceptably low risk of developing secondary malignancies including s-AML.

O421

Recombinant Adeno-associated Virus 2 Suicide Vectors for the Treatment of Human Sarcomas and Mesotheliomas

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Although great efforts have been made to improve conventional therapy for sarcoma and malignant mesothelioma, the median survival time of these entities after appearance of clinical symptoms stays poor. Effective locoregional therapy using viral vectors that contain a suicide gene may be an alternative treatment strategy. For sarcoma, we previously reported the highest susceptibility for recombinant adeno-associated virus 2 (rAAV-2) vectors in human connective tissue sarcoma cells. Now we confirm our findings in five further human sarcoma cell lines; fibrosarcoma, Ewing’s sarcoma, Askin’s tumor, rhabdomyosarcoma and soft tissue sarcoma. Furthermore, we found that rAAV-2 also achieved both high transduction rates and GFP expression levels in three human mesothelioma cell lines. Among rAAV-2 constructs containing different promoters, after transduction, the vector with the elongation factor 1-alpha (EF1α) promoter showed the highest expression rates in both the sarcoma and mesothelioma cell lines. To ensure the use of constant vector particles number, all stocks were titrated using both the functional and our real-time PCR-based titration assay.

Several new thymidine kinase (TK) gene-containing vectors under control of either the Cytomegalovirus or the EF1α promoter showed the highest expression rates in both the sarcoma and mesothelioma cell lines. To ensure the use of constant vector particles number, all stocks were titrated using both the functional and our real-time PCR-based titration assay.

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Xenotransplantation tumor models for both the human sarcoma (i.p., s.c.) and mesothelioma cell lines (i.p.) were established in NOD/SCID mice. In proof-of-principle experiments, mice transplanted with rAAV-TK/eGFP-transduced and ganciclovir-exposed sarcoma or mesothelioma tumor cells survived >5 months while in the non-transduced group all mice had died approximately 1 month after inoculation. Currently, further animal experiments using this suicide system are ongoing.

The data shown here hold promise for further development of AAV-2-based suicide gene therapy of both soft tissue sarcoma and mesothelioma towards a future clinical application.

O422
Ifosfamide and Liposomal Daunorubicin (IDx) is a Well Tolerated and Active First Line Chemotherapy Regimen in Advanced Soft Tissue Sarcoma – Results of a Phase II Study

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Purpose: Anthracycline/ifosfamide combination is the most effective chemotherapy in soft tissue sarcoma. To improve tolerability and potentially efficacy of this combination we combined high dose continuous infusion ifosfamide with liposomal daunorubicin (L-Dauno). Patients and Methods: In a single-arm phase II study 40 patients were enrolled with a median age of 57 years (19 to 78 years). Treatment regimen was ifosfamide 5 g/m² over 24 h and L-Dauno 100 mg/m² every 4 weeks with G-CSF support. Initially, 5 infusion at a dosage of 1000 mg/m² on day 1, 8 and 15 every 4 weeks. All patients (pts) with metastatic soft tissue sarcoma (STS) is limited, especially in elderly patients. Randomized studies with this regimen are warranted.

Results: Eleven (31 %) out of 35 anthracycline/ifosfamide naïve patients achieved a PR/CR, all after 2 treatment cycles, 6 patients (17 %) had stable disease and 13 patients (37 %) progressed; 5 patients were not evaluable. PR with respect to histology was: 4/7 PNET, 1/6 leiomyosarcoma, 2/4 liposarcoma, 1/2 synovial sarcoma, 1/3 pleomorphic sarcoma, 1/1 malignant histiocytoma and 1/2 carcinosarcoma. Median time to progression was 6 months, median overall survival 14 months and median time to treatment failure 15 months. Toxicity was tolerable with 5 episodes of ifosfamide related CNS toxicity grade II, one ifosfamide related acute renal failure and 11 episodes of neutropenic fever. One patient died due to a neutropenic sepsis. No other non-hematological toxicity exceeding grade II was observed. Conclusion: IDx is a well tolerated and highly active chemotherapy regimen for first line treatment of soft tissue sarcoma, even in elderly patients. Randomized studies with this regimen are warranted.

O423
An Open Label, Non-Comparative Phase II Study of Gemcitabine as Salvage Treatment for Patients with Pretreated Advanced Soft Tissue Sarcoma

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Purpose: The number of effective cytotoxic agents for the treatment of patients (pts) with metastatic soft tissue sarcoma (STS) is limited, especially when pts have failed anthracycline-based chemotherapy. The aim of this trial was to evaluate the efficacy of gemcitabine inpretreated pts with STS. Methods: Between 8/01 and 3/03 19 pts were eligible to enter this open-label, non-comparative study. Gemcitabine was administered as a 30-minutes infusion at a dosage of 1000 mg/m² on day 1, 8 and 15 every 4 weeks. All patients had progressive disease during or shortly after an anthracycline/ifosfamide-based regimen. The primary objective of the study was to determine the overall response rate (CR/PR). A two-stage study design was used. Results: Four of 19 pts did not start study treatment because of fulminant progression. Fifteen pts, median age 47 years (32 – 72), with various histological subtypes of STS were assessable. All pts had received at least one prior treatment regimen (1 – 6) for metastatic disease containing anthracyclines (14 pts/93%) and ifosfamide (12 pts/80%). Extent of disease at study entry included local recurrence (n=12), lung mets (n=12), lymph node involvement (n=5) and liver as well as bone mets (n=2 each). To date, a total of 62+ cycles have been applied (median: 3 = 1 – 18+). Seven pts (47%) had progressive disease at the first response assessment. One pt (6%) attained a PR, and 7 pts (47%) achieved disease stabilisations (SD). The reason for treatment stop was tumor progression in 13 pts (87%). Two pts are still on treatment (6, respective 18. cycle). The median progression-free rate (PFR) was 3 mos (1–18+) and the median overall survival 6 mos (3–18+). Toxicity profile was favourable. 87% of the cycles have been applied without dose modification or delay. Grade 3 toxicities consisted of thrombocytopenia in 5 pts (33%), leukopenia (2 pts/13%) as well as anemia, neutropenic infection, alopecia and flu-like symptoms (1 pts/7% each). Conclusions: This series confirms that a considerable number of disease stabilisations in pretreated adult STS patients can be achieved with gemcitabine. The calculated PFR at 3 and 6 mos was 46.7% (CI95%, 21.4-71.9) and 13.3% (CI95%, 0-30.5). Considering response and progression-free rate as primary endpoints for phase II trials in STS, gemcitabine has moderate but defined efficacy in pretreated adult STS.

O424
Treatment of Malignant Peritoneal Mesothelioma with Pemetrexed

Karthaus M., Pliall A., Frölicher F., Wirt N., Mahler G., Metzner D.

Malignant pleural mesothelioma (MPM) is a rare and aggressive neoplasm of the lining of the lung. Rare reports are available for treatment and outcome of peritoneal mesothelioma only (AbM). Pemetrexed (ALIMTA), is a novel antifolate targeting key enzymes in purine and pyrimidine synthesis. Recently data from a randomized phase III have shown superior efficacy of pemetrexed/DDP vs DDP alone in MPM. No data of pemetrexed in AbM are available. In an open label trial efficacy and safety data of pemetrexed (500 mg/m²) +/-DDP (75 mg/m²) or carboplatin (AUC 5) were studied in malignant peritoneal mesothelioma. A total of 49 pts with mesothelioma with stage III/IV were observed between 12/02 and 04/04. Pts received pemetrexed based therapy including dexamethasone prophylaxis for skin rash on day –1 to+2 additionally. Folic acid 400 µg po daily, prior to and during study, and vitamin B12 1000 µg i.m. q 9 wks in addition was administered to prevent adverse events. Study endpoints were time to progression (TTP), best response and safety. Results: Four pts (1 AbM, 1 MPM, 2 MPM+AbM) were excluded due to renal impairment (n=1) or death prior to CTX (n=3). 45 mesothelioma pts (34 m/11 f) were treated from 12/2002 until 11/2003. Staging revealed AbM in 10 pts, MPM in 30 pts, while 5 pts had malignant mesothelioma on both sites of the diaphragma. Initial combination was with DDP in 34 pts and carboplatin in 11 pts. Pemetrexed was administered in a median of 6 cycles (range 1 –13). Major toxicity (WHO >III/IV) was nausea and neutropenia. Response are presented in the following table.

<table>
<thead>
<tr>
<th></th>
<th>AbM (n=10)</th>
<th>MPM + AbM (n=5)</th>
<th>MPM (n=30)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male/Female</td>
<td>8/2</td>
<td>4/1</td>
<td>22/8</td>
</tr>
<tr>
<td>Mean Age</td>
<td>64.2</td>
<td>60.8</td>
<td>69.4</td>
</tr>
<tr>
<td>Pretreated Pts</td>
<td>3</td>
<td>3</td>
<td>13</td>
</tr>
<tr>
<td>Pemetrexed cycles/median</td>
<td>70/6</td>
<td>39/8</td>
<td>156/6</td>
</tr>
<tr>
<td>RR (CR+PR/SD)</td>
<td>3 (30%)</td>
<td>3 (60%)</td>
<td>10 (33%)/9 (27%)</td>
</tr>
</tbody>
</table>

Data regarding TTP, best response and safety will be presented at this meeting. Conclusion: Pemetrexed in combination with platinum is a well tolerable and active regimen for advanced peritoneal malignant mesothelioma.
Phase-II Study with Capcetabinate and Celecoxib (CC) in Patients with Advanced Solid Tumors

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Purpose: Cytotoxic agents are effecting tumor cells as well endothelial cells. The application of low dose continuous (metronomic) chemotherapy may optimize the effect on the tumor endothelial cells, acting like an unspecific anti-angiogenic regimen. Capcetabinate is a prodrug of 5-FU. Because of high concentrations of thymidylatesynthase in proliferating endothelial cells, metronomic application of capcetabinate might inhibit angiogenesis. Celecoxib, a selective cyclooxygenase-2 (COX-2) inhibitor, is known to have an apoptotic effect on tumor cells as well as an anti-angiogenic effect on tumor endothelial cells. The objectives of the trial was to evaluate the antitumor activity and toxicity of continuous treatment with the combination of CC in patients (pts) with advanced cancer.

Methods: Pts were treated with capcetabinate 500 mg bid and celecoxib 400 mg bid continuously until progressive disease or toxicity occurred. Pts with advanced or metastatic cancer were included in the trial. 36 patients (16 female, 20 male) were treated with CC. Mean age was 58.3 years. 1 cycle was defined as a period of 4 weeks. Assessment of tumor size was performed before, after 1 and 3 cycles and then every 2nd cycle. Dynamic contrast-enhanced MRI (DCE-MRI) was used for two reasons: 1. to evaluate tumor vessel permeability and thus the anti-angiogenic effect of CC; 2. to assess the tumor size. Pts were evaluable for response when they were treated at least 3 cycles. Results: 2 pts had stable disease (SD) for 6 cycles, 1 pt had SD for 4 cycles, and 1 pt had SD for 3 cycles, then progressive disease (PD). 8 pts had PD after 3 cycles. 13 pts did not complete 3 cycles because of PD and therefore were not evaluable for response. 10 pts are ongoing in cycle 3 to 2 weeks. Evaluation of response in these pts will be performed after completion of 3 cycles. Regarding toxicity, 2 pts had an increase in creatinine levels, possibly due to celecoxib treatment, and were excluded from the study. 2 pts developed a mild hand-foot-syndrome. All toxicities were reversible.

Conclusions: In general, therapy with CC was well tolerated and showed potential antitumour activity. Cancer control (no change, stable disease) is the main goal of a successful anti-angiogenic therapy. Although CC is able to reach cancer control in some patients, patients with aggressive tumor progression doesn’t seem to profit from this drug combination. The DCE-MRI was a valuable tool to track antiangiogenic effects of the CC treatment.

Poster Session: Angiogenesis

Small Rho GTP Binding Proteins Regulate Vascular Endothelial Growth Factor-2 Expression IN Endothelial Cells

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Recent evidence suggests, that rho-family small GTPases play a significant role in the regulation of different endothelial cell functions, implicated in capillary network formation. Whereas rho-family small GTPases are primarily known to control actin-based motility processes, rhoA, rac1 and cdc42 have been shown to regulate other cellular activities as well, such as membrane transport and gene transcription. As vascular endothelial growth factor receptor-2 (VEGFR2) expression is necessary for angiogenic responses to occur, we hypothesized that rho-family small GTPases may affect VEGF2 expression by cultured endothelial cells (ECs). Overexpression of dominant negative mutants N17Rac1 and N19RhoA were seen to significantly inhibit VEGF2 protein expression by HUVEC, whereas transfection of mutant N17Cdc42 failed to affect VEGF2 levels. As N17Rac1 and N19RhoA also supressed VEGF2 mRNA accumulation, we subsequently examined their effects on VEGFR2 transcriptional activation. Analyses of a different 5’-deletional VEGF2 promoter-based reporter gene constructs revealed that inhibition by N17Rac1 and N19RhoA is conveyed by distinct gene regulatory elements. Whereas N17 Rac1-mediated suppression is confined to a GC-rich region between bp -77 and -100, N19RhoA-mediated inhibition appears to be conferred by an element located between bp -225 and -160, harboring a consensus E-box binding site. We were able to show by EMSA analysis, that constitutive Sp1-dependent DNA binding to the GC-rich region is decreased by N17Rac1 transfection, indicating that inhibition of rac1 may interfere with Sp1-dependent VEGFR2 transcription. Therefore, different members of the rho-family small GTPases exert diverse effects on VEGF2 expression. Significantly, inhibition of VEGF2 transcription by N17Rac1 and N19RhoA involves distinct molecular mechanisms as different elements seem to be engaged. In conclusion, control of capillary network formation by rho-family small GTPases may likely be mediated in part via modulation of VEGFR2 expression.

Imatinib and Microvascular Endothelial Cell Function

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Purpose: Imatinib is a tyrosine kinase inhibitor with c-kit, c-Abl, and PDGF beta-receptor activity of that has been shown to reduce increased bone marrow vascularization and Bcr-Abl mediated VEGF secretion in patients with chronic myeloid leukaemia (CML). Microvascular endothelium is involved in the
regulation of trafficking, differentiation, and homing of stem cells within the haematopoietic microenvironment. It has been suggested that imatinib influences angiogenesis through an anti-migratory and anti-proliferative effect on vascular smooth muscle cells via the inhibition of the PDGF beta-receptor phosphorylation. In our study we analysed the effect of imatinib on proliferation and function of microvascular endothelial cells. Methods: The effects of imatinib at concentrations varying between 0.1 and 50mM on human microvascular endothelial cells (HMVEC) were observed in 4 in vitro assays. Endothelial proliferation was detected with a cell viability assay based on the ability of living cells to convert a redox dye (resazurin) into a fluorescent red product (resoruvin). Endothelial function was assessed by the ability of HMVEC to form tube networks in growth factor reduced matrigel. Tube formation was detected by phase contrast microscopy and recorded with a digital camera. Endothelial cell migration was analysed using the scratch wound lesion assay and the Boyden chamber chemotaxis assay. Endothelial cell basal medium was supplemented with the growth factors FGF-2, VEGF, IGF-1, EGF. Endothelial cells that migrated through the pores of the membrane to the bottom chamber were stained and counted in 20 random fields. Tube formation and scratch wound healing were quantified with image analysis software. Results: Imatinib treatment showed no significant effect on HMVEC proliferation in therapeutic concentrations (0.1 - 10mM). Interestingly, we could observe that imatinib increased the endothelial tube and network formation on matrigel. However, the migration of HMVEC was clearly suppressed in the wound healing migration as well as in the Boyden chamber chemotaxis assay. Conclusions: These data suggest that the angiogenic static property of imatinib also involves anti-migratory effects on endothelial cells without affecting their ability to proliferate and to form tube networks. Therefore, imatinib in addition to its efficacy in the antineoplastic treatment of CML and certain cancers might also prove to be clinically useful in diseases characterized by unregulated angiogenesis.

### Poster Session: Molecular Genetics

**P456 Localization of BCR-ABL in Philadelphia-Chromosom Positive Blasts is Dependent on its Aberrant Kinase Activity**

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**Purpose:** The chromosomal translocation t(9;22) giving rise to the Philadelphia (Ph) chromosome aberrantly fuses BCR sequences to the non-receptor tyrosine kinase c-ABL. The presence of the BCR oligomerisation interface in the fusion protein leads to its tetramerisation and to a constitutive activation of the ABL tyrosine kinase. Multiple signalling pathways such as the Ras pathway or JAK-STAT kinases activated by BCR-ABL transform haematopoietic cells and lead to the malignant phenotype of Ph-chromosome positive CMLs and ALLs. BCR-ABL is expressed in about 95% of CML and 20-25% of adult ALL cases. The specific ABL kinase inhibitor STI571 induces apoptosis in BCR-ABL transformed cells, leads to complete remission in chronic phase CML-patients and has a remarkable but not durable effect in CML-blast crisis or in Ph-positive ALL patients. In this study we investigated the cellular localization of BCR-ABL and its dependency on the kinase activity and the colocalization with the BCR-ABL adaptor proteins GRB-2 and SHC.

**Methods:** We treated cell lines endogenously expressing BCR-ABL (BV173, Sup-B15) and cell lines infected with BCR-ABL constructs (BaFa3, Ba) as well as primary cells from a patient with CML blast crisis with 0.5 μM STI571. As a control we investigated cells infected with a kinase deficient BCR-ABL mutant. At different time points the cells were fixed on cover slides. The subcellular localization of BCR-ABL was analysed by immunofluorescence with an ABL specific antibody. Colocalization of ABL with GRB-2 or SHC were studied by co-immunofluorescent stainings with ABL and GRB-2 or SHC specific antibodies. Pictures of the cells were taken by confocal laser scan microscope.

**Results:** We found that i) BCR-ABL is localized in dots whereas wildtype ABL is distributed diffusely among cytoplasm ii) the typical BCR-ABL staining pattern is detected in endogenously BCR-ABL expressing cell lines, in cells infected with BCR-ABL constructs and in primary Ph-chromosome positive cells iii) the localization of BCR-ABL is perinuclear iv) treatment of Ph-chromosome positive cells with STI571 leads to redistribution of ABL from BCR-ABL dots to microspeckled pattern v) the same effect can be achieved in transfected cells with a BCR-ABL construct lacking the tyrosine kinase domain vi) there is no colocalization of BCR-ABL with the adaptor proteins GRB-2 or SHC before or after treatment with STI571 and vii) the effect of STI571 is irreversible.

**Conclusions:** The results of the localization studies indicate that the staining pattern of ABL and BCR-ABL differ completely and that the typical BCR-ABL pattern strictly depends on the aberrant tyrosine kinase activity of the fusion protein. The responsible mechanism is unknown but further investigations may elucidate the formation of BCR-ABL dots and involvement of the kinase activity and its downstream targets.
tively. Results: Telomere erosion was observed leading to a delayed proliferation arrest in a all selected subclones (n=10). Interestingly, the lag period varied based on the genetic background: whereas p53+ cells (n=3) stopped proliferation uniformly after 40 population doublings (PDs), growth arrest occurred between 38 and 59 PDs in p53- cells (n=4) and between 40 to 59 PDs in p21+ cells (n=3). Although Q-FISH and M-FISH analysis revealed that all subclones showed increased rates of telomere dysfunction such as signal free ends and end-to-end fusions, the level was strikingly pronounced in p53- and p21+ cells, respectively. Furthermore, we observed a higher rate of hyper-diploidy in checkpoint deficient cells without telomerase activity. Conclusions: These data suggest that the p53 and p21 checkpoint status is critical for the genetic stability in tumor cells following telomerase dysfunction. Importantly, the resulting genetic crisis appears to be incompatible with cell proliferation which justifies the concept of telomerase inhibition for cancer therapy.

P459 Enhancers of the RUNX2 Gene Active in Human Prostate Cancer

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Purpose: The transcription factor RUNX2 has been accepted to be the key regulator of osteoblastic development. Recently however, several reports have been published suggesting a role for RUNX2 in different types of tumors, in particular in those that can form bone metastases. Prostate cancer is such a type of tumour. We focused our studies on the signalling pathways that lead to ectopic RUNX2 expression. Our knowledge of the control of RUNX2 gene expression is mainly confined to external signals and to trans acting factors. cis acting genetic elements however, are poorly understood. Thus far studies on the two promoters of the RUNX2 gene explain only a fraction of its observed physiological expression pattern. Hence, we conclude that there are additional regulatory elements required for expression control of RUNX2 at least in some tissues. Methods: For sequence comparison NCBI’s Blast programmes were used. Mouse counterparts of the conserved genetic elements CR3, CR4, and CR5 were amplified by PCR from genomic mouse DNA and cloned into pGL-3 promoter (Promega). RUNX2 expression was assayed by RT-PCR. Mesenchymal cells were transfected with Eugene 6 transfection reagent (Roche), prostate cancer cells were transfected with Transfast (Promega). Enhancer activity was measured applying Dual Luciferase Assay System from Promega according to the manufacturer’s protocol. Results: We previously screened the RUNX2 loci of human and Fugu rubripes for highly conserved non-coding sequences that might represent regulatory elements. This screen revealed the two promoter sequences (here termed CR1 and CR2) and further three conserved sequences: CR3, CR4 and CR5. In the present study we functionally analysed CR3-5 by generating reporter constructs as enhancers and a firefly luciferase gene driven by the respective elements were analysed in mesenchymal and prostate cancer cells. This study provides first data supporting the assumption that CR3 and CR5 in contrast to CR4 are functional enhancer elements. While CR3 displays its enhancer activity in a constitutinal way, CR5 enhancer activity is linked to RUNX2 expression. Conclusions: We conclude, that CR5 is a good candidate for a RUNX2-specific enhancer active in prostate cancer cells. CR3 seems to be a constitutional enhancer of transcription.

P460 Analysis of CALM/AF10 Fusion Gene Positive Leukemias: HOX Gene Derelegation and High Incidence of CALM/AF10 Fusions in T-ALL with TCR γδ Rearrangement

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The t(10;11)(p13;q14) resulting in the CALM/AF10 fusion gene is found in undifferentiated leukemia, acute myeloid leukemia, acute lymphoblastic leukemia and malignant lymphoma. The CALM/AF10 fusion protein was reported to be the most common fusion protein in T-ALL with TCR γδ rearrangement. CALM/AF10 is associated with a poor prognosis.

We have analyzed 10 patients with T-ALL with the different types of leukemia: case 1 (AML/M2), case 2 (AML-M0), case 3 (Pre-T-ALL), case 4 (AL, unclear), case 5 and case 6 (ALL), case 7 and 8 (Pro-T-ALL), with a t(10;11) which did not show involvement of the MLL gene. In addition, we analysed a series ten patients with T-ALL with TCR γδ rearrangement. The samples were analysed for the presence of the CALM/AF10 and AF10/CALM mRNA by RT-PCR and sequence analysis. cDNA of five patients was analysed using oligonucleotide microarrays representing 33000 different genes.

All ten patients with reported t(10;11) were positive for the CALM/AF10 fusion. We found two different breakpoints in CALM at nucleotide 1926 and 2091. In addition, a new exon, with 106 bases after nt 2091 was found. In AF10 four breakpoints were identified: at position 424, 589, 883 and 979. Of the ten T-ALL patients with TCR γδ rearrangement from which the karyotype was unknown, two were positive for the CALM/AF10 transcript, confirming previous results (Asnafi et al, 2003). In these two patients the breakpoint in CALM was at nucleotide 1926 and 2091. In AF10 the breakpoint was at nucleotide 883. In five patients it was also possible to amplify the reciprocal AF10/CALM fusion transcript (case 1, 3, 4, 5 and 6). There was no correlation between disease phenotype and breakpoint location. Preliminary analysis of microarray expression profiling of five of these cases revealed high expression levels of the HOX genes. The overexpression of HOX genes seen in those CALM/AF10 positive leukemias is reminiscent of the pattern seen in leukemias with rearrangements of the MLL gene, normal karyotypes and complex aberrant karyotypes suggesting a common effector pathway (i.e. HOX gene deregulation) for these diverse leukemias. It is known that alhambra, the Drosophila homologue of AF10, can act on polycomb group responsive elements. It is thus conceivable that the CALM/AF10 fusion proteins acts in a dominant negative fashion on wildtype AF10 function, relieving the repression that is presumably normally exerted by AF10 on the expression of the HOX genes.

P461 The Multidomain Bcl-2 Homolog Bax but not Bak Mediates Synergistic Induction of Apoptosis by TRAIL and 5-FU through the Mitochondrial Apoptosis Pathway

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The death ligand TRAIL has been suggested as a suitable biological agent for the selective induction of cell death in cancer cells. Moreover, TRAIL synergizes with DNA damaging therapies such as chemotherapeutic drugs or ionising irradiation. Here, we show that synergy of TRAIL and 5-fluorouracil (5-FU), i.e. cross-sensitization between TRAIL and 5-FU for induction of apoptosis, entirely depends on Bax proficiency in human DU145 and HCT116 carcinoma cells. DU145 prostate carcinoma cells that have lost Bax protein expression due to mutation fail to release cytochrome c and to activate caspase-3 and -9 when exposed to TRAIL and 5-FU. In contrast, TRAIL sensitized for 5-FU-induced apoptosis and vice versa upon reconstitution of Bax expression. Sensitization occurred through a caspase-8 dependent pathway and was associated with Bid cleavage. Isoisolobographic analyses of ED50 doses for 5-FU at increasing TRAIL concentrations showed a clear synergism of TRAIL and 5-FU in Bax-expressing cells. In contrast, the effect was merely additive in DU145 cells lacking Bax. Notably, DU145 and other cells having lost Bax such as the frequently employed HCT116 Bax k.o. cell line still express Bax. This indicated that Bax is not sufficient to mediate cross-sensitization and synergism between 5-FU and TRAIL. Stable overexpression of Bax in DU145 sensitized for etoposide-induced apoptosis but failed to confer synergy between TRAIL and 5-FU. Moreover, we show by the use of EGFP-tagged Bax and Bak that TRAIL and 5-FU synergistically trigger oligomerization and clustering of Bax but not Bak. These data clearly establish distinct roles for Bax and Bak in linking the TRAIL death receptor pathway to the mitochondrial apoptosis signaling cascade and delineate a higher degree of specificity in signaling for cell death by multidomain Bcl-2 homologs.
P462
No Influence of NOD2/CARD 15 Mutations with Transplant-Related Mortality and Acute GVHD in Patients who Received an Intestinal Bacterial Decontamination using Metronidazole and Ciprofloxacin after allogeneic Stem Cell Transplantation

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Mutations of the NOD2/CARD14 gene have been associated with an increased incidence of Crohn’s disease due to a diminished NF-kB response to bacterial cell wall products. Moreover, it was reported that mutations of this gene loci might be associated also with an increased risk for TRM and severe GVHD in patients who underwent allogeneic blood stem cell transplantation. Here we analyzed 201 patients and their respective donors for NOD2/CARD15 mutations and correlated the results with the incidence of acute GVHD. Moreover, we evaluated if the occurrence of NOD2/CARD15 gene mutations are accompanied with an increased risk for transplant-related mortality. Mutated alleles were observed in 17% of the patients and 16% of the patients. The estimates for one-year transplant-related mortality did not differ significantly between patient/donor pairs without mutations (27%), patients with mutated alleles (13%), donors with mutated alleles (22%), or donor/patient with mutated alleles (18%). Also no significant differences in the incidence of acute GVHD (grade 4–4) and gastrointestinal GVHD were seen in the four groups. But patient/donor pairs with mutated alleles seemed to have a slightly higher incidence of severe acute GVHD (grade 3–4) compared to patient/donor pairs without had mutated alleles (n=6 of 11, 55%) versus patient/donor pairs without mutations (35/145, 24%). Multivariate analyses including all potential factors, which might have influence on the outcome, revealed that mutations of NOD2/CARD15 had no influence either on the occurrence of acute GVHD, nor on the occurrence of transplant-related mortality. Since all patients had received an intestinal bacterial decontamination using Metronidazole and Ciprofloxacin after allogeneic stem cell transplantation it might be speculative if the reduction of concentrations of anaerobic bacteria in the intestine might have protected patients from the occurrence of increased TRM or incidence of GVHD. Higher concentrations of anaerobic bacteria are associated with an increased risk for severe GVHD as reported earlier.

P463
The 638 G>A Polymorphism of the Sulfotransferase 1A1 Gene and Breast Cancer Risk

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Purpose: Sulfotransferase 1A1 (SULT1A1) is involved in the bioactivation and detoxification of a variety of xenobiotic compounds. A common arginine (R) to histidine (H) variation at amino acid position 213 influences SULT1A1 activity and has been suggested as a risk factor for a variety of cancers. Methods: To investigate the role of this polymorphism for breast cancer risk, SULT1A1 genotype was determined in 500 women with clinically verified breast cancer and 500 female age-matched healthy control subjects. Genotyping was done by a 5'-nuclease assay (TaqMan®). Primer and probe sets for amplification were designed and manufactured using Applied Biosystems ‘Assay-by-Design’ custom service. P-values were calculated by χ² test using SPSS 10.0. All tests were two-sided, threshold for significance was P < 0.05. Results: Frequencies of heterozygous (controls: 42.5%; patients: 50.2%) or homozygous (controls: 12.6%; patients: 9.4%) carriers of the 213H variant were not significantly different between groups. The odds ratio of the SULT1A1 rs213H polymorphism compared to the wild type (SULT1A1 213RH) was 0.83 (95% confidence interval 0.54 – 1.26). Conclusion: We conclude that the SULT1A1 R213H polymorphism is not a general risk factor for breast cancer, but may be involved in lymph node metastasizing in breast cancer patients. Further prospective studies should be performed to analyze this potential role of the SULT1A1 polymorphism for lymph node metastasis development.

P464
In Multiple Myeloma, Chromosomal Abnormalities Involving the DKK1 Gene Locus (10q11) are Rarely Identified by FISH

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Introduction: In multiple myeloma (MM), a number of secreted factors, such as RANKL and osteoprotegerin, are involved in the pathogenesis of bone destruction. In a recent gene expression profiling study, overexpression of DKK1, an inhibitor of osteoblast differentiation, has been demonstrated to be associated with the presence of lytic bone lesions in patients (pts.) with MM (Tian et al., 2003). The mechanisms leading to DKK1 overexpression in this disease remain unclear. The DKK1 gene resides to chromosome band 10q11. In chromosome banding or comparative genomic hybridization studies, chromosome 10 has not been reported to be a frequent site of genomic changes, such as genomic imbalances or reciprocal translocations. However, both techniques have limitations, especially with respect to their spatial resolution. To our knowledge, chromosome band 10q11 has not been studied yet for chromosomal aberrations using interphase FISH. Aim: To determine whether or not interphase FISH can reveal chromosomal abnormalities, e.g. high-level amplifications or chromosomal translocations, involving the DKK1 locus in clonal plasma cell disorders. Material and methods: Bone marrow specimen from 47 pts. (MM n=40, MGUS n=7) obtained during routine diagnostic procedures have been analyzed in this study so far. Among the MM pts., 18 had advanced focal bone lesions as determined by conventional radiographs, while 22 pts. had no or only one osteolysis. Tri-color FISH using BAC probe RP11-346D6 (181 kbp) containing the DKK1 gene was applied as previously described. Results: No genomic change involving chromosome band 10q11 was identified among the 7 pts. with MGUS. Among the 40 pts. with MM, no high-level amplifications were found, while 5 pts. (12.5%) exhibited extra copies involving chromosome band 10q11 (trisomy n=4, tetrasomy n=1). Additional FISH data indicated tetraploidy in 2 of these 5 pts. In 2 of 40 MM pts. (5%), a monoleptic deletion of chromosome band 10q11 was found. There was no evidence for translocations breakpoints involving the DKK1 locus, i.e. disruption of BAC probe RP11-346D6. Conclusion: In MM, chromosomal alterations detectable by FISH rarely involve the DKK1 gene locus at chromosome band 10q11.

P465
Phenotype Analysis of Mice Deficient in TLE3 Expression

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AML/RUNX transcription factors are involved in many developmental processes in the organism. Three members of AML/RUNX family have been described. One of them, RUNX1/AML1, takes part in hematopoietic development. Haplosufficiency of this transcription factor leads to the autosomal dominant heritable "familial platelet disorder with a predisposition to acute myeloid leukemia" (FPD/AML). It is also involved in chromosomal translocations in acute leukemias (e.g. t(8;21)). The second member of the family, RUNX2, is a key factor of osteogenesis, and the third one, RUNX3, is important for neuronal development. All RUNX proteins are considered to be transcriptional activators, however they can be converted to transcriptional repressors by interaction with corepressor proteins, e.g. members of TLE family. It was shown that TLE family members interact with RUNX transcription factors in vitro and downregulate the expression of their target genes. They are also coexpressed in certain tissues. The restricted expression pattern of one member of TLE family, TLE3, points to a defined, specific interactions of this protein with RUNX transcription factors in vivo. To investigate the physiological relevance of TLE3 with respect to RUNX function we have generated a mouse strain deficient of TLE3 protein. Preliminary studies have shown that TLE-3 knockout embryos are underdeveloped and die between e14.5 and e15.5. Additionally, for the disruption of the Tle3 gene we have used the targeting cassette containing a beta-galactosidase reporter gene. This strategy allowed us to map the expression pattern of Tle3 gene during embryogenesis and compare it with known expression domains of RUNX transcription factors.
Gene Expression Profiling of Granulocytes of CML Patients Using 30k-Arrays

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Abstract

Purpose: Approximately 95 % of patients with chronic myeloid leukemia (CML), a clonal hematopoietic stem cell disorder, show the reciprocal translocation t(9;22)(q34;q11). This so called Philadelphia chromosome leads to a constitutive activation of the abnormal tyrosine kinase resulting from the bcr-abl fusion-gene. Course of this disease is divided in three phases: chronic phase, accelerated phase and finally blast crisis. Although increase of bcr-abl genes on the transition to blast crisis can be well detected by real-time PCR, molecular events leading to the progression of CML are still poorly understood. Therefore gene expression studies using array technology could be a powerful method for finding further prognostic and therapeutic targets.

Methods: Granulocytes of peripheral blood from five untreated patients with primary diagnosis of CML and the molecular marker bcr-abl, as well as from control patients, were separated by CD 33/CD15 positive antibody conjugated magnetically beads. Following RNA isolation using a guanidinium isothiocyanate method and amplification according to Eberwein, cRNA was fragmented, labeled and hybridized to 30 oligo glass arrays. Fluorescence was detected with a GenePix® array reader and the results were analyzed using GenePix pro and Acuity® software.

Results: The expression profile of transcriptional active genes shows a fivefold over or under expression of 78 genes on all arrays. Highly over expressed genes are involved in a variety of immune defense reactions like degrading connective tissue, chemotaxis, phagocytosis, an overexpression of p53 in monocytes and inflammatory response. Regulatory genes for cell growth, proliferation, differentiation and apoptosis are more than tenfold increased in all CML patients. Calcium regulating genes, adipose metabolism relevant genes as well as genes with unknown function or hypothetical genes are also regulated. Candidate genes which might play a role in development and progress of CML will be investigated on 800 samples from 160 patients by qPCR.

Conclusions: By DNA microarray technology, 78 specific differentially expressed genes could be detected in CML patients. Candidate genes of prognostic interest should be found by further screening of clinical samples of patients treated with various therapies by real-time PCR.

Poster Session: Proteomics

Proteomic Pathway Discovery Reveals that C/EBPalpha Disperses PML Nuclear Bodies to Recruit its own Coactivators in a MAPK-Dependent Manner

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The tumor suppressor protein CEBPalpha plays a major role in the terminal differentiation of various tissue types, while inactivation of CEBPalpha through different mechanisms is a critical event during tumorigenesis. Down-regulation of CEBPalpha turned out to be the case in nearly 50% of all primary lung cancer specimen. In lung cancer cell lines, a total loss of CEBPalpha expression was reported in even 80% of the cases. In contrast, restoration of its expression leads to differentiation and ultimately apoptosis.

To investigate the mechanisms mediating CEBPalpha’s antiproliferative and differentiation-inducing activity in lung cancer, we performed a proteome-wide screen for CEBPalpha target proteins using 2-D gel electrophoresis and MALDI-TOF mass spectrometry. Besides 80 other target proteins, we identified PML as a protein being regulated by CEBPalpha. In fluorescence microscopy studies we could show that the induction of CEBPalpha leads to a dramatic decrease in the number of PML nuclear bodies. We found out that these changes occur due to a reduction in the slower migrating sumoylated isoforms of PML. In reporter assay, overexpression of PML was increasing the transactivation capacity of CEBPalpha. CBP could further augment this coactivating effect. Interestingly, Cadmium, which is like CEBPalpha desumoylating the PML protein and dispersing the PML nuclear bodies, was having the same effect. In earlier studies the MAP-kinases ERK 1/2 and p38 were suspected to be involved in the desumoylation of PML. Consistent with these results CEBPalpha is also activating ERK and p38. The p38 inhibitor SB203580 could partially abrogate the CEBPalpha-induced effects on PML nuclear bodies. Taken together, our investigations could identify a mechanism, how CEBPalpha is targeting PML and CBP to sites of active transcription.

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expression was low in the Burkitt lines. Expression of BCL-2 and BAX, was weak or undetectable in the 3 Burkitt cell lines. Protein levels of the cell cycle regulators CDK4, CYCLIN-D1, CDK4 and E2F and lower expression of CYCLIN-D3 when compared to the B-CLL line EHEB. SURVIVIN expression was higher in the Burkitt lines. CDKN2A and JURKAT in relation to EHEB and JVM. Strong expression of AID was seen in the Burkitt cell lines BL60 and NAMALWA whereas the other cell lines showed no or weak signals. In conclusion, characteristic protein expression patterns in the cell lines were identified. EHEB (B-CLL) and GRANTA-519 (MCL) could be differentiated by the cell cycle regulators CDK4, CYCLIN-D1/2, SURVIVIN and E2F1 indicating enhanced proliferation in GRANTA. No remarkable differences in protein expression were seen between EHEB (B-CLL) and JVM-2 (B-PLL) confirming the pathogenic similarity of both diseases. Remarkably, the characteristic findings in the Burkitt cell lines were reduced levels of the proliferation associated proteins CDK4, CYCLIN-D1, CYCLIN-D2, but a high level of CYCLIN-D3 and of AID in line with a germinal center origin of Burkitt lymphoma. In summary, characteristic protein expression patterns were observed in cell lines derived from distinct lymphoma entities. Therefore, the cell lines appear to reflect the biological behavior of the lymphomas they were obtained from and may provide a basis to elucidate the pathogenesis of different lymphomas.

P470 Identification of BCR-ABL Dependent Gene Regulation By Using A Combined Proteomics/Phosphoproteomics Approach

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Introduction: The selective tyrosine kinase inhibitor imatinib (formerly STI571, Gleevec,) has been shown to block phosphorylation of tyrosine residues by occupying the ATP binding site of the Abl tyrosine kinases as well as platelet-derived growth factor receptor (PDGFR) alpha and beta and of the receptor for human stem cell factor (SCF) c-kit. We chose a combined proteomics/phosphoproteomics approach to identify novel downstream targets of imatinib. Material and Methods: Protein expression analysis of K562 leukemic cells was performed using 2-dimensional gel electrophoresis after treatment with imatinib (4 µM) or DMSO for 24 hours and 48 hours. Phosphoproteomics was performed by comparison of large scale phosphoryrosine-immunoprecipitation of imatinib (10 mM/2 hours) versus DMSO treated K562 cells with subsequent one-dimensional polyacrylamide gel electrophoresis. Resulting differentially expressed or differentially phosphorylated proteins were analyzed using MALDI-TOF and ESI-MS/MS. Protein identification was performed using Mascot search tool and NCBI database. Differential expression or phosphorylation was confirmed by western blot or combined immunoprecipitation and western blot analysis of selected candidate proteins. Results: Unique changes in protein expression levels were observed over a 24- and 48-hour time course and most of them could be classified based on their known biological function in cell cycle regulation and proliferation control (e.g. nucleophosmin), focal adhesion and cytoskeletal organization (e.g. vinculin). Two proteins play a role in nuclear import/export (e.g. RanBP1), two proteins are involved in amino acid/purine metabolism and the function of two other proteins is still unknown. Phosphoproteomics revealed 8 differentially phosphorylated proteins after two hour treatment of BCR-ABL positive K562 cells, including the recently identified c-abl and BCR-ABL itself, the latter confirming autophosphorylation of BCR-ABL. The remaining candidates were involved in signal transduction, protein folding or were associated with diverse cellular activities. Discussion: We could detect significant differences in protein expression levels as well as in the phosphorylation pattern of BCR-ABL-dependent cell line K562 upon treatment with imatinib. Ongoing studies are aimed at the analysis of the role of the identified proteins for BCR-ABL induced signal transduction as well as for the development of resistance to imatinib.
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We have developed a method based on capillary electrophoresis (CE) and mass spectrometry (MS); which allows us to detect and identify all polypeptides in the urine and serum of patients and to compare these to healthy controls. Here we report the application of this method in the screening of 34 patients (25 AML, 3 ALL, 2 CML high risk, 4 other diseases) after allogeneic HSCT, healthy individuals. Eleven patients developing GvHD between day 10 and 28 post HSCT and 1 received an autologous transplantation. GvHD prophylaxis was in 20 patients cyclosporin A and in 3 patients T-cell depletion. After transplantation all patients were treated with methotrexat or mycophenolate (MMF).

Here we report the application of this method in the screening of 34 patients after allogeneic HSCT. Nineteen patients were transplanted from unrelated donors (18 MUD, 1 mismatch), 12 received stem cells from family donors, 3 received haplo-HSCT and 1 received an autologous transplantation. GvHD prophylaxis was methotrexat or mycophenolate (MMF) and cyclosporin A in 20 patients and T-cell depletion in 3. After transplantation and serum samples were collected twice a week, starting before conditioning until discharge from the ward. Screening of the patients’ urine with CE-MS yielded between 700 and 2500 polypeptides defined via their mass, charge, and migration time in the CE-MS. These polypeptides were depicted as a three dimensional picture (contour plot, "Diaspat", Fig. 1) and the data for each individual patient were stored in a database, allowing comparison of the different samples of one individual patient as well as comparison within patient groups and controls. Patients with no major complications during the observation time (n=16) gave DiasPats similar to the controls (autologous HSCT, healthy individuals). Eleven patients developing GvHD between day +14 and day +28 showed significant differences with completely new polypeptides appearing and significant changes in concentration of the proteins excreted. Comparison of the DiasPats of all patients with GvHD yielded about 25 polypeptides only present in these patients, while they were never seen in any of the other patient groups. Polypeptides “significant” for GvHD could be detected at least 6 days prior to clinical parameters like skin rash or increase of liver enzymes. Comparison of the “GvHD pattern” to patients with sepsis (n=5), acute renal failure (n=2) or other complications will be discussed. Acute renal failure patients excrete significantly different polypeptides than patients with GvHD. Proteomics may serve as a useful tool for the identification and early diagnosis of complications associated with HSCT.

Poster Session: Cytokines
P474
Functional Activity of in Vivo Primed Granulocytes: A Comparative Study
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Recombinant granulocyte colony-stimulating factor (G-CSF) has been widely used in the treatment of chemotherapy-induced neutropenia as well as in mobilisation of peripheral blood stem cells in context with autologous bone marrow transplantation. Beside recombinant G-CSF expressed in E.coli (Filgrastim) and G-CSF expressed in CHO-cells (Lenograstim), pegylated filgrastim (Pegfilgrastim) has been introduced for single-administration into clinical use. Here we study the effects of different G-CSF on functional activity of granulocytes including chemotaxis, oxidative burst and antigen expression as well as potential correlation to G-CSF serum level. Granulocytes were obtained from patients with hematological malignancies before and after the administration of one of the three G-CSFs and isolated using a polymorph prep density gradient. Chemotactic properties were assessed using a Boyden chamber assay in combination with an under agarose assay, both using IMLP as chemotactic stimulus. Release of superoxide anions served as measure of the oxidative burst after stimulation with PMA using a chemiluminescence assay. The viability and surface antigen expression were assessed by FACS. In addition serum samples have been stored at -80°C for ELISA tests.

FACS analysis showed a decrease in CD10, CD11b and CD62L of the selectin family contrary to an increased expression of the VLA-5 alpha chain CD49, the LPS-receptor CD14 and the IgG receptor FcRI (CD64). A stronger effect of lenograstim on CD11b and CD14 could be assessed contrary to filgrastim showing a stronger effect on CD62 and CD64. Further data on pegfilgrastim is to be evaluated.

We observed a decrease of chemotactic activity and non-directed random migration in patients receiving filgrastim, as opposed to the results in patients receiving lenograstim. No obvious differences were found in production of superoxide anions. Present data shows differences between the commonly used G-CSFs Filgrastim and Lenograstim in granulocyte antigen expression and chemotaxis, further data on patients receiving Pegfilgrastim has to be evaluated.
analysis for nuclear disintegration as typical signs of apoptosis. The detection of soluble TNF and IL-1 was performed by ELISA according to the manufactur- ers’ instructions. Results: Whereas IRSF and infliximab suppress both, the release of the LPS-induced endothelial cell apoptotic factor and proinflamma-
atory cytokines, etanercept only protected against the LPS-triggered apoptosis activity, but left the LPS-induced cytokine release unchanged. Conclusions: Though it remains to be elucidated whether reverse signaling also occurs in vivo, the data provided could explain a number of clinical observations, including the increased occurrence of fungal infections after infliximab therapy as compared to etanercept treatment of GVHD. Based on our results we would hypothesize that infliximab completely anergizes monocytes and even blocks desirable anti-inflammatory monocyte responses against secondary infections. Our study strongly suggests to individually re-evaluate cytokine antagonist-based therapeutic approaches in various inflammatory disorders.

P476 Overexpression of BMP-2 (Bone Morphogenetic Protein 2) in Breast Carcinoma Cells Supports Tumor Formation and Ossification

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Purpose: Bone morphogenetic proteins (BMPs) are expressed in various normal tissues as well as in tumor cells and are involved in the regulation of intracellular processes that support cell survival such as cell cycle control. Therefore, we analysed how BMP-2 modulates the behaviour of tumor cells. Materials and Methods: An BMP-2 overexpressing cell line (MCF7/BMP2) was created by transfecting MCF-7 with a pcDNA3.1- vector containing full-length BMP-2. Cell lines were cultivated with DMEM + 10% fetal calf serum. BMP-2 mRNA levels were determined by RT-PCR. Protein levels were estimated by western blot and immunohistochemistry. Tumorigenicity assays were performed with MCF7/BMP2 and MCF7/3.1 (for control) using athymic NuNu mice. Results: Overexpression of BMP-2 resulting in a continuous high level of BMP-2 in the breast cancer cell line MCF-7 (MCF7/BMP2) enhanced the in vitro migratory properties of these cells in comparison to controls (MCF7/BMP2: 15.75 ± 4.09 SD; MCF7/3.1: 8.42 ± 4.03; p<0.004, MCF7: 7.58 ± 3.09; p<0.001; n=12). In a xenograft model without estrogen supplementation MCF7/BMP2 cells formed tumors (9/10), whereas no tumors were found with MCF7/3.1 cells (0/10). MCF7/BMP2 tumors were characterised by the human-specific epithelial membrane antigen (EMA), an increased level of phospho-SMAD1, indicating an elevated activity of the BMP/Smad pathway and a slightly enhanced expres-
sion of K67. The MCF7/BMP2 cells formed nests surrounded by connective tissue, which contained chondroid and osseous structures. Conclusion: We could demonstrate that elevated levels of BMP-2 enhance the tumorigenic properties of breast carcinoma cells and drive the cells towards a more aggressive phenotype.

This work was supported in part by the Dr. Rainald-Stromeyer-Foundation and the Deutsche Krebshilfe e.V.

P477 Rapid Succession of Stem Cell Mobilisation Cycles in Patients with Chronic Heart Failure – Effects on Haematopoietic and Non-Haematopoietic Organs

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Purpose: Circulating (bone marrow deriv ed) stem cells may contribute to the regeneration of non-haematopoietic organs. We subjected patients with chronic heart failure to granulocyte-colony stimulating factor (G-CSF) induced stem cell mobilisation cycles. Herein we report the effects on haematopoietic and non-haematopoietic organs. The impact on cardiac func-
tion has been described elsewhere. Methods: 14 male patients with chronic heart failure of New York Heart Association (NYHA) functional class III or IV due to dilated or ischemic cardiomyopathy were subjected to four G-CSF treatment periods of 10 days’ duration separated by three 10-day treatment-
free intervals followed by monthly control visits until day 180 after the start of therapy. The dose of G-CSF (starting dose: 480 µg sc bid) was adjusted on a daily basis to reach a leukocyte count of 45.000 per µl by day 4 and main-
tained a level of 45.000 – 50.000 per µl until day 10. Analyses included physical examination, ultrasonography of the spleen and laboratory investigations. Results: In the haematopoietic system, G-CSF induced a rapid increase in cells of all leukocyte lineages with return to levels equal to (granulocytes) or lower than those before treatment (monocytes, lymphocytes) during the treatment-
ment-free intervals. Red cells remained unchanged, and platelets decreased followed by rebound thrombocytosis. Increase in CD34+ cells was highly variable with low values in patients receiving amiodarone. For each patient, the changes induced were identical through all cycles, but the G-CSF dose in the first cycle was significantly higher than in subsequent cycles. Between patients, an inverse correlation was observed between the leukocyte level reached and the dose of G-CSF administered. Serum levels of bilirubin decreased, while creatinine and urea nitrogen increased. Conclusions: Sequential stem cell mobilisation is feasible and well-tolerated, inducing identical alterations in all treatment cycles. G-CSF responsiveness varies among patients and is increased by pre-treatment with G-CSF. Notably, repetitive CD34+ cell mobilisation in 10-day intervals is feasible in the absence of leukapheresis, indicating that the size of the stem cell pool is maintained despite excessive G-CSF stimulation.

Poster Session: Gene Therapy

P478 The Formation of Hybrid TCRs can be Averted by the Single Chain TCR Concept in Adoptive Immunotherapy

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Purpose: The T-cell receptor (TCR) is able to recognize human tumour associ-
ated antigens (TAA) and subsequently triggers a complex signaling cascade that results in the initiation of cytotoxic effector functions in CD8+ T-cells. In human gene therapy, high-affinity TCRs have been retrovirally introduced into T cells to eradicatetumour cells in vivo. In order to circumvent self-toler-
ance to human TAA, high-affinity T cells were generated in various HLA-
A2.1 transgenic mouse models to induce HLA-A2.1-restricted immune responses directed against human TAA, such as peptides originating from p53 and MDM2. A2.1-transgenic mice served as a tool to create CD8-inde-
dependent p53-specific high-affinity TCRs as compared to HucDF8x2A2-
transgenic mice that resulted in CD8-dependent MDM2-specific TCRs. These TCRs were ideally suited for studying the contribution of the core-
ceptor CD8 on cytolytic efficacy. Methods: A major concern was to deter-
mine as to whether or not murine TCRs are able to form hybrid TCRs with the endogenous ones potentially raising the issue of autoimmunity. Amino acid substitutions that affect interchain affinity will have an impact on surface expression, the formation of hybrid TCRs and functionality in terms of cytokine secretion and cytolsis in chromium release assays. A multitude of TCR constructs, designed as either murine and partially humanized double chain TCRs (dTCR) or as single chain TCRs (sTCR), have been assayed on their cellular outcomes and consequences for the endogenous TCRs. Single chain TCR constructs have been designed by covalently hooking up the vari-
able Vα and Vβ domains as major determinants for antigen recognition via a linker to the invariant Cβ domain for membrane anchoring. This strategy enables the irreversible linkage of the variable domains in order to abrogate potential pairing with endogenous TCR chains. Results: The expression of single murine TCR chains documents their capability to form hybrid TCRs a tendency that can be further increased by the humanization of the constant domains. Point mutations that impair pairing as deduced from protein struc-
ture reduce their surface expression and inversly accumulate in the cyto-
domain. Point mutations that impair pairing as deduced from protein struc-
ture enable the irreversible linkage of the variable domains in order to abrogate pairing as deduced from protein structure.
domain, a truncated TCRζ was generated comprising the Cζ domain preceded by the TCRζ signal peptide. This construct was coexpressed with either the p53- or the MDM2-specific scTCR to provide them with the missing domain that is believed to harbor a binding site for CD8, the α-CPM consensus motif, FETDxLN/L. We found that both scTCRs of different CD8-dependency require the Cζ domain for function at all while only the CD8-independent p53-specific scTCR gave rise to substantial cytotoxicity comparable to that of wildtype TCR, even for MDM2-silenced CT2x construct. Furthermore exclusively the CD8 independent scTCR/Cζ construct provided sufficient structural avidity to stain both CD8⁺ as well as CD4⁺-T cells in tetramer analysis. Formation of the mandatory disulfide bridge linkage has been proven for them by western blotting. Conclusions: We do have clear evidences that the scTCR concept is comparable to wildtype TCRs in terms of functional avidity despite some impairment in structural avidity making them an amenable tool in adoptive cancer therapy.

P479

Function of HSV-TK Suicide Gene Modified Canine T Lymphocytes

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Purpose: Adaptive immunotherapy with donor lymphocyte infusion after stem cell transplantation is the treatment of choice for a number of hematological disorders. The transfer of donor T cells is limited by the occurrence of graft versus host disease (GvHD). A promising way to control GvHD is the transfer of donor T cells transduced with the HSV-Tk suicide gene. Methods: Transduction was performed using virus supernatant generated with the PG13 packaging cell line. After 72h stimulation with 5 µg/ml PHA-M and 100 U/ml IL-2 transduction of T cells was performed by spin-inoculation on fibronectin coated-plates. On day 8 the transduced T cells were purified with immunomagnetic beads against the LNGFR marker gene. More than 95% of the cells were positive for LNGFR expression. Cells were susceptible to the in vitro treatment with ganciclovir (GCV) in therapeutic concentrations. Allreactivity of gene modified cells was comparable to non-transduced T cells. Results: In vivo results were obtained in a DLA-identical setting. The animals were transplanted with T-cell depleted bone marrow from a DLA-identical littermate. 60-80 days after BMT gene modified T cells were infused into the host. The functionality of these cells was assayed by the conversion of haematological chimerism. Gene modified cells were also capable of transferring immunity to a foreign antigen from the donor to the host. The persistence of gene modified T cells in the blood was shown by FACS and PCR. In the in vivo ablation of gene modified cells by 4 doses of GCV (10 mg/kg/d) was shown by FACS and PCR. We generated gene modified CTLs against DLA and tested the cells in vitro. The capacity of gene modified CTLs to suppress the growth of PHA-blasts was analyzed in a Delta-Assay. The results showed a highly specific suppression of the growth of PHA-blasts incubated with gene modified CTLs. At an E/T ratio of 1.25 to 1 we measured a growth suppression of 96.7% (± 2.88%) compared to the control. CTLs not transduced with the HSV-TK suicide gene but otherwise treated in the same way as transduced cells resulted in a growth suppression of 97.3% (± 0.48%). Conclusions: We have shown the transduction and adoptive transfer of canine T cells and antigen-specific T cells and showed their function in vitro and in vivo. On the basis of these results we will develop an experimental canine GvHD model with gene modified T cells.

P480

Unmodified Oligodeoxynucleotides Require Single-Strandedness to Induce Targeted Repair of a Chromosomal EGFP Gene

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Purpose: A number of genetic defects in humans are due to point mutations in a single, often tightly regulated gene. Genetic treatment of such defects is preferably done by correcting only the altered base pair at the endogenous locus rather than by a gene replacement strategy involving viral vectors. Promisingly high repair rates have been achieved in some systems with the non-viral approach of transferring chimeric RNA/DNA oligonucleotides (chimeraplasts). However, since this technique does not yet perform robustly, several parameters thought to be important in oligonucleotide-mediated gene repair were examined. Methods: A series of transgenic HEK-293 cell clones has been established harbouring high or low copy numbers of a point-mutated ‘Enhanced Green Fluorescent Protein’ (EGFP) gene as the target. At the level of single living cells, repair efficiencies were measured by FACS regarding topology (single-stranded, double-stranded), exonuclease protection (four phosphorothioate linkages at both ends), polarity (sense, antisense), and length (13mer, 19mer, 35mer, 69mer) of the oligonucleotide. Results: When targeting chromosomal loci, up to 0.2% corrected cells were obtained with single-stranded unmodified oligodeoxynucleotides, whereas a chimeraplast, its DNA analogue, and double-stranded DNA fragments were practically non-functional. Correction efficiencies correlated with target gene copy numbers. Modifying exonuclease resistance, polarity or length of single-stranded oligodeoxynucleotides did not enhance repair efficacy above the sub-percentage range. Conclusions: Successful chromosomal reporter gene repair in HEK-293 cells required an oligodeoxynucleotide to be single-stranded. In concert with the gene copy number correlation, functional interaction between the repair molecule and the target site seems to be one bottleneck in targeted gene repair.

P481

Tumor Cells Escape Suicide Gene Therapy by Genetic and Epigenetic Instability

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Purpose: Transfer and expression of suicide genes is one cornerstone of cancer gene therapy and is also considered as a proactive tool to enhance the safety of somatic transgenesis. Using viral or physicochemical gene transfer procedures, numerous preclinical and some clinical trials have been conducted, identifying insufficient gene delivery as a major limitation. We addressed whether retrovirus-mediated suicide gene therapy would result in a predictable anti-tumor immunity, given that problems related to gene transfer are solved or that the suicide gene is used in a proactive approach. Methods: We induced an experimental tumor by transplanting retrovirally engineered murine EL-4 lymphoma cells in congenic C57Bl/6 mice. The vectors encoded the thymidine kinase (TK) gene of herpes simplex virus and the neomycin resistance gene. Results: Systemic administration of the produrg ganciclovir (GCV) resulted in reversal of transduced clonal and polyclonal tumors in vivo. However, both uncloned and cloned EL-4 tumors gave rise to GCV-resistant subclones, eventually causing tumor relapse. GCV-resistant tumors showed postinsertional alterations of transgene structure, or loss of the entire transgene. A complete loss of a fusion chromosome that contained the retroviral suicide gene was confirmed by spectral karyotyping (SKY analysis). Transgene silencing occurred in another EL-4 tumor clone. Conclusions: We conclude that genetic as well as epigenetic instability related to biological features of the tumor, the insertion site and the gene transfer system limit the efficiency of retroviral suicide gene therapy.
P482
Additive Effects in the Induction of Apoptosis in Leukemic Cells by Wt1 and Bcr-Abl Specific siRNAs
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We studied the effect of siRNA targeting wt1 in leukemic cells and normal CD34+ positive cells with regard to proliferation, induction of apoptosis, and cell differentiation. Furthermore, we evaluated if the additional use of bcr-abl siRNA can augment the antileukemic effects of wt1 siRNA in CML cells. A reduction of wt1 gene expression measured by real-time RT-PCR was observed in all studied cell lines: K-562, Kasumi-1, MV 4-11 and NB-4, as well as in cells of AML- and CML patients. We found a two-fold increase of induced apoptosis in MV4-11 cells, NB-4 cells and Kasumi-1 cells (p<0.01) and a moderate increase in K-562 after transfection with wt1 siRNA versus controls (p<0.02). Proliferation was strongly inhibited in all studied leukemic cell lines and leukemic cells of patients by wt1 siRNA. In normal CD34+ positive cells, the proliferation was only slightly inhibited by about 20% and no induction of apoptosis was found. Transfection with both siRNAs together inhibited the proliferation rate additionally compared to transfection with bcr-abl siRNA or wt1 siRNA alone (p<0.01) in the K-562 cell line and CML cells. The rate of induced apoptosis was more announced than transfection with each siRNA alone (p<0.01). We performed a microarray analysis and found that mostly genes involved with DNA synthesis or metabolism, transcriptional regulation, cell signaling and small molecule metabolism, were regulated by the silencing of wt1 gene in K562 cells. Wt1 specific siRNAs had no effect on expression of surface differentiation markers. Wt1 seems to be an interesting target in leukemic cells for new therapeutic strategies.

P483
Efficient Stimulation of Virus-Specific CD4+ and CD8+ T-Cells by T-Cells Modified to Express a Viral Candidate Antigen
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Adaptive transfer of T-cells expressing the herpes simplex thymidine kinase induces an strong immune response against the transgene in HIV patients and in allogeneic SCT recipients resulting in eradication of transferred cells. The elimination is mediated by a vigorous T-cell response against the viral thymidine kinase protein. Therefore we reasoned that human T-cells if presenting a viral antigen might represent a novel class of antigen presenting cells. Human T-cells were retrovirally modified with the viral model antigen CMVpp65 creating T-APC CMVpp65 that in turn were used to stimulated a CMV-specific T-cell response. In the first set of experiments T-APC CMVpp65 could activated CMV pp65 T-cell clones as efficiently as CD40L-activated B-cells modified to express CMV pp65 (B-APC CMVpp65). In addition if autologous fresh PBMC were used as effectors population both T-APC CMVpp65 and B-APC CMVpp65 could stimulated a combined CD4+ and CD8+ CMV-specific T-cell responses from CMV-seropositive donors. In vitro stimulation for 8 days with both T-APC CMVpp65 and B-APC CMVpp65 resulted on average in a 80-fold expansion of virus-specific T-cells, which in turn could kill efficiently both CMV pp65 modified LCL’s as well as CMV-infected fibroblasts. To address the question why T-APC CMVpp65 can stimulated such a vigorous T-cell response, the costimulatory ligands CD70, CD80, CD86, 4BB-L and CD40L on T-APC CMVpp65 were evaluated and compared to CD40L-activated B-cells. All costimulatory ligands were expressed by T-APC CMVpp65 at a lower level than B-APC CMVpp65. To determine, if lower expression of costimulatory ligands in T-APC CMVpp65 resulted in a different phenotype of generated CMV-specific T-cell lines, the expression of corresponding costimulatory receptors and homing molecules were compared. Both generated T-cell lines displayed a similar mixed population of peripheral memory and effectors antigen-specific T-cells with same percentage of central memory T-cells (< 25%) based on expression of the chemokine receptor CCR-7.

In summary, T-APC modified to express viral antigen can induce a strong combined CD4+ and CD8+ viral T-cell response. T-cell lines do not differ in expansion and phenotype from virus-specific T-cells generated by professional antigen presenting such as CD40L-B-cells. Adoptive transfer of T-APC modified to express viral antigens may therefore present a new potential vaccination strategy for inducing viral immunity in the immunocompromised host.

P484
Oncolytic Adenoviruses for Acute Myeloid Leukemia
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Tumor-selective replicating viruses are a promising new approach for the treatment of human cancer, as they may offer appealing advantages over conventional cancer therapy. While chemotherapeutic drugs distribute more or less evenly in the human body and follow log kinetics of cell killing, local replication of administered oncolytic viruses amplifies the input dose and creates a high concentration of a therapeutic agent at the target site. This may provide increased potency, while limiting side effects. Although intravenous virus delivery for metastatic or systemic disease has been found to be safe, several major hurdles remain unsolved.

Among the adenovirus serotypes, group C viruses (particularly serotypes 2 and 5) have been extensively studied. Their attachment to the cell is mediated by the coxsackie-adenovirus-receptor (CAR), while virus endocytosis is dependent on α, integrins. However, many advanced tumors loose CAR expression, preventing efficient adenovirus attachment and infection. On the contrary, group B adenoviruses have recently been shown to attach and infect cells in a CD46-dependent manner, independently of CAR. We chose to examine the natural tropism of group B adenoviruses for CAR-negative malignancies. Here, we demonstrated that myeloid leukemia cell lines as well as primary AML blasts expressed little or no CAR on their cell surface. However, we observed strong expression of CD46 (and αβ, integrins) on the surface of myeloid cell lines and primary AML blasts. Furthermore, myeloid leukemia cells were readily infected by adenovirus serotype 35 co-expressing GFP (a group B adenovirus) at multiplicities of infection of 10 and 50, while serotype 5 (group C) was not. The susceptibility of myeloid leukemia cells to group B adenoviruses were confirmed by immunofluorescence for a panel of wildtype group B serotypes. We studied CD46-expression in myeloid differentiation, and observed marked changes in overall expression, as well as splice variants, in the majority of cell systems examined. Importantly, myeloid leukemia cells supported efficient viral DNA synthesis of group B adenoviruses and efficient viral replication was observed in the majority of cell lines examined.

We conclude that the use of group B adenoviruses may be useful for designing oncolytic adenoviruses for CAR-negative tumors, such as myeloid leukemia. Furthermore, primary AML blasts may be a more suitable host for testing viral infection and replication in primary tumor cells, circumventing the limitations of xenograft animal models for human viruses. Current efforts concentrate on achieving tumor-selective replication and several strategies will be discussed.

P485
Enhanced Tumor Cell-Specific Expression of Nbk/Bik by Combining the CEA Promoter and the Tet off System in An Adenoviral Vector
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Adenovirus vectors are considered as good gene delivery vectors because they achieve transient high-level transgene expression and high gene transfer efficiency. Recombinant adenovirus vectors carrying proapoptotic genes are a promising approach in the therapy of human malignant tumors. To investigate the antitumor activity of the proapoptotic BH3-only protein Nbk/Bik, we constructed a single recombinant adenoviral vector for the regulated Nbk/Bik expression based on the Tet off system. The E1 and E3 region of the adenovirus was replaced by expression cassettes for Nbk/Bik and for the reverse tetracycline regulated transactivator (tTA), respectively. Whereas Nbk/Bik was under control of the tetracycline responsive element, a constitutive CMV...
promoter drove the tetracycline-regulated transactivator. Thus, addition or withdrawal of tetracycline can regulate Ad-Nbk expression. Ad-Nbk expression induced cell death in a variety of human cancer cell lines examined including breast cancer, colon cancer, liver cancer, neuroblastoma and osteosarcoma cells, indicating a possible role of Nbk/Bik as a therapeutic gene in cancer gene therapy. Nevertheless, in this CMV driven expression system, expression of Nbk/Bik is not limited to cancer cells. To restrict expression of suicide genes to cancer cells, tissue and tumor-specific promoters have been widely used. However the expression levels of these promoters are generally low.

Carcinoembryonic antigen (CEA) is expressed by many tumors of the colorectal origin, and it is a clinically useful parameter for colorectal carcinoma recurrence. To achieve tumor specific and regulated expression of Nbk/Bik and to enhance CEA driven expression of Nbk/Bik we replaced the CMV promoter in the recombinant adenovirus by the CEA promoter. In this vector system the CEA promoter is driving the expression of the tTA which then can activate transcription of Nbk/Bik from the tetracycline responsive element. In contrast to the CMV vector system, transduction of cells with the CEA adenovirus vector induces Nbk/Bik expression and cell death only in CEA positive cells. Thus, this vector is a model for targeting suicide gene expression to tumor cells and can be adapted to other human tumors in conjugation with different cell type and tumor-specific promoters.

P486
Real-Time Quantitative PCR-Based Method for Rapid Titration of Adeno- Associated Virus Serotypes 1 – 6

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Adeno-associated virus (AAV) vectors are a promising model for future approaches in gene therapies. Recombinant AAV-2 (rAAV-2) vectors are currently used in clinical gene therapy studies for hemophilia, cystic fibrosis and preclinical studies. Still, some cell types and tissues do not seem to be susceptible to rAAV-2 vectors. Here, recombinant vectors based on other AAV serotypes could help to overcome this limitation.

Using a two plasmid production system established by Grimm and colleagues (Mol. Ther., 7, 2003), recombinant vector stocks of the AAV serotypes 1-6 can now readily be produced. Since the differences in susceptibility of the reference cell lines to the serotypes used for titration do not allow comparison between titers, a cell-free assay is imperative.

Previously, we established a highly standardized cell-free and high throughput titration assay with low variability (C.V.< 0.10), based on the quantitative PCR (Veldwijk et al, Mol .Ther., 6, 2002). Now, the assay has been modified to allow titration of rAAV stocks based on serotypes 1-6. For this purpose, the primers and probe were placed between the poly-A and 3’ ITR present in all our rAAV vectors. The rAAV-2 plasmid pTR-UF5 was used as standard, thereby allowing absolute quantification.

The highly standardized titration by Q-PCR described here is our application of choice for rAAV serotype titration, as it allows the determination of the amount of vector particles for any serotype, where differences in susceptibility of the reference cell lines used in the functional titration assay formerly set the limit.

P487
Small Interfering RNAs Directed against Growth Factor Independence 1B Gene (GFI-1B) Inhibit Proliferation and Induce Apoptosis in Leukemic Cell Lines

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GFI-1B is a zinc finger protein which is expressed exclusively in hematopoietic cells. GFI-1B regulates transcription during erythropoiesis and is also involved in regulating the process of hematopoietic cell differentiation and megakaryopoiesis. We studied the effect of transfection with small interfering RNA (siRNA) targeting GFI-1B in leukemic cells and normal CD34+ positive progenitor cells in regard of proliferation, inducing apoptosis, and cell differentiation. Further, we evaluated if the post-transcriptional gene silencing of GFI-1B mRNA can be augmented by use of two further siRNAs targeting the bcr-abl hybridogene or targeting the wt-1 gene expression in the cell line K-562. A reduction of GFI-1B gene expression measured by real-time RT-PCR to the 50-70% (mean) was observed in the K562 and Hel cell lines compared to controls (controls were set up to 100%). We found a two-fold increase of induced apoptosis in MV4-11 cells, NB4 cells and a four-fold increase of apoptosis in Hel, Kasumi-1, K-562 cell lines 24 hours after transfection with GFI-1B siRNA versus controls. Proliferation was strongly inhibited (of about 80%) in cell lines: Hel, Kasumi-1, MV4-11 by GFI-1B siRNA and moderate decreased (of about 25%) in NB4 and K-562 leukemic cells. In normal CD34-positive cells, the proliferation was inhibited too (by about 70%). In contrast to leukemia cells, no induced apoptosis was found in CD34+ cells after GFI-1B siRNA transfection. Cotransfection with GFI-1B siRNA, wt-1 siRNA and bcr-abl siRNA did not inhibit the proliferation rate more effectively than transfection with GFI-1B siRNA alone. The rate of induced apoptosis was constant to the transfection with each siRNA. No synergistic effects of GFI-1B siRNA with bcr-abl siRNA or wt1 siRNA was measured. The transfection of GFI-1B siRNA in CD34+ cells had no influence on differentiation markers CD13, CD14, CD33, CD34, CD45, CD117 (c-kit). Necropsy A. Glycophorin A expression increased after transfection with GFI-1B siRNA in the Hel cell line, whereas CD64 positive monocytes increased in the K-562 cell line after the transfection with GFI-1B siRNA. These findings suggest that GFI-1B seems to be a promising target for new therapeutic strategies with siRNAs in the treatment of erythroleukemic cells.

P488
Efficient Transfer of siRNAs and Plasmids in CD34+ Hematopoietic Stem and Progenitor Cells using Nucleofection

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Gene silencing using small interfering RNAs (siRNAs) has become a powerful method for studying gene function. The use of siRNAs in human primary cells continues to accelerate applications such as target validation and therapeutic approaches that could lead to new gene-specific siRNA-based therapeutics. However, previously leukemic cells and CD34+ hematopoietic stem and progenitor cells have been demonstrated to be resistant to most of non-viral gene transfer methods, which has limited their use for siRNA experiments. The aim of this study was to evaluate a new method for transfection of primary CD34+ cells and leukaemia cell lines and to improve the efficiency of siRNA delivery. We used a novel electroperoration based technique called nucleofection. This novel technique uses a combination of special electrical parameters and specific solutions to deliver siRNA directly to the cell nucleus under mild conditions.

1-2x10^6 CD34+ hematopoietic stem and progenitor cells immunomagnetically enriched from peripheral blood mononuclear cells and the human chronic myeloid leukemia cell line K562 were nucleofected using the human CD34+ cell Nucleofection™ Kit and the cell line Nucleofector™ KitV. The cells were nucleofected with 10 µg non-silencing rhodamine-labelled control siRNA or with 2.5 µg plasmid pmaxGFP encoding for the green fluorescent protein (GFP). At different time points post nucleofection the cells were analyzed by flow cytometry and fluorescence microscopy. Dead cells and CD34+ cells were excluded by propidium iodide (PI) staining and gating. We obtained a transfection efficiency in CD34+ cells of 84,4% for rhodamine-labelled siRNAs and of 32% for pmaxGFP as assessed 4h following nucleofection. PI staining 48h post nucleofection showed that 19% of cells were dead. In K562 transfection efficiency was 58% for rhodamine-labelled siRNAs and 86% for pmaxGFP as assessed 3h and 12h following nucleofection. In K562 a greater toxicity was found in comparison with CD34+ cells as 26.9% ± 11.3% of cells were dead 48h post nucleofection.

In conclusion, nucleofection is a useful method for delivering of siRNAs and plasmids into primary CD34+ hematopoietic stem and progenitor cells. The study provides the basis for knock-down experiments for target validation using siRNAs.
In order to investigate a retroviral transduction protocol for the genetic modification of NOD/SCID repopulating human umbilical cord blood (UCB) cells, we initially compared four different cytokine cocktails for their ability to enable efficient transduction. CD34+ enriched UCB cells (n=4) were prestimulated with different cytokine cocktails followed by a quadruple transduction procedure within 48 hours in fibronection coated dishes, all in presence of the same cytokine cocktail used for prestimulation. A gibbon ape leukemia virus (GALV) pseudotyped murine stem cell virus (MSCV) based retroviral vector encoding the enhanced green fluorescence protein (EGFP) marker was used for transduction. FACS analysis of CD45+/EGFP+ cells revealed transduction rates of 72.7±2.9% for cytokine combination I (SCF, IL3, IL6), 49.6±21.8% for combination II (SCF, TPO, Flt-3), 60.7±16.6% for combination III (SCF, TPO, G-CSF), and 64.9±12.8% for combination IV (SCF, TPO, Flt-3). Repopulation rates of CD34+ ranged in between 29.3±3.8% and 38.0%, revealing no statistical significance between the combinations tested. In further experiments duration of incubation period as well as number of transduction rounds were evaluated for their influence on engraftment of EGFP+ UCB cells in NOD/SCID mice. Best results were obtained with a 24 hour prestimulation period and a triple transduction procedure within 72 hours, followed by 24 hours of maintenance. Therefore, in subsequent experiments (n=8) cytokine combination IV as well as the modified transduction protocol was applied in combination with the high titer GALV pseudotyped EGFP containing MES/SFFV hybrid vector SBFeta1-I-EGFP. These experiments resulted in a mean transduction rate for CD45+ of 40.9±17.8% and 40.4±3.7% for CD34+ cells. Transplantation of 9.3-8.9x10^6 transduced cells into NOD/SCID mice resulted in a percentage of 17.2±4.6% human CD45+ cells in engrafted animals when analyzed by FACS four weeks after transplantation. 33.5±10.5% of engrafted human CD45+ cells were EGFP positive. Subpopulation analysis of the engrafted EGFP+ cells revealed 22.8±8.0% CD19+/CD45+ cells and 22.6±4.4% CD34+/CD45+ cells, the predominant subtype being CD33+/CD45+ with 45.7±12.3%. In conclusion, our gene transfer protocol results in sufficient transduction of both, lymphoid and myeloid differentiated cells.

Poster Session: Coagulation

P489
Thrombophilia and Spontaneous Abortion

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Introduction: Antithrombin (AT) and prothrombin (G20210A) are known to be risk factors for abortion. Moreover, other thrombophilic risk factors like Factor V Leiden (FVL)-mutation seem to play an important role in this context. Incidence of APA syndrome (13%) was comparable to data from earlier studies.

Results: Fifty out of 136 women who had experienced (recurrent) abortion(s) were examined for thrombophilia and spontaneous abortion. Thrombophilic risk factors were determined: In 49/136 patients MTHFR-Polymorphism (10 homozygous, 39 heterozygous) were detected, 16/49 MTHFR-Polymorphism patients additionally presented with FVL-Mutation. In 35/136 patients APC-resistance (FVL-mutation) were detected (1 homozygous, 34 heterozygous), 16/35 FVL-patients additionally presented with MTHFR-Mutation. 18/136 patients had APA-syndrome, 2/136 patients presented with Prothrombin Polymorphisms (2 heterozygous), 2/136 patients had Protein-S-deficiency, 2/136 patients presented with Antithrombin (AT3)-deficiency, 2/136 patients presented with Thrombocytopenia, 1/136 patients presented with Factor XII deficiency, 1/136 patients presented with Plasmaiminogen defecy. Conclusion: In women who had experienced (recurrent) abortion(s), thrombophilic risk factors were detected very frequently – even more frequently than usually detected in venous thromboembolism. Our data show, that especially Factor V Leiden mutation and MTHFR polymorphism seem to play an important role in this context. Incidence of APA syndrome (13%) was comparable to data from earlier studies.

P490
Thrombophilia and Spontaneous Abortion

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Introduction: Antiphospholipid (APA)-syndrome is well known to be a risk factor for abortion. Moreover, other thrombophilic risk factors like Factor V Leiden mutation are discussed to enhance the risk of spontaneous abortion. Moreover, other thrombophilic risk factors like Factor V Leiden mutation are discussed to enhance the risk of spontaneous abortion.

Patients and methods: 136 women who had experienced abortion(s) were examined for thrombophilic risk factors. Median age at time of first abortion was 29.9 years (range: 18-42 years). In all patients, in addition to routine laboratory tests, the following parameters were determined: PT, aPTT, fibrinogen, antithrombin (AT), αPC ratio, protein C, protein S, plasminogen, factor XII, factor VIIIc, lipoprotein (a), antiphospholipid-antibodies, cardiolipin-antibodies (ELISA IgM and IgG) and β2-glycoprotein-antibodies. Moreover, patients were tested for Factor V Leiden (FVL)-mutation, MTHFR (677T) and prothrombin-polymerisms (G20210A). Results: 1136 women experienced 314 miscarriages in total. 47/136 patients had nonrecurrent, 89/136 had recurrent abortions. 75% of abortions occurred during the first trimester of pregnancy. 2. In 96/136 (70.6 %) patients, thrombophilic risk factors were determined: In 49/136 patients MTHFR-Polymorphism (10 homozygous, 39 heterozygous) were detected, 16/49 MTHFR-Polymorphism patients additionally presented with FVL-Mutation. In 35/136 patients APC-resistance (FVL-mutation) were detected (1 homozygous, 34 heterozygous), 16/35 FVL-patients additionally presented with MTHFR-Mutation. 18/136 patients had APA-syndrome, 2/136 patients presented with Prothrombin Polymorphisms (2 heterozygous), 2/136 patients had Protein-S-deficiency, 2/136 patients presented with Antithrombin (AT3)-deficiency, 2/136 patients presented with Thrombocytopenia, 1/136 patients presented with Factor XII deficiency, 1/136 patients presented with Plasmaiminogen defecy. Conclusion: In women who had experienced (recurrent) abortion(s), thrombophilic risk factors were detected very frequently – even more frequently than usually detected in venous thromboembolism. Our data show, that especially Factor V Leiden mutation and MTHFR polymorphism seem to play an important role in this context. Incidence of APA syndrome (13%) was comparable to data from earlier studies.
Our hypothesis is that activated thrombin within malignant effusions results in increased proliferation of tumor cells mediated through cleavage of PAR-1. We suggest that these cells might have a survival benefit. We harvested primary cancer cells from the pleural and peritoneal effusions of patients with pancreatic carcinoma, colon carcinoma, lung cancer (non-small cell lung cancer as well as small cell lung cancer), carcinoid and pleural mesothelioma. We found that 55.6% of primary cancer cells were PAR-1 positive using RT-PCR assays. Correspondingly, these cells were positive using the FCM (flow cytometry) assay.

In this paper, we show for the first time that half of the primary cancer cells exhibit a positive PAR-1 expression. Also, thrombin is activated in most malignant effusions as shown by prothrombin fragment 1,2 elevation. These cell-biological mechanisms might aggravate cellular resistance in primary tumor cells found in malignant effusion in end-stage cancer.

**Poster Session: Stem Cell Transplantation**

**P493 Functional and Phenotypic Characterization of NK Cells in AML Patients after Allogeneic Stem Cell Transplantation (SCT)**

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**Purpose:** Hsp70 is frequently found on the plasma membrane of bone marrow-derived leukemic blasts but not on normal bone marrow cells. In vitro Hsp70-peptide activated NK cells have been found to lyse autologous Hsp70 membrane-positive leukemic blasts (Gehrmann et al. 2003). Granzyme B release provides a surrogate marker for estimating the cytolytic response of NK cells against Hsp70 membrane-positive tumor target cells (Gross et al. 2003). Here, we studied the phenotype and cytolytic capacity of NK cells derived from AML patients at different time points (days 14–20, 45, 90, 6 months, 12 months) after allogeneic stem cell transplantation (SCT).

**Methods:** HLA class I, HLA-E and Hsp70 surface expression was determined on patient-derived leukemic blasts by flow cytometry. The amount of NK and T cells and their phenotype was investigated by multicolor flow cytometry using the following antibody-combinations detecting classical NK cell specific markers and activatory killer cell receptors: CD3/CD16/CD56, CD56/CD94, CD161/CD69, NKp30, NKp44 and NKp46. Concomitantly, the cell biological mechanisms might aggravate cellular resistance in primary tumor cells found in malignant effusion in end-stage cancer.

**Results:** Leukemic blasts were positive for HLA class I, HLA-E and Hsp70. A significant amount of CD3-negative, CD56/CD94-positive NK cells, but hardly any CD3-positive T cells, were detectable between days 14 and 45 after allogeneic SCT. Although only few patients were tested so far, in all cases the lytic capacity, after in vitro stimulation with Hsp70-peptide, correlated with the expression of CD56/CD94 on autologous NK cells. **Conclusions:** In addition to autologous NK cells, also patient-derived, allogeneic Hsp70-peptide activated NK cells, obtained between days 14 to 45 days post transplant, efficiently kill Hsp70 membrane-positive leukemic blasts. Further kinetic analysis should elucidate the graft-versus-leukemia effect of NK cells at later time points after allogeneic stem cell transplantation. Project funded by EU-TRANS-EUROPE grant QLK3-CT-2002-01936.


**P494 Veno-Occlusive Disease of the Liver is Uncommon after Conditioning with Low-Dose Total Body Irradiation**


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**Purpose:** Hepatic veno-occlusive disease (VOD) of the liver is a life-threatening complication after allogeneic haemopoietic cell transplantation (HCT). It occurs in 10 to 60% of patients undergoing high-dose chemotherapy and HCT. Severity ranges from a mild reversible to a severe disease culminating in multigang failure (MOF) and death. Reduced-intensity conditioning (RIC) regimens for allogeneic HCT were introduced to reduce the morbidity and mortality of conventional HCT. VOD was until now uncommonly reported after RIC.

**Methods:** In a series of 215 patients transplanted after conditioning with low-dose total body irradiation (TBI) and fludarabine (FLU) we observed 2 patients (pt.) who developed VOD. Patient 1, 51 years, and pt. 2, 59 years, received RIC because of Ph-neg CML, advanced phase, and high-risk-MDS-relapse after first conventional HCT respectively. The donors were unrelated in pt. 1 and related in pt. 2. Conditioning regimen consisted of fludarabine 30 mg/m² from day -4 to -3 and 2 Gy TBI (pt. 1) or 3 Gy TBI (pt. 2) on day 0. Immunosuppressive therapy included cyclosporine and mycophenolate-mofetil.

**Results:** Pt. 1 and pt. 2 developed the classical symptoms of VOD [painful hepatomegaly, jaundice (bilirubin 838.3 µmol/l in pt.1 and 119.9 µmol/l in pt. 2, normal range 2.0 - 20.3 µmol/l), and weight gain] 6 and 40 days after HCT respectively. Pt. 1 died because of MOF on day 13. Pt. 2 was successfully treated with defibrotide. He improved from moderate VOD after 34 days of treatment. **Conclusions:** We conclude that VOD is an uncommon complication after RIC-HCT. In our series of 215 patients, the incidence was < 1%. This is much lower than that reported after conventional HCT. Although rare, VOD must be considered in patients with typical symptoms after non-myeloablative HCT.
P496

Effects of Prior Irradiation and Chemotherapy on the Mobilisation of PBPCs in Patients with Multiple Myeloma

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Purpose: Irradiation of bone marrow prior to mobilisation of autologous peripheral blood progenitor cells (PBPC) may lead to reduced yield of mobilized CD34+ cells in patients with multiple myeloma. Furthermore, clinical parameters like previous chemotherapy regimens and patient’s characteristics were taken into account. Patients and Methods: 122 patients (76 male, 46 female) were analysed retrospectively. The median age was 52 years (range 30-62). All patients underwent mobilisation with G-CSF at stage II or III of the WHO classification (Macdonald and Durie) by the time of diagnosis. 53 patients had been irradiated prior to mobilisation chemotherapy. Most patients (87%) were mobilised with high-dose cyclophosphamide followed by G-CSF. Conventional-dose chemotherapy prior to mobilisation consisted of vincristin, adriamycin and dexamethasone (VAD) in 51 patients, VAD + cyclophosphamide (VACD) in 38 patients, melphalan (17 patients) or no chemotherapy (9 patients). According to hematological toxicity, the previous cytotoxic treatment was divided into 3 groups: moderately myelotoxic (<5 cycles, no melphalan) intermediate (>5 cycles, no melphalan) and highly myelotoxic chemotherapy (prior treatment with melphalan). Dose and fractionation of irradiation, volume of the irradiated bone marrow, effective biological dose and time between radiation therapy and mobilisation were taken into account.

Results: 114 patients were analysed, 8 had to be excluded due to partially missing data files. The median volume of irradiated bone marrow was 8.5% (range 1-30%) of the hematopoietic bone marrow and the median dose was 36 Gy (19-60 Gy). Correlation of irradiated bone marrow volume and number of CD34+ cells in peripheral blood (p=0.78), dose of bone marrow irradiation and CD34+ cells (p=0.4) showed no significant result. Multivariate analysis showed significant differences when comparing the different chemotherapeutic groups (p=0.001) and the patient’s age (p=0.031). Conclusions: In this study local irradiation of bone marrow prior to PBPC mobilisation did not lead to a reduced level of circulating CD34+ cells in the peripheral blood on the first-day of leukapheresis. However, there seems to be a trend for low CD34+ cell counts in patients with a higher proportion of irradiated bone marrow volume. The dose of irradiation had no impact on CD34+ cell count. Thus, we could not show any significant negative impact induced by prior radiotherapy on the mobilisation of PBPCs in patients with multiple myeloma.

P497

CNS Involvement Of Tropheryma Whipple: The First Description of a Late Complication after Allogeneic Bone Marrow Transplantation

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Background: Whipple’s disease (WD) is an extremely rare systemic disease of infectious etiology. About 120 cases of WD with nervous system involvement are reported in literature. We describe the first case of a cerebral manifestation after allogeneic bone marrow transplantation (BMT). Case Report: A 36 year old man undergoing matched related allogeneic BMT for CML developed 42 months after transplantation signs of motorical and sensorical deficit on the right hand side (Nervus ulnaris). MRI showed three intracerebral mass lesions in the left hemisphere with typical signs suggestive of toxoplasmic encephalitis. Despite immediately starting toxoplasmosis therapy with Dara- prime, Sulfadiazine and Clindamycin the neurologic symptoms worsened and lesions increased. Therefore a stereotactic biopsy was performed. Histology showed PAS-positive macrophages as typical signs of a Tropheryma whippelii infection. The diagnosis was confirmed by electron microscopy. The PCR diagnosis is pending. No signs of other infectious diseases or malignancy were observed. Immediately after starting a combination therapy of ceftazidime and streptomycin intracerebral lesions regressed and the patient resolved of all neurological symptoms. Summary: Neurologic presentation of WD is very rare. This is the first case of a cerebral WD after BMT. To avoid a delay of therapy the stereotactic biopsy is indispensable. Antibiotic treatment for at least 1 year is recommended.

P498

NKT cells are Significantly Increased up to one Year after Allogeneic as Compared to Autologous Stem Cell Transplantation and thus May Contribute to Graft-versus-Leukemia Activity

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Purpose: NKT cells are distinct cell populations sharing characteristics of both T cells and natural killer (NK) cells. Since experimental and clinical data indicate that they are involved in tumor surveillance, we compared the number of different types of NKT cells (%NKT, %CD3+CD56+ NKT, and %Vα24Vβ11 NKT) at defined time points after autologous and allogeneic HSCT. Methods: In a prospective study, the absolute and relative number of NKT subsets from pts with AML, ALL (n=11), NHL, HD, MM (n=15), and CML (n=5) were determined by three colour flow cytometry and compared with classical T- and NK-cell subsets immediately prior to and at day +30, +100, and day +300 following autologous (n=15) or allogeneic (n=16) HSCT. Results: The median number of %NKT, %CD3+CD56+ NKT, and %Vα24Vβ11 NKT was significantly higher in stem cell donors than in pts prior to HSCT. After autologous transplantation, CD4+ and CD3+ cells improved rapidly, whereas all types of NKT cells remained at extremely low levels until day +300. In contrast, percentages of allografts demonstrated a steady increase in the number of all NKT cell subtypes. At day 30, %NKT, %CD3+CD56+ NKT, and %Vα24Vβ11 NKT were significantly higher in the allografted vs. autografted patients (2.8 v. 17.8%, p < 0.05; 3.5 vs. 17.2%, p < 0.50; 5.2 vs. 110.5%, p < 0.05). The decrease was even more pronounced at day +300. No significant differences were detected in the number of CD56 cells and in the percentage of NK cells expressing the activation markers NKp30, NKp44, and Nkp46. Conclusion: NKT cell subsets are decreased in pts with different types of hematological malignancies. Whereas their number remains extremely low after autologous transplantation, allografting resulted in increasing numbers of all NKT subsets. Whereas this difference may contribute to GvL activity and whether the number of NKT cells is related to GVHD or other clinical parameters will be determined in a more homogenous population of AML patients after allogeneic HSCT.

P499

Cyclosporine A and Mycophenolate Mofetil Versus Cyclosporine A and Methotrexate for Graft versus Host Prophylaxis after Stem Cell Transplantation from HLA-Identical Siblings

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Purpose: The combination of Cyclosporine A (CSA) and Methotrexate (MTX) is an effective and well established combination for the prevention of graft versus host disease (GVHD) after allogeneic stem cell transplantation (SCT). Mycophenolate Mofetil (MMF) is an inhibitor of the purine nucleotide synthesis and inhibits proliferation of activated lymphocytes. We retrospectively compared the combination of CSA and MTX versus CSA and MMF in patients with acute and chronic leukemia after allogeneic SCT from HLA identical siblings (HS). Methods: One hundred eleven patients (median age 34, male 58, female 53) with acute myeloid (n=48) and acute lymphoblastic (n=21) as well as chronic myeloid leukemia (n=42) received either CSA/MMF (n=30) or CSA/MTX (n=81) as GVHD prophylaxis following high dose chemotherapy alone (n=46) or in combination with TBI (n=71) and consecutive allogeneic SCT from HIS. Eighty-eight patients were...
in complete remission (CR) or chronic phase (CP) while 23 patients had a progressive disease. Median follow up time after transplantation was 12 months (range 1-67 months) in the CSA/MMF group and 42 months (range 0.5-166) in the CSA/MTX group. Results: Although results showed a trend in favour of the CSA/MMF combination, no statistically significant difference was found in the overall survival of the two treatment groups with 2 year survival rates between 65% (CSA/MTX) and 70% (CSA/MMF) in patients with CR or CP, and 29% in the group of patients with advanced disease, respectively. As well no differences in relapse rate, treatment related mortality or death from disease were evident. Moreover, the incidence of acute GVHD and chronic GVHD was the same among the two regimens. Interestingly, the time until leukocyte reconstitution differed significantly within the two treatment groups with 11 days (median, range 3-17) in patients receiving CSA/MMF in CR or CP and 18 days (median, range 12-62) in the CSA/MTX group. This difference was also found in patients with advanced disease (CSA/MMF median 14 days, range 1-102, CSA/MTX median 18,5 days, range 17-27). Conclusion: results from this retrospective single centre analyses suggest that the combination CSA/MMF is at least equivalent to CSA/MTX for prophylaxis of GVHD for patients receiving SCT from HES with no change in overall survival, relapse rate or treatment related mortality. The time to leukocyte recovery is reduced by the use of MMF in stead of MTX in combination with CSA.

P500
Second Line Treatment of Acute GvHD with Cytokine Blocking Agents May Improve Short Term Outcome - A Retrospective Analysis in 169 Consecutive Patients

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Purpose: Whereas corticosteroids can be considered as standard for first line treatment of acute GVHD following allogeneic stem cell transplantation, there is no clearly defined and recommended second line treatment. Therefore, we now analysed treatment related mortality and outcome in relation to first and second line treatment of GVHD. Methods: Records of a consecutive cohort of 169 patients receiving an allogeneic stem cell transplant at the university of Regensburg between 6/1998 and 10/2003 were analysed. Due to a high rate of mainly infectious toxicity of second line treatment using T cell depleting agents like ATG or complete TNF blocking agents like infliximab (group I) we switched our strategy to the combined use of cytokine blocking agents daclizumab and etanercept (group II) in 2002. Results: First line treatment with corticosteroids could be considered as standard for first line treatment of acute GVHD following allogeneic stem cell transplantation, there is no clearly defined and recommended second line treatment. Therefore, we now analysed treatment related mortality and outcome in relation to first and second line treatment of GVHD. Methods: Records of a consecutive cohort of 169 patients receiving an allogeneic stem cell transplant at the university of Regensburg between 6/1998 and 10/2003 were analysed. Due to a high rate of mainly infectious toxicity of second line treatment using T cell depleting agents like ATG or complete TNF blocking agents like infliximab (group I) we switched our strategy to the combined use of cytokine blocking agents daclizumab and etanercept (group II) in 2002. Results: First line treatment with corticosteroids was given in 125 of 166 evaluable patients (75%) whereas a total of 44 (27%) patients required second line treatment due to progression of GVHD. TRM at 6 months was 19% in pts not receiving corticosteroids and 18% in those receiving corticosteroids alone but increased to 57% in patients receiving 2nd line treatment. Within this group, 180d TRM was lower in group II than in patients receiving depleting antibodies (57%) (p < 0.01). However, due to an increased incidence of late complications and relapses overall actuarial survival after a median follow up of 19 months was 27% in group I and 22% in group II. Conclusions: Our data suggest that cytokine blocking agents might be less toxic but clearly support the need of randomised and standardized comparisons of 2nd line strategies. Therefore, a multicenter trial comparing both approaches in steroid-resistant GVHD has been initiated.

P501
Acute Graft-versus-host Disease Prophylaxis Using a Combination of Alemtuzumab and Ciclosporin: Comparison Between Two Dose Levels of Alemtuzumab

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Alemtuzumab (Campath-1H), a humanized monoclonal antibody directed against the CD52 antigen, has demonstrated particular high efficacy in the prevention of acute graft-versus-host disease (GVHD). It is generally thought that its major mechanism is in-vivo donor T-cell depletion (TCD). In addition, Alemtuzumab can act on host antigen-presenting cells (APCs) and may thereby inhibit the induction phase of acute GVHD. At the commonly applied dose level of 100 mg Alemtuzumab, both mechanisms appear to be involved, while after low-dose Alemtuzumab (i.e. 50 mg total dose) its effect on host APCs appears to prevail. To evaluate the clinical efficacy of the two dose levels, two cohorts of patients (pts) received either standard dose (n=36) or low dose (n=34) Alemtuzumab in combination with ciclosporin (3 mg/kg/day IV). Both cohorts were comparable in terms of underlying diseases as well as patient, donor and treatment characteristics (advanced donor age and sex, underlying disease, and donor age 38 yrs, female to male transplants n=13, matched unrelated donors n=60, sibling donors n=10, myeloablative conditioning regimen n=70). Alemtuzumab was applied as a two-hour infusion on days -6 to -2. No serious adverse effects were noted during the total of 350 antibody infusions. The clinical grades of severity of acute GVHD were nearly identical between the two Alemtuzumab dose levels (50 mg/100 mg: grades 0: 85%/80%, 1: 9%/14%, 2: 3%/3%, III-IV: 3%/3%). Consequently, transplant-related mortality (TRM) at day 100 was comparatively low with 8% ± 5% (32/34 survivors) after 50 mg and 11% ± 5% (32/36 survivors) after 100 mg. The 1-yr survival estimate for pts in early disease stages is 83% ± 15% (9/10 survivors [90%]). This estimate is 50% ± 13% (44/60 survivors [73%]) in advanced disease stages. Causes of TRM were bacterial/mykotic infections (n=6 [9%]), multi-organ failure (n=3 [4%]), EBV-LPD, acute cardic arrest, and leukemaphenolopy (n=1 [1.4%]), while 5 pts have died from disease recurrence (n=5 [7%]). In conclusion, both investigated total dose levels of Alemtuzumab are comparatively effective in preventing acute GVHD and are associated with a reasonable short-term outcome in a predominantly unfavorable patient population. Further, our experience indirectly supports that the inactivation of host APCs by Alemtuzumab is the dominant mechanism in preventing acute GVHD.

P502
T-cell Chimerism after Transplantation of Allogeneic Highly Purified Peripheral Blood CD34+ Cells from HLA-identical Sibling Donors in Chronic Myeloproliferative Syndromes Compared to Acute Leukemias

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Purpose: In the setting of transplantation using highly purified peripheral blood CD34+ cells from HLA-identical sibling donors without any other prophylactic immunosupression, we studied the development of chimerism using fluorescence in situ hybridization for the X and Y chromosomes. Methods: Seventy one adult patients were transplanted for malignant hemato-logic diseases, 56 suffering from chronic myeloproliferative syndromes (MPS) mainly CML, 8 acute leukemias (AL), and 7 lymphomas or multiple myelomas. Severe acute graft-versus-host disease did not occur post transplant. Fifty eight patients at least 3 months after the first donor lymphocyte infusion (DLI) received a median of 3 DLI starting on day 93 (44 – 986) in a median maximal dose of 3 x 10^6 CD3+ per kg body weight. In the last 36 DLI patients, DLI were started as a programmed T-cell-addback (TCA) on days +90 +135 in doses of 0.33 x 10^6 and 1 x 10^6 CD3+ per kg. Results: NK-cells recovered within the first month. Numbers of B-cells and CD8+ cytotoxic T-cells normalized in the 2nd month post transplant whereas T-helper cell numbers stayed low beyond the first year (Beelen et al., 2000). Twenty nine patients had a donor patient gender disparity and a follow up of at least 3 months, 16 of these received donor lymphocytes as TCA. In contrast to a rapid complete chimerism of the NK- and B-cells in all patients, overall and T-cell chimerism developed differently in patients suffering from MPS compared to AL. Overall chimerism stayed in the MPS group at 90% in the first year after transplant, whereas overall chimerism in the AL group was >99% at all time points analyzed (p<0.05 at 3.6 and 9 months, p=0.065 at 12 months). T-cell chimerism in the MPS group was low with 5% at 3 months, increasing to 40% at 6 months, 48% at 9 months, 62% at 12 months, reaching 87% at 18 months. AL patients were completely chimeric in the T-cell population shortly after transplant (p=0.006 at 3 months). Early T-cell addback did not make a difference. The T-cell chimerism in the groups with TCA vs. late DLI was 38% vs. 41% (p=0.52) at 6 months, 40% vs. 71% (p=0.60) at 9 months and 45% vs. 87% (p=0.51) at 12 months. Conclusions: Expansion of patient clones is responsible for the early recovery of CD8+ T-cells in patients suffering from chronic myeloproliferative syndromes in contrast to acute leukemia patients. This might explain the known increased risk of secondary graft failure in this patient group.
In vitro measurement of GrA and GrB production levels significantly corre-

investigated. GrA and GrB production levels with the development of acute GvHD was

tive responses (RR) of MLC and with HLA class II mismatches. In a second,

selected cohort of 37 potential patient/donor pairs were correlated with rela-

immunosorbant assay (ELISA). GrA and GrB production levels from a

hours pre-transplant mixed lymphocyte cultures (MLC) by enzyme linked

granzymes (Gr) which are involved in the pathogenesis of GvHD. Therefore,

Activated donor cytotoxic T lymphocytes and natural killer cells produce

human leukocyte antigen (HLA)-identical SCT when CTLp and HTLP frequencies

are analyzed in PBMC of the respective stem cell graft (bone marrow (BMMC) or granulocyte colony-stimulating factor (G-CSF)-mobi-

zized PBMC) and compared to PBMC of PB. Host-specific CTLp frequencies

measured in 25 patients and HTLP frequencies analyzed in six patients were

low in all responder cell sources. CTLp and HTLP frequencies seen against

HLA-mismatched unrelated third-party cells were high, but third-party-

specific CTLp and HTLP frequencies were lower in G-CSF-PBMC than in

PBMC (p=0.047 for CTLp frequencies). Host-specific CTLp frequencies

analyzed in all responder cell sources did not predict acute or chronic GVHD.

Lower CTLp frequencies were detected in all responder cell sources from

patients who relapsed after SCT than in patients without relapse, but the

differences between both groups were statistically significant only in PBMC.

In conclusion, a significant correlation was detected only between relapse and

CTLP frequencies measured in PBMC. The lower frequency of third-party-

specific cells in G-CSF-PBMC indicates that the mobilization procedure with

G-CSF itself may influence results.

Graft-versus-host disease (GvHD) due to host-reactive antigen differences

remains an important cause of morbidity and mortality after allogeneic stem

cell transplantation (SCT). Currently, there is no reliable test system available

to predict the development of this serious complication. Activated donor cytotoxic T lymphocytes and natural killer cells produce

granzymes (Gr) which are involved in the pathogenesis of GvHD. Therefore,

we investigated a potential role of GrA and GrB production levels both for

the selection of the best stem cell donor and the prediction of the development of

acute GvHD prior to SCT.

We measured the GrA and GrB production levels in the supernatants of 96

hours pre-transplant mixed lymphocyte cultures (MLC) by enzyme linked

immunosorbant assay (ELISA). GrA and GrB production levels from a

selected cohort of 37 potential patient/donor pairs were correlated with rela-

tive responses (RR) of MLC and with HLA class II mismatches. In a second,

consecutive cohort of 20 sibling SCT recipients an association of enhanced

GrA and GrB production levels with the development of acute GvHD was

investigated. In vitro measurement of GrA and GrB production levels significantly corre-

lated with the RR of pre-transplant MLC (r=0.492, p≤0.01 and r=0.853,
randomised prospective analysis of defibrotide as “pre-emptive therapy” in 100 treatment cases and 90 patients with signs of liver toxicity after stem cell transplantation between October 1999 and January 2004. Pre-emptive DF was initiated with a bilirubin-increase above 3,0mg/dl, independent of other criteria. Bleeding was correlated to the bleeding score according to Nevo (Blood 91: 1469-1477, 1998). Median age was 31 years (range 0-65 y), 25 were younger than 18 years and 12 of them even younger than 10 years. Diagnosis were haematological malignancies (CML=27, AML=20, ALL=6, MM=7, MDS=7 and others n=23). 42% received sibling and 58% unrelated stem cells. Defibrotide was given intravenously, doses ranged from 30 to 60 mg/kg/d. Clinical diagnosis of VOD was defined by the Jones Criteria (bilirubin > 2,0mg/dl and two of the following criteria: hepatomegaly (usually painful), ascites or weight gain greater than 5% above admission weight).

Results: 55% had a complete response of bilirubin, 3% developed severe VOD under DF-therapy. In 45y we had to discontinue treatment because of increasing bilirubin or for attributable adverse events. In 43% bleeding episodes occurred (score II 26%, score III 12%, score IV 5%) and in 4 cases they stopped after discontinuation. The most important kind of severe bleedings were pulmonary (score II+IV 5%) and cerebral haemorrhages (score IV 1%). 57% of the 100 cases had bleeding scores score 0-1. Mortality rate 100 days after SCT was 20%. Conclusions: DF is an effective pre-emptive treatment to prevent severe VOD. Despite this positive outcome careful monitoring for bleeding is necessary.

P508 The Impact Of Donor Type on the Survival of Patients with CLL, who Received Reduced-Intensity Conditioning and Allogeneic Stem Cell Transplantation

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Purpose: To study the impact of the donor type (related versus unrelated) for patients with chronic lymphocytic leukemia (CLL) who receive hematopoietic stem cell transplantation (HSCT) after reduced-intensity conditioning.

Methods: 60 patients with advanced CLL were included. The median age was 52 years (range 12-68). A median number of 3 chemotherapy regimens had been given before HSCT. After conditioning with fludarabine, busulfan and ATG (n=40) or Campath (n=20) patients received HSCT from related (23 patients) or unrelated donors (37 patients). Graft-versus-host disease (GVHD) prophylaxis consisted of cyclosporine alone or a combination with methotrexate or mycophenolate mofetil. Results: After a median follow-up period of 32 months (range 2-70), 39 patients are alive. Acute GVHD Grade II-IV occurred in 26% of patients with related compared to 70% of patients with unrelated donors (p=0.007). Extensive chronic GVHD occurred in 9% and 25% of patients (p=0.063), respectively. At three years the probability of overall survival was 62% (95% CI, 48% to 86%) for patients with related donors and 57% (95% CI, 39% to 75%) for patients with unrelated donors (log-rank, p=0.3267). The 3-year probability of progression-free survival was 52% (95% CI, 30% to 74%) for patients with related donors and 51% (95% CI, 33% to 69%) for patients with unrelated donors (log-rank, p=0.8366).

Relapse mortality was 32% (95% CI, 8% to 56%) for patients with related donors and 36% (95% CI, 18% to 56%) for patients with unrelated donors (log-rank, p=0.2354), and the probability of non-relapse mortality was 9% (95% CI, 0% to 21%) for patients with related donors and 37% (95% CI, 19% to 55%) for patients with unrelated donors (log-rank, p=0.0393).

Conclusions: Overall and progression-free survival of patients with advanced CLL after allogeneic stem cell transplantation did not differ with respect to the type of donor, although causes of death differed largely. Searching for unrelated donors is therefore suggested in patients with advanced CLL who do not have a family donor. Future transplant strategies should focus on improved disease control in patients with family donors and on further reduction of treatment related mortality in patients with unrelated donors.

P507 Results and Safety of Open or Stereotactic Brain Biopsies In Patients With Intracerebral Lesions after Allogeneic Hematopoietic Stem Cell Transplantation

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Purpose: To date there is no standardized diagnostic approach in patients (pts) with intracerebral lesions who have undergone allogeneic hematopoietic stem cell transplantation. This study was undertaken to evaluate the safety and the diagnostic potential of invasive methods, i.e. open or stereotactic brain biopsies in comparison to less invasive methods, like CSF-analysis and MRI. Methods: The records and neuro-imaging studies of 6 pts with post-transplant CNS lesions in which diagnostic tissue has been obtained by open or stereotactic brain biopsy between 2002 and 2004 were reviewed retrospectively. Results: Five pts received an allotransplant from an unrelated donor and one from a HLA-identical sibling. The underlying diseases were AML (n=4), OMF (n=1) and CML (n=1). At the time of biopsy the median patient age was 48.5y (36-61y) and 2 of the 6 pts suffered from limited chronic GVHD requiring corticosteroid treatment. The median time from transplant to the onset of neurological symptoms was 247d (120-1017d) and the interval between the onset of symptoms and biopsy was 27.5d (9-36d). Four pts underwent open and 2 pts stereotactic biopsy. Besides the occurrence of post-operative seizures in 2 pts the procedure was well tolerated and there were no bleeding complications. The biopsy results are compared to CSF and MRI findings in table 1. Three pts (narcosids, whipple disease, cavernoma) were successfully treated and are alive and well at a median of 75d (63-835d) after biopsy. The pt with JC-PML died 49d after biopsy due to progressive PML, successfully treated and are alive and well at a median of 75d (63-835d) after biopsy. Conclusions: Open or stereotactic brain biopsies can safely be performed after allogeneic stem cell transplantation in pts with brain lesions of unknown origin and may uncover rather unexpected and possibly treatable conditions.

Table 1:

<table>
<thead>
<tr>
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<td>CSF</td>
<td>normal</td>
<td>normal</td>
<td>normal</td>
<td>n.d.</td>
<td>normal</td>
<td>mild pleocytosis, EBV-PCR pos</td>
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<td>MRI (sugg- estive of)</td>
<td>Aspergil- lousis</td>
<td>Toxoplas- mosis</td>
<td>Toxoplas- mosis</td>
<td>Lymph- oma</td>
<td>Toxoplas- mosis</td>
<td>Aspergil- lousis</td>
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<td>Biopsy result</td>
<td>Nocard- ia</td>
<td>JC-PML</td>
<td>Toxoplasmosis</td>
<td>Localized AML- relapse</td>
<td>Cavernoma</td>
<td>EBV- Lymphoma</td>
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P509 Expression of HA-1 and HA-2 in Patients with Hematological Malignancies and in their HLA-Matched Stem Cell Donors: A Retrospective Analysis

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Purpose: Minor histocompatibility antigens (mHAs) are peptides derived from polymorphic proteins, which can be responsible for graft versus host disease (GVHD) as well as for graft versus leukaemia (GvL) reactions in patients after allogeneic stem cell transplantation. Immune responses against mHAs with broad tissue expression can result in a high incidence of GVHD, whereas mHAs with restricted tissue distribution seem to be ideal candidates for effective GVL reactions. In order to estimate the applicability of mHAs specific immunotherapies after stem cell transplantation, we analyzed the frequencies of allelic mismatches between recipients and donors, with regard to the HLA-A2 restricted mHAs HA-1 and HA-2. Both mHAs are expressed by hematopoietic cells. HA-1 has also been described on several types of solid tumors, including breast and renal cancer. There are two allelic counter- parts, HA-1H and HA-1R and HA-2V and HA-2M, respectively. The HA-1H and HA-2V alleles are the immunogenic variants, which can elicit T cell responses after stem cell transplantation. Methods: In our analysis 62 HLA- A2+ patients with different hematological malignancies were included. These patients underwent allogeneic stem cell transplantation with reduced conditioning. Using an allele specific PCR for HA-1 and HA-2 we performed a

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Mycophenolate Mofetil (MMF) is an effective immunosuppressive drug and will established prevent graft rejection in solid organ transplantation. Studies in recipients of solid organ grafts have shown that MPA trough plasma levels are not sufficient to predict the effectiveness of therapy. Further investigations provided some evidence that a MPA-AUC0-12 (area under concentration-time curve) between 30-60 µg/ml*h are necessary for effective immunosuppression in renal transplantation. In contrast, studies by our group demonstrate low MPA plasma levels in the early phase after conditioning therapy and HCT. Most likely as a result of this a high rate of acute and hyperacut GVHD was observed. Methods: We launched a study for patients with MDS or AML receiving conditioning with fludarabine and busulfan and hematopoetic stem cells from HLA-compatible siblings or unrelated donors using a combination of tacrolimus and MPA-AUC targeted iv MMF as GVHD prophylaxis. The target range of MPA-AUC0-12 was 30-45µg/ml*h. 12 h AUC measurements were performed once a week on three occasions. For detection of MMP and MPA we used an isocratic RP-HPLC-system with fluorescence-Spectroscopy. MPP doses were adjusted in steps of 500 mg/d calculated by taking into account the relative difference between measured and target MPA-AUC0-12. Results: Preliminary data in six patients indicate that targeted dose adjustment (up to 4.5g/d) is feasible to achieve therapeutic MPA-AUC (median MPA-AUC0-12 after second dose adjustment = 33µg/ml*h) and is well tolerated by most of the pts (2/6 pts mild gastrointestinal, 1 pt mild renal toxicity). Acute extramedullary toxicity seems to be less compared to standard prophylaxis with CSA/MTX. In the evaluated patients the rate of acute extramedullary toxicity seems to be less compared to standard regimen (2 pts with grade II GVHD). Conclusions: Since approximately 25% of the analyzed donorrecipient pairs show a mismatch in the HA-1 locus, this mHA could be a useful tool in the treatment of haematological malignancies relapsing after stem cell transplantation. In contrast, allelic mismatches in the HA-2 locus are present in less than 5% of the couples so that this antigen does not seem a useful tool for immunotherapy in most cases of transplantation between HLA-identical individuals.

P510

Mycophenolate Mofetil AUC Targeting for GvHD Prophylaxis after Allogeneic Hematopoetic Stem Cell Transplantation

Freiberg-Richter J., Jenke A., Pursche S., Bonin M., Schleyer E., Ehninger G., Bornhäuser M.
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Purpose: Mycophenolate mofetil (MMF) is an effective immunosuppressive drug and well established to prevent graft rejection in solid organ transplantation. Studies in recipients of solid organ grafts have shown that MPA trough plasma levels are not sufficient to predict the effectiveness of therapy. Further investigations provided some evidence that a MPA-AUC0-12 (area under concentration-time curve) between 30-60 µg/ml*h are necessary for effective immunosuppression in renal transplantation. In contrast, studies by our group demonstrate low MPA plasma levels in the early phase after conditioning therapy and HCT. Most likely as a result of this a high rate of acute and hyperacute GVHD was observed. Methods: We launched a study for patients with MDS or AML receiving conditioning with fludarabine and busulfan and hematopoetic stem cells from HLA-compatible siblings or unrelated donors using a combination of tacrolimus and MPA-AUC targeted iv MMF as GVHD prophylaxis. The target range of MPA-AUC0-12 was 30-45µg/ml*h. 12 h AUC measurements were performed once a week on three occasions. For detection of MMP and MPA we used an isocratic RP-HPLC-system with fluorescence-Spectroscopy. MPP doses were adjusted in steps of 500 mg/d calculated by taking into account the relative difference between measured and target MPA-AUC0-12. Results: Preliminary data in six patients indicate that targeted dose adjustment (up to 4.5g/d) is feasible to achieve therapeutic MPA-AUC (median MPA-AUC0-12 after second dose adjustment = 33µg/ml*h) and is well tolerated by most of the pts (2/6 pts mild gastrointestinal, 1 pt mild renal toxicity). Acute extramedullary toxicity seems to be less compared to standard prophylaxis with CSA/MTX. In the evaluated patients the rate of acute extramedullary toxicity seems to be less compared to standard regimen (2 pts with grade II GVHD). Conclusions: MMF adjusted according to MPA-AUC seems to be a feasible way to achieve therapeutic drug levels, which might result in effective control of GVHD and are tolerated well by the patients. Further accrual and follow-up is necessary to confirm the efficacy of this approach.

P511

Clobazame (CLB) for the Prevention of Seizures Associated with High-Dose Oral and Intravenous Busulfan (HDBU) in Patients Receiving an Allogeneic Hematopoetic Stem Cell Graft (alloHSCT)

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Background: Conditioning with HDBU requires prophylactic anticonvulsant therapy. Phentoin (DPH) is an effective and widely used agent for this purpose. It is however associated with adverse reactions in 1/3 of patients (pts), some of them severe. Daily monitoring of blood DPH concentration is mandatory to ensure therapeutic levels. DPH induces an increased clearance of BU and Cyclophosphamide (CY) from the blood and thereby may influence transplant results. Clobazame (CLB), a 1,5-benzodiazepine effective in treating and preventing seizures has no significant side effects or interaction with other drugs. After oral administration it is well absorbed and well tolerated. Here we review our experience using exclusively CLB for seizure prophylaxis in 177 adults receiving HDBU orally (n=144) and intravenously (n=33) as part of their preparative regimen for alloHSCT. Patients and methods: 177 pts (n=13074), median age 38 (16-65) were treated with 16mg/kg bw BU po (3 mg/kg q 6 hrs for 4 days) and 12.8 mg/kg bw (n=27) or 9.6 mg/kg bw (n=60) BU iv (16 and 12 doses of 0.8 mg/kg q 6 hrs resp. followed by CY (120 or 200 mg/kg) (n=166) or Cy plus VP16 (n=11). 83 pts received a graft from a matched related and 94 from an unrelated donor. Median body weight was 72 kg (49-110). Indication for HSCT was AML (50), ALL (2), MDS (12), CML (109), OMH (3), PNH (1). In all pts CLB was started the day before the first BU dose and continued for the following 9 days. The exact protocol was as follows: CLB 5, 5, 10mg (i.e. three doses each day) starting from day before the first BU dose through the 1st day after the last BU dose, 5, 10 mg/day on the 2nd, 0, 0, 10mg/day on the 3rd and 0, 0, 5 mg/day on the 4th and 5th day after the last BU dose. Antiemetic prophylaxis consisted of a 5 HT antagonist. Results: All 177 pts received the CLB dose prescribed. CLB was well tolerated. Somnolence was absent or minimal. No serious side effects like respiratory depression or sedation attributable to CLB were observed. No generalized seizure or any other sign of neurotoxicity were seen during or after HDBU. Conclusion: In pts receiving HDBU as conditioning for alloHSCT CLB safely prevents seizures. Using the described protocol clinically effective CLB plasma levels are achieved with no need for expensive and time consuming drug monitoring like in DPH prophylaxis. Anticonvulsive prophylaxis with CLB is therefore easy to perform, well tolerated and cost effective.

P512

Differential Sensitivity of Primary Leukemia Blasts to Perforin/Granzyme B

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Purpose: CTL induced apoptosis in leukemic blasts is the molecular mechanism involved in the Graft versus leukemia (GvL) reaction following allogeneic bone marrow (BMT) and peripheral stem cell transplantation (PB SCT). This effect is being taken advantage of by using donor lymphocyte infusion (DLI) following post transplant relapse. Many groups have reported remission induction by DLI. However, many patients do not respond to DLI or become refractory. Thus, it is important to further identify mechanisms of resistance to CTL induced apoptosis. We have investigated the sensitivity of primary leukemic cells to the main molecular effectors of CTL for induction of apoptosis: the pore forming protein perforin and the serine protease granzyme B. Methods: Cytotoxic Granules rich in perforin/granzyme B were purified using the human NK cell line YT. Briefly, cells were lysed by hypotonic swelling and granules were isolated by sequential centrifugation to remove nuclei, heavy membrane and mitochondria fractions. Lysates were tested for apoptosis induction in HeLa cells. Granzyme B uptake was measured by western blot. Death receptor caspase pathway induced apoptosis was excluded using caspase inhibitors IETD and ZVAD. Cell lines and primary cells were incubated with granules and membrane permeabilization was measured after 4 hours by PI uptake using a FACS Calibur FACScan. Results: Primary cells from 21 patients were investigated with AML (n=8), ALL (n=6) and CLL (n=7). We found strong interindividual variation in sensitivity to perforin. Between different entities a trend could be shown towards weaker responses in AML cells, intermediate responses in ALL cells and the strongest responses in CLL cells. Conclusions: We could show that leukemic cells show differential sensitivities to purified cytotoxic granule proteins perforin/granzyme B. Thus, resistance mechanisms at this level of apoptosis induction by CTL must exist. It remains to be further investigated how sensitivity to perforin/granzyme B correlates to clinical outcome following allogenic BMT/PBSCT and DLI after relapse.
Background: After alloSCT, oral VALGCV is a promising alternative to IV GCV against CMV but its pharmacokinetics (PK) have not been studied. Methods: We investigated the PK of GCV after oral VALGCV in a randomized, crossover phase II study of 48 patients (pts) after alloSCT. Pts who had >400 copies/ml of CMV DNA in plasma by quantitative PCR were randomized to receive oral VALGCV 900 mg BID for 7 days, followed by IV GCV as 1-h-infusion at 5mg/kg every 12 h for another 7 days, or to the inverse sequence of study drug administration. The PK of GCV were assessed on days 4 and 11. Safety monitoring was done until day 84 after SCT. Results: 28 pts were fully assessable for PK analyses. Among the 22 pts without intestinal graft-versus-host disease (GVHD), the mean±SD AUC0-12 (mg/L*h) was 53.8±18.0 on oral VALGCV vs 39.5±13.9 on IV GCV (mean difference 14.3 [95% CI 7.6 to 20.9]; Cmax (mg/L) was 8.8±2.4 vs 10.2±2.1; tmax (h) was 2.7±0.8 vs 1.1±0.6; and t1/2 (h) was 4.2±1.1 vs 3.4±1.8. Among the 6 pts with gut GVHD, the mean±SD AUC0-12 (mg/L*h) was 46.6±24.9 on oral VALGCV vs 35.3±12.8 on IV GCV (mean difference 11.3 [95% CI -13.4 to 35.9]). Cmax (mg/L) was 7.1±3.6 vs 11.1±3.1; tmax (h) was 2.7±0.8 vs 1.2±0.4; and t1/2 (h) was 5.6±2.0 vs 3.3±0.7. Clearance of viral DNA was equally effective in both arms. Nonfatal CMV pneumonia developed in 2 pts during follow-up after antiviral therapy, and neutropenia <500/µl occurred in 3 pts. Conclusions: After alloSCT, systemic exposure to GCV with oral VALGCV treatment is superior to that with IV GCV in pts without gut GVHD, and is within the range seen with IV GCV in pts with gut GVHD. Oral VALGCV could replace preemptive IV GCV after alloSCT if shown similarly efficacious.

P515 Haploidentical Peripheral Blood Stem Cell Transplantation: A Single Centre Experience of 24 Cases

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Purpose: Over the past 8 years we have performed 24 haploidentical peripheral blood stem cell transplants on patients (pts) with incurable malignancies and no prospect of a matched unrelated donor within adequate time period. Patients and methods: We treated 12 females and 12 males with a median age of 37 years (range 20 – 57 years). The diagnoses and remission status were as follows: AML not in CR (n=9), AML in CR2 (n=4), MCL (n=7), CML in CP1 (n=3) and ALL. Philadelphia chromosome positive in CML (n=1). Conditioning consisted of ATG, TBI, thiopeta, cyclophosphamide in most patients and additional radioimmunotherapy with an Yttrium-90 or a Rhenium-188 labelled anti-CD66 antibody was given in 15 patients. All patients received G-CSF mobilized peripheral blood stem cell grafts. GVHD prophylaxis consisted of T cell depletion by CD34+ selection in all but one pt, no posttransplant immunosuppression was given in 21 pts. Results: Stable engraftment was achieved in 23 pts. 1 case of acute graft rejection was observed. 11 patients developed grade II-IV acute GVHD, we observed no cases of grade III-IV disease. 11 pts have developed chronic GVHD. 5 pts have suffered a relapse of their disease, 1.2 pts died of transplant related mortality. Infections were the most significant problem after transplant, in particular aspergillus infections. After a median follow-up of 39 months (2-59 months) 7 pts are surviving in hematological remission, 1 pt with CML has minimal residual disease and is treated with STI-571. Conclusion: Haploidentical peripheral blood stem cell transplantation is a possible alternative to a MUD transplant. Maintenance of the infection prophylaxes for a prolonged period of time and frequent screening for infections are mandatory to reduce transplant-related mortality.

P516 Rituximab-Monotherapy in Patients with Follicular Lymphoma Relapsing after ASCT

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The prognosis of patients with follicular lymphoma (FLC), relapsing after autologous stem-cell-transplantation (ASCT) is poor. These patients have chemo-resistant disease and sometimes limited bone marrow capacity, therefore antibody treatment may afford an alternative option. We initiated a monocentric phase IV study of rituximab for patients with CD 20 pos. FCL, relapsing more than 6 months after ASCT. Initial post-transplant treatment-regimen consisted of rituximab (375mg/m²) weekly. After completion of fourth cycle patients entered an observation period. Results: 12 patients ( 9 male, 3 female ), 28-61 years old (median: 48,5) were treated. Median time from diagnosis to ASCT was 30 months (range: 9-65) and median time from ASCT to rituximab 18 months (range 6-52). Treatment was well tolerated, in 4/8 therapy cycles grade III-IV toxicity ( 2 FUO, 1 bronchopulmonary infection, 1 chillis+pain ) was observed only. No delayed granulocytopenia occurred. Responses were achieved in 8/12 (66%) patients - 5 CR, 3 PR -, 4 patients were refractory to rituximab (NC); 2/4 NC died within 1 month, one patient was treated with chemotherapy and lives in PR for 23 months; 1 NC-patient was treated with rituximab (RIC-alloSCT) and is alive in CCR.
for 54 months. 3/8 responders relapsed and died after 20, 43 and 44 months despite chemotherapy. 5 patients are alive (4 in CCR – 2 after RIC-alloSCT, 1 in PR) 18, 24, 29 and 46 months after initiating rituximab. Conclusion: Rituximab is an effective and safe therapy for patients with FCL relapsing after ASCT with a 66% response rate. But to achieve a continuous complete remission additional chemotherapy or RIC-alloSCT was needed in one third of our patients.

P517
High Dose Chemotherapy And Autologous Stem Cell Transplantation For Relapsed Chemosensitive Hodgkin’s Disease – A Single Center Experience

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Background: High dose chemotherapy (HDC) followed by autologous stem cell transplantation represents an effective treatment modality in relapsed Hodgkin’s disease (HD). We evaluated efficacy and toxicity of this therapy in patients (pts) with relapsed HD, who were treated in our institution between 1997 and 2003. Patients and Methods: 18 pts. were treated within this period, median age at transplantation was 34 yrs. (range, 19-61). 16 pts. were treated in first relapse, median time to relapse was 33 months (range, 2-95). 2 pts. had a primarily resistant disease. As induction chemotherapy pts. received different regimens including DEXA-EBAM, High-Dose (HD)-Etoposide, HD-Cyclophosphamide, HD-Methotrexat, DHAP, IEV. All patients achieving at least a PR proceeded to transplantation (100%). The most frequently used high-dose regimen was BEAM. In 2 pts. with early relapse and 1 pt. with resistant disease during the first induction regimen a double transplantation with TCM and BEAM was performed. Median time from relapse to transplantation was 5 months (range, 2-20). Results: Median follow up was 42 months (range, 5-82), 17 of 18 (94%) transplanted pts achieved CR, 1 pt. who was primarily resistant achieved PR. 2 (11%) pts. relapsed at 20 and 59 months. Disease free survival at 3 years was 89%, overall survival at 3 years were 100%. Out of 11 pts. with a follow up more than 3 yrs., only 1 pt. relapsed after 5.9 yrs. and died from second relapse. Transplantation related toxicity was as expected: 67% (n=11) suffered > grade II and 27% (n=5) > grade III toxicity. 1 pt. developed aspergillosis pneumonia and 1 pt. thrombosis of V. cephalica. There was no transplant-related death. Conclusion: Our data confirm that High Dose Chemotherapy with autologous stem cell transplantation is associated with a high response rate resulting in durable disease free survival. Transplantation associated toxicity was acceptable.

P518
Autologous DC Transfected with in Vitro Transcribed mRNA for the Analysis of CMV-pp65-Specific T Cell Reconstitution after Allogeneic Stem Cell Transplantation

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Purpose: CMV-specific T cell reconstitution after allogeneic stem cell transplantation has been examined by several investigators. Most analyses used known peptide epitopes derived from CMV-proteins (mainly of pp65) to examine the frequency of CMV-specific CD8+ T cells with either HLA/peptide multimers in flow cytometry or with peptide loaded antigen presenting cells (APC) in cytokine ELISPOT assays. The use of peptides, however, is limited to known epitopes and to the presence of their restricting HLA-alleles. We have previously shown with peripheral blood lymphocytes (PBL) of healthy donors, that RNA-transfected autologous dendritic cells (DC) can be applied to detect CMVpp65-specific T cells in IFN-gamma ELISPOT assays. We have also demonstrated that the sensitivity of this approach is similar to the use of peptide loaded APC. Methods: Here we report on four patients who underwent allogeneic stem cell transplantation for haematological malignancies. All patients and their donors were CMV-positive and experienced at least two CMV-reactivations. PBL were analyzed prior to, and at least at two timepoints after transplantation. For two HLA-B7-positive patients the immunodominant HLA-B7-restricted pp65-peptide TPRTVVGGGA (pp65404-412) was used as a positive control. Results: Specific CD8+ T-lymphocytes pre-transplant ranged between 0.15 and 1.0% of peripheral blood CD8+ cells. Using pp65-RNA transfected autologous DC increasing frequencies of pp65-specific CD8+ T-cells were detected after transplantation. The course of CMV-specific reconstitution differed significantly between individual patients. In both HLA-B7-positive patients pp65404-412-specific T cells were detected in a similar range as RNA-specific CD8+ positive cells, underlying the comparable sensitivity of this approach. In three of four patients CD4-positive cells specifically recognizing pp65-RNA transfected autologous DC were also detected. The frequencies of specific CD4+ T-cells ranged between 14 and 80% of those of specific CD8+ lymphocytes. Conclusions: Our data indicate the feasibility of using RNA-transfected autologous DC as antigen-presenting cells in IFN-gamma ELISPOT assays for the sensitive and HLA-independent monitoring of CMV-specific CD8+ and CD4+ immune reconstitution. RNA-transfected DC might be also used for other viruses, such as HSV, RSV and adenovirus, where immunodominant epitopes have been poorly described.
A 45-year-old male patient with chronic idiopathic myelofibrosis and consecutively anaemia, leukaemia and thrombocytosis received an allogeneic peripheral blood stem cell transplantation from an HLA-identical female sibling after conditioning with TBI/cyclophosphamide. He had a rapid take of donor hematopoiesis and only moderate complications, four and a half years later, relapse was diagnosed with leukocytosis of 11.260/µl, an elevated hemoglobin level of 20.4 g/dl, thrombocytosis of 616.000/µl and a marked reduction in donor chimerism, resembling pretransplant level of myelofibrosis.

**Methods:** The patient received two DLI with increasing cell dose (1 x 10^6 CD3+ cells per kg and 3 x 10^6 CD3+ cells per kg bw., respectively). The patient developed no significant graft versus host disease (GVHD) of the skin or the gut. DLI were stopped due to suspected GVHD disease of the liver. However, elevated liver enzymes decreased after one year. Within 5 months after the last DLI, donor hematopoiesis was fully restored and white blood count, hemoglobin and platelets returned to normal. At present, the patient is alive and well without further relapse or GVHD almost one year after his last DLI.

**Conclusion:** This case report indicates that DLI can be an effective treatment for patients with a late relapse after BSCT.

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**Purpose:** Immune reconstitution was studied in 154 patients transplanted from May 1999 to April 2004 using either highly purified peripheral blood CD34+ cells without any other prophylactic immunosuppression (72) or in vivo T-cell depletion with 100 mg (47) or recently 50 mg (35) Alemtuzumab and cyclosporine A (CsA) for 3 months post transplant.

**Methods:** All patients having received 50 mg Alemtuzumab are too early to analyze. In very short term, there is no significant difference of the two dosages in regard to the important clinical end points including acute GVHD and to immune reconstitution. Nineteen patients with a follow up of at least half a year have received 100 mg Alemtuzumab (group A). Seventeen patients have been transplanted using CD34 enriched grafts including all 8 non-CML patients with a sufficient follow up period (group B). These are compared amongst each other and with a cohort of 20 successive patients transplanted after myeloablative conditioning with a standard immunosuppression (CsA and short course methotrexate) in the year 2002 (group C). Absolute numbers of CD3+CD4+RO+, CD3+CD4+RA+, CD3+CD8+, CD16/56CD3- and CD19+ cells were measured in the peripheral blood by flow cytometry at 3 and 6 months post transplantation. The correlation coefficient between dose corrections and resulted risk of RRT. To improve this time and labor intensive procedure we evaluated a TMD of oral BU is a feasible procedure in patients receiving BSCT to minimize the risk of RRT.

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**Conclusion:** This case report indicates that DLI can be an effective treatment for patients with a late relapse after BSCT.

**Purpose:** Immune reconstitution was studied in 154 patients transplanted from May 1999 to April 2004 using either highly purified peripheral blood CD34+ cells without any other prophylactic immunosuppression (72) or in vivo T-cell depletion with 100 mg (47) or recently 50 mg (35) Alemtuzumab and cyclosporine A (CsA) for 3 months post transplant.

**Methods:** All patients having received 50 mg Alemtuzumab are too early to analyze. In very short term, there is no significant difference of the two dosages in regard to the important clinical end points including acute GVHD and to immune reconstitution. Nineteen patients with a follow up of at least half a year have received 100 mg Alemtuzumab (group A). Seventeen patients have been transplanted using CD34 enriched grafts including all 8 non-CML patients with a sufficient follow up period (group B). These are compared amongst each other and with a cohort of 20 successive patients transplanted after myeloablative conditioning with a standard immunosuppression (CsA and short course methotrexate) in the year 2002 (group C). Absolute numbers of CD3+CD4+RO+, CD3+CD4+RA+, CD3+CD8+, CD16/56CD3- and CD19+ cells were measured in the peripheral blood by flow cytometry at 3 and 6 months post transplantation. The correlation coefficient between dose corrections and resulted risk of RRT. To improve this time and labor intensive procedure we evaluated a TMD of oral BU is a feasible procedure in patients receiving BSCT to minimize the risk of RRT.

**Results:** The patient received two DLI with increasing cell dose (1 x 10^6 CD3+ cells per kg and 3 x 10^6 CD3+ cells per kg bw., respectively). The patient developed no significant graft versus host disease (GVHD) of the skin or the gut. DLI were stopped due to suspected GVHD disease of the liver. However, elevated liver enzymes decreased after one year. Within 5 months after the last DLI, donor hematopoiesis was fully restored and white blood count, hemoglobin and platelets returned to normal. At present, the patient is alive and well without further relapse or GVHD almost one year after his last DLI.

**Conclusion:** This case report indicates that DLI can be an effective treatment for patients with a late relapse after BSCT.
Transplants contained a median of 8.02 X10^6 CD34+ cells/kg BW. GVHD prophylaxis consisted 12 CSA/MTX, 4 CSA/MTX/ATG, one CSA only, and 4 ATG only. Twenty one pts engrafted for granulocytes (ANC>0.5/ul) at median of 16.8 days (9-26) and 16 pts for platelets at 20.7 days (13-33), respectively. One pt had a primary transplant failure with progressive disease and an other pt had a secondary transplant failure. The most non hematological side effect grade ≥4 was mucositis. Early complete donor chimerism (>95%) was observed in 20/22 pts. Acute GVHD of grade 2 II occurred in eleven pts and chronic GVHD in seven pts (two limited and five extensive). Respectively, TRM was observed in 5 cases: MOF (n=1), sepsis (n=2), enterale GVHD (n=1), and opportunistic infection (n=1). Three pts died of relapse. 14 pts (63.6%) are still alive at median survival time of 479 days. In conclusion, in our series of pts with imatinib resistant CML treated with regorafenib therapeutic allogenic HSCT using related or unrelated donor may be cure pts with this disease without severe TRM.

**Poster Session: Stem Cell Biology**

**P524 Resistance of Human Bone Marrow Mesenchymal Stem Cells (hMSC) for Cytotoxic Treatment Is Associated with Low Levels of Caspase Activation**


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**Purpose:** MSC participate in the bone marrow microenvironment which sustains hematopoiesis. Recent reports suggest, that autologous human MSC might be of interest for various therapeutic approaches. However the biology and ability of in vitro expansion of these cells still remains obscure. In the present study we investigated hMSC from healthy as well as affected human bone marrow regarding their sensitivity for cytotoxic treatment. **Methods:** MSC were isolated from BM aspirates of patients with proven malignant BM infiltration or with a history of chemotherapy as well as from healthy donors. Phenotypical analysis was performed by flow cytometry. Single cell cultures were analyzed for their in vitro osteogenic and adipogenic differentiation potential (cytochemistry, RT-PCR). Sensitivity against cisplatin, vincristin and etoposide was investigated by SRB cytotoxicity assays determining IC90 values. Caspase activation was analysed in cytosolic extracts by colorimetric assays using specific substrates. Presence of active caspase fragments was analysed by western blotting. **Results:** MSC with typical phenotype were isolated from all patients and showed mesodermal multipotent differentiation capacity in single cell derived cultures. IC90 values for vincristin, cisplatin and etoposide were comparable to those of established resistant tumor cell lines but considerably higher than in pluripotent sensitive embryonal carcinoma cells (ECC). Treatment for 24 h with IC90 cisplatin doses resulted in a high degree of morphologically apoptotic cells. However little activity of caspase 2 and 3 and almost no activity of caspase 8 and 9 – which are all typically activated by cisplatin - was observed. Western blot confirmed the lack of caspase 8 and 9 activation. **Conclusions:** Cultured hMSC show a resistance to cytotoxic treatment. However, this seems not to be the result of an in vitro artefact as we demonstrated previously that MSC are present in BM of patients after high dose chemotherapy. Thus, hMSC appear to have an intrinsically high apoptotic threshold. The reduced amount or entire lack of caspase activation after cisplatin treatment suggests that distinct apoptotic pathways are suppressed in hMSC. This not only offers an explanation for their persistence in BM after cytotoxic therapy but also gives new insights into the biology and degree of differentiation of these cells.

**P525 Analysis of Genexpression of Primitive Hematopoietic Progenitor Cells**

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**Purpose:** Stem cells are characterized by their dual ability to self-renew and to differentiate into multiple cell types. A genome wide gene expression analysis could shed light on the molecular mechanisms that regulate hematopoietic stem cells. We have provided evidence that divisional kinetics could be exploited to separate stem cells into two fractions: a slow dividing fraction (SDF) that is predominantly associated with primitive function and self-renewal, and a fast dividing fraction (FDF) that divides symmetrically and predominantly proceeds to differentiation. In continuation with this line of research we have analyzed the global gene expression profiles of these two populations. **Methods:** The CD34+/CD38- fraction and the CD34+/CD38+ fraction were isolated from human umbilical cord blood. We have then separated the CD34+/CD38- population into a slow dividing fraction (SDF) and a fast dividing fraction (FDF) as described in detail previously. Gene expression analysis was performed with a novel Human Transcriptome Microarray (Winfier U., Ansorte W. et al., manuscript in preparation) that represents 51,145 UnigeneSet-RZPD3 cDNA clones. To obtain additional information on primitive versus committed progenitor cells, gene expression profile of CD34+/CD38- cells, which are associated with more primitive function was compared with that of the CD34+/CD38+ cells that are committed to differentiation. Flow cytometry and semi-quantitative RT-PCR confirmed the differential expression of several genes. **Results:** In the CD34+/CD38- fraction 96 sequences were found to be at least two-fold over-expressed and 119 were suppressed in comparison to CD34+/CD38+. In contrast in the SDF versus the FDF a larger proportion of genes was differentially expressed (942 sequences up-regulated and 794 down-regulated). Among the genes showing the highest expression levels in the SDF were the following: CD133, erg, cyclin g2, MDR1, osteopontin, clqr1, ifi16, jak3, Ifd6 and hoxa9, a pattern compatible with their primitive function and self-renewal capacity. In comparison to three other published microarray studies we identified several genes that were up-regulated in different fractions of murine and human HSC including: HoxA9, Jak3, Ifd6, mdr1 and rbpms. Furthermore we observed more podia-formation in the SDF and CD133 is located on the tip of these podia. Time-laps analysis of the cells in co-cultivation with a stromal feeder-layer showed that hematopoietic progenitor cells were often tightly attached to AFT024 cells. The impact of cell-cell contact on gene expression profiles of primitive HSC is concurrently analyzed. **Conclusion:** We have demonstrated that the quiescent population of CD34+/CD38- cells represents a highly enriched stem cell population on a molecular level. These results have provided the foundation for further analysis of genes that are responsible for self-renewal and will contribute to a detailed understanding of the asymmetric division of stem cells.

**P526 Acquision of CD34 Correlates with Increased Hematopoietic and Self-Renewal Activity of CD34 CD133+ Cord Blood Cells**

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CD34 is a sialomucin expressed on hematopoietic cells, endothelial cells, and muscle satellite cells. Within the hematopoietic system, CD34 expression has been associated with very immature progenitor cells as well as hematopoietic stem cells (HSC), and it is widely used to assess cell activity in clinical protocols. In the past, HSC activity was thought to be retained exclusively in the subset of cells expressing CD34. This view has been challenged by recent observations in mice, in which HSC activity was also found in the CD34-negative cell fraction. These findings have since been reproduced using
human marrow and cord blood cells. However, the exact relationship between CD34+ and CD34- stem cells remains unclear. We investigated the regulation of CD34 expression as dependent on cell division history. To follow cell division, human cord blood cells were labeled with the fluorescent dye CFSE. Lin-CD34-CD133+CFSE- (CD34+) and CD34- cells were almost indistinguishable in their ability to produce CAFcres, content. After 3 days of serum-free culture with stem cell factor, FLt3 ligand and thrombopoietin, almost all initially CD34+ cells had acquired expression of CD34, including all undivided cells. In cultures initiated from CD34+ cells, virtually all CAFcres+ were produced from the divided CD34+ cells, indicating these cells had self-renewed. In contrast, similar cultures from initially CD34+ positive cells demonstrated that hematopoietic activity associated with the undivided cell fraction. Analysis of mRNA expression showed that CD34+ and CD34- cells expressed almost equal levels of CD34, AC133, FLt3, Flk1, and Flt4, while CD34+ cells expressed significantly lower levels of Tie1 and Tie2 than CD34+ cells. CD34 cells that did not express CD34 on their cell surface exhibited intracellular CD34, suggesting these cells were "primed" to express CD34. In conclusion, Lin-CD34-CD133+ cells acquire the expression of CD34, even in the absence of cell divisions. These CD34- cells self-renew more rapidly in vitro than cells initially expressing CD34, and self-renewal is preceded by acquisition of CD34 antigen.

P527
Expression and Regulation of the Cyclosporin A-sensitive Transcription Factor NFAT (Nuclear Factor of Activated T cells) in CD34+ Cells

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Transcription factors of the NFAT (Nuclear Factors of Activated T cells) family are established as one of the key elements of inducible gene expression in activated T cells and are considered to be the main molecular targets of the immunosuppressive drugs cyclosporin A and FK506. While regulation and function of NFAT proteins in lymphocytes has been extensively studied, little is known about their expression and potential biological relevance in myeloid cells. We have recently shown that NFATc2 (NFAT1) protein is expressed in CD34+ cells, but not in peripheral blood neutrophil granulocytes and monocytes. In the current study, we analyzed the expression of all five known NFAT family members on the mRNA level in CD34+ cells of cord blood and G-CSF-mobilized peripheral blood, in peripheral blood CD34+ cell subsets, and during the differentiation of CD34+ cells into neutrophil granulocytes. In CD34+ populations of both peripheral blood and cord blood, NFATc3 was the NFAT family member found to be most prominently expressed by quantitative RT-PCR. Transcript levels for all NFAT family members were present at equal or higher levels in the immature CD34+CD38+ subset of peripheral blood CD34+ cells, as compared to CD34+CD38- or bulk CD34+ populations. Cytokine-induced in vitro differentiation of CD34+ cells into neutrophil granulocytes resulted in the rapid and almost complete suppression of NFATc2, but not NFATc3 transcripts. Dephosphorylation/rephosphorylation as well as nuclear/cytoplasmic translocation of NFAT in CD34+ cells followed the same calcineurin-dependent pattern as in T lymphocytes, suggesting that NFAT activation in these cells is equally sensitive to inhibition with cyclosporin A. Finally, in vitro proliferation of CD34+ cells cultured in the presence of FL-T3-L, SCF, GM-CSF, IL-3 and G-CSF was profoundly inhibited by treatment with cyclosporin A in a dose-dependent manner; in contrast, their differentiation into neutrophil granulocytes remained unaffected. These results suggest a novel and unexpected role for members of the NFAT transcription factor family in the hematopoietic system.

P528
Homing and Long-Term Engraftment of Hematopoietic Stem Cells in Various In Vitro Niches – Expression pattern of Adhesive Molecules and Ligands of the Notch-Pathway as Predictive Factor

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Purpose: Several transplantation studies in animal and human models demonstrated preferential homing and long term engraftment of bone marrow-derived stem cells in hematopoietic environments and to a lesser extent in non-hematopoietic tissues like skin, gut, lung or brain. We have recently demonstrated the vital role of cell-cell contacts, especially between hematopoietic stem cells (HSC) and the stromal environment, not only for stem cell homing, but also on the long-term fate of HSC. In this study we have quantified and compared the homing and stem cell supporting properties of various in vitro microenvironments. Characterization of the environments for the expression of adhesive and cell-fate regulating molecules revealed a strong correlation between the expression pattern and their functional properties. Methods and Results: CD34+/133+ HSC were cocultured for up to 24h with the murine stromal cell line AFT24, human mesenchymal stem cells (MSC), a murine neuronal (NFL) and a murine astrocyte feeder layer (AFL). Determination of adhesion kinetics revealed significant differences in the affinity of HSC towards the different feeder layers. A high and stable adhesion for at least 12h occurred only in coculture with AFT204 (37.6 ± 12.7% adherent cells) and MSC (31.7 ± 6.1% adherent cells). Adhesion to non-hematopoietic environments was either low with 10.3 ± 4.2% adherent cells for NFL and instable with initially 33.6 ± 2.8% adherent cells and 15.3 ± 3.5% adherent cells after 12h for AFL. Nevertheless HSC adhered firmly (≥ 100 pN) to the various microenvironments as measured by an optical trap. To assess the support of the various feeder layers for repopulating HSC a LTC-IC assay in limiting dilution was performed. Conservation of long-term initiating cells was 59.7% in coculture with AFT204 and 60.2% in coculture with MSC, whereas astrocytes and neurons did not support LTC-IC at all. Characterization of the feeder layers by means of immunophenotyping, western blotting and immunofluorescence microscopy for main adhesive ligands (CD29, CD54, fibronectin, heparan sulfate proteoglycan perlecan) and cell fate regulating molecules revealed a distinct expression profile correlating highly with the different functional properties of AFT204, MSC, NFL and AFL. Conclusion: These data demonstrate superior homing and stem cell supporting properties of AFT204 and MSC compared with the neuronal and astrocyte feeder layer. Besides that they show that the different functional properties of the various in vitro environments correlate highly with the expression pattern of certain adhesive and cell fate regulating molecules.

P529
Characterization of Cellular Contacts in Human Mesenchymal Stem Cells

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Purpose: Mesenchymal stem cells (MSC) are a population of adherent, fibroblast-like cells from human bone marrow, that are capable to differentiate into bone, cartilage and adipose tissues and are therefore of high interest for regenerative medicine. They can be grown to confluency on plastic surfaces and form multiple intercellular contacts. Thus far, neither the isolation and culturing methods of these cells have been standardized, nor have the intercellular junctional complexes been characterized. Methods: We have systematically analyzed the molecular composition of cellular contacts between human MSC obtained from bone marrow aspirates from healthy voluntary donors, using a panel of antibodies specific for various components of tight junctions, gap junctions, adherence junctions and desmosomes, by light and electron microscopy and by biochemical analysis, including immunoprecipitation and RT-PCR. Results: In these studies we have made two surprising observations of fundamental significance. The MSC are inter-
connected by occasional gap junctions and two types of frequent adhering
junctions: (i) typical puncta adherentia (PA), often clustered or even fused, and
(ii) a system of extended, slender, tentacle-like cell processes (processus
adhaerentes) bearing one or several groups of PA, often in terminal or preter-
minal position. Some of these processus adhaerentes can be remarkably long
(20 to 400 μm). The molecular composition of these adhering junctions is
also unique, as they comprise the transmembrane glycoproteins cadherin-11,
N-cadherin, and β-catenin, together with cytoplasmic plaque proteins
α-actinin and β-catenin and p-120. These junctions have not been described in human
MSC before and bear similarity to those described by us in primary mesenchymal
stem cells of day 7 to 8.5 mouse embryos (Franke et al., 1982, 1983).
Conclusions: Our data indicate that MSC communicate with each other
through junctions and junctional complexes. We hypothesize that MSC
can embark on alternative differentiation pathways with different junctional
and cytoskeletal patterns. Characterization of and understanding the role of
such intercellular contacts and their correlation to specific differentiation
programs and stages are being elucidated.

P530
Impact of Stem Cell Factor (SCF) on the Engraftment and
Differentiation Capacity of Human Hematopoietic Stem
Cells Transplanted in NOD/SCID Mice
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Purpose: The transplantation into irradiated immunodeficient NOD/SCID
mice is a valid assay to detect pluripotent human stem cells with the ability of
multilineage hematopoietic reconstitution. In this study, we investigated the
impact of SCF on the engraftment of lineage depleted (lin-) peripheral blood
and cord blood-derived mononuclear cells, and analysed the organ
distribution of the transplanted cord blood cells with or without the addition
of SCF. Methods and Results: In two experiments, 16 NOD/SCID mice
were injected with lin- peripheral blood cells ± SCF. Six weeks later the bone
marrow of the animals was analysed by flow cytometry and PCR. 8 out of 8 mice in the SCF group showed multilineage hematopoietic engraft-
ment, while in the control group no human cells could be detected. Then, cord
blood cells were injected a SCF into irradiated NOD/SCID mice. Mice that
received SCF showed a 2.7 fold higher proportion of human CD45+CD11b+ cells
within the bone marrow six weeks after transplantation as compared to the
control group. In addition, human cells were detected by immunohistochem-
istry in liver and kidneys, but not in neural tissue and testes of the trans-
planted animals. The percentage of human cells increased by the addition
of SCF within the liver from 0.06 % to 0.13 % and within the kidneys from
0.02 % to 0.26 %. Conclusions: Our data indicate a profound increase in
the engraftment capacity of the transplanted cells. The organ specific differentiation
of the human cells detected in liver and kidneys of the recipients has to
be determined.

P531
Mesenchymal Stem Cells of Patients with Chronic Myeloid
Leukemia do Not Carry the Philadelphia Translocation
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Purpose: Mesenchymal stem cells (MSC) play a crucial role in the regenera-
tion of mesenchymal tissues and in the microenvironment of bone marrow
cells. The role of MSC in hematological malignancies, however, remains to be
further elucidated. Moreover little is known about the influence of MSC in
the development and maintenance of the malignant clone in chronic myeloid
leukemia (CML) patients. This disease is characterized by a reciprocal
translocation between chromosomes 9 and 24, which gives rise to the BCR-
ABL fusion protein, called Philadelphia (Ph) chromosome. Early works
showed that hepatocytes precursors, found in the liver of CML patients can be
Ph positive. Our intent was to elucidate if MSC isolated from patients with
CML in different stages of the disease carry the Ph translocation. Results:
MSC were isolated from bone marrow aspirates of 11 patients with CML at
different stages of disease. Five patients were analyzed at diagnosis, two after
allogenic stem cell transplantation, three on treatment with the tyrosine kinase
inhibitor imatinib and one on treatment with interferon alpha in combination
with hydroxyurea. Cells were isolated by density gradient methods, resus-
pended in RPMI1640 medium containing 10% fetal bovine serum and plated
in culture flasks to adhere. After 4-5 weeks of culture the expression of
several surface markers were detected by fluorescence activated cell sorter
(FACS). The presence of the Ph chromosome was assessed by fluorescence in
situ hybridization (FISH) and polymerase chain reaction (PCR). Results were
then compared with those obtained by analyzing the whole bone marrow.
Isolated cells grew to confluence after a few weeks of culture, appearing as
an adherent, spindle shaped cell layer. MSC were defined by expression of the
typical surface markers SH2 and SH2, as well as by absence of CD34, CD45
and CD14. MSC were then analyzed by FISH using probes for BCR-ABL.
We could not detect the Ph translocation in any of the analyzed patients, even
if it was present in the remnant bone marrow cells. Moreover, results did not
show that expression of BCR-ABL was measured by high sensitivity RT-
PCR. Conclusions: MSC of patients with CML are Philadelphia negative
irrespective of the stage of disease and the treatment given, suggesting that
these cells are not involved in the development of the malignancy.

P532
Preclinical Production of Human Lineage-Negative Cells
from Nonmobilized Blood for Regenerative Stem Cell
Therapy
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Purpose: Regenerative stem cell therapy (SCT) is currently tested in clinical
tests based on the hypothesis that adult SC can repair ischemic and metabolic
organ injury. The ideal type and source of cells for regenerative SCT are not
yet defined. Lineage (Lin)-depletion is an experimental procedure capable to
enrich all recently recognized SC types with regenerative potency. This study
was performed to define a practicable MoAb cocktail for Lin-depletion and to
test whether clinical scale Lin-depletion is possible. Methods: MoAbs
(CD2/14/15/19/56) GlycoporphinA were selected to specifically mark seven mature hematopoietic lineages. Lin7-negative (Lin7NEG) cells were
analyzed in peripheral blood (PB, n=9), mobilized PB (MPB, n=5), umbilical
cord blood (UCB, n=5) and bone marrow aspirates (BM, n=4) by flow cytom-
etry. The hematopoietic, mesenchymal and endothelial differentiation potential
of sorted Lin7NEG cells was evaluated under standardized in vitro culture
conditions. Preclinical Lin-depletion was tested with leukapheresis products
from PB following good manufacturing practice (GMP) principles. Results:
Lin7NEG cells comprised 0.23±0.04%, 0.27±0.03%, 0.53±0.07% and
0.49±0.03% of PB, MPB, UCB and BM, respectively. Basophil, CD34+ and
dendritic cells constituted the major Lin7NEG subpopulations (84±2%, 90±3%,
40±3%, 80±3% in PB, MPB, UCB, BM). Minor populations included CD7-
and CD45+ cells. In vitro differentiation analysis revealed the propagation of
endothelial and mesenchymal phenotypes from Lin7NEG SC products.
Conclusions: MSC of patients with CML are Philadelphia negative
irrespective of the stage of disease and the treatment given, suggesting that
these cells are not involved in the development of the malignancy.

P533
Functional Activity of Receptors of the Hypothalamic
Peptides Corticotropin-Releasing Hormone (CRH) and
Orexins A/B in Primary Human CD34+ Stem and
Progenitor Cells
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Recently, we have shown that primary human CD34+ stem and progenitor
cells express numerous neurobiological genes, among them G protein-
coupled receptors such as corticotropin-releasing hormone (CRH) 1 and 2
receptors, orexin/hypocretin 1 and 2 receptors and GABA A receptor. Most

Abstracts
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P535

**Expression of a Human Endogenous Retrovirus, HerV-K in Hematopoietic Cells**


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**Purpose:** In contrast to other human endogenous retrovirus families which are highly diverse, HerV-K proviruses possess all types of viral genes which are known in exogenous retroviruses. In malignancy (tumor cell lines, breast cancer, melanoma as well as leukemias and lymphomas) but also in many normal tissues, including placenta HerV-K mRNA expression has been detected. In hematopoietic stem cells, an interaction of HerV-K with elements from vectors which are used for gene therapy may lead to unforeseen consequences. Thus, the purpose of this study was to localize HerV-K mRNA expression in hematopoietic stem cells of different sources.

**Methods:** Quantitative real time PCR (RTQPCR) and RNA- in situ hybridization (RISH) for HerV-K was applied for detecting HerV-K expression in cytopsins and smears of blood and bone marrow samples of patients with lymphomas (9 blood samples and 3 lymph node), myelodysplastic syndromes MDS (10) and acute leukemias AL. In addition, 8 blood samples and 2 bone marrow samples from healthy donors as well as 9 cord blood samples and 20 apheresates from lymphoma patients containing PBSC were analyzed. From cord blood, CD34 positive cells were enriched using the DYNSAL system. Analysis of the same HerV-K gene (pol) in genomic DNA from above mentioned sources served as control.

**Results:** Relative mRNA levels of HerV-K in aphereses as well as in blood and bone marrow samples of patients with MDS, MDP and AML from subtypes with less than 1% CD34 positive cells were in the range of less than 2% in relation to housekeeping genes. However, in bone marrow samples of two AML and a CML blast crisis (all of them with more than 50% CD34+ cells) as well as in CD34+ cells from cord blood (but not from aphereses or normal bone marrow) as well as in three lymph nodes of patients with B-cell lymphomas, an overexpression of HerV-K was detected and confirmed by RISH. Interestingly, an enhanced expression of HerV-K was detected in cord blood derived apoptotic CD34+ cells, whereas the relative amount of HERV in genomic DNA did not change in samples from different sources.

**Conclusions:** Our data suggest that retroviral genes may be integrated in hematopoietic stem cells of different sources. Methods: Quantitative real time PCR (RTQPCR) and RNA- in situ hybridization (RISH) for HERV-K was applied for detecting HERV-K expression in cytopsins and smears of blood and bone marrow samples of patients with lymphomas (9 blood samples and 3 lymph node), myelodysplastic syndromes MDS (10) and acute leukemias AL. In addition, 8 blood samples and 2 bone marrow samples from healthy donors as well as 9 cord blood samples and 20 apheresates from lymphoma patients containing PBSC were analyzed. From cord blood, CD34 positive cells were enriched using the DYNSAL system. Analysis of the same HERV-K gene (pol) in genomic DNA from above mentioned sources served as control.

**Results:** Relative mRNA levels of HERV-K in aphereses as well as in blood and bone marrow samples of patients with MDS, MDP and AML from subtypes with less than 1% CD34 positive cells were in the range of less than 2% in relation to housekeeping genes. However, in bone marrow samples of two AML and a CML blast crisis (all of them with more than 50% CD34+ cells) as well as in CD34+ cells from cord blood (but not from aphereses or normal bone marrow) as well as in three lymph nodes of patients with B-cell lymphomas, an overexpression of HERV-K was detected and confirmed by RISH. Interestingly, an enhanced expression of HERV-K was detected in cord blood derived apoptotic CD34+ cells, whereas the relative amount of HERV in genomic DNA did not change in samples from different sources.

**Conclusions:** Our data suggest that retroviral genes may be activated in CD34+ cells from bone marrow of leukemia patients and in lymph nodes of lymphomas. In cord blood, it appears to be associated with apoptosis. This may support a critical stance regarding the use of cord blood derived stem cells as an alternative to allogeneic bone marrow transplantation. Similar amounts of HERV-K in different samples of genomic DNA of the same cells and the absence of HERV-K activation in normal bone marrow and PBSC indicates that activation of HERV-K is the result of regulation of gene expression rather than amplification.

Supported by Jubiläumsfonds der Österreichischen Nationalbank.
genes including inflammation related cytokines (i.e. IL1 and IL8) and cell-cycle related proteins (i.e. BTG2, DUSP2) are up regulated in comparison to cultures without the fibrils. **Conclusions:** Contact with collagen seems to increase the potential of CD34+ cells to form colonies without increasing the number of cells. This could be correlated with the enhanced expression of cytokines and proteins with antiproliferative characteristics on RNA level. All together the results suggest that contact with collagen results in a delay in cell cycle progression and reduced further differentiation into more committed cells.

**P537**

**Short Term Repopulating Cells Obtained from Cultures of Human Cord Blood CD34+ Cells Retain Multi-Lineage Activity with a Low Output of Megakaryocytic and Erythroid Cells in Vivo**

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**Purpose:** The clinical use of human hematopoietic cord blood (CB) transplants is hampered by a frequently associated delayed platelet recovery. We have previously shown that two types of human short term repopulating cells (STRC) can be enumerated by their ability to selectively engraft NOD/SCID-β2m-/- mice but not NOD/SCID mice and that these STRC may play an important role in the early neutrophil recovery seen in patients transplanted with cultured mobilized peripheral blood cells. Previous studies also showed that the delayed platelet reconstitution by CB transplants was likely due both to a low number of STRC in CB transplants and a reduced megakaryocytic and erythroid differentiation of individual CB STRC as compared to STRC in other types of transplants. **Methods:** To determine whether the numbers and in vitro differentiation activity of CB STRC could be modified in vitro, we have now analyzed the STRC present in suspensions of CD34+ CB cells before and after being cultured for up to 21 days in serum-free medium supplemented with EPO, flt-3 ligand, TPO, SCF, IL-6, IL-11, and IL-3. **Results:** Under these conditions, the number of CD41+ cells increased 40-fold at day 14, total colony-forming cells increased 30-fold at day 8 and megakaryocytic colony-forming cells increased 14-fold at day 8. In contrast, serial bone marrow aspirates performed on a total of 172 NOD/SCID-β2m-/- mice transplanted with cells from the same experiments showed an initial 2 to 10-fold decrease of STRC activity after 4 days in culture, followed by a recovery of STRC-M and STRC-ML numbers to 25% and 80% of input values, respectively, by day 8. Analysis of the size and lineage content of the STRC-M and STRC-ML clones generated in the mice showed no differences between the initial and culture-derived STRC. There was also no evidence of STRC that had become restricted to a particular myeloid lineage. **Conclusions:** Our findings demonstrate that the generation of STRC in cultures of CB CD34+ cells under conditions that support erythroid or megakaryocytic differentiation does not modify either their proliferative or their differentiation activity in vivo. This, in turn, suggests that such differences are developmentally regulated by mechanisms that cannot be readily activated by exposure to these conditions. The striking differences in the kinetics of in vitro defined progenitor populations and STRC also provide evidence that these assays detect distinct populations.

**P538**

**Microanatomy of Peripheral Collected Hemopoietic Stem Cells: Mitochondria and C-Kit**

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**Purpose:** Binding of stem cell factor (SCF) to c-kit can initiate intracellular Ca2+ release. Mitochondria are cellular Ca2+ buffer and play an important role in regulation of survival / apoptosis of haemopoietic cells. In excitable cells it has been shown that mitochondria are located on sides of Ca2+ release. In order to get more insight into mitochondrial location and activity and effects of progenitor cell mobilization in haemopoietic progenitor cells we investigated the mitochondrial network of peripheral collected CD34+ hemo-

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**Conclusions:** C-kit is expressed in clusters in HSCs and shows no colocalization with the mitochondrial networks of HSCs.
by material examination of a great number of transplanted patients. Oral
scraps are easily and non-invasive obtained from pts and can be evaluated
for degree of epithelial chimerism with the method described here. In order to
further elucidate the mechanisms of this biological phenomenon we recently
established PCR-based methods of laser-microdissected nucleated. Currently, we
are evaluating microsatellite STR markers, DNA rearrangement, SNPs or
germline DNA configuration in nuclei isolated from single or 5-10 pooled
Y+CK+/CD45- cells.

P540
Different Types of Transplantable Human Cord Blood Cells Display Different Aldehyde Dehydrogenase Activities

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Cells expressing aldehyde dehydrogenase (ALDH+) cells can be isolated by
FACS based on their ability to convert freely diffusible BODIPY-aminoc-
etaldehyde (BAAA) into an intracellularly-retained, negatively-charged-fluo-
rescent derivative (BODIPY aminocacetate, BAA). We now report that 0.9±0.1 %
of low-density cord blood (CB) cells are ALDH+ (11 exp’s) and removal of mature cells expressing lineage markers increases this proportion
~10-fold. 81±1% of ALDH+ CB cells with low light side scattering charac-
teristics (SSCLow) were found to be CD34+ and most of these cells did not
express CD38. ALDH+ SSClow CB cells are also highly enriched in their
content of primitive progenitors defined functionally; i.e., CFCs and LTC-ICs
(enriched 80-fold and 220-fold, respectively). Transplantable cells able to
regenerate human lympho-myelopoiesis for at least 16 weeks after intra-
venous injection into sublethally irradiated immunodeficient NOD/SCID mice
were found exclusively in the ALDH+ SSClow fraction of CB cells at a
frequency of 1/4,700. Additional removal of the ALDH+ SSClow popula-
tion of cells expressing markers not characteristic of transplantable human
hematopoietic cells allowed these to be purified a further 10-fold. In contrast,
CB cells that produce a transient output of myeloid progeny for 3-6 weeks in
the same mice appeared to be equally distributed between the ALDH+ and
ALDH- subsets of SSClow CB cells. These results provide further support for
the view that human CB contains biologically distinct and prospectively sepa-
rate subpopulations of cells that regenerate mature blood cells in vivo at
different rates and for different periods of time, and that these cell types can
be distinguished by measurable differences in their ALDH activity.

P541
Fetal Calf Serum (FCS) Free Isolation and Expansion of Human Mesenchymal Stem Cells (MSC) for Clinical Use
(Osteogenic Differentiation)

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Introduction: Mesenchymal stem cells (MSC) according to Caplan or
Prockop et al. are isolated and expanded in FCS-containing media and are
multipotent. MSC have already been used in the clinic for children with
osteogenesis imperfecta or metabolic diseases, and immunomodulation in the
prevention and treatment of GVHD in allogeneic stem cell transplantation.
Further applications in cell therapy are being developed in animal models and
may enter the clinic soon. One major hurdle are problems related to FCS,
such as viral infections, prions (BSE) and allergic reactions, which cannot be
excluded by several washings after expansion. Therefore, considerable efforts are
directed towards the development of FCS-free media for clinical use. In
particular, isolation of MSC from bone marrow has so far proven elusive
without the use of FCS. Methods: Nine donors gave 5 ml of bone marrow
aspirate each. Isolation of MSC was conducted according to Caplan et al. and
expansion used a modified protocol with low-density seeding. Medium D
consisted of DMEM low glucose with 10% of our selected FCS (gold stan-
dard), while media A, B and C were FCS-free. Isolation and expansion were
performed on surface-treated 24-well plates and in flasks. Cells were counted
under the microscope daily or every 3 days. Osteogenic differentiation was
induced by a standard protocol with β-glucosyrophosphate being added after
9 days. Adipogenic differentiation was likewise induced by a standard protocol
repeated twice. Differentiation was evaluated semiquantitatively with the use of
the microscope and standardized scales. Results: Isolation of MSC-like
cells was best in media C with C > D > B > A. Proliferation was exponential with
C > D > B > A. Morphologically, MSC isolated and expanded in medium C were indistinguishable from those in medium D. Phenotypic
markers of MSC grown in medium C and analyzed by flow cytometry were:
CD34-, CD45-, MHC class I-, CD90+, CD105+, MHC class II+, similar to
MSC isolated and grown in medium D. Moreover, MSC from medium C
showed more osteogenic potential in the von Kossa assay than those from
medium D in all cases: C ++, D ++, B +, A 0. Cells retained their immaturity
as shown by adipogenic differentiation: D ++, C +++, B +, A 0. Conclusions:
We were able to demonstrate that growth of MSC in a FCS-free medium is
feasible without addition of FGF (fibroblast growth factor). Based on our
data, we conclude that medium C is as good as our 10% FCS-containing
medium D with regard to both isolation and expansion of MSC, and in partic-
ular for osteogenic differentition, while media A and B are inferior. Seventy
million MSC can be grown within 4 weeks. Details of media A, B and C will
be provided at the meeting.

Poster Session: Acute Lymphoblastic Leukemia

P542
Combination of Imatinib mesylate (Glivec®) and Interferon-α (IFN-α) maintains complete hematologic remission in minimal residual disease positive (MRD+)
Philadelphia-positive acute lymphoblastic leukemia (Ph+ALL)

For the GMALL Study Group

Background: Remission duration in patients with advanced Ph+ALL treated
with single agent Imatinib is brief due to rapidly acquired resistance. IFN-α
may enhance the antileukemic activity of Imatinib. We evaluated the impact
of combined treatment with Imatinib and low-dose IFN-α in patients with
Ph+ALL who either were in complete hematologic but not molecular remis-
sion (CHR) during Imatinib monotherapy or were refractory to single-agent
Imatinib. Patients: Median age of the 15 pts. in CHR who were MRD+ with
Imatinib alone (group I) was 64 years. Imatinib was given for relapsed or
refractory Ph+ALL (n=2), persisting bcr/abl positivity after chemotherapy
(n=4), 1° line therapy in elderly patients (n=5) or nonhematologic toxicity
precluding further chemotherapy (n=2). Four pts. had previously undergone
allogenic (n=3) or autologous (n=1) SCT. Subcutaneous IFN-α was added a
median of 4.0 months after starting Imatinib, with dose escalation to a target
dose of 3 MU 3x/week if tolerated. Six pts. with Ph+ALL were enrolled
because of refractoriness to single-agent Imatinib (group II) administered for
relapse (n=5) or bcr/abl positivity after a 2° alloSCT (n=1). All pts. in this
group had received previous SCT (allogenic n=5, autologous n=1). Results:
Nine of 15 pts. (60%) enrolled in the setting of MRD positivity (Group I)
remain in CHR a median of 10 months (range 1.4-29.3+ mo.) after adding
IFN-α, two pts. converted to MRD negativity. Three pts. developed BM
relapse within 2.9 mo. after adding IFN-α, 2 pts. without prior CNS-irradia-
tion developed CNS-relapse during combination or shortly after its
discontinuation, one pt. died in CHR from septicaemia. Estimated progres-
sion-free survival (PFS) at 24 mo. was 58.4±14.4% and OS at 12 and 24 mo. was
68.9±13.1% and 51.7±17.9%. In contrast, 5 of 6 pts. with Imatinib refractory
disease (group II) progressed within 2.4 mo. (range 0.2-5.0 mo.) after IFN-α-
addition. One pt. was censored after 1.5 mo. at the time of alloSCT. Estimated
PFS at 5.0 months and OS at 13.9 months are 0%. Conclusion: Combination
treatment with Imatinib and low-dose IFN-α sustains prolonged hematologic
remissions in patients with Ph+ALL if started in the setting of minimal
residual disease. Conversely, additional IFN-α has limited or no benefit in
Ph+ALL patients who are refractory to single agent Imatinib at the hemat-
ologic rather than the molecular level.
P543
Treatment of Childhood Acute Lymphatic Leukemia in Mongolia according to the BFM95 Protocol
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Purpose: Acute lymphoblastic leukemia is the most common leukemia in children in Mongolia. The BFM95 protocol was introduced in 2002 to improve survival rate which was poor in the past. Methods: 17 patient were diagnosed in 2003 at the only treatment center for childhood leukemia at the Maternal and Child Medical Research Center of Mongolia in Ulaan Baatar. Median age of 6 male and 11 female patients was 9.5 years (2-17 years). Results: Patients suffered from bleedings (6), fatigue (7), bone pain (4) and had elevated white blood counts of median 13 x 10^9/l (range 2-270). Diagnosis was confirmed by typical lymphoblasts in bone marrow (median 62 , range 11-93 percent). and peripheral blood ( median 35, range 1-77 percent). 13 of 17 patients responded to induction therapy. 7 patients finished block I, 5 patients block II, 2 patients finished block II and one patient entered maintenance therapy. To this day, 7 patients are alive and well, 4 patients left therapy, 5 patients died of sepsis and other infections, 1 patient died of bleedings. Conclusions: These results are encouraging by showing that the BFM95 has been introduced successfully in Mongolia. Main problems arose from severe infections and from the abortion of the therapy by the patients or their parents. In the future efforts are necessary to improve the management of infections and to improve the patients compliance.

P544
Aleukemic AML Presenting as Nonimmune Hemolytic Anemia with Progressive Hepatosplenomegaly
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Purpose: Presentation of an uncommon case of aleukemic ALL. Case Report: Aleukemic myeloid leukemia in childhood often leads to diagnostic difficulties. Both in the pediatric and the adult age group, localized infiltrates of AML are most typically found in the gingiva or the skin. Involvement of liver and spleen, but without overt leukemia disease, is rare. Hemolytic anemia as paraneoplastic condition in leukemia has more often been described in AML, however, occasionally it is also seen in AML. In most cases it is immune-mediated. Here we describe a 3 years, 3 months old boy presenting with severe nonimmune hemolytic anemia, followed by massive hepatosplenomegaly. Sequential bone marrow examinations first showed from severe infections and from the abortion of the therapy by the patients or their parents.

P545
Treatment of Patients with ALL and Burkitt’s Lymphoma According to the “GALL/ALL Protocol 05/93” at the Department of Hematology & Oncology, Innsbruck. A Unicenter Experience
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Purpose: We performed a retrospective analysis on 43 newly diagnosed ALL-patients (pts) and 8 pts with Burkitt’s lymphoma treated at our Depart- ment during 1996 – 2002. Methods: The latest update was performed in December 2002 with a median follow-up of 20.5 months. Results: Median age was 38 years (range:16 to 63 yrs). All pts received potentially curative treatment following GMALL protocol 05/93. 76.5% achieved a complete remission (CR) after first induction therapy cycle, 12% had a PR and 4% did not respond to first induction chemotherapy cycle. The early death rate was 7.8%. After the 1st year of therapy (including induction, consolidation and reinduction) 86% achieved a CR. The remission duration, however, was short (median 6 months) with 17/51 patients (33%) even relapsing during ongoing therapy (both, on consolidation/reinduction or maintenance). The 5-years-OS of the ALL-pts was 33% (median OS 20 months) and 62.5% in the Burkitt’s lymphoma pts (24 months median OS). The OS of the 8 ALL-pts with a proven bcr-abl fusion gene in their initial cytogenetics was 18% at 4 years in comparison to 39% at 7 years in pts not harbouring the bcr-abl fusion gene. According to different therapy-arms patients within the B-ALL-therapy group (including pts with Burkitt’s lymphoma; n=9) had the best OS of 55.6 % at 5 years followed by pts that were treated according to the standard risk (SR) group with an OS of 45.5% (n=25). High risk pts (B-precursor-ALL with WBC >30000/µl, pre-pre-B-ALL or bcr-abl pos.; n=10) and the T-ALL-pts (n=7) had a similar OS of 18.4% and 17.9% at 3 years. 19/51 patients (37%; incl. 2 pts with Burkitt’s lymphoma) received an alloSCT (9 siblings, 10 MUD) due to relapse/refractoriness of the disease (8 pts) or due to a high risk constellation (10 pts), one was treated with an alloSCT from a sibling at SR. The 3-years-OS of the pts transplanted due to high risk was 33%, those of pts transplanted due to relapse/refractoriness was 18%. Main causes of deaths (60%) for all pts were fatal infections, 22% experienced disease progression, bleeding complications occurred in 3.7% (n=1), toxic drug effects occurred in 7.4% and other/unknown causes of death were found in 7.4%. Conclusions: Our data are comparable with published data. An initial high CR rate in ALL pts is followed by frequent relapses. Future therapy protocols should there- fore focus on improving maintenance strategies rather than on the induction phase. If possible, an alloSCT should be offered to all patients with high-risk ALL.

Poster Session: Acute Myeloid Leukemia
P546
High BAALC Expression Predicts Adverse Outcome in Acute Myeloid Leukemia with Normal Cytogenetics: Results of the SHG’96 Study
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Purpose: High mRNA expression levels of the human gene BAALC (Brain And Acute Leukemia, Cytoplasmic) have been shown to be an adverse risk factor in newly diagnosed acute myeloid leukemia (AML) patients (pts) (Blood 2003;102:1613). The first study included 86 de novo AML pts under the age of 60 with normal cytogenetics and a more favorable FLT3 mutations status, which were uniformly treated on the Cancer And Leukemia Group B protocol 9621. An independent confirmation of this data is indicated to validate and expand the initial results, so that BAALC expression can be exploited for risk adapted treatment stratification of intermediate risk AML pts. Methods: Here we present the results of a Süddeutscher Hämoblastoseruppe (SHG) study including 315 acute AML pts with normal cytogenetics, age <60 years and uniformly treated on the SHG 96 protocol. Median follow- up for all patients was 14 months (range 0-86 months) and for the patients
alive 27 months. BAALC expression was measured in pretreatment peripheral blood blasts by comparative real-time RT-PCR. Pts having BAALC expres-
sion values within the lower 75% were classified as low BAALC and pts having BAALC expression values within the upper 25% were classified as high BAALC. Results: There was no difference in the frequency of FLT3 internal tandem duplications between the high and the low BAALC group. Pts with high BAALC expression levels had an inferior overall survival (OS, median: 32 months vs. 45 months, P<0.03), event-free survival (EFS, median: 9 months vs. 14 months, P=0.0026), and disease-free survival (DFS, median: 9 months vs. 34 months, P=0.025). A multivariate analysis is in process to confirm that BAALC is an independent prognostic factor. Conclusions: This independent and larger study strongly supports the relevance of high BAALC expression as an adverse prognostic risk factor in intermediate risk AML pts with normal cytogenetics.

P547
Proteomic Pathway Discovery of C/EBPζ-p30 Target Proteins in Acute Myeloid Leukemia: The C/EBPζ-p30 AML Mutant Interferes with nuclear mRNA Processing
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Recently, it has been reported that mutations in the transcription factor C/EBPζ occur in 10% of patients with acute myeloid leukaemia (AML), which often leads to the expression of an N-terminal, truncated, 30kDa isoform. The mutant protein blocks the wild type C/EBPζ DNA binding and transactivation of granulocyte target genes in dominant negative manner and fails to induce granulocytic differentiation. In the present study, we applied a global proteomics approach (2D gel electrophoresis and MALDI-TOF mass spectrometry) to identify the target proteins of C/EBPζ-p30. Furthermore, we demonstrate protein pathway discovery on a global level using the ingenuity protein pathway finder software. We used K562 cells stably transfected with a C/EBPζ-p30 expression plasmid under the control of a beta estradiol inducible promoter as model system. Our preliminary results suggest that C/EBPζ-p30 modulates the expression of many proteins including signal transduction regulators, nucleic receptors, metabolic enzymes, oncopgenic proteins, heat shock proteins, proteins involved in oxidation and reduction, structural proteins and nuclear mRNA processing and export proteins such as hnRNP A1, A2 and JKBTP1del6, activated RNA polymeraseII transcriptional co-activator p15(TCP4) and translation elongation factor EF-Tu(p43). These data together with data from primary patients with N-terminal mutation of C/EBPζ suggest that C/EBPζ-p30 target proteins could be novel biomarker for the treatment of leukemia. Furthermore, it reveals protein networks in human granulopoiesis and how the balance between proliferation and differentiation can be disrupted in AML.

P548
C-RAF Mutations in Therapy-Related Acute Myeloid Leukemia
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Purpose: Constitutive activation of the RAS-RAF-MEK-ERK signaling cascade plays an important role in the pathogenesis of several malignancies including acute myeloid leukaemia (AML). We were interested whether abnormalities of the RAF proto-oncogenes occur in AML and whether they constitute potential targets for small molecule inhibitors and antisense strategies. Methods: cDNA expression of A-, B-, and C-RAF was assessed by real-time quantitative (rtq) PCR in 102 patient samples of AML, three AML cell lines and normal CD34+ cells of seven individuals. The results were corre-
lated with the phosphorylation status of ERK, as measured by immunoblot anal-
alysis. Furthermore, we screened for gene amplifications by rtq PCR and mutations by PCR and sequence analysis. In samples with RAF mutations we additionally searched for N-RAS point mutations and FLT3 internal tandem duplications repeats. Finally, frequency of RAF germline mutations was determined in the general population by performing a denaturing HPLC of 200 healthy individ-
uals. Results: Forty-five of 82 (54.9%) AML cases showed ERK phosphory-
lation indicating constitutive activation of the RAS-RAF-MEK-ERK pathway. A statistically significant correlation among RAF isoform expres-
sions could be shown indicating their common upstream regulation. However, there was no correlation between ERK phosphorylation and the expression levels of either A-, B- or C-RAF. Deletions of the B-RAF gene were observed in 20(9%) cases and were in concordance with cytoge-
etic results. Interestingly, in the samples of therapy-related AML one A-
RAF gene duplication and two novel C-RAF nonsense mutations were detected: S427G in one and 1448V in the other patient. Both mutations are located within the protein kinase domain and are associated with strong ERK activation, however, no RAS or FLT3 abnormalities were detected in these samples. Screening constitutional and primary tumor DNA revealed these mutations of germline origin with a frequency in the healthy population of <1/400. Conclusions: We have confirmed the high prevalence of a constitu-
tively activated RAS-RAF-MEK-ERK pathway in AML and furthermore identified two novel C-RAF germline missense mutations in therapy-related AML. Since both C-RAF mutations are associated with constitutive activa-
tion of the pathway, they may represent a novel target for specific therapeutic interventions in patients with therapy-related AML.

P549
Identification of Genomic Imbalances in AML with Complex Karyotype using Matrix-Based Comparative Genomic Hybridization
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Approximately 10 to 15 % of acute myeloid leukaemia (AML) exhibit complex karyotypes, i.e. three or more chromosome abnormalities without presence of a specific fusion transcript. Using chromosome banding analysis, the majority of such cases cannot be completely described due to the low resolution of this method. Comparative genomic hybridization to microarrays (matrix-CGH) is a novel technique that allows genome-wide screening for genomic imbalances at high resolution and thus may facilitate the identification of new regions harboring potential disease-related genes. We designed a microarray consisting of 2799 different human genomic DNA fragments cloned in bacterial artificial chromosome (BAC) or P1-derived artificial chro-
mosome (PAC) vectors. A set of 1502 of these clones covers the whole human genome with a physical distance of approximately 2 Mb. The remaining 1297 clones either continguously span genomic regions known to be frequently involved in hematologic malignancies (e.g. 1p, 2p, 3q, 7q, 9p, 11q, 12q, 13q, 17p, 18q) or contain oncogenes or tumor suppressor genes (n=687). In a first series we studied 30 AML cases with complex karyotypes. Genomic losses were more frequent than gains. The most frequent aberration was deletion of chromosome 5q (26 of 30 cases), followed by genomic losses affecting chromosomes 7a, 7q (53,3%), 17p (50%, 2q, 4p, 6,7%, 8p, 12p, 16q, and 18q (26,7% each). Genomic amplifications were identified in 12 cases. These amplifications were located in chromosomal bands 8q24, 9p21-
p24, 10p13-p15, 11q13-q23, 12p13, 13q12-q14, 20q11.1, 21q22, and 22q11-q22. Our preliminary data demonstrate the potential of matrix-CGH with regard to spatial resolution and sensitive detection of genomic imbal-
cances. Analysis of a large series of AML cases with complex karyotype may lead to the identification of novel candidate regions and pathogenetically rele-
vant genes.

P550
Activating Mutations of the SHP-2 Protein Tyrosine Phosphatase in Adult Patients with Acute Myeloid Leukemia
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Purpose: Acute myeloid leukaemia (AML) represents a heterogeneous group of early stem cell malignancies characterized by an uncontrolled expansion of malignant cells blocked at certain stages of myeloid differentiation. Several
alterations have been described, including translocations involving genes of the CBF family, as well as activating mutations of signal transduction proteins, e.g. N-RAS, K-RAS or the F35 receptor tyrosine kinase (RTK). More recently, mutations in the SHP-2 protein, a tyrosine phosphatase involved in RTK signal transduction and regulation, have been described in patients with juvenile myelomonocytic leukemia (JMML) as well as paediatric patients with AML. In order to investigate the prevalence and the prognostic relevance of this gene in adults, we analyzed the mutational status of SHP-2 in a cohort of patients with AML. Patients and Methods: DNA prepared from bone marrow or peripheral blood samples taken at diagnosis from 426 patients treated in the AML96 protocol of the SHG Dresden was analyzed. Screening for mutations in the mutational hot spot in exon 3 of the PTPN11 gene coding for SHP-2 was performed on a dHPLC system (Transgenomic). Samples with aberrant dHPLC chromatograms were sequenced. Results: In the 426 patients investigated so far, 13 PTPN11 mutations were found (3.1%). All mutations consisted of single base pair exchanges in codons 60-76, most mutations occurred in codons 69 (3) and 72 (3). No association with age and other clinical parameters was found. Seven patients had a normal karyotype, while in six patients cytogenetic aberrations were identified. Interestingly 3/13 patients with PTPN11 mutations had FAB M5b morphology compared to only 12/413 PTPN11 neg. cases (P = .008). The CR rate was comparable in SHP-2 mutation positive and negative cases, while in patients < 60 years, however, the overall outcome of patients carrying this alteration was poor, worses prognosis of patients with SHP-2 alterations. We are currently investigating the potential role of this alteration.

P552
Proteomic Identification of the C/EBPalpha Multiprotein Complex Reveals that Downregulation of c-Jun N-Terminal Kinase 1 (JNK1) Activity Leads to loss of C/EBPalpha Function in Patients with Acute Myeloid Leukemia
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Acute myeloid leukemia (AML) is characterized by prominent myeloid blasts and genetic aberrations hampering protein function. Functional inactivation of transcription factor C/EBPalpha leads to AML, whereas activation of C/EBPalpha restores normal myeloid stem cell differentiation. We and others have shown that protein-protein interactions of C/EBPalpha plays a pivotal role in myeloid differentiation and AML. Systematic identification and characterization of proteins at a global proteome-wide level using proteomics has emerged as a novel tool. We hypothesized that the identification and functional characterization of all C/EBPalpha interacting proteins will lead to novel insights into the systems biology of C/EBPalpha. Thus, the GST-DNA-Binding domain of C/EBP was incubated with nuclear extracts of U937 cells, interacting proteins were identified by two-dimensional gel electrophoresis and MALDI-TOF mass spectrometry, and functionally characterized by bioinformatic pathway discovery with subsequent biological and mechanistic confirmation of the discovered pathway. We were able to identify known as well as novel interacting proteins of C/EBPalpha which include p21 activated kinase 6, MAPK-1, huntingtin protein, transcriptional coactivator sp110, and c-Jun N-terminal kinase 1 (JNK1). JNK1 binds to the C/EBPalpha DNA binding domain in GST-pull down and in co-immunoprecipitation assays in vitro and in vivo, respectively. JNK1 phosphorylates and enhances the half life of C/EBPalpha protein. Therefore, JNK1 enhances the transactivation and DNA binding activity of C/EBPalpha which in turn leads to G0-G1 arrest in cell cycle. JNK mRNA expression as well as its kinase activity is low in AML patients which supports the hypothesis that C/EBP alpha might be inactive in AML because of lack of JNK1 activity. Thus, we propose JNK1 phosphorylates C/EBPalpha and stabilizes the half life of C/EBPalpha and thereby activates it. In AML, however, JNK1 activity is downregulated leading to a loss of C/EBPalpha function.

P551
Identification of Methylation-Silenced Genes in Acute Myeloid Leukemia
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Alterations of chromatin structure play a predominant role in the pathogenesis of acute myeloid leukemia (AML). These conformational changes are mainly caused by mechanisms like promoter methylation and histone acetylation. For example, methylation of tumor suppressor genes such as p15 ranks among the most common aberrations found in AML. In addition, the translocation product PML-RARA has been demonstrated to induce methylation of the differentiation-associated RARbeta2 gene. However, no systematic knowledge exists, which specific genes are silenced by methylation-dependent mechanisms as a consequence of fusion protein expression. Here, we applied a microarray-based approach to screen for such genes. As a cell line model, we used U937 cells inducibly transfected with AML1-ETO or PML-RARA. In the absence of the fusion proteins, cells were exposed to the demethylating agent 5-azacytidine for 6 days. Genome wide changes in gene expression levels were analyzed by high-density microarray analyses. In a first step, we analyzed changes in the expression profiles before and after 5-azacytidine exposure. Overall, 118 genes were consistently induced at least 5-fold by demethylation treatment in the absence of translocation products. Among these genes encoding S100 proteins, transcription factors and chemokines. More than 90% of the induced genes identified in the microarray analyses were confirmed by real-time RT-PCR. Bisulfite analyses for some of the identified genes verified Cpg-island methylation of the genes’ promoters in vivo, establishing the overall validity of the approach. Subsequently, the cells were released from 5-aza-didin, and the effects of the fusion proteins AML1-ETO or PML-RARA were studied. This approach allowed us to identify genes that were repressed by DNA methylation in a fusion-protein-dependent manner. Here, several unexpected findings were obtained. For example, a myeloid transcription factor and growth suppressive genes were repressed by PML-RARA. Taken together, these data indicate that genes involved in differentiation and growth suppression are silenced by methylation in leukemia cell lines. Some of these genes were shown to be specifically repressed by AML fusion proteins, presumably by mechanisms involving DNA methylation and histone acetylation. In summary, our approach provides a novel link between DNA methylation and the identification of genes that were repressed by DNA methylation in a fusion-protein-dependent manner.

P553
Loss of the Short Arm of Chromosome 17 in Patients with de Novo Acute Myeloid Leukemia is Indicating Inferior Prognosis even in the Absence of Additional Cytogenetic Aberrations
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for the German multi-centre treatment trial of the SHG AML96 study group

Purpose: Loss of the short arm of chromosome 17 is a rare cytogenetic abnormality in patients with AML. A deletion of 17p can be of variable extent but usually involves the tumour suppressor gene p53 (Sonen et al. 1998) by allelic loss. The tumour suppressor protein p53 plays an important role in the control of genetic stability and it is well described that loss of p53 function contributes to malignant progression. In AML p53 inactivation is accompanied by chromosomal instability leading to complex aberrant karyotypes, where the outcome is poor. Results: Out of the 1331 patients with de novo AML included into the multicentric SHG-AML96 study 111 patients had a complex aberrant karyotype. 38 (34 %) of these patients were shown to have a loss of 17p. There were only 12 (1 %) patients with a loss of 17p as a single aberration or accompanied by only one other abnormality. We compared the clinical course of patients with singular loss of p53 with patients showing instable karyotypes as defined by additional (multiple) aberrations. Five out of the 12 patients (41.7 %) achieved CR after the double induction therapy of the SHG96 study including intermediate dose Ara-C (MAV-MAMAC). However, outcome was poor with 2 early relapses after 224 and 282 days, respectively, the other 3 patients succumbed due to early death. Generally, overall survival and event free survival of the 12 patients were inferior as compared to patients with a normal karyotype (p<0.001) and not different from patients with a complex aberrant karyotype. After the period of 2 – 417 days (median survival 109.5 days) no patients was alive. Conclusion: In conclusion, patients with a loss of the short arm of chromosome 17 are a high-risk group in patients with de novo AML.
Risk Adapted Therapy in Acute Promyelocytic Leukemia: AIDA 2000 - A DSIL Study Group (Deutsche Studieninitiative Leukämie) Treatment Protocol

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Purpose: Since November 2000 we registered 41 patients (23 pat. ≤ 60 ys, 18 pat. > 60 ys) with newly diagnosed acute promyelocytic leukemia (M3 / M3v; M3v = 33 / 8 pat.). Based on different international studies we established a risk adapted treatment protocol according to age (≤ 60 ys / > 60 ys) and initial leucocyte count (≤ / > 10 GPT/l). The main objectives of the treatment protocol are to investigate 1. the efficiency and toxicity of the treatment plan, 2. the patients outcome with the prognosis dependent reduction of the intensity of consolidation therapy and whether the high risk group of patients seems to benefit from more intensive therapy, 3. the detection of kinetics of the PML-RARα transcript for further therapeutical decisions and 4. correlating other pretreatment factors with the risk of relapse.

Therapy: In the induction therapy ATRA 45 mg/m²/d until CR but not more than 90 days and idarubicin 12 mg/m²/d for 4 days were administered. The 1. consolidation therapy consists of daunorubicin 60 mg/m²/d for 3 days in patients ≤ 60 ys, 40 mg/m²/d for 3 days in patients > 60 ys and additionally Ara-C in patients with initially leucocytes > 20 GPT/l 200 mg/m² as continuous infusion for 7 days in patients ≤ 60 ys and 100 mg/m² in patients > 60 ys. As 2. consolidation course mitoxantrone 10 mg/m²/d for 3 days and Ara-C in patients with initially leucocytes > 20 GPT/l twice daily 3 g/m²/d for 4 days in patients ≤ 60 ys and 1 g/m²/d in patients > 60 ys were administered. As maintenance therapy over 2 years patients are treated with 6-mercaptopurin daily 90 mg/m²/d, methotrexate weekly 15 mg/m²/d and intermittend ATRA 45 mg/m²/d over 15 days every 3 months. Results: At time of evaluation 90 % (35/39 pat.) of all patients and 100 % of patients ≤ 60 ys achieved complete remission. 6 patients died (1 patient ≤ 60 ys, 5 patients > 60 ys), 1 patient during induction therapy due to tumor lysis syndrome and haemorrhagic disorder, 4 patients after induction therapy due to sepsis, pneumonia and multiorgan failure, another during consolidation therapy due to sepsis. The overall survival was 85 % in all patients, there was a significant difference in overall survival due to age (pat. ≤ 60 ys: 95 %, pat. > 60 ys: 72 %, P = 0.04). No significant difference in survival was shown in patients with M3 / M3v, leucocyte count ≤ / > 10 GPT/l, t (15;17) with or without other chromosomal abnormalities. 2 patients relapsed. Conclusion: Comparable to other trials these results indicate a high effectiveness in the treatment of acute promyeloctic leukemia.

Hyperdiploidy in Acute Leukemias and MDS is Associated with Poor Prognosis

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Purpose: In order to determine the frequency and prognostic impact of hyperdiploidy, we reviewed 332 cases of acute myeloid leukemia (AML), 718 cases of myelodysplastic syndrome (MDS) and 214 cases of acute lymphoblastic leukemia (ALL) that were karyotyped and followed up at our institutions.

Results: Hyperdiploidy (>48 chromosomes) was found in 14 of 214 (6.5%) patients with ALL. Twelve patients had massive hyperdiploidy with >250 chromosomes (median 52, range 49-82). Three of those patients were also Philadelphia-chromosome positive. All 14 patients received induction chemotherapy. Complete remission (CR) was achieved by 11 patients, 9 of whom relapsed. Median survival was 13 months. Hyperdiploidy was present in 4 of 332 (1.2%) patients with AML. Three had a massive hyperdiploidy (50, 54, and 77 chromosomes, respectively). Two of them achieved CR, but both relapsed within three months. None of the patients is alive, and median survival was only 5 months. A hyperdiploid karyotype was documented in 12 of 718 (1.7%) patients with MDS. The diagnosis was refractory anemia (RA) in 4, and RAEB or RAEB-T in 8 patients. Seven patients had a massive hyperdiploidy (median 52, range 50 to 93 chromosomes). In 4 patients, the disease showed progression to AML. Among 5 patients who received induction chemotherapy 3 achieved CR but had an early relapse. Median survival in MDS patients with hyperdiploidy was 10 months (5-18).

While it is known that hyperdiploidy is a complex karyotype, which is an important adverse risk factor in MDS and AML, the prognostic impact of hyperdiploidy in hematological malignancies is not well established. In childhood ALL, hyperdiploidy is found in up to 27% of patients and is associated with a favourable prognosis, which appears to be different in adult ALL. In myelodysplastic syndromes, we are not aware of any previous investigations addressing this issue. For patients with AML, Iyer et al recently reported that hyperdiploidy is associated with a poor prognosis. Conclusions: Our results indicate that hyperdiploidy is not a rare event in adult ALL, where it is associated with an unfavorable prognosis. Only two patients, both with T-ALL, remain alive, after 43 and 46 months of follow-up, respectively. Hyperdiploidy was rare in our patients with AML and MDS, but clearly associated with a poor outcome. In summary, we found that hyperdiploidy is an unfavorable prognostic factor in adult patients with hematological malignancies such as ALL, AML, and MDS.
During the last years FLT3 mutations became important prognostic factors in acute myeloid leukaemia (AML). Considering the frequent expression of wild type FLT3 in leukaemic and normal bone marrow and its function as regulator of differentiation, apoptosis and modulation of the immune system, the role of the wild type FLT3 transcript level in adult AML is not fully understood. In the presented work FLT3 transcript levels of 186 AML and 7 healthy donors were assessed by real time PCR patients and correlated to cytogenetics, FAB, mutation status, as well as other clinical relevant factors. We found that FLT3 expression significantly correlated with the number of blasts (p=0.005) and leukocytes (p=0.005). FLT3 expression levels were not distributed equally within different FAB subtypes (M3 < M2 < M6 < M4 < M0, M1 < M5). Thus, the lowest median levels were assessed in FAB M3/M4 (n=22) and the highest mean levels in FAB M5a (n=7). According to cytogenetics the lowest levels were detected in t(15;17) and t(8;21) and the highest in t(11q23) and normal karyotype. Differences between the lowest (t(15;17) and highest expressers (t(1q23)) were significant (p=0.001). No difference could be shown within the group of secondary AMLs and therapy associated AMLs compared to idiopathic AMLs. In contrast to previous publications no difference of FLT3 expression could be detected between AMLs with FLT3 length mutations (FLT3-LM) (n=52), FLT3 point mutations (n=11) and without any FLT3 mutation (n=114). In addition, FLT3 mRNA expression as assesses by real time PCR was correlated to expression measured by microarray analysis (Affymetrix) and FLT3 protein expression (CD135) as assess by flow cytometry and was found to significantly correlate with both parameters (p=0.001 and p=0.003, respectively). Using Cox-regression analysis a significant influence of expression level to overall and event free survival could not be shown yet. Further analysis are needed to evaluate the importance of FLT3 expression to identify new possible risk groups, which might have implication on therapy stratification.

**P559**

**Outcome of Patients with AML after Potential Curative Treatment at the Department of Hematology & Oncology, Innsbruck**

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**Purpose:** We performed a retrospective analysis on 73 newly diagnosed patients (pts) with AML (74% de-novo-AML), treated at our Institution during 1996–2002. **Methods:** The latest update was performed in December 2002 with a median follow-up of 19 months. **Results:** Median age was 57 years. 46 patients were ≤ 60 years and 27 pts > 60 years. All pts received potentially curative treatment regimens. After first cycle of induction therapy 63% achieved CR (≤ 60 yrs: 70%, >60 yrs: 52%), 10% PR and 16% had no response. Early-death rate was 9.6% (≤ 60 yrs: 4.3%, >60 yrs: 18.5%). Median remission duration for all 73 pts was 9.6 months, the median overall survival (OS) was 19.1 months (cumulative-OS: 33% at 4 years). The median OS for patients ≤ 60 yrs. was 22.3 months in comparison to 13.7 months for elderly patients > 60 years. The OS at 4 years in patients ≤ 60 years was almost twice as high as in elderly patients (39% vs. 22%). Within the group of elderly patients major differences in survival rates were seen. The majority of patients ≥ 65 years relapsed early and their OS at 3 years was 16% (n=14) in comparison to 29% for patients between 61 and 64 years (n=13). According to cytogenetic risk groups the patients ≤ 60 years with low risk cytogenetics (t(8;21), t(15;17), inv16) had a five-year-OS of 57% (n=7), with intermediate risk (n=27). So far no patient in the high risk group survived longer than 2 years; the cumulative survival after one year was merely 13% (n=10). Patients receiving at least one course of intermediate-dose-cytarabine during consolidation therapy (1 g/m²/d over 5 days) or high-dose cytarabine (≥ 2 g/m²/d over 5 days) performed better (OS 58.8%, n=12 and 42.2%, n=27 at 5 years, respectively) than patients receiving standard doses of cytarabine (100–200 mg/m²/d over 7 to 10 days; 3 years-OS: 16%, n=34). The administration of (high or intermediate)-dose cytarabine instead of standard doses prior to alloSCT had no influence on OS. **Conclusions:** Our data suggest, that the application of intermediate and high doses of cytarabine definitely improves OS in patients not receiving an allotransplant. In contrast, the cumulative dose of cytarabine applied before alloSCT had no impact on the outcome of alloSCT. In addition, for most pts between 61 and 64 years intensive treatment with curative intent should be performed whereas for the majority of patients ≥ 65 years intensive chemotherapy should be considered to palliative treatment options or best supportive care.
otype anomaly (11/15/15), (t8/21), (inv)(16) achieved cytogentic remission. The CCR rate was substantial lower in patients with intermediate-risk cyto-
genetic group (25/56) and in patients with complex karyotype (11/32). Median survival in the group achieving CCR was 42 months as compared to 13 months in patients with persisting abnormal karyotype after induction (p=0.00005). The difference remained highly significant when calculated only for patients with complex or intermediate-risk karyotype (median survival 31 vs 12 months). Conclusions: The examination of the karyotype after induction is useful to detect a persisting malignant clone and gives addi-
tional information to cytology. Achieving cytogentic remission after induc-
tion therapy is strongly correlated with a better long-term outcome. Patients with a persisting abnormal karyotype can be recognised as high-risk patients and should therefore receive intensified treatment.

P561 Feasibility of Fast Search Diagnostics for AML Patients in a Multi-Center Study – First Experience within the DSIL AML2003 Trial

Schäich D.

Induction Therapy in Peripheral Blood Stem Cell Transplantation (pBSC) of Acute myeloid Leukemia – First Experience within the DSIL AML2003 study group

Since December 2003 the AML2003-Study of the German Study Initiative Leukemia (“DSIL” former “SHG Dresden”) is activated. It represents the follow-up study of the AML96 protocol for patients <= 60 years. AML2003 is a prospective randomized trial, to investigate the value of an intensified treatment strategy, i.e. early allogeneic stem cell transplantation in aplasia after induction therapy, for high-risk patients. Therefore, a rapid analysis of risk factors (cytogenetics, FLT3 status and clearance of blasts after first induction) and the donor situation is of utmost importance. This “fast search diagnostcs” together with routine analyses of morphology and immunophe-
notyping is accomplished centrally in all enclosed patients. Within the first 5 months 67 patients with a median age of 48 (17-60) years were included in the study. Fast search diagnostics including the information about possible related or unrelated donors was complete with a median of 14 days after arrival of the bone marrow samples for all patients. 39/67 patients were upfront randomized into the intensified treatment arms. Out of these 19 (49%) patients with high-risk have been identified. Seven high-risk patients (37%) received early allogeneic stem cell transplantation in aplasia after the first (n=3) or the second (n=4) induction therapy course within the protocol. Three were transplanted with stem cells of related and four of unrelated donors. In three patients early allogeneic transplantation was not possible due to the absence of an available donor. One patient has not finished the first induction course yet. Eight patients showed refractory disease after the first induction course and were referred to salvage therapy. Two of them already received an allogeneic transplant. These encouraging preliminary results show that fast search diagnostics is feasible in a large multi-center study. This leads to a significant proportion of early allogeneic stem cell transplants in aplasia after induction therapy within the group of high-risk AML patients.

P562 Case Report: Detection of Minimal Residual Disease after Induction Therapy in Peripheral Blood Stem Cell Apheresis by Flow Cytometry and FISH in a Patient with AML FAB – M7

Schöndube D., Matyis A., Ratei R., Einsele H., Schoch C., Haferlach T., Karawajew L., Rhein P., Ludwig W.-D.

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Detection of minimal residual disease (MRD) in peripheral blood or bone marrow is a critical factor for treatment stratification and prognosis for patients with AML in complete remission after induction chemotherapy. In recent years autologous peripheral blood stem cell transplantation (aPBSCT) after consolidation therapy has been used as maintenance therapy in AML patients, but until now few studies have addressed the issue of MRD-detection in apheresis products in these patients. We report a 43 years old female with anemia, mild thrombocytopenia and blasts in the peripheral blood. Immunophenotyping showed a blast population characterized by the expression of CDA3, HLA-DR, CD117, CD133+ and CD61 so that AML FAB M7 was diagnosed. Bone marrow showed severe fibrosis. Cytogenetic analysis revealed a complex karyotype with a 7q31 deletion detected by FISH in 90% of blast cells. The patient was treated according to the AMLCG 99 – protocol with two induction courses of high dose Cytarabine and Mitoxantrone resulting in complete morphological remis-
sion in peripheral blood, although bone marrow cytology was not representa-
tive due to persisting fibrosis. Allogeneic bone marrow transplantation would have been the therapeutic option of choice for this patient. But as no HLA-
identical donor was available we collected autologous peripheral stem cells (aPBSCT) after induction therapy in order to perform high dose chemotherapy. PBSCT apheresis has then been analysed with FISH and resulted to be nega-
tive for the described 7q31 deletion. Subsequently, the PBSCT apheresis product was sorted by flow cytometry based on CD34 expression and FISH was performed again on CD34+ sorted cells. 7q31 deletion could now be clearly detected, demonstrating the persistence of the initial leukemic clone. Ration treatment intensification requires knowledge about the parameter that is to be intensified. I.e. there must exist a clearly established relation between that parameter and treatment effect (e.g. CR rate). A correlation of such parameters such as total drug dose, dose intensity or area under the curve (AUC) (esp. of cytarabine) and treatment effect has so far never been system-
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tically been demonstrated in AML. Esp. AUC is often considered to be-
Conclusions: 1) We established the concentration coefficient N of cytarabine in AML, which is a useful parameter to formally quantify the relative relevance for the clinical effect of exposure time versus concentration. 2) N-AUC of cytarabine is highly correlated with the clinical effect, which suggests that it might be a better parameter for "treatment intensification" than dose, dose intensity or AUC. 3) The concept of N-AUC allows the realistic comparison of pharmacokinetically widely diverse applications of cytarabine and immediately suggests rational ways of treatment intensification.

P564
The Bisphosphonate Zoledronate Shows Cytotoxic Effects in AML, but is Not Synergistic with Cytarabine

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Purpose: The third generation bisphosphonate zoledronate (ZOL) (Zometa®, Novartis), a heterocyclic imidazole, has been shown to exert antitumorigenic activity in various cell lines including breast cancer, multiple myeloma and chronic myeloid leukemia. The main mechanism of action is supposed to be inhibition of ras protein signaling by inhibition of both farnesylation and geranylgeranylation. In this study we investigated the anti-leukemic activity of single ZOL and in combination with Cytarabin in Acute Myeloid Leukemia (AML). Methods: HL60 cell lines (n=5) and fresh blasts (n=4) from patients with AML were studied. For assessment of cytotoxicity and possible interactive effects of ZOL and AraC the WST-1 assay was used. The assay is based on cleavage of the tetrazolium salt WST-1 to a coloured formazan dye by mitochondrial dehydrogenase of viable cells. This signal can be detected spectrophotometrically and directly correlates to the number of viable cells. The assay was performed in 96 well plates with increasing concentrations of ZOL and AraC over 24 hours. All experiments were carried out at least in triplicate for HL60. Interactions were assessed by isobologram analysis. Results: ZOL shows significant anti-leukemic activity in HL60 cell lines and AML blasts. Mean LC50 (lethal concentration for 50 % of cells) in HL60 cells was 222 µg/ml after 24. In cells from patients the mean LC50 was 606 µg/ml (range: 366.8 – 855.5 µg/ml; n=4) after 24 hours. Using isobologram-analysis for evaluation of interactions, concubation of ZOL with AraC for 24 hours in HL60 model could be shown to result in subadditive cell kill. Conclusions: These data show that ZOL is active in HL60 cells as an AML model but also in primary AML blasts in vitro. Although the incubation of ZOL alone resulted in substantial cell kill, no synergistic but rather subadditive effect interactions with AraC were observed in HL60 cells.

P565
Treatment of Acute Myeloid Leukemia (AML) in patients above the age of 60 years: A report of the AML97#38 study of the East German Hematology and Oncology Study Group

For the East German Hematology and Oncology Study Group (OHSO), Germany

Purpose: Patient selection still plays an important role in curative treatment trials of elderly patients with AML. In the present AML97 study all patients above the age of 60 years with AML were reported and treated using either a curative, palliative or supportive protocol. Methods: Since March 1998 a total of 520 patients were enrolled in this protocol (curative 375, palliative 112 and supportive 33). In the curative arm patients were treated with one or two courses of induction therapy (AraC 2 g/m² iv day 1, 3, 5 and mitoxantrone 10 mg/m² iv day 1-3) followed by 2 consolidation courses (AraC 240 mg/m² iv day 1-5 and mitoxantrone 10 mg/m² iv day 1-2). Palliative therapy consisted of idarubicin 10 mg po day 1 in combination with either thioguanine 40 mg po day 1-5, or AraC 80 mg po day 1-5 or etoposide 100mg po day 1-5. Results: Cytogenetics at diagnosis was the most important prognostic factor for CR (p=0.0005, multivariate analysis). 93 % of the patients with favourable, 80 % with normal, 79 % with other aberrations and only 46 % with unfavourable karyotype achieved CR. The EFS at 2 years was 0.39 ± 0.14 (median 463 d), 0.24 ± 0.05 (median 240 d), 0.18 ± 0.07 (median 206 d) and 0.08 ± 0.04 (median 84d) for patients with favourable, normal, other and unfavourable cytogenetics respectively. Furthermore with a median follow up of 283 days (range 33 – 1688) the actual OS was 0.58 ± 0.15 (median 762 d), 0.38 ± 0.06 (median 421 d), 0.39 ± 0.09 (median 411 d) and 0.14 ± 0.05 (median 165) for patients with favourable, normal, other and unfavourable cytogenetics respectively. The median survival for patients treated with palliative Therapy was 54 days and only 12 days for patients treated with supportive care. Conclusions: Our used curative arm of the protocol is able to induce CR in a high proportion of elderly patients with AML with an acceptable early death rate. Despite high CR rate, EFS is still low. The treatment results with palliative chemotherapy are disappointing. These results suggest the need to develop novel therapeutic strategies for these elderly patients and a risk-adapted treatment. To improve the EFS a new AML phase III study will start in July 2004 within the “Kompetenzzentrum – Acute leukaemia”. One aim is the improvement of consolidation therapy by using low dose TBI based conditioning regimen and maintenance treatment with ciclosporine and MMF followed allogenic PBSC (MRD or MUD) or application of more intensive consolidation chemotherapy.

P566
High-Dose Melphalan is an Effective Salvage Therapy in Patients with Acute Myeloid Leukemia who Relapse after an Autograft

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Purpose: Recurrent disease remains the most frequent reason for treatment failure in patients with acute myeloid leukemia (AML). In our center, salvage chemotherapy includes mitoxantrone, topotecan and cytarabine. In this study we investigated the antileukemic activity of high-dose melphalan followed by an autologous peripheral stem cell transplantation (auto-PBSC) in patients with untreated relapse. Methods: We therefore applied high-dose melphalan (HD-Mel, 140-200 mg/m²) and auto-PBSC to eight patients (median age 57, range 46-60 years) with recurrent AML: 2/8 patient had relapsed after conventional chemotherapy and 6/8 after auto-PBSC performed in previous CR. At the time of HD-Mel salvage therapy, 2/8 patients were in untreated relapse, while 6/8 had patients received one course of MTC 27-41 days prior to HD-Mel, leading to subsequent CR in one patient only. Five patients proved to be refractory to MTC. Results: All 8 patients, including those not responding to MTC, achieved CR after salvage therapy with HD-Mel and auto-PBSC. Median cumulative duration of neutropenia was 56 days in the 5 patients refractory to MTC but only 2 weeks in patients with untreated relapse. CR lasted 2–7 months, thereby facilitating further allogeneic PBSC: 5 patients proceeded to a matched unrelated donor (MUD)-PBSC after 1.7–3.1 months. Two of five patients remained in CCR after 6 and 28 months and 3/5 days after MUD-PBSC in CR due to infection and/or GVHD. Three patients did not receive a consolidating allogeneic PBSC, relapsed again after 2, 5 and 7 months and eventually died. Conclusions: We therefore conclude that HD-Mel can safely be administered to intensively pretreated patients. It may induce second or subsequent remission even in patients refractory to conventional chemotherapy regimens with reasonable toxicity and a low rate of infectious complications, thereby facilitating allogeneic PBSC in the majority of patients. Thus we propose to use HD-Mel as first-line salvage therapy in relapsed AML patients with stored autologous stem cells.

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Onkologie 2004;27(suppl 3):1–230 123

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Abstracts

P567

Treatment of Acute Myeloid Leukemia (AML) in Patients Below and Above the Age of 60 Years: Influence Factors for CR and Hematological Recovery. Study of the East German Hematology Oncology Study Group (OSHOT)


For the East German Hematology Oncology Study Group (OSHO), Germany

Purpose: The OSHOT performed AML - phase III study’s in patients < 60 years (AML 96) and > 60 years (AML 97). Methods: All patients were treated with one or two courses of induction therapy (AraC 2 g/m² iv on day 1,3,5,7 in combination with idarubicine 12 mg/m² day 1-3 in AML 96 or mitoxantrone 10 mg/m² day 1-3 in AML 97) followed by 2 consolidation courses. Consolidation courses were different in both study’s. Due to identical chemotherapeutical regimes for younger and elderly patients, we analysed the whole patient population regarding CR rates and hematological recovery of leukocytes and platelets after induction therapy. Altogether we analysed 522 patients, 323 below and 209 above the age of 60 years. We performed a multivariate analyse (logistic regression model) to identify factors of influence for CR rate. We checked cytogentic at diagnose, classification (de novo or secondary AML), WBC (<2/2-9/0 x 10⁹/l), sex, FAB-classification (FAB-M0/M1/M2/M3/M4/M5/M6/M7) and age. Results: The most important and highly significant parameter for CR rate was cytogentic at diagnose (p<10⁻⁴). Classification (de novo or secondary AML) was significantly different (p<0001) too. In contrast, we could not demonstrate any significant influence of age (p=0.267). Significant factors for the recovery of leukocytes were cytogentic (p<10⁻⁴), CR yes/no (p=0.025) and classification (de novo or secondary AML) (p=0.034). There was no influence of age (p=0.9).

Regarding platelets recovery we identified the following factors to be significant: CR yes/no (p<10⁻⁴), classification (de novo or secondary AML) (p<10⁻⁴) and cytogentic (p=0.0014). There was no influence of age too (p=0.85).

Conclusions: Cytogentic at diagnose was the most important parameter for CR and highly significant in the performed multivariate analyse. We could not demonstrate any influence of age regarding CR rate and hematological recovery. Although this is a retrospective analyses only of an induction therapy, these data may suggest that the biology of AML below and above the age of 60 years is not as different as described before.

P568

Prognosis of Adult Patients with CBF Leukemias - a Single-Center Experience

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CBF leukemias (carrying the AML1/ETO or CBFB/MYH11 rearrangement) belong to subgroups of acute myeloid leukemia (AML) characterized by a distinct genotype/phenotype relationship.

Table 1: Patient characteristics

<table>
<thead>
<tr>
<th>t(8;21): n = 14 (5 with additional chromosomal abnormalities)</th>
<th>n = 11 (2 with additional chromosomal abnormalities)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median age (years)</td>
<td>21 (70)</td>
</tr>
<tr>
<td>Male: female (%)</td>
<td>16:9 (64:36)</td>
</tr>
<tr>
<td>Preceding MDS (%)</td>
<td>5 (20)</td>
</tr>
<tr>
<td>Choroma at AML diagnosis (%)</td>
<td>2 (8)</td>
</tr>
<tr>
<td>Median WBC (p/μl) at all pts (t(8;21)) / inv(16)</td>
<td>1650 (8250 / 4200)</td>
</tr>
<tr>
<td>Median LDH (U/l) at all pts (t(8;21)) / inv(16)</td>
<td>506 / 394 / 635</td>
</tr>
<tr>
<td>Median bone marrow blasts (%) at all pts / t(8;21) / inv(16)</td>
<td>77 / 65 / 88</td>
</tr>
<tr>
<td>Median peripheral blood blasts (%) at all pts / t(8;21) / inv(16)</td>
<td>52 / 31 / 78</td>
</tr>
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They are frequently associated with a favorable prognosis, particularly after high-dose cytarabine (HiDAC) consolidation, showing among all non-M3 AML a high response rate and often long remission durations. However, very few single-center studies have reported the outcome of CBF leukemias in unsellected patients. Between June 1993 and October 2003, 25 consecutive adult patients (pts) with CBF leukemias were treated at our center. 23 pts received 1-2 induction courses with either IVA (16 pts, Heil et al., Ann Hematol. 2004, 83:336-334), or other induction protocols. 1 additional pt (35 yr) with inv(16) died of hyperleukocytosis and massive DIC prior to induction, 1 other pt (70 yr) with t(8;21) received low-dose Decitabine. 15/23 pts (65%) achieved CR. Induction death occurred in 3/23 pts (13%), due to hyperleukocytosis in 1 pt (25 yr) with inv(16), due to sepsis in 2 others. 5/23 pts did not achieve remission after two courses of induction chemotherapy, 4 of them were allografted. 11/15 CR pts (73%) received consolidation with HiDAC. Median overall survival was 19 months (mths). Median relapse-free survival was 12.8 mths with a relapse rate of 33%. 12/25 pts (48%) are still alive with 4 pts (16%) living more than 4 years, 3 of them have received HiDAC. Conclusion: This single-center analysis confirms that long-term remissions can be achieved in cytogenetically defined subgroups of AML using HiDAC consolidation with allografting being indicated for non-responders. We noted an almost 5-fold higher median WBC in inv(16) compared to t(8;21) pts in 2 cases associated with fatal, early hyperleukocytosis complications.

P569

Myeloblasts in Acute Myeloid Leukemia (AML) Produce and Secrete an Active Form of Tryptase

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Purpose: Several blast-derived growth regulators are considered to be involved in the pathogenesis of acute myeloid leukemia (AML). Tryptase is a highly potent mitogen for mesenchymal cells that has recently been implicated in fibroblast proliferation and angiogenesis. We have recently shown that myeloblasts produce significant amounts of tryptase in a group (roughly 40%) of patients with AML (Sperr et al, Blood 98:2200, 2001). Methods: In the present study we asked whether soluble and functionally active tryptase is secreted by AML blasts and could serve as a relevant molecule in the pathogenesis of AML. Results: In all patients with tryptase+ AML examined (n=5), the tryptase protein determined by FIA, was found to accumulate over time in supernatants of isolated unstimulated blast cells in vitro. Serum samples in these patients contained high tryptase concentrations (>50 ng/ml; normal range <15 ng/ml) and were found to promote uptake of H-thymidine in cultured bone marrow fibroblasts in a similar way compared to bFGF or recombinant human tryptase. To elucidate the role of tryptase in the pathogenesis of AML, we screened for expression of the tryptase receptor PAR2 using a monoclonal anti-PAR2 antibody. As determined by multicolor flow cytometry, PAR2 was found to be expressed on blood monocytes and the AML cell line KG1. In addition, myeloblasts in three patients with AML were found to react with anti-PAR2 antibody. Conclusion: In summary, our data show that AML blast cells in tryptase+ AML constitutively produce and release a functionally active form of tryptase. Whether blast cell-derived tryptase acts as an autocrine or/and paracrine growth regulator in patients with AML remains to be determined.

P570

Chemotherapy-Induced t-MDS/AML-Associated Genetic Aberrations

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Purpose: Therapy-related solid and hematological neoplasias occur according to the treatment regimen and intensity. The cumulative incidence of therapy related myelodysplastic syndromes and acute myeloid leukemias(t-MDS/AML) ranges between 5 – 15% in non-myeloablative and myeloablative treatment protocols respectively. t-MDS/AML share characteristics of cytogenetically defined subgroups of AML. Methods: We have recently shown that soluble and functionally active tryptase is secreted by AML blasts and could serve as a relevant molecule in the pathogenesis of AML. Results: In all patients with tryptase+ AML examined (n=5), the tryptase protein determined by FIA, was found to accumulate over time in supernatants of isolated unstimulated blast cells in vitro. Serum samples in these patients contained high tryptase concentrations (>50 ng/ml; normal range <15 ng/ml) and were found to promote uptake of H-thymidine in cultured bone marrow fibroblasts in a similar way compared to bFGF or recombinant human tryptase. To elucidate the role of tryptase in the pathogenesis of AML, we screened for expression of the tryptase receptor PAR2 using a monoclonal anti-PAR2 antibody. As determined by multicolor flow cytometry, PAR2 was found to be expressed on blood monocytes and the AML cell line KG1. In addition, myeloblasts in three patients with AML were found to react with anti-PAR2 antibody. Conclusion: In summary, our data show that AML blast cells in tryptase+ AML constitutively produce and release a functionally active form of tryptase. Whether blast cell-derived tryptase acts as an autocrine or/and paracrine growth regulator in patients with AML remains to be determined.
Abstracts

Onkologie 2004;27(suppl 3):1–230

P571 Detection of Functionally Active Granulocytes in AML
Patients Receiving Intermediate or High Dose ARA-C for Consolidation

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Purpose: High dose- (HiDAC) and intermediate dose- (iDAC) ARA-C have recently been introduced as effective post remission treatment for pts with AML. These regimens have been reported as a safe approach with a relatively low rate of infections and a relatively short time of absolute neutropenia.

Methods/Results: In this study, we analyzed the number, phenotype, and functional properties of neutrophils in AML pts during consolidation with HiDAC (n=8) or iDAC (n=8). We found that absolute neutrophil counts (ANC) remained at relatively high levels until day 10-14. In a majority of the pts receiving iDAC or HiDAC, ANC on day 10 were >5000/µl. These cells were found to be mature neutrophils by Wright-Giemsa staining and electron microscopy (EM). They contained secondary granules and phagolysosomes similar to neutrophils in healthy controls. In addition, these cells exhibited oxidative burst activity and expressed phagocytosis-related and activation-linked cell surface antigens including the C3biR (CD11b), CR1 (CD35), C5aR (CD88), FcγRI (CD64), FcγRII (CD32), FcγRIII (CD16), G-CSF-R (CD114), GM-CSF-R (CD116), and slL (CD15). The biologic significance of neutrophils persisting until day 10 in AML pts was confirmed in functional analyses using fluorescence labeled, heat-killed E.coli bacteria, flow cytometry, and EM. In these experiments, day 10 neutrophils were found to take up bacteria into phagolysosomes in the same way as neutrophils in healthy controls.

Conclusions: In summary, our data show that considerable numbers of functionally active neutrophils survive until day 10 in AML pts treated with HiDAC or iDAC. The consecutive delay in occurrence of neutropenia may be responsible for the relatively low rate of severe infections in these pts compared to other consolidation regimens.

P572 Making the Diagnosis in Acute Leukaemia: Role of Peripheral Blood Smear, Bone Marrow Aspirate, Immunophenotyping of Peripheral Blood and Marrow Aspirate, and Bone Marrow Trephine Biopsy

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Purpose: Which diagnostic procedures contribute rapidly and reliably to confirming acute leukaemia.

Methods: From 05/2000 to 5/2004 82 patients were diagnosed with acute leukaemia in our institution (20 to 90 years of age, median 59 years). 67 patients were diagnosed with AML, 13 with ALL, one with plasmacell-leukaemia, and one with blast transformation of Sézary syndrome. Initial haematological work-up enclosed peripheral blood smears, bone marrow aspirate and trephine biopsy with immunocytochemistry, and immunophenotyping of peripheral blood and bone marrow aspirate.

Results: In 37 of 67 patients AML could be confirmed by peripheral blood smears and immunophenotyping. 13 patients presented with leucocytosis and blast counts of less than 20%. 15 patients displayed leukaemia, but 4 of these patients showed blast counts > 20%, confirming AML. 2 patients presented with a normal blood count. Bone marrow aspirate and cytochemistry was applicable in 58 out of 67 patients confirming AML in 52 patients and revealing AUL (acute undifferentiated leukaemia) in 6 cases. 2 patients had punctio sicca. Immunophenotyping was diagnostic in 55 patients and not evaluable in 3 patients. Bone marrow trephine biopsy was evaluable in 57 patients confirming AML in 49 and displaying AUL in 7. Major differences in diagnosis were noted with respect to bone marrow aspirates and immunophenotyping in 6 patients (myelodysplastic or myeloproliferative syndrome, acute lymphoblastic leukaemia, high grade - lymphoma). In ALL, work-up of blood count, blood smear, and immunophenotyping was diagnostic in 7 patients. 3 patients presented with leukaemia/prancytopenia, and 3 patients displayed normal blood counts. Bone marrow aspirate and cytochemistry confirmed AML in 6 patients, showed AUL in 5, and was misleading or not diagnostic in 2. Bone marrow trephine biopsy was applicable in 12 patients and compatible with ALL in 9 patients, revealing AUL in 2, and showing bone marrow contamination < 20% from lymphoblastic lymphoma in one. Careful work-up of peripheral blood smear confirmed AML in 61/67 (41/67) and ALL in 54/71. In suitable patients, combining blood smear and immunophenotyping of peripheral blood rendered AML in 87% (42/48) and ALL in 78% (7/9). Bone marrow aspirate and cytochemistry confirmed AML in 87% (52/60) and ALL in 46% (6/13) only. In ALL, combination of bone marrow aspirate and immunophenotyping was diagnostic in 92% (11/12). Bone marrow trephine biopsy confirmed AML in 86% (49/57) and ALL in 75% (9/12), and was misleading in 10% (6/67). Conclusions: Bone marrow aspirate in combination with immunophenotyping of marrow cells and/or peripheral blood cell confirm rapidly and reliably acute leukaemia.

Poster Session: Non-Hodgkin’s Lymphoma I

P573 Purine Antagonists for Chronic Lymphocytic Leukaemia: Preliminary Results of a Comprehensive Meta-Analysis

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Purpose: Recent trials suggest improved response rates for purine antagonists compared to alkylator-based regimens in the treatment of CLL. However, none was able to show a survival advantage, which may be due to study design or sample size. The aim of this study was to determine, if there is any advantage of purine antagonists compared to alkylating agents (alone or in combination) in the treatment of patients (pts) with CLL. Methods: Randomised controlled trials comparing purine antagonists with alkylator-based regimens in pts with previously untreated B-CLL were included. Endpoints included overall survival (OS), overall response (OR), complete
remissions (CR), time to progression (TPP), treatment-related morbidity and mortality. Medical databases (Cochrane Library, MEDLINE, EMBASE) and conference proceedings were searched (1990-2003) as well as internet-based registers of current clinical trials. We included full-text and abstract publications as well as unpublished data. Data extraction and quality assessment were done in duplicate. Authors of original publications were contacted to obtain missing data. Results: Out of 9 eligible studies, 5 trials with 2026 randomised pts were included, 2 trials are still ongoing, 1 trial has not yet been fully evaluated and the results of 1 trial were published including data from non-randomised patients. Compared to alkylators, treatment with purine antagonists (fludarabine, cladribine) significantly improved the relative risk for achieving an OR (RR 1.22 [95% CI 1.13-1.30]; 5 trials, n=1746) and resulted in a significantly higher proportion of CRs (RR 1.93 [95% CI 1.65-2.27]; 5 trials, n=1746). Incidence of grade III/IV infections was significantly increased in patients receiving treatment with purine antagonists (RR 1.97 [95% CI 1.33-2.91]; 3 trials, n=1515). There was no significant difference concerning the RR for grade III/IV neutropenia (RR 1.14 [95% CI 0.96-1.35]; 3 trials, n=1515) and therapy-related death (RR 1.05 [95% CI 0.84-2.29]; 2 trials, n=1296). Incidence of hemolytic anemia was low, but significantly increased in the purine antagonist group (RR 3.18 [95% CI 1.13-8.92]; 2 trials, n=1153). Raw data from investigators concerning OS and TTP is pending. Conclusions: In patients with previously untreated B-CLL, therapy with purine antagonists results in significantly increased OR and CR rates compared to alkylator-based regimens. However, treatment with purine antagonists also augments the risk for grade III/IV infections. Results for OS and TTP will be presented at the meeting.

P574

**Aberrant Promoter Methylation Patterns of CDK Inhibitor, p57KIP2, and Cell Cycle Regulation in MCL**

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Introduction: Mantle cell lymphoma (MCL) is characterized by the chromosomal translocation t(11;14)(q13,q32), resulting in the overexpression of cyclin D1. However, additional genetic alterations are detectable in the vast majority of cases. The hypermethylation of CpG islands of the promoter region represents an alternative selective mechanism of gene inactivation. So far little is known about the differential effect and the hierarchy of promoter methylation of INK4 and p57KIP2. Methods: MSP analysis with subsequent cloning and sequencing of PCR products were applied to determine the methylation pattern of p57KIP2. Results: were correlated to genomic alterations in the INK4a gene cluster, p53 gene inactivation and cell proliferation. RNA expression of the p57KIP2 was investigated by RT-PCR in cell lines. Results: By analysing p57KIP2 promoter methylation and RNA expression in different hematological cell lines a promoter region of the gene could be identified which is functionally relevant for the expression of the gene product. The identified promoter region was methylated in 4 out of 10 tested MCL patient samples but neither in 4 control samples from healthy volunteers nor in CD34+ cells. Currently, multivariate analysis of the p57KIP2 gene methylation being performed in additional MCL patient samples to analyze its correlation to other genomic alterations in the INK4a gene cluster, p53 gene inactivation and cell proliferation to identify the functional relevance in malignant transformation of MCL. Conclusion: Our study results proof methylation of a specific promoter region of p57KIP2 is functionally relevant for gene expression. Analysis of a small number of MCL patient sample suggests that methylation of this promoter region is characteristic for MCL. Analysis of additional patient samples is planned to identify the clinical relevance of p57KIP2 methylation in MCL.

P575

**Off-study Use of Alemtuzumab is as Effective as Reported in Clinical Trials in Heavily Pretreated B-CLL: A Retrospective Analysis**

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Purpose: Alemtuzumab (MabCampath) is the most effective monoclonal antibody in single-agent use for B-cell chronic lymphocytic leukemias (B-CLL). Response rates in heavily pretreated patients range between 33 and 52%. Methods: The results of a retrospective survey on the therapeutic efficacy of alemtuzumab in CLL given in a series of more than 70 patients treated in several Austrian hospitals are reported. 5% of patients showed features of secondary PLL and Richter’s transformation, respectively. Mabcampath was given alone and, in some cases, in combination with fludarabine phosphate after alemtuzumab monotherapy. After dose escalation, alemtuzumab was administered at 30 mg, three times weekly for up to 30 weeks. Results: The median age was 63 ys, and most patients were in Rai stages III and IV disease. All patients have been heavily pretreated (median 4 prior regimens, range 1-7). All patients had previously received fludarabine phosphate, and the majority was considered fludarabine phosphate-refractory. Using National Cancer Institute (NCI) Workshop criteria, objective responses to alemtuzumab in nearly 40% of pts were recorded, with CR in 10% of responders. Stable disease (SD, >2 months) was seen in another 40%, whereas about 15% of pts showed immediate progressive disease. Peripheral blood response rates were much higher, with 40% CRs and near 50% PRs, whereas nodal responses included about 5% CRs, and 35% PRs. The overall response to alemtuzumab was not worse in fludarabine phosphate-refractory disease (near 40%). Fludarabine phosphate/alemtuzumab in combination was not more potent, probably because these patients represented a unfavourable subgroup. Available survival data suggest a profound survival prolongation when compared to published series with the about 8 months’ survival in fludarabine-refractory disease. CMV reactivations occurred in 10%, as to be expected; interestingly, we observed two tuberculosis reactivations and one second primary tumor (Hodgkins disease), the propagation of which might have been triggered by CLL- and/or treatment-associated immunosuppression. Conclusions: Alemtuzumab is effective in these unfavourable CLL patients in terms of response and prolongation of survival. Toxicities and complications occurred in the expected range suggesting correct supportive therapies according to guidelines.

P576

**BCL2 as an Independent Predictor of Survival in Diffuse Large B-Cell Lymphomas (DLBCL) – a Tissue microarray (TMA) Based Study**

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Purpose: Gene expression profiling of DLBCL revealed three distinct phenotypic variants: germinal center B-cell-like (GC), activated B-cell-like (ABC) and a „third“ type. The present study was designed to analyze the relationship between immunohistophenotypes and clinical features and outcome in DLBCL with respect to their protein expression profile. Methods: A TMA comprising 113 different cases of DLBCL with complete clinical follow-up (mean observation period 40 months) was constructed. Expression of CD10, CD20, CD44, bcl-2 and bcl-6 was assessed by immunohistochemistry. Discrfit in specific survival (DSS) was analyzed by univariate Kaplan-Meier method and compared by the log-rank test. Nonparametric tests were applied to demonstrate correlations between immunoprofiles and stage, IPI, LDH, β2-microglobulin.
microglobulin, age, sex and primary site of involvement. Multivariate analysis for the effect of each expressed factor as well as for clinical parameters was performed using a Cox stepwise regression analysis. Samples were segregated into GC (bcl-2+/ bcl-6+ and/or CD10+/CD44−, n=42), ABC (bcl-2+/ bcl-6−/CD10−/CD44−, n=18), type three (all other immunophenotypes, n=37) and secondary transformed DLBCL (all CD44+, n=13). For statistical analysis, secondary transformed DLBCL were excluded. Results: Multistep Cox regression analysis showed IPI (p=0.0001), B-symptoms (p=0.012) and BCL-2 (p=0.023) to be of independent prognostic significance for DSS in DLBCL. Multivariate analysis of all patients with an IPI score of 1 to 3, i.e. 0 to 3 risk factors, showed BCL-2 to be the only significant risk factor considering DSS (p=0.023, IPI: p=0.066, Fig.1). Conclusions: In low to high intermediate-grade DLBCL patients BCL-2 is the only significant risk factor considering DSS. The predictive value of BCL-2 is superior to that of IPI. This finding might be important in stratifying patients with DLBCL in regard to risk-adjusted treatment.

Fig. 1: Kaplan-Meier plot showing BCL-2-dependent survival in patients with IPI 1-3.


P577 Double Induction Combined with Tandem Autologous Stem Cell Transplantation in Relapsed and Refractory Aggressive Lymphomas
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Purpose: Feasibility and high efficacy of repetitive dose-intensive chemo-immuno-therapy in relapsed and refractory aggressive NHL (adjusted IPI at relapse 2 and 3) was proven by double-induction followed by tandem stem cell transplantation including a treosulfan-based conditioning regimen.

Methods: For cytoreduction and stem cell mobilisation, 2 cycles VIPE were applied followed by two identical cycles of high-dose chemotherapy (HD-CT) consisting of treosulfan 14 g/m² iv day –4 to day –2, carbolipatin 300 mg/m² iv day –4 to day –2 and etoposide 500 mg/m² iv day –2 to day –4. In B-NHLs each cycle was combined with rituximab 375 mg/m².

Results: So far, 26 patients (pts) mean 48 years (range 22-65), stage III: n=7, stage IV: n=19, have been enrolled, 5 pts with early relapse (within 6 months), 12 with refractory disease and no available matched related or unrelated donor, 7 pts with late relapse (> 6 months). All patients with NHL received previously CHOP-based CT. Histology revealed diffuse-large cell lymphoma (n=19), Hodgkin’s lymphoma (n=3), and plenomorphic T-cell lymphoma (n=4). Only one stem cell mobilization was necessary to collect sufficient CD34+ cells for two transplantations. Median hematologic recovery (> 1 leukocytes/nl and platelets >200k) after 1st and 2nd HD-CT was achieved by day 10 (8-11). No therapy-related death occurred. CTC “III” and “IV” non-hematologic toxicities were as follows: 11 of 26 pts after 1st HD-CT had “III” toxicities (infection, vomiting, enteritis, stomatitis, diarhoea), after 2nd HD-CT 9 of 23 pts, respectively. Complete remission (21 of 26 pts, 81%) was achieved after double-induction (n=2), 1st HD-CT (n=7), and 2nd HD-CT (n=12), PR in 4 pts, and no pt had progressive disease. CR after double-induction and 1st HD-CT was followed by continuous CR (cCR, 2 to 26 months, mean 12,5 months) in 9 of 10 cases (90%), after 2nd HD-CT in 6 of 12 cases (50%). At a median observation time of 12,5 months 18 pts (69%) are alive. Conclusions: Tandem treosulfan-based high-dose CT is feasible with manageable toxicity profile. CR and cCR rates argue in favor for a dose-response relationship even in high-risk patients with aggressive lymphomas. This trial has now been extended as an OSHO multicenter trial.

P578 Protein Expression Analysis of Candidate Genes in Chronic Lymphocytic Leukemia with Trisomy 12
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The pathogenic role of candidate genes involved in CLL with trisomy 12, one of the most common aberrations in CLL, is unclear. While the critical region of most aberrations in CLL could be narrowed down to a set of candidate genes, this has been difficult in the case of trisomy 12. Aim of this study was to identify specific protein expression patterns of genes located on chromosome 12 when comparing CLL cases with trisomy 12 to cases with a normal karyotype and to cell lines. 75 CLL cases (tumor load > 70 %) showing either a trisomy 12 (59 cases) or a normal karyotype (16 cases) as detected with a comprehensive FISH probe set were selected. In addition to trisomy 12 one case had a 17p deletion, one an 11q deletion and 12 cases a 13q deletion. In the trisomy 12 group 26 cases were VH unmutated, in the group with a normal karyotype 11 cases. Western blotting was performed to quantify the expression of the following proteins: CDK4, CDK2, CYCLIN-D2, p27, SMAC, MDM-2, STAT6, ARF3, GLI, APAF-1, AID. Expression was compared to three lymphoma cell lines (JVM-2, EHEB, JURKAT).

The most striking differences in expression levels among the cell lines were found for CYCLIN-D2, p27, STAT6 and MDM-2. CYCLIN-D2 was not expressed by JURKAT but clearly detectable in EHEB and JVM. JURKAT showed higher expression of p27 in relation to EHEB and JVM. JURKAT cells showed a weak expression of STAT6 while EHEB and JVM demonstrated strong signals. MDM-2 detection revealed a highly specific pattern for each cell line with a lower expression of the p57 band in JURKAT as compared to JVM-2 and EHEB who showed all three MDM-2 bands (p90, p76, p57). EHEB had the highest expression of the p57 band of the three cell lines. No clear difference in the expression levels could be found between the CLL subgroups with or without trisomy 12 for the 11 investigated proteins. As expected CDK4, CDK2 and CYCLIN-D2 were generally expressed at lower levels in CLL irrespective of the trisomy 12 status when compared to the cell lines. Expression of p27 was higher in all CLL cases as compared to JVM-2 and EHEB but as strong as in JURKAT. In conclusion, when comparing CLL with or without trisomy 12 the expression of the investigated proteins was independent of the trisomy 12 status as well as of the VH mutation status. In contrast to a previous study which associated a high p27 expression with inferior outcome in CLL we were not able to detect different p27 expression levels in genetic subgroups of CLL.

P579 Expression Pattern of MLT/MALT1 m-RNA in B-Cell Non-Hodgkin’s Lymphomas Detected by Real-Time Quantitative Polymerase Chain Reaction (PCR)
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The MLT/MALT1 gene located on chromosome 18q21 was discovered due to its involvement in the translocations t(11;18)(q21;q21) and t(14;18)(q32;q21), which characterize about one third of extranodal marginal zone B-cell lymphomas of the MALT-type. The t(11;18) leads to a fusion of the apoptosis inhibitor gene API2 on 11q21 and the MLT/MALT1 gene, a
human paracaspase. The t(14;18)(q32;q21) fuses the MLT/MALT1 gene and the immunoglobulin heavy chain (IGH) locus. In addition to translocations, amplifications of MLT/MALT1 have been reported as a possible mechanism in the pathogenesis of B-cell non Hodgkin’s lymphomas (NHL). Recent studies have shown that MLT/MALT1 is a crucial signaling component in the NF-kappaB activation in response to TCR induction. But the pathogenic consequences of API2-MLT/MALT1, IGH-IGH-MALT1 and MLT/MALT1 amplifications remain uncertain.

Using a real-time PCR (LightCycler, Roche) we determined the expression of MLT/MALT1 m-RNA in a variety of B-cell NHL including follicular lymphoma (FL n=15), mantle cell lymphoma (MCL n=9), extranodal MALT lymphoma (MALT n=6), chronic lymphatic leukemia (CLL n=10), diffuse large B-cell lymphoma (DLBCL n=14), and three Burkitt lymphoma cell lines (Namalwa, CA-46, Raji). Reactive lymph nodes from 10 healthy donors were used as negative controls. 2µg of total RNA was transcribed with Superscript II reverse transcriptase and oligo (dT)-primers. Primers for MLT/MALT1 encompassed parts of exons 16 and 17, respectively, giving rise to a product of 360 bp. MLT/MALT1 expression was normalized by using GAPDH and PBGD housekeeping gene products as internal references and plasmid DNA as standard for all products.

MLT/MALT1 m-RNA was found to be expressed in all 54 patients, 3 cell lines, and 10 reactive lymph nodes analyzed. The highest levels of expression were observed in the MALT lymphomas and the reactive lymph nodes. Moderate expression was found in FL, MCL, high-grade NHL, CLL, and anaplastic large B-cell lymphomas. The Burkitt lymphoma cell lines showed a low expression of MLT/MALT1. However, in all lymphoma subtypes there was a considerable interindividual variation of MLT/MALT1 expression.

Similar results were obtained with GAPDH and PBGD normalization. We conclude that MLT/MALT1 is widely expressed in B-cell NHL, with a high expression in MALT lymphoma and that the quantitative PCR method applied in this study is an effective and reliable method for the assessment of MLT/MALT1 expression.

P580 Prognostic Significance of Combined ZAP-70 and CD38 Expression Analysis in B-Cell Chronic Lymphocytic Leukemia

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B-cell chronic lymphocytic leukemia (B-CLL) represents a heterogeneous disease with a highly variable clinical course. Previous studies have demonstrated that CD38 cell surface expression and immunoglobulin heavy-chain variable-region gene (IgVH) mutation status are important prognostic markers in B-CLL. CD38 expression is associated with unmutated IgVH genes; however, results are discordant in approximately 30% of cases. Thus, combined IgVH and CD38 expression status of CLL cells seems to be a useful predictor of disease progression, response to therapy, and overall survival. Recently, expression of the protein tyrosine kinase ZAP-70 has been identified as a prognostic marker in B-CLL correctly predicting the IgVH mutational status in 92-93% of patients. Since intracellular ZAP-70 expression can be readily determined in a flow cytometry assay, ZAP-70 analysis is now increasingly used as a convenient surrogate marker of IgVH status. Recently, we have shown that ZAP-70 and CD38 expression correlated in 36 out of 76 patients (83%), while the remaining 13 patients (17%) revealed discordant results. A majority (97%; 12%) showing a ZAP-70+/CD38- pattern, while the remaining 13 patients (17%) revealed discordant results. Further, we investigated whether the ZAP-70+/CD38- patients were characterized by a lower progression-free survival than the ZAP-70+/CD38+ patients. To further characterize the prognostic significance of combined ZAP-70 and CD38 expression, we extended our previous study to a larger cohort of B-CLL patients (n=195). To this end, a discordance of ZAP-70 and CD38 expression performed in a subgroup of B-CLL patients will be presented.
To gain a better understanding of the kinetics and persistence of aberrant translocation-bearing cell clones, we decided to perform an epidemiologic study on the occurrence of aberrant B cell clones in pre-morbid blood samples of individuals who later developed non-Hodgkin’s B-cell lymphomas (NHL), either associated with HIV infection or not. We identified NHL patients from large prospective studies on HIV-positive individuals, using the AIDS Cancer Cohort Study (ACCS), the Multicenter Hemophilia Cohort Studies I and II (MCHCS), and the NY/DC Homosexual Male Study (DCG). We also identified HIV-negative individuals from the Prostate, Lung, Colorectal and Ovarian cancer etiology study (PLCO) from which tumor tissues and pre-morbid blood specimens were available.

In a small pilot study, we examined 14 tissue sections with lymphoma from 12 patients. We were able to isolate sufficient amounts of DNA in 9 of 14 tissues. In 7 cases, we were able to determine clonotypic VDJ rearrangements using DNA sequencing. One patient showed two distinct VDJ rearrangements, possibly indicating a biclonal tumor. We then designed allele-specific oligonucleotide primers (ASO) that were combined in some cases with consensus family framework region 3 primers. Using ASO PCR primers, we were able to develop real-time quantitative PCR methods for lymphoma-associated VDJ rearrangements for four patients. This finding encouraged us that it is possible to identify the clonal VDJ rearrangement from lymphoma slides and to use this as marker for the specific detection of clonotypic cells in pre-morbid samples.

Purpose: We have previously shown that PKC delta is constitutively activated in B-CLL cells. In the present study, we have analyzed the mechanism of apoptosis induction as well as the effects of PKC delta in the presence of chemotherapeutic agents. Methods: The mitochondrial membrane potential, presence of active caspase 3, conformational status of antiapoptotic proteins BCL-2, BCL-xL, and XIAP, and expression of proapoptotic proteins BAX and BAD in B-CLL cells were determined by flow cytometric analysis. Expression of BCL-2 family proteins and XIAP was measured by Western blotting. Results: Rottlerin enhanced the cytotoxicity of the chemotherapeutic agents Fludarabine, Vincristine, and Daunorubicin. Inhibition of PKC delta by expression of dominant-negative PKC delta resulted in a significant increase in the rate of apoptosis in B-CLL cells. Conclusion: Inhibition of PKC delta might be a powerful treatment option for B-CLL given its potential to induce apoptosis, inhibit antiproliferative signals, and increase the cytotoxicity of chemotherapeutic agents.

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In elderly patients with relapsed aggressive non-Hodgkin lymphomas (NHL), no efficient standard therapies are available. In this study, we used a combination therapy with rituximab and bendamustine (R-Bendamustine) for patients with relapsed aggressive NHL, who were not eligible for a salvage therapy. We have previously shown that PKC delta is constitutively activated in B-CLL cells. In the present study, we have analyzed the mechanism of apoptosis induction as well as the effects of PKC delta in the presence of chemotherapeutic agents. Methods: The mitochondrial membrane potential, presence of active caspase 3, conformational status of Bax and apoptosis assays (Annexin V stain, Tunel assay) were performed using flow cytometric analysis. Expression of BCL-2 family proteins and XIAP was assessed by Western blotting. Results: Rottlerin at 5μM (which is not toxic for normal lymphocytes) induced apoptosis in a large amount of B-CLL samples. Rottlerin was equally effective in zap70-positive and negative samples. Rottlerin induced apoptosis was in part caspase dependent and involved processing of caspase 3, 8 and 9. Expression of antiapoptotic proteins MCL-1 and XIAP was reduced in Rottlerin treated cells and BAX conformation change to its proapoptotic form. Rottlerin was also very effective in inhibiting survival signal like IL-4 or stromal cell contact. Treatment with Rottlerin enhanced the cytotoxicity of the chemotherapeutic agents Fludarabine, Vincristine and Daunorubicin. Conclusion: Inhibition of PKC delta might be a powerful treatment option for B-CLL given its potential to induce apoptosis, inhibit antiproliferative signals, and augment the cytotoxicity of chemotherapeutic agents.
Relapsing indolent NHL is associated with a poor prognosis. The disease course is characterized by consecutive shortening of treatment success and terminally refractoriness. The introduction of mAB has improved results, both of first line and relapse therapies. However, even with the addition of this new tool, most patients finally die of their disease. In order to further improve results, radioimmunoconjugates have been developed. Yttrium-90-ibritumomab-tiuxetan (IT) (Zevalin©), the combination of a murine CD 20-antibody and the radiosotope Yttrium-90, proved to be efficacious in phase I to II clinical trials. After FDA approval 2 years ago, the drug now became available in the EU for the treatment of relapsed indolent B-NHL. Therefore, there are very limited experiences of the practical use of this new modality in Germany. Here we report on our first experiences of 10 patients treated in 5 centres outside clinical trials on an individual basis.

Patients: 10 patients with relapsed indolent NHL (FCI 9, SLL 1) up to 95 years old, median number of pretreatments 2 (range: 1-6), received a single dose of IT, for the following reasons: availability of other treatment options/refractoriness, intercurrent medical problems not allowing conventional therapy, or social reasons. In selected patients dual therapy with Indium 111-IT revealed median doses to BM, liver and spleen of 103, 788, 258 cGy, respectively. Radiochemical purity of IT was about 98% and mean treatment activity was 1038 MBq (range 825-1184 MBq).

Importantly, no acute treatment associated side effects occurred. Main side effects during follow up were mild cytopenias mainly in weeks 4-7 after treatment. However, none of patients dropped to platelet counts below 50 and only one patient had a leukocyte nadir below 2 n/l. No transfusion support was needed, and no severe infections were noticed. Most patients accepted the treatment as comfortable and not cumbersome. At this time, treatment response has been evaluated in 9/10 patients. Overall 3/9 patients achieved CR, 2/9 achieved PR, resulting in an ORR of 55%. In two patients staging revealed one MR and one SD. Two patients with bulky disease showed transient responses, however thereafter progressed rapidly.

IT proved to be a safe and easy-to-perform treatment in routine clinical practice. Response rates have been substantial, even in heavily pre-treated patients. Data of an extended survey and follow-up will be presented during the meeting.

**Purpose:** Combination chemotherapy can cure patients (pts) with Non-Hodgkin’s lymphoma (NHL), but those with relapse still have a poor prognosis. High-dose chemotherapy (HDCT) with autologous stem cell support (ASCT) can improve the outcome of these pts as shown in the precursor study with response rates of 46% (32% CR, 14% PR) and FFPFOS of 20%/40% at the final evaluation (Data recently published). Chemosensitivity and achievement of minimal disease status prior to HDCT are important prognostic factors in NHL pts treated with HDCT. Rituximab demonstrated encouraging activity in aggressive NHL and showed low toxicity in the setting of combined immunochemotherapy. Methods: Eligibility criteria include pts with age 18-65 years and eligible for HDCT with histologically proven CD20+ relapsed NHL. Treatment program consists of two cycles DHAP (dexamethasone, cytarabine, cisplatin) plus rituximab (375mg/m²); pts with PR or CR receive cyclophosphamide (4g/m²) plus rituximab followed by PBSC harvest; methotrexate 5g/m² and vincristine 1,4mg/m² plus rituximab; and etoposide 2g/m² plus rituximab. The final myeloablative course is BEAM plus rituximab followed by ASCT. Results: 20 pts (median age 57 years, range 22-65) with relapsed (16 pts) and refractory (4 pts) aggressive NHL have been enrolled (stage II/III: 6, stage III/IV: 11, NA: 3). 18 pts had CHOP or CHOP-like regimens as first-line therapy, one pt. was treated with fludarabine + cyclophosphamide + alemtuzumab and one with BEACOPP. The median time to progression was 9 months. This chemoinmunotherapy combination regimen was well tolerated in all pts without side effects exceeding the toxicity expected from chemotherapy alone. 19/20 pts were available for restaging after 2 cycles DHAP with 2 CR, 12 PR, 2 SD and 3 PD. One patient died on myocardial infarction. At the final response evaluation from 9/13 pts were in CR, 3/13 in PR and 1/13 was not available. Treatment was discontinued in one pt after HD-cyclophosphamide due to severe heart failure. Conclusion: The preliminary results suggest feasibility and safety of this regimen with a overall response rate of 60% (45% complete remission and 15% partial remission); toxicity was tolerable. The combination regimen allows effective mobilization of stem cells and the tolerability of the final myeloablative BEAM was not affected by rapid sequential administration of DHAP and high doses of cyclophosphamide, methotrexate and etoposide, each in combination with rituximab.
patients. (2) In 34/67 follow up investigations after PBSCT all CD19+/CD5+ were negative for CD79b implicating the presence of CLL cells. This was also the case in 1/18 patients with a decreased percentage (<5%) of total B cells. (3) Both, CLL cells (CD79b+) and normal B cells (CD79b+) coexpressing CD19/CD5+ could be detected in 10/67 measurements. (4) In further 13 samples only normal B (CD79b+) cells coexpressing CD19/CD5- were detectable. This cohort contained 4 patients with up to 46% of total B cells. The remaining 8 samples did not show any CD19+/CD5- cells.

Results: In summary, the proposed 4-color B-CLL immunophenotyping is a feasible tool for routine flow cytometry discriminating subpopulations of CD19+/CD5+ B cells. Correlation of flow cytometric results with PCR investigations and clinical course of the patients is in process.

P590
Maintenance Therapy after Autologous Stem Cell Transplantation Using the Anti-CD20 Antibody Rituximab in Patients with Follicular Non-Hodgkin Lymphoma

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Purpose: Rituximab is a monoclonal antibody which binds to the CD20 antigen inducing apoptosis and complement as well as effector cell mediated lysis. CD20 is present on the malignant cells of more than 95% of patients with B-cell lymphoma. In clinical trials, treatment of B-cell non-Hodgkin lymphomas (NHL) at different stages with Rituximab in combination with cytotoxic chemotherapy or alone was shown to be efficacious. In this study, we compared a group of 11 patients with stage II-IV NHL who received Rituximab as maintenance therapy with a historical group of patients who did not receive therapy following high dose chemotherapy and autologous stem cell transplantation (PBSCT). Rituximab was given at a dose of 375 mg/m² for a median number of 7 infusions (range 1-13) in time intervals ranging between 4 and 12 weeks. Molecular monitoring of t(14:18) was performed from samples of peripheral blood and bone marrow using nested as well as quantitative real time PCR (qPCR) based on the LightCycler technology. The median time of follow up after transplantation was 29.4 months (range 4-77 months) for patients who received Rituximab and 35 months (range 5.5-106) for the control group. Except for one patient in the Rituximab group all patients were in complete remission at the time of transplantation. The groups were similar with regard to age, previous therapy, disease stage and remission status at the time of transplantation. Results: Maintenance therapy was well tolerated and there was no case of opportunistic infections. The major finding is a statistically significant difference concerning the event (p=0.02) and the progression free survival (p=0.02) indicating a therapeutic advantage of rituximab over the control group. Conclusions: Maintenance therapy with rituximab after high dose therapy and PBSCT is well tolerated and may improve event free and progression free survival in patients with follicular lymphoma.

P591
The mRNA Expression of Tumor Associated Antigens (TAAs) in CLL Patients

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Purpose: B-cell chronic lymphocyte leukemia (B-CLL) is the most common leukemia in Western countries. The disease is characterized by a long natural course, which might allow the development of immune responses against tumor cells. Moreover, most patients with B-CLL are advanced in age and therefore not eligible for aggressive chemotherapy. The antileukemic effect obtained by graft vs. leukemia reaction or by donor lymphocyte infusion (DLI) suggests the existence of immunogenic antigens in leukemias. Some antigens are shared in different types of cancers and leukemias. Identification of immunogenic leukemia/tumor associated antigens (L/TAA) as target structures might open the way to mono- or polyvalent vaccines against leukemia including dendritic cell vaccination as well as immunotherapies using specific antibodies. Methods: We investigated the mRNA expression of eighteen L/TAA from the literature (survivin, O-raf, BAGE, G250, Mage-1, Prame, prostate, Syntaxin, H-TERT and WT-1), as well as L/TAA defined previously by SEREX analysis of AML/CML patients by our group (PincH, HS2, MAZ, MPP1, RHAMm and its isoforms RHAMm& and RHAMm&-a) and by others (NewRen60). We examined PBMM from 30 CLL patients by conventional RT-PCR. Results: No expression of WT-1, H-TERT, BAGE, G250, Mage-1 and survivin was observed. Low (2-20%) expression frequency of MPP1, PINCH, PRAME and prostate was observed. We found 55%-90% expression frequency of RHAMm&-a, survivin and NewRen60 and 90-100% of HS2, MAZ and O-raf. Moreover the expression of O-raf ILP was very strong. We did not find any correlation between stage of disease, previous therapy and TAA mRNA expression frequency. Interestingly we found increased expression of only one RHAMm isotype - RHAMm&-a, RHAMm and its splice variants RHAMm& and RHAMm&-a are not expressed on CD34+ stem cells nor in PBMM of healthy volunteers. Conclusions: RHAMm&-a is an interesting target for future immunotherapy of CLL patients.
complete and 35 partial remissions. 53 of 60 pts (88%) showed a documented remission as shown in this case.

P593
Primary CNS Lymphoma Treated with HD-Methotrexate, HD-Busulfan/Thiotepa, Autologous Stem Cell Transplantation and Response-Adapted Whole-Brain Radiotherapy: Preliminary Results of the OSHO-53 Study

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Purpose: High-dose methotrexate (HD-MTX) is the single most effective agent in the therapy of primary CNS lymphoma (PCNSL). The optimal number of MTX-cycles remains to be determined, but even after multiple courses of MTX about 50% of the patients do not achieve a complete remission. Therefore, additional therapy is necessary, i.e. in most studies wholebrain radiotherapy (WBRT). But radiation grossly contributes to neurotoxicity, especially in the elderly. We wanted to investigate whether WBRT could be omitted in patients in CR after high-dose chemotherapy (HDC) with lipophilic substances, e.g. busulfan and thiotepa in combination with autologous peripheral blood stem cell transplantation (PBSCRT). Methods: Our phase II study was designed accordingly. Once diagnosis is ascertained by histology HD-MTX (8g/m², 4h, 6g/m² >60y) is given on d1 and d10 followed by leucapheresis. Then patients were stratified: SD/PD receive WBRT (45Gy) reference therapy, those in CR/PR continue with HD-busulfan (16mg/kg BW) /thiotepa (10mg/kg BW ) (HD-Bu/TT) and PBSCRT. In the case of CR treatment is finished, patients in PR receive WBRT (45gy). Time on treatment is very short (2-3mo). Results: We report the results of 19 patients at a median follow up of 9 months. Median age is 56 years (18-69), 9 females and 10 males, Karnofsky-Index 70% (30-100). HD-MTX toxicity prevented HDC in 3 patients (1 renal tox, 1 hepatic tox, 1 embolic death). 2 patients showed NC after HD-MTX and didn’t proceed to HDC. 4 of these patients underwent WBRT. The remaining 14 patients tolerated HDC with low toxicity. One patient died after HDC due to infectious complications. One patient received HDC despite SD after HD-MTX (protocol violation). Median survival of all 19 patients is 10 months (0-54mo). 10 of the 14 patients treated with HDC achieved a CR, 1 died, 2 PR, 1 PD. The latter three patients underwent WBRT resulting in an additional 2 CR and 1 PR. In the HDC-group of patients median survival is 30months. Clinical neurotoxicity was not observed in patients receiving HDC only, but two of the three patients undergoing WBRT after HDC died of leukencephalopathy. One patient developed an abdominal and testicular lymphoma 6 month post transplantation. After 8xR-CHOP14 this patient is in ongoing remission. Conclusions: Two cycles HD-MTX followed by HD-Bu/TT and PBSCRT resulted in a 53% CR rate in patients with PCNSL. These patients showed no neurotoxicity and resumed their daily activities. Response-adapted WBRT in the other patients resulted in an overall response rate (CR/PR) of 68%. These promising data have to be confirmed by further patients treated.

P594
Successful Treatment of Angioimmunoblastic Lymphadenopathy with Dysproteininaemia (AILD) with Alpha-Interferon: A Case Report

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Purpose: Angioimmunoblastic lymphadenopathy with dysproteininaemia (AILD) is recognized as a T-cell lymphoma which in most cases runs an aggressive course. The diagnosis is often difficult because of the varying clinico-pathological picture. Less than a third of the patients can be expected to have long-term remissions even after multagent chemotherapy. We experienced a remarkable effect of interferon alpha in a case of AILD which was refractory to steroids and combination chemotherapy. Methods and Results: A 56-year old man was admitted to the intensive care unit because of high fever, pneumonia, fatigue, night sweats, lymphadenopathy and progressive weight loss. The patient presented in a very bad general condition with generalized lymphadenopathy, hepatosplenomegaly, haemolytic anaemia, thrombocytopenia, polyklonal hypergammaglobulinaemia, disseminated intravascular coagulation, skin rash and acute renal failure. The coombs’ test was positive, serology revealed an acute EBV-infection. He responded well to an immunosuppressive and cytoreductive treatment with prednisolone (100mg daily for two weeks) and cyclophosphamide (500mg). Lymph node- and bone marrow biopsy were performed and AILD was diagnosed. The patient received combination chemotherapy with CHOP (cyclophosphamide, doxorubicin, vincristine, etoposide and prednisolone). He responded well initially but became refractory after 6 cycles of this regimen. Alpha-Interferon was started with 2 million IU/day s.c. As a result lymphadenopathy gradually disappeared, he remained afebrile and all blood values turned to normal ranges. The bone marrow (smear a trepne biopsy) showed no more infiltration by T-cell lymphoma. Maintenance therapy with 1 million IU/three times weekly is still continued. The patient did not experience any therapy-associated side effects and is able to lead a normal life. He is free from disease for more than two years. Conclusions: Alpha-Interferon is a well tolerated treatment option in patients with AILD refractory to combination chemotherapy and is able to induce long term remission as shown in this case.

P595
Dendritic Cells are Significantly Reduced in Non-Hodgkin’s Lymphoma and Express Less CCR7 and CD62L

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Adaptive immune responses are considered to be crucial for the development and control of cancer. Various tumor entities are characterized by a specific cellular infiltrate comprising T-, B-, NK- and antigen presenting cells. A correlation between clinical outcome and tumor infiltration has been demonstrated for solid and hematologic cancers. Among these lymphoma represents an important model to understand such tumor / immune interaction since the neoplasia develops at the primary site of induction of immune responses. Dendritic cells (DC) play a pivotal role in the induction and control of such immune responses. To address the lack of tumor control in clinically apparent non-Hodgkin lymphomas (NHL) we sought to determine, whether DC in tumor lymph nodes differ from DC in normal, reactive lymph nodes. Using flow cytometry 55 non-Hodgkin lymphomas and 33 reactive lymph nodes (RL) were analyzed for myeloid DC (mDC) and plasmacytoid DC (pDC). Overall frequency of DC in normal LN was higher than in NHL (0.63% vs. 0.15%; p<0.001) while the pDC/mDC ratio was comparable. Interestingly, the pDC/mDC ratio significantly differed between distinct histologies (FL, DLCL and MCL). Immunohistochemistry revealed that DC were reduced in tumor lymph nodes and distribution patterns were altered. To address, whether the reduced number of DC in NHL was due to altered homing or migratory properties we determined the expression of relevant chemokine receptors and adhesion molecules. Interestingly CCR7 and CD62L, both implicated in homing to lymph nodes, were determined to be significantly reduced in plasmacytoid DC of NHL compared to reactive lymph nodes. Correspondingly expression of CD62L was 4.2-fold lower in mDC. To further elucidate why induction of tumor specific T-cell responses is insufficient in NHL we determined the expression of molecules relevant for antigen presentation. Interestingly, DC-SIGN and CD80 were expressed at lower frequency on mDC of NHL than in normal lymph nodes. Taken together, the number of DC in non-Hodgkin lymphoma is reduced compared to normal, reactive lymph nodes and markers implicated in DC-homing and T-cell activation are expressed significantly less. These features potentially contribute to the lack of tumor control in NHL.
Purpose: Fludarabine-based combination therapies such as Fludarabine/Cyclophosphamide (FC) are the most active treatment options for CLL. New chemoimmunotherapy approaches with the addition of monoclonal antibodies have even shown more promising results. We have recently reported a very high remission rate of 86 % with a new 4-weekly schedule combining Fludarabine and the anti-CD52 antibody Alemtuzumab (FluCam) in 28 heavily pretreated relapsed CLL patients (Elter et al., ASCO 2004; Abstract 2113). To evaluate if the efficacy of FluCam can be further improved we are investigating the FC-Cam regimen which combines the most potent chemotherapy FC with Alemtuzumab (A). Methods: The FC-Cam schedule consists of an escalation phase (Phase A) of A up to 30 mg within 3 to 14 days, followed by the FC-Cam therapy (Phase B). F was administered at a dosage of 25 mg/m²/d (d 1-3) and C at a dosage of 200 mg/m²/d (d 1-3) immediately before the antibody-infusion (30 mg absolute) and repeated on day 29 for 6 cycles. Co-trimoxazole and valacyclovir were administered at a dosage of 25 mg/m²/d (d 1-3) and C at a dosage of 200 mg/m²/d (d 1-3) immediately before the antibody-infusion (30 mg absolute) and repeated on day 29 for 6 cycles. Co-trimoxazole and valacyclovir were given during treatment and up to a minimum of 2 months thereafter. Results: Four CLL patients (Binet C) with a median age of 56 years have been treated. All patients were heavily pretreated (2–4 prior regimens, median: 3) and had received and responded to FluCam before FC-Cam treatment. All four patients responded to FC-Cam (1 CR, 1 CRu, 2 PR). Side effects consisted of exanthema in 2 patients and FUO, Hepatitis (non-viral) and Arrhythmia in 1 patient. The symptoms resolved completely. No major side effects were seen given during treatment and up to a minimum of 2 months thereafter. These very preliminary data suggest that FC-Cam is feasible and highly effective in CLL and warrants further investigation. Therefore the GCLLSG has recently initiated a phase-II study which is testing the FC-Cam regimen in less pretreated CLL patients (CLL2L-trial). To further simplify the treatment a subcutaneous administration of A has been chosen for this phase-II study. For further information see www.dclls.de. Detailed treatment data will be presented.

P597
Radioimmunotherapy with I-131-Rituximab: Partially Maintains Life Quality in Lymphoma Patients with End Stage Disease
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Introduction: At our university we have gained experience in Radioimmunotherapy using CD20 radiolabeled antibodies during the past 4 years. We have treated 10 patients with relapsed CD20-positive B-NHL who received non myeloablative treatment with I-131-Rituximab. 7 of the 10 patients have been observed for a minimum of 6 months after radioimmunotherapy and are reported herein. The aim was to monitor therapeutic responses, overall survival and side effects. Methods: Patients with low-grade follicular lymphoma (2), immunocytoma (1), mantle cell lymphoma (4) and diffuse large cell lymphoma (3) included in the study between 3/2000 and 11/2003 underwent dosimetric measurements 0.5-96 hours after a preinjection of 1.5 mg/Kg cold antibody and infusion of 185 MBq (5 mCi) I-131 labelled 10 mg Rituximab. Total Body residence time and organ dose were calculated and entered in the MIRDOS3 program. The maximum total body activity was 40 cGy. Results: None of our patients had significant red marrow I-131-Rituximab uptake on dosimetric measurements, the uptake being below background measurement. Among the 7 patients who have been observed for at least 6 months after radioimmunotherapy one patient achieved a complete remission, one patient achieved a partial remission, one patient had a disease stabilization. 4 patients had progressive disease, meanwhile 2 of them have died. As side effects, a severe drop in platelet counts occurred in most patients between 4 and 6 weeks after therapy requiring platelet transfusions in 2 cases. There was no correlation between dosimetric estimations and hematotoxicity. Conclusions: even in severely pretreated patients radioimmunotherapy is a feasible option, that can lead to significant life prolongation with an acceptable toxicity profile. Careful patient selection is mandatory.

P598
Use of Zevalin for Lymphoma Radioimmunotherapy: Preliminary Berlin Results
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Zevalin has been released 04/04 for the treatment of refractory/relapsed CD20-positive FCL after Rituximab treatment. This group of NHL includes aggressive grade III FCL as well as secondary transformed FCL. Moreover, it can be assumed that in a significant amount of DLCL a follicular background is present. At the Charité 6 pts (age 60-71 y) have been treated so far with Zevalin. 3 pts were treated during hospitalization, 3 as outpatients. Acute toxicity was not observed but as expected cytopenias occurred 3 to 6 weeks after therapy in all pts. A heavily pretreated patient with transformed NHL needed antibiotic therapy due to neutropenic fever, none of the other pts presented with complications such as infections, bleeding, or severe anemia. Responses to therapy occurred as long as several weeks after Zevalin administration, showing that delay in responses must be taken into account in pts with large tumor masses or aggressive disease. A patient with transformed, high-grade NHL who had experienced a relapse only few weeks after high-dose chemotherapy showed PD for several weeks after Zevalin and required a temporary palliative treatment with weekly Dexamethasone/Vindesine but eventually achieved a remarkable sustained PR 16 weeks after therapy. Altogether 3 pts achieved a PR, 2 pts achieved a CR. one patient with transformed NHL and bulky disease had a fast PD already at the timepoint of Zevalin administration and died 6 weeks after therapy. In these pts dose-activity was measured at 1 meter distance immediately after injection of a max. of 1.2 Gbq 90Y-Zevalin. The values recorded were below 40 mSv/h, which demonstrate lack of radioprotection requirements for pts and their relatives. Conclusions: accurate patient selection, time scheduling as well as good cooperation between hematology and nuclear medicine are crucial for therapy. Zevalin is well tolerated by pts, including older pts. Hospitalization is not required for radioactivity protection, the hassles for the patient are limited, frequent blood counts are mandatory between 3 and 6 weeks after therapy. Delays in responses require that evaluation of treatment efficacy should not be performed too early. A maintenance therapy such as Dexamethasone/Vindesine appears to be feasible shortly after therapy if treatment is required until response to Zevalin occurs. Because of good feasibility and limited toxicity Zevalin should not be reserved as a “last-line” therapy to patients with large tumor burden or a fast PD.

P599
Randomized trial on the Impact of Minimal Residual Disease on Disease Free Survival in Hairy Cell Leukaemia Treated with Cladribine Alone or Cladribine Followed by Consolidation Therapy with Rituximab
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Background: Hairy cell leukaemia is a rare indolent lymphoma with an incidence of about 1.5-2.1/100,000. Only patients with recurrent infection due to neutropenia, the frequent need for substitution of thrombocytes or erythrocytes, and patients with symptomatic splenomegaly have to be treated. For a long time, therapy with interferon-a has been the standard treatment, not being able to achieve durable complete remissions. Since the introduction of purine analogues cladribine and pentostatin, up to 90% of patients can be assumed that in a significant amount of DLCL a follicular background be achieved durable complete remissions. Since the introduction of purine analogues cladribine and pentostatin, up to 90% of patients can achieve remission. Long term follow-up has shown that the rate of relapse in these patients is about 35% to 50% in five years. More recently published data focused on the impact of minimal residual disease on the probability of relapse: Several investigators demonstrated residual hairy cell in CR patients in 13% to 100%. This wide range may partly be explained by the application of different techniques to detect hairy cells (IHC, FACS, PCR). Patients...
refractory to 1st line treatment, or relapsing after purine analogue therapy have been shown to potentially achieve durable remissions with the anti-CD20-antibody rituximab; here, the tumor load before the start of immunotherapy was a critical parameter for achieving CR (Nieva et al. 2003, Thomas et al. 2004). We initiated a randomized Phase II trial to investigate the efficacy of rituximab as consolidation therapy with regard to disease free survival and MRD status. Methods: Cladribine will be administered s.c. at a dose of 0.14 mg/kg for five consecutive days. Four months later, remission and MRD status will be determined with FACS and/or IHC. Patients with PR, NC or PD will be treated with a salvage regimen. All patients achieving CR or CR-RD (i.e. CR with up to 5% hairy cells in the bone marrow or detectable hairy cells in the peripheral blood) will be randomized into two arms: (A) treatment with rituximab (375 mg/m²) with a loading dose (weeks 17,18,19,20) and a subsequent consolidation therapy (weeks 24, 28, 32, 36); and (B) follow-up. MRD will be re-evaluated at week 40. Purpose: Primary endpoint is disease free survival of treated patients. Secondary endpoints are minimal residual disease after conventional chemotherapy and after immunotherapy with rituximab, and overall survival. Inclusion of 120 pts is planned within 48 months. Recruitment is to be started in June 2004. Patients are welcome to our facility, and other centers are invited to join our study.

P600

Autologous Stem Cell Transplantation for Non Hodgkin's Lymphoma – A Single Center Report

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Purpose: Relapse rate, disease free and overall survival as well as long term side effects after autologous stem cell transplantation (ASCT) performed between 8/1984 and 9/2003 were evaluated in 102 patients (47 f, 55 m, age 15–65, median: 44.5 years) diagnosed as follicular lymphoma (n=29), as aggressive B-NHL (n=59), as aggressive T-NHL (n=6) or as mantle cell lymphoma (n=8). Status at transplantation for follicular lymphoma was CR 1 (stage III/IV): 8pts., CR 2: 8 pts. or sensitive disease (PR, CR>R or responding relapse): 13 patients and for aggressive lymphoma including MCL CR 1: 25 pts., CR 2: 16 pts., sensitive disease: 29 pts. or refractory disease: 3 patients. Results: Relapse rate for FCL was 38 % within the median observation period of 40 months (1–113 mo), occurring in patients transplanted in CR 1: 12.5 %, in CR 2: 37.5 % and in sensitive disease: 53 %. Overall disease free survival was 46 % without reaching a plateau cure yet (CR 1 vs CR 2 vs sensitive disease: 75 %, 36 % and 31 %, resp.). Overall survival was 52 % (CR 1 vs CR 2 vs sensitive disease: 100 %, 57 % and 26 %, resp.). Patients with aggressive lymphoma relapsed in 37 % within a median observation time of 31 months (1–229 mo), occurring in patients transplanted in CR 1: 24 %, in CR 2: 37.5 % and in sensitive disease: 41 %. All 3 patients with chemotherapy refractory disease showed progression shortly after ASCT and died within 7 months. Overall disease free survival post transplant was 56 % with a plateau after the last relapse at 56 months (CR 1 vs CR 2 vs sensitive disease: 72 %, 60 % and 48 %, resp.). Overall survival was 66 % (CR 1 vs CR 2 vs sensitive disease: 77 %, 92 % and 49 % resp.). Overall survival, disease free survival and relapse rate correlates to the status of disease at time of transplantation in follicular lymphoma as well as in aggressive lymphoma. Treatment related mortality occurred in 4/102 patients (3.9 %), i.e. in 3/42 sensitive disease due to bacterial and/or fungal infections and in 1/33 CR 1 due to viral hepatitis. Following late complications were diagnosed: one patient with cancer of the stomach (d=782), 1 patient with secondary lymphoma (d=2719), 2 patients with autoimmune disorders (d=2038, d=2745), 5 patients with hyphophysitis (d=258 – d=1248) and 3 patients with cataract after TBI. No secondary AML/MLDS was seen so far. Conclusion: ASCT for non-Hodgkin’s lymphoma with increased risk for relapse was a manageable and a beneficial treatment that led in more than half of the patients to a continuing disease free survival.
**Purpose:** Long term survivors of successfully treated Hodgkin's disease (HD) are at risk for late complications. Among these, for female patients, infertility is of major importance affecting many young women. The subject of this analysis is to evaluate the menstrual status and to define variables having influence on amenorrhoea in young women after HD therapy.

**Methods:** From 1994-1998, the German Hodgkin Lymphoma Study Group (GHSG) conducted one generation of clinical trials for early, intermediate and advanced stages HD (HD7-HD9) involving a total of 3186 patients. A questionnaire to document the menstrual status in young female patients after therapy was sent out by the GHSG, the presented data were updated in May 2004. **Results:** A total of 405 female patients (66.6%) aged <40 years answered the questionnaire referring the menstrual status before and after HD therapy. For most patients (89.6%), menstruation before the beginning of therapy was regular. 

**Conclusions:** Most female patients who are treated for advanced-stage HD experience amenorrhoea after therapy. Amenorrhoea is significantly higher in women with advanced-stage HD, in patients receiving 8 cycles of dose-escalated BEACOPP than in patients treated with ABVD alone, COPP/ABVD or standard BEACOPP (p<0.0066). Moreover, amenorrhoea after therapy was higher in patients with advanced-stage HD (p<0.0001), in patients older than 30 years at treatment (p=0.0065) and in patients who did not take oral contraceptives during therapy (p=0.0002).

**P605 Lymphocyte-predominant and Classical Hodgkin's Disease: Comparison of Outcomes in three Study Generations of the German Hodgkin Lymphoma Study Group (GHSG)**

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**Purpose:** The pathology and clinical presentation of Lymphocyte-predominant Hodgkin's disease (LPHD) differs from other forms of Hodgkin's disease (HD), the classical type of HD (cHD) included. The European Task Force on Lymphoma project found that treatment of LPHD patients (pts) using standard HD protocol can lead to CR in more than 95% of pts. However, survival and freedom from treatment failure (FFTF) are substantially worse in advanced stage pts compared with early stage pts. Since there are no randomized studies, the GHSG reviewed all LPHD-cases registered in the last studies and compared treatment outcome with cHD pts. **Methods:** We retrospectively analysed 401 LPHD pts and 8196 cHD pts treated within the GHSG trials (HD4 to HD12). From 401 LPHD pts 43.9% were in clinical stage (CS) I, 33.2% in CS II, 17.7% in CS III and 5.2% in CS IV. Of the 8196 cHD pts analysed, 13.1% were in CS I, 49.9% in CS II, 23.8% in CS III and 13.2% in CS IV. **Results:** 87.1% LPHD pts reached CR/Cru compared to 80.7% cHD pts. 0.4% LPHD pts developed progressive disease compared to 3.4% cHD. The relapse rate of LPHD pts was very similar to cHD (6.9%). 4.0% LPHD pts and 8.2% cHD pts died. FFTF rates according to clinical stages in LPHD pts were following: 93% for CS I (median observation time (MT) 40 months), 88% for CS II (MT 39 months), 87% for CS III (MT 44 months) and 70% for CS IV (MT 43). Appropriate OS rates were: 99% for CS I, 95% for CS II, 95% for CS III and 79% for CS IV. Full analysis of data will be presented. **Conclusions:** cHD pts present more frequently with advanced stages compared with LPHD pts. FFTF and OS rates for LPHD pts showed significant differences in early and advanced stages. Comparing LPHD and cHD pts we found differences in treatment outcome in
**Abstracts**

**P601**

**Gender-Specific Aspects and Factors Influencing the Treatment and Outcome of Males and Females with Hodgkin’s Lymphoma**

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For the German Hodgkin Lymphoma Study Group (GHSG), University Hospital Cologne, Germany

**Purpose:** Male gender has been identified as adverse prognostic factor in patients with advanced-stage Hodgkin’s lymphoma (HL). The purpose of this analysis is to investigate sex differences regarding pre-treatment and therapy-related variables. The further aim is to analyze the influence of these gender-specific factors on the outcome of males and females in all stages of Hodgkin’s lymphoma.

**Methods:** Between 1988 and 1998 the GHSG conducted two trial generations for early, intermediate and advanced HL (HD4 - HD9). The present analysis comprises 4626 patients of all stages, aged 15 to 75 years who were enrolled into these multicenter studies and registered in the GHSG database. Patients were treated according to the GHSG-study protocols.

**Results:** 2050 female and 2576 male patients were suitable for this retrospective comparison. The median observation time was 5 years (HD7-9) and 7 years (HD4-6), respectively. Patients in each group had comparable profiles in terms of age, performance status, stage, histological subtype, clinical risk factors and prognostic factors of the International Prognostic Score. There were slightly more nodular sclerosis subtypes and mediastinal masses in women, and a few more mixed cellularity subtypes in men. Acute toxicities from chemotherapy were distributed almost equally except for hematotoxicity: females showed higher rates and grades of anemia and in particular, leukopenia. Disease response to treatment was similar in both groups: 90.7% of females (versus 89.7% of males) reached a complete remission, 2.0% (versus 2.8%) a partial remission, 0.4% (versus 0.2%) had no remission and in particular, leukopenia. Disease response to treatment was similar in both groups: 90.7% of females (versus 89.7% of males) reached a complete remission, 2.0% (versus 2.8%) a partial remission, 0.4% (versus 0.2%) had no change, and 5.0% (versus 5.3%) had progressive disease. However, a lower rate of relapse (9.1% vs 11.8%) and death (10.3% vs 14.6%) was observed in females. Univariate analysis revealed significant better rates in terms of FFTF and OS for females. For multivariate analysis the following variables were included: sex, age, stage of disease, B-symptoms, mediastinal mass, and leukopenia grade III/IV. Sex was not identified as an independent prognostic factor in terms of FFTF (p=0.1665), however lower stages of disease (p<0.001), less B-symptoms (p=0.0002), younger age (p<0.0001), and leukopenia grade III/IV (p=0.0001) were significant variables to which better outcome in females can be related. Particularly, leukopenia grade III/IV has a great impact on better FFTF in females: when eliminating the factor leukopenia from the model, an almost significant p-value can be observed for gender (p=0.0620).

**Conclusions:** In this large retrospective analysis of the GHSG database, female patients had better FFTF and OS compared to male patients. The protective role of severe leukopenia in females supports the rationale for a more individualized, response-adapted therapy.

**P607**

**The CC Thymus and Activation Related Chemokine (TARC) Is a Prognostic Factor in Primary Hodgkin’s Disease**

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**Purpose:** The CC thymus and activation related chemokine (TARC) is a protein, which is highly expressed by Reed-Sternberg cells in Hodgkin’s disease (HD) and is found in the majority of HD patients. **Methods:** Within several trials conducted by the German Hodgkin-study group 63 HD patients were elected based on clinical response to study serum TARC levels by ELISA. TARC levels from 34 patients with continuous complete response (CCR), 15 patients with relapse, and 14 patients with progressive disease (PD) were correlated with freedom from treatment failure (FFTF) and survival.

**Results:** As defined in healthy donors (mean value + 2x standard deviation), a TARC level greater than 500 pg/ml was considered as elevated. The mean TARC level of all patients at baseline and after completed primary treatment was 10.68±4.17 pg/ml and 1.86±4.51 pg/ml, respectively. Baseline and post-treatment TARC levels of patients with PD were significantly higher than those of patients with CCR (p=0.0005 and p=0.002) and of patients with relapse (p=0.026 and p=0.025). While pre-treatment levels of TARC correlated significantly with FFTF (p=0.0003) but not survival, post-treatment levels correlated significantly with FFTF (p=0.0003) and survival (p=0.0001). A TARC level greater than 2000 pg/ml after completed treatment was a significant risk-factor for poorer survival (p=0.020), but not for relapse.

**Conclusions:** Monitoring serum TARC levels in HD patients adds valuable information about therapy success in HD patients, especially those with PD and should therefore be further evaluated in future trials.

**P608**

**Elderly Patients with Intermediate Stage Hodgkin’s Disease (HD) have a Poorer Outcome Compared to Younger Patients Particularly when Treated with Combined Modal- ity Treatment and Extended Field Radiotherapy (EF)**

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**Purpose:** The HD8 study of the German Hodgkin Study Group (GHSG) demonstrated that involved field (IF) radiotherapy is equally effective when compared with EF radiotherapy after four cycles of chemotherapy (2 x COPP/ABVD). Since there are indications that elderly patients with HD might fare worse depending on the type of treatment applied, we revisited the HD8 data for possible differences between younger and older patients.**Methods and Results:** A total of 1204 patients were randomised to receive two double cycles of COPP/ABVD and either 30 Gy EF + 10 Gy bulk or 30 Gy IF + 10 Gy bulk. Of these, 98 evaluable patients were older than 60 years and 1038 patients were younger than 60 years. In general, there were more risk factors such as B-symptoms, elevated ESR, and poorer Karnofski index in the elderly group. On the other hand, there were fewer bulky tumours, large mediastinal tumours and a lower number of lymph node areas involved in elderly patients. The toxicity of treatment was more pronounced in elderly patients with 76 of 96 patients experiencing chemotoxicity Grade III or IV (79%) compared with 69 of 1018 (69%) in those younger than 60 years. After a median follow up of 52 months, the 5-year-FFTF was 85% in younger patients and 63% in patients older than 60 years (p <0.001). The 5-year-overall survival was 94% for patients younger than 60 years and 66% for patients older than 60 years (p < 0.001). In addition, patients older than 60 years treated with EF had a trend for worse FFTF and overall survival compared to those receiving IF radiotherapy. **Conclusions:** Event-free and overall survival of patients older than 60 years old are worse compared with younger patients. In particular, patients older than 60 years receiving EF radiotherapy had a poorer prognosis.

**P609**

**Phase I/II Study of a Fully Human Anti-CD30 Monoclonal Antibody (MDX-060) in CD30 positive Lymphoma**

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**Purpose:** CD30 is constitutively expressed on Reed-Sternberg cells in HD and on ALCL cells and represents an attractive target for monoclonal antibody (mAb) therapy. MDX-060 is a fully human IgG1mAb that recognizes CD30 with nanomolar affinity and mediates killing of HD and ALCL cell lines in vitro and in xenograft tumor models. MDX-060 cross-reacts with activated T cells from cynomolgous monkeys; in preclinical studies high doses of MDX-060 (30 mg/kg x 3) were well tolerated, with no drug-related toxicities. **Methods and Results:** A total of 15 patients (11 with cHL and 4 with ALCL) and 13 with PD were enrolled. Doses of MDX-060 (30 mg/kg x 3) were well tolerated, with no drug-related toxicities. **Conclusions:** Monitoring serum TARC levels in HD patients adds valuable information about therapy success in HD patients, especially those with PD and should therefore be further evaluated in future trials.
mg/kg. All patients are to be assessed for toxicity; assessment of response is to be made at Month 2 of the study. Results: To date, 21 patients (HD=16, ALCL=3; Other=2) have been treated. MDX-060 has been well tolerated and no maximum tolerated dose has been identified. There have been no significant infusion-related reactions and no opportunistic infections have occurred. There has been 1 episode of possible drug-related toxicity in the 1 mg/kg dosing cohort. This patient, who had a history of chronic GVHD, developed elevated (Grade 3) liver transaminase levels. While efficacy assessments have not yet been completed in all patients, 1 patient with ALCL in the 1 mg/kg cohort had a complete response to therapy of 4 months duration and 1 patient with Hodgkin’s disease showed a CR in the 15 mg/kg cohort. Plasma concentrations of MDX-060 were determined using a CD30 binding assay. In the 0.1 mg/kg cohort, MDX-060 concentrations were below the quantitative limit of the assay. In the 1 and 5 mg/kg cohorts, the MDX-060 trough and peak levels ranged from approximately 4-27 μg/mL and 28-140μg/mL, respectively. The half-life has not yet been determined. No patients tested thus far have demonstrated antibody responses to MDX-060. Conclusions: In conclusion, preliminary evidence indicates MDX-060 to be well tolerated, with minimal toxicity. In addition, we have seen clinical activity with complete responses observed in 2 patients so far. The study continues to accrue patients and response assessment is ongoing. Updated data will be presented.

P610 Fertility in Male Patients with Hodgkin's Disease after Therapy – Results from the German Hodgkin Lymphoma Study Group (GHSG)
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Purpose: Treatment results in Hodgkin Disease (HD) have improved tremendously over the last two decades. Therefore long term side effects of therapy are of growing importance. Infertility after therapy of HD is considered as a side effect of chemotherapy and radiotherapy. However patients with HD have increased risk for inadequate semen quality even prior to treatment. To investigate the influence of therapy on the fertility status in patients with HD we performed semen analysis before and after treatment. Methods: Semen quality were evaluated in patients with first diagnosis of HD enrolled into trials of the GHSG between 1988 and 2002, in 40 centers in Europe. Patients had no history of chemotherapy or radiotherapy. All semen analysis were evaluated according to WHO-guidelines. Results: We included 111 male patients with a median age of 26 years (range 16-52 years). At first diagnosis 10 patients were in clinical stage (CS) I, 60 in CS II, 60 in CS III and 8 in CS IV; systemic symptoms were present in 52 patients. In 9 patients therapy regimens consisted only of chemotherapy, in 12 of radiotherapy and in 90 of combined modality. 71 patients underwent fertility screening before therapy; normospermia was diagnosed by 19 patients, other 52 had inadequate semen quality. All 111 patients underwent at last once a fertility screening after therapy; in 38 patients (34%) a recovery of spermatogenesis was observed. Table 1 shows time of onset of spermatogenesis after the end of therapy.

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<td>Patients</td>
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Table 1 shows time of onset of spermatogenesis after the end of therapy.

From 12 patients treated with radiotherapy 11 (92%) recovered from azoospermia, from 9 patients treated only with chemotherapy only 1 patient recovered and from 90 patient treated with chemotherapy and radiotherapy recovered 26 (29%). Recover rate from azoospermia was lower in patients treated with BEACOPP chemotherapy, with systemic symptoms and elevated BSG. Conclusions: We confirmed that HD patients had inadequate semen quality even prior to treatment. The majority of patients had azoospermia after treatment, but recovery of spermatogenesis was observed, in general after 2 years after the end of therapy. Patient treated only with radiotherapy recovered in higher frequency. Further studies on this area are urgently warranted.

P611 Identification of Target Genes of Tyrophostin AG17 in Treated Hodgkin and Reed Sternberg Cells by Gene Expression Profiling

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Constitutive activation of signal transducer and activator of transcription 3 (STAT3) is observed in a variety of hematopoietic and solid tumors. Classical Hodgkin Lymphoma (cHL) is a malignancy of unknown pathogenesis. The malignant Hodgkin and Reed/Sternberg cells derive mainly from germinal center B cells but have a heterogeneous and largely uncharacterized phenotype and thus make it difficult to design targeted drugs. Tyrophostins are Tyk2 kinase inhibitors and were proven to be potent inhibitors of cell proliferation and cell survival of different tumor entities including cHL. Recently we showed that treatment of cHL cells with tyrophostin AG17 went along with decreased levels of constitutive STAT3 phosphorylation as well as reduced DNA-binding. For a comprehensive description of AG17 action in cHL a gene expression profiling containing 32,000 genes was performed. 143 transcripts were found to be affected of which 70 show a downregulated and 73 an increased expression. The remaining transcripts are of so far unknown function (expressed sequence tag/EST). A selected number of genes were analysed by Taqman-Realime-PCR. The identified AG17 affected genes may play an important role in the pathogenesis of cHL and may be considered for therapeutic targeting in tumors with deregulated STAT3 signalling.

P612 Sequential Application of Chemotherapy and Monoclonal CD 20 Antibody: Successful Treatment of Advanced Composite-Lymphoma

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We describe successful treatment of a 38-year old patient with composite lymphoma stage IVa, who presented with multifocal enlarged lymph nodes. The lymph node histology showed classic morphometric features of Hodgkin’s lymphoma, mixed cellularity subtype and follicular B-cell lymphoma. Immunophenotypic analysis showed immunoreactivity for CD20, CD10 and Ki-67 in the malignant small cell population. The areas of Hodgkin’s lymphoma demonstrated positive immunoreactivity for CD30 and CD20 in the Hodgkin’s cells. Both cell populations were bcl2-oncoprotein positive. Eight courses of dose-escalated BEACOPP (Cyclophosphamide 1250mg/sqm d1, Doxorubicine 35mg/sqm d1, Etoposide 200mg/sqm d1 to 3, Bleomycin 10mg/sqm and Vincristine 2mg d8) were administered. Staging after chemotherapy showed a partial remission radiologically. Histology confirmed persisting follicular B-cell lymphoma without bone marrow infiltration and no evidence of Hodgkin’s lymphoma. Mobilisation of peripheral hematopoietic stem cells failed, therefore an innovative therapeutic option was needed in order to obtain the most favourable results in this patient. He was treated with monoclonal CD 20-antibody (Rituximab) 10mg/kg bw weekly for eight consecutive weeks due to a marked positivity of CD 20-antigen in follicular lymphoma cells. This procedure was well tolerated and final staging showed a complete remission of the composite lymphoma, namely Hodgkin’s lymphoma and marginal B-cell lymphoma. This patient is in CR 28 months after treatment. In conclusion, in the rare event of a composite lymphoma the combination of chemotherapy and subsequent immunotherapy might be considered as a promising therapeutic option to achieve a long lasting remission.

Abstracts
Poster Session: Lung Cancer

P613 Positive Endothelial Progenitor Cells Contribute to the Tumor Vasculature in Non-Small Cell Lung Cancer

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Purpose: Recent results generated in a mouse model postulate that tumor angiogenesis/vasculogenesis can be initiated and maintained by bone marrow-derived endothelial progenitor cells. The present study investigated the distribution and frequency of CD133-positive endothelial progenitor cells in non-small cell lung cancer (NSCLC) patients (tumor tissue and tumor-free lung regions) and healthy controls using fresh-frozen specimens. The novel marker CD133 identifies human hematopoetic precursor cells as well as human endothelial progenitor cells. Methods: 79 lung cancer specimens and 66 adjacent histologically tumor-free tissues of the same patient cohort were analyzed; 11 autopsy specimens from control patients who did not suffer from any malignant disease served as control. Cryostat sections were stained against CD133, CD31, VEGFR-2 (KDR), p53 and the proliferation marker Ki-67 and correlations analysed. Results: 43/63 (68%) valuable tumor specimens revealed elevated expression of CD133 and in some cases capillary forming positive structures were detectable. Additionally, 48% showed elevated expression of KDR and 46% increased MVD. Increased CD133 expression marginally correlated with elevated KDR expression but not with p53 and Ki-67. Conclusions: A significant increase in CD133 positive cells was documented in NSCLC patients suggesting an involvement of endothelial progenitor cells in tumor vasculogenesis and tumor growth in NSCLC patients.

P614 Low grade MammaglobinB mRNA Expression in Non-Small Cell Lung Cancer

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Purpose: Recently, our group reported that mammaglobin B (hMAM-B) is regularly expressed in lung cancer, as detected by reverse-transcriptase polymerase chain reaction (RT-PCR). The present study investigated frequency and levels of hMAM-B expression in an extended cohort of NSCLC patients and correlated hMAM-B expression with clinical and immunohistochemical parameters. Methods: 73 native, fresh-frozen tumor-infiltrated lung cancer specimens and 58 adjacent, histologically tumour-free specimens were analysed. Furthermore, 10 lung specimens from patients who died of diseases other than cancer served as controls. Cell suspensions of all specimens were analysed. Furthermore, 10 lung specimens from patients who died of diseases other than cancer served as controls. Cryostat sections were stained for hMAM-B expression but not with p53 and Ki-67. Conclusions: A significant increase in CD133 positive cells was documented in NSCLC patients suggesting an involvement of endothelial progenitor cells in tumor vasculogenesis and tumor growth in NSCLC patients.

P615 Efficient in Vitro Expression of Human Reverse Transcriptase (hTERT) in Dendritic Cells of Lung Cancer Patients

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Purpose: Patients with advanced lung cancer have a poor prognosis after standard radiochemotherapy. An innovative treatment strategy is the targeting of tumor associated antigens with cellular effector cells. The telomerase catalytic subunit (hTERT) is an attractive target for cytotoxic T cells that is highly expressed in both NSCLC and SCLC cells. The aim of the study was the expression of hTERT in dendritic cells (DC) that are the most powerful antigen presenting cells. Methods: We used electroporation of DCs with hTERT mRNA that enables an HLA independent whole antigen approach. Results: A significant increase of hTERT expression was observed in 61.8% of the SCLC tumors. The fragile histidine triad (FHIT) protein, a putative tumor suppressor in humans, is frequently lost in human lung cancer. So far, there are no data available on prognostic impact of FHIT protein expression in small cell lung cancers (SCLC). In this report, 225 patients with SCLC were retrospectively analyzed for FHIT protein expression and prognosis using formalin-fixed, paraffin-embedded tissue sections. As assessed by immunohistochemistry, FHIT protein expression was observed in 61.8% of the SCLC tumors. The Kaplan-Meier survival analysis with log-rank and Chi-Square tests was performed in order to identify prognostic factors for better overall survival. A lack of FHIT protein was significantly associated with poor survival with a median survival of 157±18 days compared to 210±18 days for those patients with FHIT protein positive tumors (p=0.0061). No significant differences were observed between the survival curves of patients with weak or strong FHIT protein staining (median survival of 208±17 vs. 234±34 days, respectively). The fragile histidine triad (FHIT) protein, a putative tumor suppressor in humans, is frequently lost in human lung cancer. So far, there are no data available on prognostic impact of FHIT protein expression in small cell lung cancers (SCLC). In this report, 225 patients with SCLC were retrospectively analyzed for FHIT protein expression and prognosis using formalin-fixed, paraffin-embedded tissue sections. As assessed by immunohistochemistry, FHIT protein expression was observed in 61.8% of the SCLC tumors. The Kaplan-Meier survival analysis with log-rank and Chi-Square tests was performed in order to identify prognostic factors for better overall survival. A lack of FHIT protein was significantly associated with poor survival with a median survival of 157±18 days compared to 210±18 days for those patients with FHIT protein positive tumors (p=0.0061). No significant differences were observed between the survival curves of patients with weak or strong FHIT protein staining (median survival of 208±17 vs. 234±34 days, respectively).
p=0.665). Further, the proportion of FHIT protein-positive tumor cells was related to survival. Patients with tumors of less than 25% FHIT protein-positive cells had the worst survival of 155±21 days compared to 217±19 days for patients with a proportion of >25% of FHIT protein-expressing tumor cells (p=0.0016). Multivariate analysis using Cox regression including 11 variables confirmed the independent prognostic significance of FHIT protein expression next to performance status, tumor stage and LDH.

P617 Vinorelbine and Carboplatin in Elderly Patients with Metastatic Non-Small Cell Lung Cancer (NSCLC): A Multicentre Phase II Study

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Purpose: More than 60% of cancer patients (pts) are older than 65 years. Lung cancer is the leading cause of cancer related mortality in industrialized countries. Vinorelbine has been known as an active and well-tolerated treatment in pts older than 70 years. In younger patients, however, platinum containing combinations are more effective than monotherapies. The aim of this phase II study was to prospectively evaluate the toxicity and efficacy of the platinum-combination vinorelbine/carboplatin in elderly patients with metastatic NSCLC. Methods: Chemotherapy-naïve pts (age >70 years, stage IV NSCLC, Karnofsky ≥ 80%) were eligible for this investigation. They received vinorelbine (25 mg/m² days 1 and 8) and carboplatin (AUC 5 day 1) every 3 weeks. Depending on response, a maximum of 6 chemotherapy cycles were administered. Results: 72 patients (female/male: 23/49) at a median age of 75 years (range 70-82) years were enrolled. Histology: 50% adenocarcinoma, 33% squamous cell, 10% large cell, 7% others. 61% of metastases were located in the lung and lymph nodes and 64% of patients had more than one metastatic site. Karnofsky Index: 100% n=6; 90% n= 50; 80%=31. A total of 262 chemotherapy cycles were administered to 71 patients. 71 pts were evaluable for toxicity and 58 pts were evaluable for response. Best overall response: 21/58 PR (36%), 24/58 SD (41%), 13/58 PD (23%). Tumor control rate 45/58 (78%). The main non-hematological toxicities were pain and fatigue. Grade 1-2 toxicities (% of pts): anemia 15%, neutropenia 33%, grade 3-4: anemia 8%, neutropenia 2%. A total of 27 patients died, 5 patients died from toxicity and 22 patients died from disease progression. Conclusion: The combination of vinorelbine and carboplatin shows a promising activity in elderly patients with stage IV NSCLC. The low toxicity profile favours outpatient treatment.

P618 Operable Stage IIIA NSCLC: Prospectively Randomized Multicenter Trial of Surgery and Postoperative Radiotherapy versus “Trimodality Treatment”-Analysis of Surgical Results and Toxicity

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Purpose: selected patients (pts) with early stage IIIA-NSCLC (operable), 1-2 LN at mediastinoscopy, no clinical N2, no bulky/extra nodal disease are still taken to upfront surgery (S) at most centers in Europe and North America. We have compared this approach to an aggressive trimodality therapy -induction chemotherapy (CTX) , preoperative CTx/RTx and S- in a multicenter randomized German Kebrshilfe trial*. Methods: Pts with operable IIIA (criteria as given above, mediastinoscopy, central T3N0-1, WHO 0.1) were stratified ( TN-group, treatment center) and randomized: arm A – S and postop RTx (50-60 gy); arm B – induction CTx (3 x cisplatin (P) 60 mg/m² d 1+7/etoposide (E) 150 mg/m² d 3,4,5) + cc CTx/RTx (45 Gy; 1.5 Gy bid; 1 x P 50 mg/m² d 2 + 9, E 100mg/m² d 4,5,6) + S (1 PCI – 15 x 2 Gy =30 Gy q 3 wks). Results: From 11/94 till 7/2001 overall 12 pts randomized, 6pts (3 per arm) not evaluable (mis-staging, pts refusal, early progression), interarm comparison (surgical study treatment). 106 pts were evaluable for complete study analysis: Pts characteristics M 90 F 16; age median 59 (37-71); histopathology SCC 49 adenoc 35 LCC 16 other 6; Arm A: n = 51 pts T3N0-1 3-T1N2 38 T3N2 10; Arm B: n = 55 pts T3N0-1 3-T1N2 45 T3N2 7. Arm A: S 51/111 prophatory thoracotomy (PT) 17/51 R1/R2 10/51 complete resection (Rb) 36/51; Arm B: S 40/55 PT 1/55 R1/R2 6/55 R0 33/55; peri- and postoperative morbidity and mortality not significantly different between both arms (rate of early deaths, infections, stump insufficiencies, bleeding, embolisms, postop duration of hospitalization, time on postop respirator, time on intensity care unit). Conclusions: This trimodality protocol was proven feasible and safe in the given multicenter setting. S following this complex bimodality induction does not show increased morbidity or mortality rates. A detailed analysis of long-term survival data will be available at the time of the meeting 10/04). Although initially planned as phase-III trial, the small number of pts in this carefully selected pts group (“operable IIIA”) makes this trial more a “multicenter randomized phase-II design” with a unique comparison (local treatment only versus aggressive trimodality (+PCI))

*This trial was sponsored by all full trials grant from GERM AN KREBS HELFE, BONN

P619 Improved Overall Survival by Second-Line Combination Chemotherapy: Follow-up Results of a Phase III Study Comparing Three-Weekly Paclitaxel/Carboplatin (PC) with Weekly PC in Patients (pts) with Advanced, Measurable Non-Small Cell Lung Cancer (NSCLC)

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Background: PC is a widely-used active regimen for stage IIIB/IV NSCLC. Previously we reported our results comparing weekly (A) with three-weekly PC (B) 1-line therapy (A: paclitaxel (P) 100 mg/m², 3h i.v. plus carboplatin (C) AUC 2, 30-60 min i.v. d 1, 5, 12, 17, 22, 29, 36 (q21w) for a maximum of two treatment blocks. B: P 200 mg/m², 3h i.v. d 1; C AUC 6, 30-60 min i.v. d 1 for max. 6 courses). Discussion: Comparison of major survival endpoints was not possible due to different treatment modalities, non-cross-overs, and the different end-points (OS, PFS). Results and Toxicity: For 104 pts (50%), physicians opted for 2nd-line mono-therapy; the other half line chemotherapy, 101 of them had received PC q3w, 107 weekly PC before. For 104 pts (50%), physicians opted for 2nd-line mono-therapy; the other half was treated by duplet combinations. With regard to former 1-line therapy, both treatment options were equally balanced in the two study arms. Results: Patients treated by 2nd-line combination therapy showed in comparison to 2nd-line mono-therapy an improved overall survival in terms of median survival (13.8 months vs. 12.8 months), 1-year (61/104, 58.6% vs. 54/104, 51.9%) and 2-years survival rates (17/104, 16.4% vs. 12/104, 11.5%). The observed survival advantage is in line with the response rates determined so far: 8% vs. 3% responses (CR/PR), 29% vs. 17% disease stabilisation. Similar results are obtained if the subgroups in the q1w and q3w study arm are compared. Discussion: In recent literature, a benefit in terms of survival as well as quality of life has been demonstrated for 2nd-line mono-therapy compared to best supportive care. Our results show that approximately one quarter of patients received 2nd-line chemotherapy after preceding PC therapy and indicate that for 2nd-line therapy the use of combination regimen compared to mono-therapy offer a distinct clinical benefit by improving 1-year and 2-years survival rates.
Bendamustin in Relapsed Small Cell Lung Cancer: A Phase II Study

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Purpose: Bendamustin has single agent activity in first line therapy of small cell lung cancer (SCLC) with a response rate of 40 – 50% with moderate toxicity. We investigated the activity of bendamustin in patients, who relapsed > 2 months after first line treatment (sensitive relapse). Methods: Bendamustin was administered at a dose 120mg/m², on day 1+2 of a three week cycle. In the absence of tumor progression a maximum of six cycles was given. Response was assessed after the second, fourth and sixth cycle. Inclusion criterias: Patients refractory to first line therapy were excluded from this study. Results: Fourteen patients are currently evaluable for interim analysis. Five out of fourteen (36%) patients had a confirmed partial response. Four (27%) had stable disease and 4 (27%) progressed. One patient was not evaluable for response analysis due to early death. Grade IV neutropenia occurred in two (14%) patients, of these one patient had fatal neutropenic sepsis. Main non-hematologic toxicity was mild nausea. No alopecia was observed. In these 14 patients median TTP was 6 months (95% CI 5.9 – 8.1). Conclusions: This interim analysis already demonstrates activity of bendamustin in relapsed SCLC. The study is ongoing until 35 patients are included.

Weekly Irinotecan in Patients with Advanced Non-Small-Cell Lung Cancer (NSCLC) after Failure of Cisplatin, Taxane and Gemcitabine Based Chemotherapy

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Purpose: Irinotecan is known to be active in patients with NSCLC since its discovery in the early 90s but failed to achieve approval or widespread use outside clinical studies. Patients and Methods: We reviewed the data of all patients of our department with advanced NSCLC who received irinotecan between 2001 and 2004, 21 patients, 20 male and one female, median age 58 years (range 42-76y), 17 with aden, 3 squamous cell and 1 mixed cell carcinoma were treated with an average of 4 different chemotherapies prior to irinotecan. Patients had progressive disease on cisplatin or carboplatin (21/21), paclitaxel or docetaxel (19/21; best response prior to PD: 5 PR, 4 SD), gemcitabine (20/21; 1 PR, 4 SD) and vinorelbin (18/21). Irinotecan was given at a weekly dose of 100mg/m² for 6 weeks with one week rest until disease progression. Results: Patients received an average of 11 weekly irinotecan doses. Four patients discontinued therapy due to toxicity and were not evaluable for response. Among the 17 evaluable patients there were 3 partial remissions (18% PR) and 7 patients with stable disease (41% SD) resulting in a clinical benefit rate of 59%. There was no grade 3 or 4 leucopenia, thrombocytopenia or stomatitis. Seven patients had grade 3 or 4 diarrhoea. Time to treatment failure was 3.6 months (range 1 to 11 months). Median survival from time of metastatic disease for these patients was 24 months. Conclusions: Weekly irinotecan is effective in patients with advanced NSCLC and response rates compared favourably to those of prior gemcitabine or taxane based chemotherapy in these heavily pretreated patients. Disease stabilization was seen in 59% of all patients. The use of the increased number of active substances for sequential treatment of patients with NSCLC achieved valuable symptom control and may lead to increased survival of patients with incurable disease.

Dynamic Fluoro-Desoxy-Glucose Positron Emission Tomography in Non Small Cell Lung Cancer

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Purpose: The Fluoro-Desoxy-Glucose positron emission tomography (FDG-PET) provides detailed information about the biology of a tumour and its change during treatment with chemotherapy. We evaluated the parameters of the FDG kinetic model in patients with metastatic NSCLC and assessed their value with regard to prediction of treatment outcome. Methods: We evaluated ten patients with advanced non small cell lung cancer (NSCLC). Nine patients received a chemotheraphy consisting of Oxalaplatin and Vinorelbine, one patient was treated with a combination of Carboplatin and Vinorelbine. The patients received chemotherapy for six cycles of chemotherapy or until progressive disease. Three FDG-PET-studies were carried out (1. prior to the chemotherapy, 2. after the first course of chemotherapy, 3. after the third course). The clinical follow-up data after three cycles chemotherapy and a WHO-based classification (progressive disease (PD), stable disease (SD), partial remission (PR), complete remission (CR)) served as reference for the PET data. The following parameters were retrieved from the dynamic PET studies: SUV, fractal dimension (FD), two compartment model with computation of k1, k2, k3, k4 and the vessel density (VB). We divided into two groups, PR/NC and SD. Results: Response rates by conventional staging were as follows: PR - one patient, SD – two patients, PD – seven patients. The best correct classification rate by FDG-PET was obtained using all kinetic data of the first study. The SUV of the first study was superior to all other parameters as a single one with respect to the overall CCR. The CCR for SUV was followed by the FD, the k3 and by the influx for the prediction of therapy outcome. Influx and SUV of the second study were comparable with respect to therapy outcome. PR/NC was associated with a higher k1 and a higher VB of the first study. Conclusions: The results of this ongoing study demonstrate, that compartmental and non-compartmental analysis of the FDG metabolism are helpful for the prediction of therapy outcome and superior to the use of SUV alone.

Systemic Treatment and Survival in Patients with Advanced and Metastatic Non-Small Cell Lung Cancer (NSCLC): A Retrospective Analysis from a Lung Cancer Center

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Purpose: Non-small cell lung cancer (NSCLC) accounts for approximately 80% of all lung cancers, with more than one million new cases worldwide each year. Most patients present with locally advanced stage III or metastatic stage IV disease. Chemotherapy for these patients has been palliative and treatment outcome remains poor. The survival rates have been improved by newer generation agents combined with cisplatin ranging from 8 to 10 months. Methods: We performed a retrospective analysis of NSCLC patients stage IIIIB and IV treated with systemic chemotherapy between 1993 and 2003 in our center. Systemic chemotherapy included following protocols: Vinorelbin/Carboplatin; Gemcitabine/Cisplatin; Taxol/Carboplatin; Taxotere/Cisplatin; Taxotere monotherapy; Taxol monotherapy; Vinorelbin monotherapy; and Gemcitabine monotherapy. Results: Eligible for evaluation were 358 patients (squamous cell carcinoma, n=108; adenocarcinoma, n=191; and other non-classified carcinoma, n=59). A total of 237 patients (66.2%) had metastatic disease. Interestingly, the rate of brain metastases were strikingly high (21.2%) including 9.2% of patients with initial metastasis and 12% following treatment course. The efficacy of chemotherapy was as follows:
The median survival for all patients was 12.9 months, for patients with squamous cell carcinoma 13.1 months, for patients with adenocarcinoma 13.7 months and for all other 10 months. **Conclusions:** In summary, multiple chemotherapy treatments for advanced and metastatic NSCLC results in survival rates which are superior compared to historic controls. Third-line chemotherapy protocols may improve the outcome in some patients. The response rates of different treatment regimes will be presented in detail.

### P625

**Measuring PKC-β Expression in NSCLC**


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**Purpose:** Intracellular signaling proteins have recently been recognized as targets in cancer. One such intracellular signaling protein is PKC-β an isoform of protein kinase C. LY317615 (Enzastaurin HCl) is a potent and selective inhibitor of PKC-β and has shown potent anti-angiogenic activity in preclinical animal models. While a number of pre-clinical studies suggest that enzastaurin has anti-tumor effect and is well tolerated in animal studies, the expression of PKC-β in NSCLC has not been extensively studied. **Methods:** We here present an immunohistochemistry (IHC) procedure and a procedure to determine mRNA levels of PKC-β protein and gene expression in paraffin-embedded material of NSCLC biopsy samples. **Results:** In 42 specimens of NSCLC of patients with stage I and II disease, 28 specimen were adenocarcinoma and 14 were squamous cell carcinoma. In 65% of these NSCLC samples, PKC-β was expressed in tumor and tumor-adjacent tissue. Interestingly, staining was higher in tumor-adjacent tissue, especially in inflammatory cell infiltrates and tumor-associated vessels. Furthermore, the same paraffin-embedded specimen used for IHC, were also employed to determine mRNA gene expression of PKC-β. The different technique confirmed that PKC-β is expressed in tumor cells and future studies will specifically look at its expression in tumor-adjacent vessels. **Conclusions:** In summary, we here show a reliable technique to determine PKC-β expression in NSCLC using IHC and compare it to detection methods using mRNA gene expression.

### P626

**Assessment of Costs and Outcomes of Chemotherapy in an Observational Setting in Patients with Advanced NSCLC (ACTION)**


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**6Zentralklinik Bad Berka**

Decision makers in the health care system are increasingly asked to include total treatment costs into their therapeutic decisions. Treatment of NSCLC patients and clinical outcome could be improved during the last years. Decision makers in the health care system are increasingly asked to include total treatment costs into their therapeutic decisions. Treatment of NSCLC, documenting every day’s use.

**ACTION** is carried out in 5 European countries with over 800 patients enrolled (status of April 2004). In Germany currently 574 patients are enrolled in 86 centers reflecting a high interest in total treatment cost data in the light of a changing health care system in the hospital domain to a diagnosis-related group (DRG) system. Analyses of baseline data will be performed and presented at the congress.

### Poster Session: Breast Cancer

**P627**

**Detection of Osteolytic Lesions in an Animal Model of Human Breast Cancer Metastasis with Flat-Panel Detector-Based Volumetric Computed Tomography**


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A major limitation in evaluating tumor progression in murine tumor models is the inability to detect and monitor tumor metastasis in detail over the course of disease. Here, implantation of human breast cancer MDA-MB-231 cells, which is a well characterized mouse model for bone metastasis, was used to perform imaging studies with a flat-panel detector-based cone beam volume computed tomograph (FPD-VCT). The present study discusses the utility of a new imaging technique of high resolution FPD-VCT in detecting bone metastasis in an animal model of breast cancer metastasis. Ten week old female SCID mice (n=8) were intracardially injected with 5 x 10^5 human breast cancer MDA-MB-231 cells. After 4 weeks, mice were sacrificed and front and hind legs were dissected. The destruction of bone tissue by metastatic cells was imaged using a FPD-VCT (General Electrics Prototype). Additionally, the legs were analysed by Faxitron X-ray radiographic inspection (Hewlett & Packard, Faxitron model 43855). For histological analysis the legs were decalcified and paraffin sections were stained with H&E. Imaging studies by FPD-VCT demonstrated the development of distinct radiolucent metastatic osteolytic lesions in distal femur, proximal tibia, and proximal humerus in six bones of three mice at day 28 after cell inoculation. These osteolytic cavities could be visualized three dimensionally allowing volumetric measurements, which were within the range of 0.35 – 0.63 mm^3_. The presence of these osteolytic lesions was confirmed by standard radiographic examination (Faxitron). Osteolysis within the diaphysal cortical bone of one humane was only detected by FPD-VCT. Histological examination revealed metastatic MDA-MB-231 breast cancer cells in the bone marrow cavity. In conclusion, FPD-VCT shows excellent sensitivity and accuracy in detecting skeletal lesions and can therefore be used to image the localisation of bone metastasis including osteolytic lesions within the diaphysal cortical bone. In comparison to standard Faxitron x-ray FPD-VCT can be applied in vivo and obtains true isotropic 3D volume image data sets for evaluation in arbitrary planes. Due to precise volumetric measurement of bone metastasis, FPD-VCT will be a powerful tool to monitor tumor progression in vivo and response to anti-cancer therapies over the course of disease.
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P628
Gemcitabine and Mitoxantrone in Metastatic Breast Cancer: A Phase I Study

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Purpose: Gemcitabine and mitoxantrone are active agents for the treatment of metastatic breast cancer. Due to different modes of action and a favorable toxicity profile they are suitable for combination therapy. This phase I trial was therefore initiated to determine the optimal doses for the combination in patients with metastatic breast cancer. Secondary objectives included the evaluation of the safety and efficacy of the regimen.

Methods: Patients with metastatic breast cancer were treated with gemcitabine (1000-1400 mg/m²) on days 1, 8 and 15 and mitoxantrone (10-14 mg/m²) on day 8. Treatment was repeated every 4 weeks for a maximum of 8 cycles. Doses were assigned at registration according to the escalation scheme.

Results: Twenty-six patients received a total of 93 cycles at 5 different dose levels. The maximum tolerated doses were 1200 mg/m² gemcitabine and 14 mg/m² mitoxantrone with grade 4 neutropenia being the dose limiting toxicity. Recommended phase II doses, however, are gemcitabine 1200 mg/m² and mitoxantrone 12 mg/m² based on a similar median dose intensity and a more favorable toxicity profile. Predominant toxicity was myelosuppression with grade 3 or 4 leukopenia, neutropenia and thrombocytopenia in 43.0%, 37.6% and 10.8% of courses, respectively. Non-hematologic toxicity was generally mild.

Conclusion: In this phase I study of gemcitabine and mitoxantrone, DLT was neutropenia. Recommended phase II doses are gemcitabine 1200 mg/m² and mitoxantrone 12 mg/m².

P629
Breast Cancer and Retroviruses: Re-examining the Postulate Using a Retrovirus-specific DNA Chip

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Purpose: We could show recently, that magnetic nanoparticles interact with viable cells in a cell-type specific manner. In order to optimize the differential labeling of tumor cells and leukocytes, we have studied the role of plasma as an important fraction of human blood. Methods: Leukocytes were prepared by erythrocyte lysis from whole blood samples of healthy volunteers for in vitro experiments and from whole blood samples from patients. The breast cancer cell line MCF-7 was kept under standard cell culture conditions. Cells were inoculated with magnetic core/carboxymethyl-dextran nanoparticles with an average magnetite/maghemite core TEM-size varying between 3 and 15 nm. Magnetically labeled cells were separated by MACS. The separated cells were analyzed by FACS and Laser Scanning Cytometry (LSC). Tumor cells were detected with anti-human epithelium-antigen (HEA)-FITC. Results: Whole blood samples from tumor patients were treated with erythrocyte lysis buffer to enrich the leukocyte fraction and to remove the plasma fraction. The remaining cells were incubated with magnetic nanoparticles in the presence of none, 1% or 5% plasma for 8 min and separated with our regime. The retained cells (positive fraction) and the flow-through cells (negative-fraction) were quantified. The leukocyte fractions showed a considerable reduction in binding of nanoparticles already after addition of 1% plasma. In the presence of 5% plasma less than 20% of the applied cells were separable by MACS. The amount of circulating epithelial cells within the positive and negative fraction was estimated by LSC. In contrast to normal leukocytes the epithelial cell fraction increased 2.5-fold in the presence of 5% plasma in comparison to the sample without supplementation of plasma. The amount of epithelial cells enriched from patients samples kept constant at 75% compared to the unseparated control.

Conclusion: We could show that human plasma affects the interaction of magnetic nanoparticles with leukocytes more seriously than with epithelial tumor cell lines. This is in concordance with previously reported results from in vitro experiments.

This work was supported by the DFG-Schwerpunktprogramm SPP1104, grant CL 2021/1

P630
Human Plasma Facilitates Enrichment of Circulating Epithelial Cells from Peripheral Blood

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Purpose: To determine the role of plasma as an important fraction of human blood.

Methods: Leukocytes were prepared by erythrocyte lysis from whole blood samples of healthy volunteers for in vitro experiments and from whole blood samples from patients. The breast cancer cell line MCF-7 was kept under standard cell culture conditions. Cells were inoculated with magnetic core/carboxymethyl-dextran nanoparticles with an average magnetite/maghemite core TEM-size varying between 3 and 15 nm. Magnetically labeled cells were separated by MACS.

Results: Whole blood samples from tumor patients were treated with erythrocyte lysis buffer to enrich the leukocyte fraction and to remove the plasma fraction. The remaining cells were incubated with magnetic nanoparticles in the presence of none, 1% or 5% plasma for 8 min and separated with our regime. The retained cells (positive fraction) and the flow-through cells (negative-fraction) were quantified. The leukocyte fractions showed a considerable reduction in binding of nanoparticles already after addition of 1% plasma. In the presence of 5% plasma less than 20% of the applied cells were separable by MACS. The amount of circulating epithelial cells within the positive and negative fraction was estimated by LSC.

Conclusion: In contrast to normal leukocytes the epithelial cell fraction increased 2.5-fold in the presence of 5% plasma in comparison to the sample without supplementation of plasma.

The amount of epithelial cells enriched from patients samples kept constant at 75% compared to the unseparated control.

P631
Radiofrequency Ablation (RFA) of Hepatic Metastases in Patients with Metastatic Breast Cancer (MBC): An Interim Analysis

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Background: A subgroup of MBC patients present hepatic metastasis without extrahepatic spread. Even with systemic therapy survival is limited. Since surgical therapy is not considered a standard of care in these patients, minimally invasive therapies as radiofrequency ablation (RFA) are gaining an increasing importance.

Methods: Between 1999 and 2003, 32 patients (median age 62 years, range 41 - 76 years) with synchronous/hepatic metastases from breast cancer were treated by RFA (Starburst XL, RITA Medical Systems, Mountainview, CA USA) subsequently/parallel to chemo and/or hormonal therapy. All procedures were performed under CT-thoracoscopic guidance applying conscious sedation and local anaesthesia. Number of lesions, primary success rate, complications, total and disease free survival were recorded and analyzed.

This work was supported by the DFG-Schwerpunktprogramm SPP1104, grant CL 2021/1

Abstracts
(by clinical, laboratory, and imaging studies) was achieved over up to 44 months. Results: RFA was performed between 0 to 138 months (median 37 months) after diagnosis of the metastasis. In 32 patients, 73 metastases were successfully treated by RFA without any major adverse event. 13/32 (40 %) patients had a solitary metastasis, the rest had 2 to 5 metastases (8 had 2, 2 had 3, 5 had 4, and 4 had 5). The size of the metastases ranged from 3 to 85 mm with a median diameter of 20.3 mm. During follow-up of 1 to 44 months (median 17.2 months), 25 patients (79 %) remained disease-free in the liver, 1 patient (3 %) developed a local tumor recurrence, and 6 patients (18 %) new hepatic metastases. 9/32 (28 %) patients died 1 to 28 months after RFA due to extrahepatic tumor spread. Among the 7 patients who developed new lesions 6 underwent repeated RFA. Up to the present time of evaluation, the median survival after RFA has not been reached. Conclusion: RFA appears to be a valuable adjunct to the classic armamentarium of endocrine- and chemotherapy in hepatic metastasis of breast cancer and needs to be investigated in comparative studies.

P632
How Can CEA and CA15-3 Be Used for Estimation of the Clinical Status and Effectiveness of Therapy During Metastatic Breast Cancer?

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Background: Tumor markers like CEA and CA 15-3 are often used in follow up care of breast cancer, but mainly due to a lack of knowledge, experience but also confidence- the actual situation is more to observe kinetins than to react on these results. Therefore we try to validate the significance of CEA and CA15-3 in the course of metastatic disease. Patients and methods: We performed our study in a group of 101 breast cancer patients who developed metastatic lesions. The patients mostly suffered from bone (n=54), liver (n=51), lung (n=35) and lymph node metastases (n=39). All patients were treated by systemic therapies. On the basis of changing therapeutic regimes we totally monitored 223 different courses of therapies including endocrine (n=29), chemotherapeutic (n=73), antibody (n=7) and combined (n=114) treatments. CEA (AxSYM/Abbott) and CA15-3 (Elecsys/Roche) were mostly determined every 3 or 4 weeks, the therapeutic response was assessed by UICC-criteria. Results: Meanwhile we evaluated CEA and CA15-3 at first diagnosis of metastases and at every further moment of progression/new metastases. At first metastatic disease CEA was in 53% above the 95th percentile of healthy individuals and CA 15-3 in 74% (19 % CEA and CA 15-3 negative) with enhancing percentage of sensitivity with increasing numbers of further events ‘progressive disease’ (PD)’ 2nd PD CEA 52% /CA15-3 69%, 3rd PD 65 % /80%, 4th PD 74%/ 93%), decreasing number of false negatives (3%/ 4th PD) and a general shift of the value levels of CEA (ng/ml)/CA15-3 69%/74% 3rd PD 65 % /80%, 4th PD 74%/ 93%). Conclusion: Our results show a clear correlation between CEA/CA 15-3 and the number of events as well as effectiveness of therapy.

P633
Ex-vivo Culture of primary Breast Cancer Specimens to Study Sensitivity to Anticancer Drugs in an Intact Tissue Environment

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Purpose: Current breast cancer models system to study drug sensitivity such as cell lines, isolated tumor cells and animal models only poorly predict clinical response. This may be caused by the fact, that the drug response of tumor cells is greatly influenced by their environment. Therefore model systems which allow to study drug effects on primary tumor cells within their tissue environment may be a great advance for research on response prediction or the study of combinatorial strategies. Results: We have developed an ex vivo culture system for studying drug effects on tumor cells within their natural tissue context. 200µm thick tissue slices from freshly excised tumor samples were prepared and maintained under cell culture conditions. Within these slices the cells remained viable and continued to proliferate for at least 3 to 7 days. The different cell compartments were distinguishable using fluorescein-labeled antibodies recognizing specific surface markers. The tissue architecture did not change significantly during the culture period. The viability of cells was assessed in culture using a confocal laser microscopy and three color fluorescence viability assay. Conclusions: This tissue culture allows to study drug effects and their molecular consequences in primary tumor samples within their natural environment. This opens the window for extensive molecular studies on the biological effects of anticancer drugs.

P634
Humoral Immune Response in Breast Carcinoma Patients against Human Endogenous Retroviral Gag Protein Identified by Recombinant Yeast Antigen Surface Expression (RAYS)

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Purpose: In order to define novel antigens which might serve as potential targets for a tumor vaccination strategy we serologically screened a cDNA-library derived from mammary gland tumor tissues. One clone which was identified, coded for a human endogenous retroviral K (HERV-K) Gag protein. HERV-Ks are relics from integration events of former exogenous retroviruses into the human genome. The Gag protein of exogenous retroviruses is part of the capsid, while the endogenous counterparts are often mutated and, therefore, not infectious. Until now, there are several indications pointing out an association of ERV-derived protein expression and tumorigenesis. In the present study we analysed the immunological response and mRNA expression of HERV-K Gag in serum and tissue samples of breast cancer patients. Methods: Cloning and expression of breast carcinoma cDNA library in yeast was done by standard methods. Detection of the IgG antibody response against HERV-K Gag was done by RAYS analysis using serum samples from 100 breast cancer patients and 50 healthy controls. mRNA expression analysis of 12 tumor and four normal breast tissue samples was carried out using Real-time PCR. Results: We observed an HERV-K Gag serological B cell response in 11% of all patients with breast cancer. Only 3% of healthy controls had a weak titer. mRNA expression analysis confirmed that the expression level of HERV-K Gag was elevated in breast cancer tissues compared to the normal breast samples. Conclusions: Antibody responses to HERV-K Gag are found in a considerable proportion of breast cancer patients and might be due to protein overexpression and tumorigenesis. Further analysis is needed to link the expression and serological data to disease stage, tumor type and extent of disease.

P635
HER2 Positive Metastatic Disease in Primary not Overexpressing HER2 Breast Cancer: Re-Evaluation of HER2 Status and Tumor Marker Guided Therapy with Vinorelbine and Trastuzumab

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Background: HER2 is overexpressed in 20-30% of breast cancers. Compared to chemotherapy alone, chemotherapy with trastuzumab improves clinical outcome in patients with HER2-positive metastatic breast cancer. In general, HER2 status in a primary lesion predicts the status of a metastatic lesion such that biopsy of metastatic lesions appears unnecessary. Patient and Method: We report a 39-year old women who was diagnosed with primary breast cancer with has shown low HER2 expression using the method and scoring system of the DAKO Hrecep Test (DAKO score 1+). After failure of several chemotherapy regimens for metastatic disease (liver, skeletal), the patient underwent CT guided needle biopsy of the liver which showed HER2 positive adenoarcarcinoma (DAKO score 3+). The patient was started on a schedule consisting of vinorelbine (30 mg/m2 d1,8,15 q4w) and trastuzumab (4 mg/kg loading dose, 2 mg/kg weekly). Results: During a
P636 Distribution of Histological Subtypes and Molecular Determinants of Treatment Response in Adult Extragonadal Germ Cell Tumours

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Purpose: To assess biological and clinical characteristics of extragonadal germ cell tumours (EGCT) in view of localization and treatment outcome. Methods: Tumour material of 52 patients (pts) with GCTs was collected from pts treated within two German multicenter trials. Clinically, serum marker elevation with evidence of a retroperitoneal or mediastinal mass is considered specific for the diagnosis without definitive histology. For this analysis, only postpubertal cases with a negative testicular biopsy or unremarkable ultrasonicography, full clinical follow-up, and material of sufficient quality and quantity were included, leading to the exclusion of 27 cases. Histological diagnosis was made by a reference pathologist (JWO). Immunohistochemistry (IHC) of proteins involved in apoptosis (bcl-xl, bcl-2, bax), cell cycle regulation (Rb, p21, p53), DNA-mismatch-repair (MLH1, MSH2), nucleotide excision-repair (XPA), and drug export or inactivation (MRP2, BCRP, LRP) was performed using standard methods. FISH analysis was performed using centromer probes for chromosome X, 1 and 12. Results: A histological diagnosis was possible in 10 retroperitoneal and 15 mediastinal EGCTs. Cure rates were high in pts with retroperitoneal EGCT (80%), whereas long-term survival was poor in pts with mediastinal EGCTs (27%). Histologies were as follows: retroperitoneal EGCTs: 2 seminomas (SE), 6 pure choriocarcinomas (CC), and 2 pure yolk sac tumours (YS); mediastinal EGCTs: 1 SE, 3 CC, 5 YS, 2 teratomas, and 4 mixed histologies. In 19 cases, IHC was performed. Compared to a historical series of gonadal GCTs, a higher number of cases showed expression of bcl-2, p21, p53, MRP2, BCRP, and LRP. Ploidy assessment using FISH was performed in 17 cases. All tumours with the exception of one diploid mediastinal teratoma were aneuploid, mainly hyperdiploid. No differences between retroperitoneal and mediastinal EGCTs were observed. Conclusions: The observation of a high proportion of pure CC in the retroperitoneum is unexpected and supports the existence of EGCTs originating in the retroperitoneum without a gonadal primary tumour. IHC results indicate that anti-apoptotic signals, ability to induce cell cycle arrest, or the expression of drug export or inactivation proteins, might confer resistance in individual cases of EGCTs. The existence of a postulated subentity of diploid mediastinal GCTs with a particularly poor outcome could not be confirmed.

P637 Testicular Tumorigenesis Reverses Cell Cycle Gene Expression Patterns of Germ Cell Maturation

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Testis cancer shows distinct clinical and biological features compared to other solid tumors. Germ cells in the testis switch from proliferative mitosis to the meiotic cell cycle, a process which is reversed in testicular tumorigenesis. The underlying molecular mechanisms of regulation are currently insufficiently characterized. The aim of our analysis was to derive a global view of the expression pattern of cell cycle regulators at the switch between mitosis and meiosis, especially their reverse regulation in tumorigenesis. Therefore, we first studied expression levels of cyclins, CDKs and CDK inhibitors during postnatal testis maturation by microarray analysis and real-time quantitative RT-PCR. We found induction of cyclins A1, B2, K and M4, CDK2, all CDK family members, CDKN2c, CDKN2d and INCA1 during testis maturation whereas cyclins A2, B1, D2, G1, G2, CDK1, CDK4 and CDK2AP1 were downregulated. The induction of cell cycle regulators in this process reflected their importance in the meiotic cell cycle and spermatogenesis whereas the other genes with decreased expression probably played a role in the mitotic cell cycle. We compared these results with expression changes between human testis tumors and normal testis tissue. To first confirm similar regulatory mechanisms between the different species, we analyzed exemplarily the activity of the human and the murine cyclin A1 promoter in testis maturation by generation of a mouse line expressing EGFP and β-galactosidase under control of the human and the murine cyclin A1 promoter, respectively. Both promoters were activated simultaneously around day 18 of post-natal development. Expression studies in normal and malignant testis samples (N=36) revealed an inverse cyclin expression pattern than in testis maturation with elevated levels of D-type cyclins and reduced expression of cyclin B2, substantiating the hypothesis that the switch back from meiosis to mitosis during tumorigenesis reverses the expression of cell cycle regulators. Taken together, we provide a comprehensive expression map of cell cycle regulators at the switch between the mitotic and the meiotic cell cycle and link previously poorly studied proteins to the regulation of meiosis and testicular tumorigenesis.
Conclusions: Although pro-apoptotic effects of immunostimulatory DNA could be demonstrated in vivo, we could not confirm these results in a TCC cell culture model. We conclude that the pro-apoptotic and potential antineoplastic effect of CpG-ODN is mediated immunologically. A direct interaction between CpG-ODN and tumor cell could not be demonstrated.

P639
The Role of Paclitaxel in the First-Line Treatment of Patients with 'Poor Prognosis' Germ Cell Tumor (GCT) Undergoing Sequential High Dose Chemotherapy

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Purpose: Paclitaxel (T) is an active agent both in cisplatin-refractory and in untreated germ cell tumor (GCT) patients (pts). In order to determine the impact of T on the survival (OS) and toxicity this analysis compares the results of two prospective trials in pts with 'poor prognosis' GCT. Methods: Paclitaxel (T) is an active agent both in cisplatin-refractory and in untreated germ cell tumor (GCT) patients (pts). In order to determine the impact of T on the survival (OS) and toxicity this analysis compares the results of two prospective trials in pts with 'poor prognosis' GCT. Results: 155 pts have been analyzed, 59 pts in HD-VIP and 96 pts in HD-VIP+T. 112 pts and 414 cycles are fully assessable for toxicity. 152 pts for OS. Pts characteristics (HD-VIP compared to HD-VIP+T): Median age 29 (16-42) vs. 31 yrs (18-54), mediastinal primary 11 vs. 24%, liver mets 40 vs. 40%, bone 7 vs. 4%, CNS 20 vs. 19%, elevated AFP, HCG, LDH; 39 vs. 30%, 61 vs. 58% and 77 vs. 77%. Dose intensity of the 3- vs. 4-drug regimen achieved have been 98% and 95%. Median time to recovery of granulocytes and thrombocytes in 17 vs. 44% of pts, and grade III in 0% vs. 3% of pts (P>.05). 88% of pts seen in terms of stomatitis (14 vs. 49%), neutropenic fever (3 vs. 20%) and 95%. Median time to recovery of granulocytes and thrombocytes achieved have been 98% and 95%. Median time to recovery of granulocytes and thrombocytes (s=500µl resp. ≥25.000µl) have been day 15 and 16 independent from the addition of T. Differences in grade III/IV toxicity in favor of HD-VIP were seen in terms of stomatitis (14 vs. 49%), neutropenic fever (3 vs. 20%) and manifest infection (3 vs. 19%) (P<0.05). Grade IIII neurotoxicity was observed in 17 vs. 44% of pts, and grade III in 0% vs. 3% of pts (P<0.05). 88% of pts attained a favorable response (NED/CR/PR/m-) to HD-VIP compared to 67% in the HD-VIP+T (P<0.05). After a median follow up period of 34 mos (range, 9-78) and 16 mos (range, 0-47) the calculated 3-yr OS rates were 69.9 (C19.5%, 54.3-83.3) for HD-VIP and 67.3% (56.3-78.3) for HD-VIP+T (P=.3). Early death rate due to progression/toxicity was 3% in both trials. Conclusions: The addition of paclitaxel to HD-VIP dose level 6 as first-line therapy was associated with a moderately elevated toxicity. No substantial impact on survival can be anticipated for HD-VIP+T.

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Late relapse in Patients with Germ Cell Tumors (GCT) after Cisplatin-Based Chemotherapy

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Purpose: Late relapses of germ cell tumors, defined as recurrence after an interval of more than 2 years from initial therapy, are rare with only a few reports published. Methods: We analyzed characteristics, therapy and outcome of patients (pts) with late relapse after cisplatin-based chemotherapy. The records of 37 pts treated at our institutions from 1983 to 2002 were reviewed. Results: 36 pts had a non-seminomatous GCT (pure teratoma in 5 cases), 1 pt a pure seminoma. The median time to late relapse was 66 months (range 25 to 191 months) with 21 pts (57%) and 5 pts (14%) relapsing after more than 5 and 10 years, respectively. HCG was elevated in 6 of 36 pts (17%), AFP in 24 pts (65%) and 20% (58%) and 77 vs. 77% (HD-VIP compared to HD-VIP+T): Median age 29 (16-42) vs. 31 yrs (18-54), mediastinal primary 11 vs. 24%, liver mets 40 vs. 40%, bone 7 vs. 4%, CNS 20 vs. 19%, elevated AFP, HCG, LDH; 39 vs. 30%, 61 vs. 58% and 77 vs. 77%. Dose intensity of the 3- vs. 4-drug regimen achieved have been 98% and 95%. Median time to recovery of granulocytes and thrombocytes in 17 vs. 44% of pts, and grade III in 0% vs. 3% of pts (P>.05). 88% of pts attained a favorable response (NED/CR/PR/m-) to HD-VIP compared to 67% in the HD-VIP+T (P<0.05). After a median follow up period of 34 mos (range, 9-78) and 16 mos (range, 0-47) the calculated 3-yr OS rates were 69.9 (C19.5%, 54.3-83.3) for HD-VIP and 67.3% (56.3-78.3) for HD-VIP+T (P=.3). Early death rate due to progression/toxicity was 3% in both trials. Conclusions: The addition of paclitaxel to HD-VIP dose level 6 as first-line therapy was associated with a moderately elevated toxicity. No substantial impact on survival can be anticipated for HD-VIP+T.

P640
Antineoplastic Effect of Immunostimulative DNA (CpG-ODN) in a Murine C57-BL6/MB-49 Translational Cell Carcinoma Model

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Purpose: Intracaval BCG installation is established and efficient in the prophylaxis of recurrent transitional cell carcinoma (TCC), but its mode of action has not yet been elucidated. However, a Th1 biased immune response is postulated. Cell culture and animal models proved the efficacy of synthetic CpG-oligodeoxynucleotides (ODN) as inducer and adjuvant for a strong Th1-response and there is evidence for a direct and/or adjuvant antineoplastic effect. Purpose of our studies was to evaluate the antineoplastic effect of locally administered CpG-ODN in an subcutaneous murine bladder cancer model. Methods: A subcutaneous murine TCC model was established with female C57Bl/6 mice and the corresponding syngeneic MB49 TCC cell line. 3 groups of 5 animals received a cell suspension standardized for 1x10^6 cells/50µl. The cells were injected s.c. into the right and left flank. Only phosphorothioate (PTO) – modified ODN were used. Group 1 received 10nmol of CpG-ODN (immunostimulative sequence: 1668CG) only into the right cell depot. Group 2 received 10 nmol of CpG ODN (1668GC) into the right cell depot to control for PTO-backbone effects. Group 3 served as untreated control and received only PBS. The animals were examined for tumor size and weight and body weight at various time points after injection until execution on day 14. Tumor or scary tissue at the injection site were excised, weighed and examined histopathologically (HE-stain). Results: Tumor sizes and weights showed no side differences. Average tumor weight on day 14 was 82mg, 62mg, and 8mg, resp. in groups 3, 2 and 1. Diameter was 5mm, 5mm and 1mm, resp. There was no measurable weight loss. Histopathology revealed solid vital epithelial tumors in group 3 without a significant inflammatory reaction. The tumors of group 2 showed reduced vital tumor mass, central necrosis and moderate mononuclear infiltration of the surrounding tissue. Group 1 showed almost complete tumor necrosis and a considerable mononuclear inflammatory response. Conclusions: Immunostimutalutic DNA does not promote the established Th2 biased immune response of the local anti-tumor activity in a murine subcutaneous TCC-model. The histological findings suggest an immunologically mediated mode of action. A PTO-backbone effect has to be considered but does not seem to be the decisive element. The results have to be confirmed in an orthotopic TCC model and the immunological response needs further elucidation.
Toll-Like Receptor Expression in MB-49 Transitional Cell Carcinoma Cells in Culture

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Purpose: Receptors of the toll-like family (TLR) seem to play an important role in initiating and mediating innate immune responses. There is hope to design agents that activate selected immune response without causing general toxicity via the TLR pathway. Immunostimululative DNA (CpG-ODN) are able to trigger antitumor, Th1 biased immune responses. In vertebrates TLR-9 detects prokaryotic DNA and synthetic CpG-ODN. To evaluate the potential antineoplastic mechanism of action of CpG-ODN in TCC, we examined the expression of the TLR – family on mRNA-level in a murine TCC cell line (MB-49). Methods: MB-49 cell culture is maintained at our laboratory under standardized conditions (DMEM+Glutamax-10%FCS+1% Pen/Strep, 37°, 5% CO2). Results: MB-49 cells express TLR-2 (E_{AUC} 1/2%= 0,0097) and TLR-4 (E_{AUC} 1/2%= 0,00257). TLR-6 and TLR-9 are not expressed by MB-49 cells in culture. Conclusions: From these in vitro data we conclude that the potential antineoplastic effects of CpG-ODN in TCC are not mediated by TLR-9 receptors on the tumor cell. The mechanism of CpG-ODN action in vivo seems to be mediated immunologically. These preliminary data need further investigations to detect the mode of CpG-ODN action in TCC.

Hyperhomocysteinemia in Patients with Gynaecological Malignancies

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Purpose: Hyperhomocysteinemia may develop secondary to B-vitamin deficiency. Recently homocysteine production was observed in tumor cell lines, and homocysteine was proposed as a tumor marker. In various diseases including coronary heart disease but also rheumatoid arthritis or neurodegenerative diseases, a close relationship exists between development of hyperhomocysteinemia and increased concentrations of immune activation markers such as neopterin. Methods: B vitamin status was compared with homocysteine and neopterin concentrations in 20 patients (age: 70 ± 12 years) suffering from various gynaecological carcinomas (e.g., 10 Npl. ov., 7 Npl. corp.). Plasma concentrations of homocysteine and cytokine were measured by HPLC on reversed phase and neopterin concentrations were determined by ELISA (BRAHMS, Berlin, Germany), folate and vitamin B12 by RIA (Chiron, Walpole, USA). Non-parametric Mann-Whitney U-test was used for group-comparisons and Spearman rank correlation analyses were performed. Results: Compared to healthy controls, homocysteine and neopterin concentrations were increased in patients (all p<0,01), folate concentrations were at the lower end of normal and vitamin B12 concentrations were rather slightly increased. Increased homocysteine only was associated with lower folate concentrations (rs = -0,605, p<0,01) and with higher cytokine concentrations (rs = 0,463, p<0,05). However, there was no any association between homocysteine and neopterin concentrations (rs = 0,095, n.s.). Conclusions: Data demonstrate that hyperhomocysteinemia in patients with malignant diseases is unrelated to immune activation phenomena. This observation is in opposite to what has been observed in several other diseases. Development of hyperhomocysteinemia in the patients with malignant diseases relates to B vitamin disturbances. Increased demand for folic acid is most probably due to tumor cell proliferation rather than to immune activation cascades. However, number of patients is still to small for a definite conclusion.

Therapeutic Options in Inoperable Desmoid Tumors – Case Report and Review of the Literature

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Background: Desmoid tumor, also known as aggressive fibromatosis (AF), is a benign tumor exhibiting fibroblastic proliferation arising from fascial or subcutaneous structures. AF infiltrates locally, but does not metastasize, and tends to recur following local excision. Despite the benign microscopic appearance, and the negligible metastatic potential, the propensity of desmoid tumours for local infiltration is potentially significant in terms of deformity, morbidity and mortality due to pressure effects and obstruction of vital structures and organs. 7-12% of AF are located at the head and neck. In the general population, desmoids are rare, accounting for 0.03 % of all neoplasms, and have an estimated incidence of 2-4 per million per year. The pathogenesis remains unclear but there may be an association with a local trauma or surgery and exposure to progesterone or estrogen. Case Report: We report the case of a 32 years old woman with an inoperable desmoid tumor in the neck, first diagnosed in November 1997. Radiation therapy was not performed because of the localization near the spine and the risk for a high transverse lesion of spinal cord. In 1998 and 1999 she was treated with non-steroidal anti-inflammatory drugs, toremifen, and later LH-RH agonist. Due to a massive tumour progression, a R2 resection was performed in November 1999. A long-term low-dose chemotherapy with vinblastine and MTX was performed in 2000 without success. Also, an oral chemotherapy with trofosfamide was unsuccessful. In 2002 no therapy was applied and the tumour growth rapidly progressed. In May 2003 the patients neck circumference was 62 cm, and the tumour showed infiltration of spine and lung. Globus sensation and dyspnea led to a tracheotomy to prevent asphyxiation. From May to July 2003 four cycles of a chemotherapy with doxorubicin and dacar-
bazine were applied, but did not reduce the tumour mass. Instability of the spine, hypoaesthesia, anosmia and a paralysis were signs for progressive disease. The patient died in October 2003. Conclusions: En-bloc resection of the tumour, is the treatment of choice, if possible. Involvement of vital structures can prevent from complete surgical resection. When surgical resection is subtotal, recurrence rates have been reported as high as 70%. Radiation therapy has been used, but a standard procedure is not established yet. Recent reports have indicated that adjuvant therapy with antieustrogens, non-steroidal anti-inflammatory drugs, warfarin, vitamin K, and testolaceton, either alone or in combination, might be useful. Antisarcoma chemotherapy (doxorubicin and dacarbazine) has shown promising results in small series, as has combination therapy with vincristine and methotrexate. Further investigation of the molecular genesis of fibroblast mitogenesis might help to develop targeted treatment strategies.

P646 Familial Retinoblastoma and Leiomyosarcoma: Case Report and Review of the Literature

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Background: Retinoblastoma is the most common primary ocular malignancy of childhood, which results from sporadic or heritable mutations in the retinoblastoma gene, RB1, located at chromosome 13q14. It is well recognized to occur in two patterns: 1) a sporadic, non-heritable form presenting with unilateral disease and no increase in risk of further malignancies and 2) a genetic, heritable form presenting with uni- or bilateral disease, which is associated with a germ line defect and greatly elevated risk of developing a second malignancy. Case report: We here report a very rare case of familial retinoblastoma and leiomyosarcoma in a father and his daughter.

Father: The 54-year old male was diagnosed with unilateral retinoblastoma of his right eye in 1950 and underwent enucleation in the age of 1, he was not treated by chemotherapy or radiation in the childhood. In 2000 a leiomyosarcoma of his right lower leg with pulmonary metastases was diagnosed. He was treated by multiple chemotherapies including ifosfamide/epirubicin, trofosfamide, doxorubicin/DTIC, and ET-743, radiotherapy and surgery of lung and soft tissue metastases. At the moment the patient is treated by a fifth line chemotherapy with gemcitabine.

Daughter: The 31-year old female was diagnosed with bilateral retinoblastoma in 1973. Both eyes were enucleated in the age of 2, followed by a radiochemotherapy including vincristine, cyclophosphamide and actinomycin. In 1980 retinoblastoma recurred in the left orbita, she was treated by surgery and radiotherapy. 17 years later in 1997 she was diagnosed with a fronto-basal leiomyosarcoma with local involvement of left orbita. She was treated by radical surgery. Only 4 months later she recurred locally. She underwent a chemotherapy with amifostine, etopubicin and ifosfamide followed by surgery. The second local recurrence of leiomyosarcoma in 2000 was treated by stereotactic radiotherapy. In 2002 she underwent R2 resection of the 3rd local recurrence. Since that time the situation remains stable as documented by MRT. Discussion: The propensity for survivors of heritable retinoblastoma to develop second nonocular malignancies is well known. It was initially reported that second tumours occur within the field of irradiation. This was the case of the here presented daughter. Subsequently, it was demonstrated that second tumours could also develop outside of field of irradiation or after chemotherapy, or failed previous radiation or chemotherapy which was the case of the father. The mostly reported second tumor after retinoblastoma is the osteosarcoma, with an incidence of up to 50%; followed by fibrosarcoma (20%). Leiomyosarcomas have been very rarely reported in soft tissues adjacent to the orbit, femur, and maxilla. Visceral leiomyosarcoma of the bladder in retinoblastoma patients were reported after cyclophosphamide containing chemotherapy. In the presented family also the grandchild is affected by retinoblastoma, fortunately it is under local control by laser therapy. With this familial history systematic screening for tumour symptoms should be performed.

P647 Entstehung von Angiosarkomen nach brusterhaltender Mammakarzinom-Therapie – Initiierung eines Registers

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Histologische Typierung von Tumoren im Strahlenfeld Studie der EORTC-STRBSG TLR 01/01

<table>
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P648 Is there an Indication for High-Dose Chemotherapy in the Treatment of Sarcomas?

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Purpose: a question still not answered, as the role of high-dose chemotherapy (HDCT) with autologous peripheral blood stem cell rescue (PBSCT) is not established as standard therapy in the treatment of bone and soft-tissue sarcomas (STS). Patients and Methods: From August 1998 to January 2004 28 patients (pts.) with bone and soft-tissue sarcomas received HDCT and PBSCT (ewing sarcoma family n=8, osteosarcoma n=6, chondrosarcoma n=1, MPNST n=4, synovial sarcoma n=3, liposarcoma n=2, leiomyosarcoma n=2, rhabdomyosarcoma n=1, meningosarcoma n=1). 16 pts. were...
female, median age at TPL was 30.4 years [range: 13 – 59]. Following conventional chemotherapy and surgery CR (n = 10), PR (n = 10), SD (n = 2) and PD (n = 6) were reached prior PSCT. Different conditioning regimens were used, most pts. received the ICE/PEI regimen containing of ifosfamide 2000 mg/m²/d 1-6, carboplatin 200 mg/m²/d 1-6 and etoposid 200 mg/m²/d 1-6 (n = 15). Results: One pt. died due to cardiac arrest after HDCT. Except hematologic complications, no WHO grade III-IV complications were observed. Four pts. died after PSCT due to PD, in another 7 pts. disease recurred and led to death, 16 pts. are alive with/without disease. For all pts., median progression-free survival (PFS) is 12.8 months [range: 0 – 61] and overall survival (OS) is 17.6 months [range: 0 – 61]. Interestingly, osteosarcomas, the ewing sarcoma family and the chondrosarcoma (n = 15) demonstrated significantly longer PFS and OS with 18.7 months [range: 0 – 61] and 23.1 months [range: 2 – 61] compared to STS (n = 13) with 6.0 months [0 – 18] and 11.2 months [range: 0 – 27], respectively. Of course, that might be due to a significant longer follow-up in the bone sarcoma group with a median of 33.6 months [range: 3 – 68] compared to 23.0 months [range: 5 – 59] in the STS patients, respectively. CR pts. (n = 10) showed a median PFS of 25.0 months [range: 3 – 61] and median OS of 39.6 months [range: 3 – 61]. Conclusion: The remission status prior HDCT seems to be a major prognostic factor for bone and soft-tissue sarcomas, as especially pts. in CR before HDCT could benefit from this treatment strategy.

Poster Session: Solid Tumors

P649

Immunocytology in the Diagnosis of Carcinomatous Effusions

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Purpose: Cytological examination is commonly used in clinical practice for clarification of effusions. The method is fast, cheap and needs only a minimal apparatus equipment. The diagnostic value of cytology, however, is often limited, being considerable depending on the investigators experience. The diagnostic sensitivity and specificity of cytology can considerably be enhanced by immunocytological methods. The immunostaining is performed directly on smears and the result can be evaluated by the conventional light microscope. Methods: Comparing the diagnostic sensitivity of cytology and immunocytology in carcinomatous effusions we investigated samples of 1112 patients. In 551 cases, the effusion was caused by various carcinomas, in 561 cases by other diseases. Results: In carcinomatous effusions, correct diagnosis was done in 292 patients (53 %) by cytology. In 23 cases (4.2 %) tumour cells without further specification were described and in 146 cases (26.5 %) the diagnosis was “effusion suspicious for tumour cells”. In 90 cases (16.3 %) the diagnosis was false negative. In 39 of the 561 nonmalignant samples, the diagnosis was falsely positive. Using immunocytological staining, correct diagnosis of a carcinomatous effusion was done in 511 patients (92.7 %) by cytology. In 4 samples (0.7 %) the diagnosis was “effusion suspicious for tumour cells”, the term “tumour cells without further specification” was not used in any of the samples. In 36 cases (6.5 %) the diagnosis was false negative. Among the 561 nonneoplastic samples, there was no false positive diagnosis. In 201 cases (36.5 %) of the carcinomatous effusions, the immunocytological staining allowed the localisation of the primary tumour site. Conclusion: Our results show, that immunocytological examination is very sensitive and specific in the diagnosis of carcinomatous effusions. The precise typing of the tumour cells often allows the localisation of the primary tumour site, which minimizes costs by reducing expensive instrumental examination.

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Inhalation Therapy of Lung Metastases with Interleukin-2

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Interleukin-2 (IL-2) treatment has been shown to be effective for several patients with metastatic hypernephroma. This immunotherapy demonstrate in lung metastases response rates of 10% to 30% when administered intra-

venously or by subcutaneous injections. Severe side effects especially during i.v. administration limit the treatment to patients with good performance status and without comorbidity. Local IL-2 application by inhalation is associated with low toxicity.

122 patients with metastatic renal cell carcinoma were treated in our department with immunotherapy since 1990. 24 of these patients received gamma interferon and 74 IL-2 subcutaneously as primary treatment for metastatic disease. 24 patients started IL-2 inhalation for unsectable lung metastases. To demonstrate the distribution of inhaled IL-2 in trachea, bronchial system and lung, we labeled IL-2 with J123. One patient with pathological proven lung metastases, responding to IL-2 inhalation previously, consented to inhale radioactive labeled IL-2. We found a homogenous perfusion of IL-2 in all parts of the lung even in peripheral areas.

In contrast to other publications we use lower doses - 7.2 x 10^4 U.I. of IL-2 are administered daily for 5 days followed by 2 days rest. Inhalation is performed by the Jetair d20c nebulizer (Hoyer, Germany) on an outpatient basis. All patients except 2 learned the technique of inhalation with the respirator, while 3 patients stopped using IL-2 inhalation due to cough within the first week. We observed in 19 patients 2 complete-, 5 partial responses and 5 patients with stable diseases. 8 patients progressed. One complete response lasted for 36 month, one patient is still relapse-free after 32 month. None of the patients experienced nausea while cough was usually mild. In this small group of patients we found even with reduced and less toxic dose of IL-2 promising results.

P651

Non-Invasive Imaging of Subcutaneous Tumors and Pulmonary Metastases in Mice Using Flat-Panel Detector-Based Volumetric Computed Tomography

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There is a strong need for non-invasive methods to monitor tumor growth and progression over time in order to improve current standard single point data tumor models in mice. This study was aimed at examining the usefulness of flat-panel detector-based cone beam volume-computed tomography (FPD-VCT) as a non-invasive technique to monitor subcutaneously growing murine melanomas and their metastatic spread at defined time intervals. C57BL/6 mice at 3 months of age were injected s.c. (1 x 10^5 cells) or i.v. (1 x 10^5 cells) with syngeneic B16F1 melanoma cells. Following tumor cell inoculation, scans of anesthetized mice were performed using FPD-VCT (General Electrics Prototype) at distinct intervals. Subcutaneous tumor volumes were also quantitated at the same time points in vivo and post mortem by standard caliper measurement procedure. The results were subsequently related to histological analyses. The experiments validated FPD-VCT of s.c. growing melanomas as a very useful and sensitive technique to reproducibly obtain three dimensional volumetric tumor measurements. The validity and reliability of accurate volumetric calculations of the tumors by FPD-VCT was confirmed by standard caliper measurements of postmortem dissected tumors. Comparative analyses also determined that subcutaneous caliper-based tumor measurements in vivo are highly variable and by far not reliable as FPD-VCT measurements. Furthermore, FPD-VCT was found to be sufficiently sensitive to detect 200 μm diameter melanoma micrometastases. However, the dignity of these smallest micrometastases could only be assessed at later stages of tumor progression. Pulmonary tumor nodules of about 500 μm in diameter could reproducibly be detected as metastases by FPD-VCT and FPD-VCT measurement of these tumors corresponded well with histological analysis. In conclusion, non-invasive imaging by FPD-VCT allows an accurate real time assessment of subcutaneously growing tumors in longitudinal studies. It is also useful for the earliest detection of pulmonary metastases. Thus, FPD-VCT analysis has the potential to establish a novel standard for real-time non-invasive tumor assessment over time in animal models.
P652 Ongoing Phase I Study of Pemetrexed Combined with Gemcitabine and Cisplatin in Patients With Locally Advanced or Metastatic Solid Tumors

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Purpose: Pemetrexed (P, Alimta®) is a novel multitrargeted antifolate with clinical activity in a variety of solid tumors, including malignant mesothelioma, lung and breast cancer. Combining P, gemcitabine (G), and cisplatin (C) may lead to synergistic activity. Methods: The aim of this ongoing phase I dose escalation study is to assess the maximum tolerated dose (MTD) and dose-limiting toxicities (DLTs) of different PGC schedules and to collect anecdotal antitumor activity data. To date, the following two schedules have been assessed: Schedule 1 (q3w): P 400 mg/m² d1 (escalated to 500 mg/m² on dose level 2), G 800 mg/m² d1+8, C 40 mg/m² d1. Schedule 2 (q4w): P 500 mg/m², G 800 mg/m², C 40 mg/m², all on d1+15. P was administered intravenously (IV) over 10 minutes (min), G IV over 30 min, and C IV over 2 hours. Standard pre- and postmedications were used. Results: On schedule 1, 12 patients (pts; 9 male, median age 58 years, ECOG Performance Status 0-1) were enrolled, 6 per dose level. Tumor types included head and neck (6), thymoma (1), bile duct (1), prostate (1), renal (1), and cancer of unknown origin (2). A total of 43 courses of Schedule 1 were administered. One pt with head and neck cancer achieved a partial response, and stable disease was observed in 7 pts (for median 10 weeks). 4 of 12 pts experienced DLTs (dose level 1: G3 skin toxicity, febrile neutropenia; dose level 2: G3 diarrhea, G3 syncope). Hematologic toxicities included G3/4 thrombocytopenia in 10 pts, G3/4 leukenopa in 8 pts, and G3/4 anemia in 4 pts. Although formal MTD criteria were not met, dose escalation was stopped early in favor of the alternative q4w schedule. Four patients (all male; median age 47 years, Performance Status 1) were enrolled into the first dose level of schedule 2. Tumor types included head and neck (1), urothelial with a second primary malignancy of pancreatic cancer (1), thymoma (1), leiomyosarcoma (1). Two patients experienced 4 DLTs: G3 fatigue (2), G3 syncope (1), G4 neutropenia requiring delay of study drug on d15 (1). With 7 cycles administered in Schedule 2 to date, there was no G3/4 thrombocytopenia or anemia, 2 of 4 pts had G3/4 neutropenia. G3/4 non-hematological toxicities included G3 fatigue (3) and G3 syncope (1). Conclusion: PGC combination treatment is feasible and has antitumor activity with 1 PR and 7 SD. However, the toxicity profiles prompts assessment of alternative q4w sequential doublet schedule which is currently under investigation.

P653 Treatment of Advanced Neuroendocrine Tumours with a Combination of Paclitaxel, Carboplatin and Etoposide

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Background: Combination chemotherapy with paclitaxel, carboplatin and etoposide has been evaluated by Hainsworth et al. (1997) in the treatment of carcinoma of unknown primary. In this study 6 patients (pts.) with undifferentiated neuroendocrine tumours were included. The protocol showed a favorable response and safety profile in comparison to cisplatin/etoposide based regimen which represents the standard treatment for undifferentiated neuroendocrine tumours. Methods: We performed a retrospective analysis of 13 pts. (5m/8f; median age 56 (range 35-75)) with moderate or poorly differentiated neuroendocrine tumours, which have been treated at our center from 1999 to 2003 with paclitaxel 200 mg/m² d1, carboplatin AUC 6 d1 plus etoposide 50 mg alternated with 100 mg orally d1–10, qd 22. 13 pts. were evaluable for toxicity and 10 for response (3 patients were lost to follow up). Results: A total of 57 cycles were administered (median 4 range 1–14). NCI-CTCAE grade III/IV toxicity was anemia 1/0 pts., thrombocytopenia 4/1 pts., leukopenia 2/1 pts., sensory neuropathy 2/0 pts. In 8 pts, a dose adjustment due to toxicity was necessary. Efficacy: 3/10 pts. showed a CR, 2/10 pts. a PR and 5/10 pts. a SD. The 2 pts. with a CR were under observation for 18 and 17 nts. with no signs of relapse. Median progression-free survival was 18+ months (range 9–47+). Conclusion: The combination of paclitaxel, carboplatin and etoposide is very active in neuroendocrine tumours and is well tolerated in an outpatient setting. The main toxicity was grade 3 thrombocytopenia. These promising results justify further evaluation of the combination of paclitaxel, carboplatin and etoposide in a prospective study.

P654 A Randomized Phase II Trial of Gemcitabine Plus Treosulfan Versus Treosulfan Alone in Metastatic Uveal Melanoma

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Purpose: Uveal melanoma is the most common primary malignant tumor of the eye. Metastases occur in 15–40% of patients and are associated with a poor prognosis and a low response rate to chemotherapy. In vitro data suggested synergistic activity of treosulfan and gemcitabine in this disease. Earlier phase I/II trials demonstrated activity of this combination and in addition suggested a survival benefit of patients treated with the maximum tolerated dose (MTD) of the gemcitabine + treosulfan (GeT) protocol when compared to lower dose levels. Methods: We therefore initiated a randomised phase II trial in chemotherapy naive patients with pathologically confirmed metastatic disease. Exclusion criteria were Karnofsky performance status (KPS) < 60%, symptomatic brain metastases, prior chemotherapy and major organ dysfunction. After stratification by KPS patients were randomly assigned to receive GeT in the MTD of 1000mg/m² gemcitabine plus 3500mg/m² treosulfan both administered on days 1 and 8 or to 3500mg/m² treosulfan alone on days 1 and 8. Treatment was repeated on day 28. Based on Simon’s optimal 2-stage minimax design for phase II trials after 9 patients per arm response rates were analysed in an interim analysis. Results: In the GeT arm 4 patients had a remission or stable disease (PR/SD). In the treosulfan alone arm one patient had a PR/SD. Therefore both arms met criteria for continuation of the protocol until 24 patients per arm will be randomised. Conclusion: Final results of this first randomised trial in metastatic uveal melanoma will be presented at the meeting.

P655 Capecitabine is Active in a Broad Variety of Solid Tumor

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Introduction: Capecitabine is an oral fluoropyrimidine carbamate that was rationally designed to be activated preferentially within malignant tumor cells. The final step of activation involves the enzyme thymidine phosphorylase (TP). This enzyme is expressed at significantly higher levels in tumor tissues than corresponding normal cells. Capecitabine has proven to be very effective in metastatic colorectal and breast cancer. A high TP activity has also been shown in a variety of other tumor tissues. In general the side effects are mild and the treatment is well tolerated. Material and Methods: We report on 22 patients with various tumor types who were treated with Capecitabine: 4 patients with pancreatic cancer, 4 patients with lung cancer, 4 patients with head and neck cancer, 2 patients with thyroid cancer, one patient with an uterine cancer, one with an urachus cancer, one with a gastric cancer, one with a cholelodsus cancer, one with a neuroendocrine tumor of the liver, and one with a carcinoma of unknown primary site. Histologically there were mainly adenocarcinomas and squamous cell carcinomas. Results: Every reported patient experienced at least a disease stabilisation over a 3 months period. Some patients had long lasting remissions with a very good quality of life. The treatment was well tolerated. More details will be presented at the meeting. Conclusion: Capecitabine is established in the treatment of metastatic colorectal and breast cancer. These selected cases of a variety of tumor types demonstrate a broad activity of Capecitabine in cancer treatment. Further clinical trials are necessary to define the role of this compound in the treatment of the different tumor entities. The oral formulation is very suitable for therapy in an out-patient setting.
The Lymphoid Oncogene TCL1 Shows a Stage-Specific Expression Pattern during both T- and B-Cell Maturation

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Purpose: Activation of nearly all T-cell oncogenes by chromosomal rearrangements produces immature blastic tumors that are arrested at a particu-
lar thymic differentiation stage. An exception occurs in the mature T-cell prolymphocytic leukemias (T-PLL) that develop after insertional activation of the T-cell leukemia-1 (TCL1) gene at the time of T-cell receptor gene rearrangement. Thus, constitutive expression of TCL1 is oncogenic but seems to allow evolution to a mature post-thymic phenotype. To help understanding the function of regulatory as well as aberrantly expressed TCL1, we focus in this report on its expression pattern in the context of lymphocyte differentiation. Methods: TCL1 protein was analyzed by Western blot and immunohis-
tochemistry on normal lymphatic cells/tissue of different maturation stages as well as in various lymphomas/leukemias and cell lines of T- and B-cell lineage. Results: In thymic T-cells, we note that TCL1 is normally expressed in only a small number of thymocytes at the earliest maturation stage (i.e. CD3-CD4-CD8-) and is silenced in later stages of T-cell maturation. Simi-
larly, in their mature T-lymphoblastoid tumor (n = 48), TCL1 was expressed only in cases with both an early (stages I and II) thymocyte immunophenotype and an absence of surface CD3 (p<0.001), except for 1 case. Among post-thymic mature T-cell tumors (n = 214), TCL1 was expressed exclusively in T-PLL (44/60; 73%), the tumor with known TCL1 rearrangements. The level of TCL1 expression in T-PLL was higher than that seen in the TCL1+ cases of stage I/I1 surface CD3-negative T-ALL/LBL. In normal B-cells, TCL1 was highly expressed in all developmental stages until the germinal center in which expression varied between different stages of follicular maturation. This pattern was replicated in a broad range of primary B-cell tumors and cell lines, of which the follicular lymphomas (n = 60) showed the most variable TCL1 expression. Here, the level of TCL1 corre-
lated with markers (i.e. CD10, CD23) reflecting the maturation stage of these germinal center derived tumors. Conclusions: In T- and B-cells and derived lymphoid tumors, TCL1 exhibits a maturation stage-specific pattern of expression, except when insertional activation of the gene occurs in T-PLL.

Clonal Precursors for both Human CD11c– Type I Interferon-Producing and CD11c+ Dendritic Cells are Contained in Lymphoid and Myeloid Restricted Hematopoietic Progenitor Populations

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Purpose: Due to different cytokine responsiveness, surface receptor, and transcription factor expression, human CD11c+ natural interferon-type I producing cells (also called plasmacytoid cells or plasmacytoid pre-dendritic cells) and CD11c– dendritic cells were thought to derive through lymphoid and myeloid hematopoietic developmental pathways, respectively. Methods: To directly test this hypothesis, we established an in vitro assay allowing simultaneous interferon-type I producing cell, dendritic cell, and B cell develop-
ment (Flt3-ligand supplemented Ac6/Sys-1 stroma cell culture), and tested hematopoietic committed progenitors isolated from cord blood for their developmental capacity. Results: Lymphoid as well as common myeloid and granulocyte/macrophage progeni-
tors were capable to develop into both functional interferon-type I producing cells and in dendritic cells. Interferon-type I producing cells expressed gene transcripts thought to be associated with lymphoid lineage development irres-
spective of their lineal origin (e.g. pre-TCR and Spi-B). Both cell types differentiated within 5-6 cell divisions from input cells as determined by CFSE dilution. However, myeloid progenitors expanded about two-fold more, and clonal progenitors for both populations were about five fold more frequent within myeloid committed compared to lymphoid committed progenitor cells (about 1 in 18 versus 1 in 92, respectively). Conclusions: CD11c+ interferon-type I producing cells, and to some extend also CD11c– dendritic cells, are important mediators of innate immunity, and, upon consecutive maturation, initiate antigen specific adaptive immune responses. Innate immunity, however, pre-dates adaptive immunity in evolution. In this view, it is conceivable that both interferon-type I producing cells and dendritic cells in humans as in mice (data not shown) robustly segregate with myeloid development; type I interferon-producing cells then might primarily use genetic programs that later were adopted in adaptive immune system development. It will be important to further characterize developmental checkpoints for both cellular populations. This might lead to identification of potential new therapeutic targets for immunomodulation, e.g. in clinical settings as allogeneic hematopoietic stem cell transplantation.
primary mixed leukocytes reactions (MLR), without affecting the reactivity against third party cells. Our aim was to examine the effects of selective depletion of CD25 and/or CD 69 positive cells in primary but also secondary MLRs in order to further characterize the mechanisms that lead to GVH or GVl effects. Results: Peripheral blood mononuclear cells (PBMC) from normal donors were co-cultured with HLA-mismatched lethally irradiated PBMC. After 24, 48 or 72 hours of culture CD25-, CD69- or double positive cells were depleted using immunomagnetic procedures. On day 5 T-thymidine was added and proliferation measured after 16 hours. For secondary MLRs irradiated original stimulator cells or third party cells were added on day 10 of culture and proliferation was measured after 24, 48 and 144 hours. Depletion of CD69 positive cells in the primary MLR (n=6) lead to a suppression of the proliferation to 25±6%, 14±3% and 9±2% compared to the controls when depletion was performed after 24, 48 and 72 hours of culture, respectively. The removal of CD25 positive cells (n=5) after 1, 2 or 3 days of culture resulted in an inhibition to 20±4%, 5±1% and 2±0.3%. Results were not different when both CD25 and CD69 cells were separated at the same time. We then examined the effects of depletion of alloreactive cells on secondary MLRs. Whereas depletion of cells resulted in an ongoing suppression of secondary responses against the original stimulators, only when CD69 and/or CD25 positive cells were separated after 48 or 72 hours of culture, reactivity against 3rd party cells was maintained. On day 10+6 we observed an increase in the proliferation rate against third party to 98±59% in double depletion cultures. Depletion of alloreactive cells results in an inhibition of primary MLRs wht the best results obtained when depletion is performed 72 hours after begin of the culture and when CD25 alone or both CD25 and CD69 positive cells are depleted. Depletion of alloreactive cells does not affect the reactivity against 3rd party cells in the secondary MLR, suggesting that this could be an interesting approach to prevent GVH reactions without affecting GVl.

P660 Telomerase Activity and Telomere Length in CD4-CD25- Regulatory T-Cells after Expansion in Vivo and in Vitro Wolf D.1, Wolf A.M.2, Koppelstätter C.1, Rumpold H.1, Gastl G.1, Mayer G.1, Gunsilius E.1, Tilg H.2

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The expandability of CD4+CD25- regulatory T-cells (Treg) has been convincingly shown in vitro and in vivo. Activation of telomerase activity is a prerequisite for clonal expansion and concomitant telomere maintenance in proliferating T-cells. Our current report corroborates recently published data showing that Treg isolated from healthy volunteers have significantly shortened telomeres when compared to their CD4+CD25- counterparts. This observation has recently led to the hypothesis that Treg are highly differentiated T-cells showing a senescent phenotype. We therefore next focused on telomere length of Treg from cancer patients, which are known to be expanded in vivo. Of note, telomere length is not shortened in Treg isolated from patients with epithelial malignancies, despite their in vivo expansion. In accordance, expanding Treg rapidly induce telomerase function after in vitro activation with anti-CD3 and IL-2. Induction of telomerase activity is likely to compensate further critical telomere loss under conditions of in vivo expansion. In contrast, sorting of in vitro proliferating Treg using dilution of carboxy-fluorescein diacetate succinimidyl ester (CFSE) revealed a significant telomere shortening in Treg with high proliferative capacity. The latter are characterized by high telomerase activity, which however seems to be insufficient to avoid telomere shortening under conditions of strong in vitro stimulation. This observation should be kept in mind when considering the application of extensively in vitro expanded Treg for the treatment of graft versus host disease (GvHD) or autoimmune diseases.

P661 Evaluation of ZAP-70 in Patients with Chronic Lymphatic Leukemia by Flow Cytometry by Routine Analysis in the Clinical Setting

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Former data indicate ZAP-70 as an excellent prognostic parameter in patients with chronic lymphocytic leukemia (CLL), as ZAP-70 is strongly associated with the mutational status of the immunoglobulin heavy chain gene (IgH). This parameter could thus be relevant for the decision for the time and the mode of treatment in patients with B-CLL. Flow-cytometry is a useful tool in determining antigen expression in a quick and easy manner. Directly conjugated antibodies directed against ZAP-70 are now available. We sought to investigate the feasibility and usefulness of this direct immunofluorescent staining and subsequent cytometric analysis because it is a rapid and easy method compared to western blotting, immunocytochemical staining, indirect intracellular immunofluorescent staining or analysis of the IgH mutational status. Methods: Peripheral blood (15) and bone marrow (1) of patients with B-CLL were first stained with PE or TC conjugated surface CD3 (Beckton Dickenson) and PE conjugated surface CD56 (Beckton Dickenson) antibodies and subsequently prepared according to the FDX and Perm protocol (An der Grub) with an additional washing step in the end, stained with the Alexa Fluor conjugated ZAP-70 antibody from Caltag. Samples were analysed by FACScan and cellquest software from Beckton Dickenson. Results: T cells and NK cells were used as reference population for positive staining. 4 of 16 (25 %) samples were positive for ZAP-70 according the cutoff of 20 % for the lymphocytes as published by Crespo et al. (N Engl J Med 2003;348:1764-75). Range of ZAP-70 expression reached from 0.02 until 73.96 %. Conclusion: Routine staining for ZAP-70 by the Caltag ZAP-70 direct antibody is easy and feasible. Together with flow-cytometric CD38 evaluation it might be a helpful diagnostic tool for evaluation of disease progression.
In conclusion we present a reliable and sensitive method that enables us to detect cytoplasmic \( \mu \)-chains by flow cytometry, thus rendering fluorescence microscopy unnecessary.

**Poster Session: Transfusion Medicine**

**P663**

**Dendritic Cells Generated from Acute Myeloid Leukemia (AML) Blasts Maintain the Expression of Leukemia Associated Antigens**

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**Purpose:** Recently, the focus is on new specific immunotherapies for AML such as cellular therapies employing dendritic cells (DCs) generated from AML blasts. AML-DCs express constitutionally leukemia associated antigens (LAAs) present in AML blasts they are generated from. Here we investigated whether the generation of AML-DCs would alter the expression level of LAAs. Moreover, we evaluated the presence of HLA and costimulatory molecules on AML blasts versus AML-DCs.

**Materials and Methods:** Quantitative real-time PCR was performed for the following LAAs: preferentially expressed antigen in melanoma (PRAME), the receptor for hyaluronic acid mediated motility (RHAMM), CD168, Wilm tumor gene 1 (WT-1) and proteinase 3. The expression of HLA-ABC, HLA-DR, CD40, CD 80, CD83 and CD86 was evaluated by flow cytometry. AML blasts and AML-DCs were evaluated in ELISPOT assays and C51 release assays for the recognition of T cell epitope peptides derived from PRAME and RHAMM recognized by CD8+ T lymphocytes.

**Results:** Quantitative real-time PCR for AML-DCs versus AML blasts showed an alteration in mRNA expression of LAAs. An elevated PCR signal for PRAME was detected in 7/12 AML-DC preparations. 6/12 AML-DC preparations showed a significant upregulation of the PCR signal for RHAMM. A stronger WT-1 and proteinase 3 signal was observed in PCR for only 2/12 respectively 1/12 AML-DCs. All preparations showed a strong expression of at least one of the LAAs examined. As demonstrated by flow cytometry, AML-DCs strongly upregulated costimulatory molecules like CD40 and CD80 in comparison with AML blasts. CD8+ T cells recognized T cell epitope peptides derived from PRAME and RHAMM on AML blasts (40-50% specific lysis) and to a higher extent on AML-DCs (100% specific lysis). Conclusion: AML-DCs might constitute a powerful tool in immunotherapy for AML. Real-time PCR allows a quick and quantitative assessment of LAAs expression with only 10^3 DCs and might be helpful for the decision whether the AML-DC vaccination strategy is favourable or not. These qRT-PCR results were confirmed by T cell assays.

**P664**

**The Predictability of the CD34+ Cell Yield Harvested by Two Automated Leukapheresis Programs: Results of a Multicenter Trial**

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**Purpose:** In a prospective multicenter trial we investigated the predictability of the yield of peripheral blood progenitor cells (PBPC) harvested by the leukapheresis programs MNC and RV-PBSC of the Fresenius cell separator COM.TEC®. Based on the preleukapheresis CD34+ cell count, the machine calculates the total yield of CD34+ cells collected during the procedure. **Methods:** A total of 166 donors (128 patients with malignant diseases and 38 healthy individuals) underwent 203 leukapheresis either with the MNC or the RV-PBSC program. The median CD34+ cell collection efficiency (CD34-CE) was significantly higher in MNC than in RV-PBSC program (p<0.001): 86% (43-99) vs. 56% (25-95) and 12.5 mL (3.8-47.7) vs. 24.9 mL (4.7-60.5), respectively. **Conclusions:** The higher CD34-CE and the more exact prediction of the CD34+ cell yield make the MNC program a safe and convenient leukapheresis procedure. However, when a PBPC concentrate with a low RBC content is required (ABO-incompatible allografts), the RV-PBSC program should be preferred.

**P665**

**Use of Paper Sheets in a Clean Room Area Category B according to GMP Guidelines: Proof Of Feasibility**

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**Background:** The feasibility of documentation using paper sheets in clean room areas category B according to GMP guidelines has been put into question recently. Concerns addressed the release of particles in unacceptably high numbers. We have developed and validated a procedure for the use of paper sheets which proved to be associated with acceptable numbers of released particles. The procedure involves the exposure of the paper to dry heat at a temperature of 90°C for at least two hours. For comparison, another set of 50 paper sheets was tested without heat exposure. The paper sheets were placed on a table in the clean room area. Particle detectors were placed in front of the table and particles were counted during the following procedures: A) room in operation, paper untouched, B) operator in front of particle detector, paper untouched, C) writing on paper using a ball pen, D) moving and turning of the paper sheets, E) vigorous pounding of the paper sheets on the table. Particles were counted per m², with the air stream moving towards the detector. The tests were performed four times in two different clean room categories B. **Results:** The particle numbers never exceeded the maximum values allowed in clean room areas category B in operation according to GMP guidelines (≥5µm: 2000 per m², ≥0,5µm 35000 per m³). However, the particle numbers were lower when using heat-exposed paper sheets than in untreated paper (table). Microbiological testing revealed no colony-forming units on the surface of heat-exposed paper. **Conclusions:** The use of paper sheets in clean room areas category B according to GMP guidelines is feasible. In view of the low particle numbers and the negative results in microbiological assays, the use of heat-exposed paper as described above should be preferred to untreated paper.

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<th></th>
<th>writing using a ball pen (particles per m², min-max)</th>
<th>moving and turning of the paper sheets (particles per m², min-max)</th>
<th>vigorous pounding of the paper sheets on the table (particles per m², min-max)</th>
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<td>≥0,5µm 1340-7370</td>
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<tr>
<td>Heat-exposed paper</td>
<td>≥0,5µm 635-6200</td>
<td>1600-12000</td>
<td>14100-28420</td>
</tr>
</tbody>
</table>

**Table:**

- **Particles per m²:**
  - ≥0,5µm: 2000 per m²
  - ≤0,5µm: 35000 per m³

**Concentrates harvested by the RV-PBSC program had a significantly higher percentage of mononuclear cells and a lower red blood cell (RBC) content than those obtained by MNC (p<0.0001): 383 mL (204.586) vs. 148 mL (95.250). Concentrates harvested by the RV-PBSC program had a significantly higher percentage of mononuclear cells and a lower red blood cell (RBC) content than those obtained by MNC (p<0.0001): 383 mL (204.586) vs. 148 mL (95.250). Concentrates harvested by the RV-PBSC program had a significantly higher percentage of mononuclear cells and a lower red blood cell (RBC) content than those obtained by MNC (p<0.0001): 383 mL (204.586) vs. 148 mL (95.250). Concentrates harvested by the RV-PBSC program had a significantly higher percentage of mononuclear cells and a lower red blood cell (RBC) content than those obtained by MNC (p<0.0001): 383 mL (204.586) vs. 148 mL (95.250). Concentrates harvested by the RV-PBSC program had a significantly higher percentage of mononuclear cells and a lower red blood cell (RBC) content than those obtained by MNC (p<0.0001): 383 mL (204.586) vs. 148 mL (95.250). Concentrates harvested by the RV-PBSC program had a significantly higher percentage of mononuclear cells and a lower red blood cell (RBC) content than those obtained by MNC (p<0.0001): 383 mL (204.586) vs. 148 mL (95.250). Concentrates harvested by the RV-PBSC program had a significantly higher percentage of mononuclear cells and a lower red blood cell (RBC) content than those obtained by MNC (p<0.0001): 383 mL (204.586) vs. 148 mL (95.250).
Abstracts

Oncologie 2004;27(suppl 3):1–230

P666
Development of Hygromas or Severe Edema during Treatment with the Tyrosine Kinase Inhibitor STI571 is not Associated with platelet-Derived Growth Factor Receptor (PDGFR) Gene Polymorphisms

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STI571 is active against Bcr/Abl-, c-KIT- and PDGFR-driven malignancies. Mild to moderate edema is common, whereas severe edema, body cavity effusions and subdural hygromas are rarely observed. These effects are generally not associated with the development of grossly visible tumors. The present study was designed to examine the influence of genetic polymorphisms in the PDGFR, BCR-Abl and c-KIT genes on the development of severe, life-threatening edema in patients treated with STI571. SNPs in intronic regions of the PDGFR and BCR-Abl genes were analyzed by direct sequencing in 43 patients treated with STI571 for a period of 6–24 months. No single SNP or SNP combination was significantly associated with the development of severe edema. However, two patients who presented with severe edema over a period of 10 months carried the c-KIT G2029S SNP (p = 0.02). This is the first study to assess the influence of genetic polymorphisms on the development of severe, life-threatening edema in patients treated with STI571.

P667
Zoledronic Acid Modulates Monocyte Function and Differentiation into Dendritic Cells

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Bisphosphonates are widely used for the treatment of osteoporosis and metastases of the skeletal system. Recent reports provided evidence that bisphosphonates may not only reduce bone loss but also exert direct anti-tumor and anti-angiogenic effects. In addition, bisphosphonates have been shown to activate T cells. Both, the anti-osteolytic as well as the T cell-activating properties depend on their affinity to phagocytosing and/or antigens-presenting cell types, such as osteoclasts and monocytes. The latter represent a major source for Dendritic Cells (DC), which are key players for the regulation of the host’s immune response. Hence, it is tempting to speculate that Zoledronic Acid (ZA), a member of the latest bisphosphonates, exerts immunomodulatory properties by modulating monocyte/macrophage function as well as their differentiation to DC from mononuclear precursor cells. Our current report demonstrates that therapeutic doses of ZA (0.1 to 10 µM) inhibit the generation of DC from CD14+ selected monocytes, as shown by an impaired upregulation of MHC II, CD83, CD86, CD40 and CD54 on DC derived from monocytes pretreated for 24 hours with ZA. This observation is not due to induction of apoptosis of monocytes by ZA. In parallel, ZA also inhibits the LPS-induced activation of NF-kappaB which is a critical factor for DC differentiation. Accordingly, the activation of allogeneic PBMC in a Mixed-Lymphocyte Reaction (MLR) by ZA-DC is significantly reduced. In addition, ZA dose-dependently inhibits the production of TNF-α by monocytes as well as the phagocytic capacity of macrophages. In contrast, ZA did not exert any effect on isolated T-cells in terms of their phenotype, cytokine profile or their proliferative capacity. Hence, therapeutic doses of ZA alter monocyte/macrophage function and might modulate T-cell activation via direct inhibition of monocyte differentiation into DC.

P668
The Inhibitory Effects of Imatinib on Monocyte Derived Dendritic Cells are not Mediated by PDGFR and c-Kit

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Purpose: Dendritic cells (DC) are the most powerful antigen presenting cells playing a decisive role for the initiation and maintenance of primary immune responses. However, signaling pathways involved in the differentiation of these cells have not been fully determined. Imatinib is a novel tyrosine kinase inhibitor effective against Ab1 kinases, c-Kit and platelet-derived growth-factor receptor (PDGFR-R) while not affecting other kinases.

Methods and Results: Using this compound, we show that human monocyte derived DC generated in the presence of therapeutic concentrations of imatinib show a concentration dependent reduced expression of CD11c, MHC class I and II and co-stimulatory molecules CD40, CD45, and Dectin-1 expression as well as decreased activation-induced secretion of chemokines and cytokines like MCP-1, RANTES, and IL-12. Moreover, exposure to imatinib reduces the capacity of DC to prime T cell responses that cannot be restored by the addition of IL-12 and is not due to induction of apoptosis or IL-10 secretion. Using Western blot analyses we found that these effects are mediated by a pronounced downregulation of nuclear localized protein levels of NF-κB family members RelB, RelA and NF-xB p50 while not affecting the phosphorylation state of p38 MAP kinase and ERK1. To further analyze pathways and molecules affected by imatinib during DC differentiation we performed gene expression profiling utilizing DNA microarrays. These experiments revealed upregulation of lysosomal genes and genes preferentially expressed in monocytes/macrophages. Importantly, utilizing blocking antibodies, tyrosine kinase inhibitors and siRNA experiments we demonstrate that the inhibitory effects of imatinib on DC differentiation are not mediated by PDGFR-R and c-Kit but most likely via c-Ab1 tyrosine kinase.

Conclusions: Our results reveal a novel signaling pathway involved in the development and function of human monocyte derived DC.

P669
The Effects of a Combination Treatment with Imatinib and Farnesyltransferase Inhibitors on Chronic Myelogenous Leukemia Cells

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Farnesyl protein transferase inhibitors (FTIs) represent a new class of anti-cancer agents. We were interested whether the farnesyltransferase inhibitors L-744,832 and LB42918 were more potent with ED50 = 4 ± 1 µM (mean ± S.D., n = 3) in the BCR-ABL+ cell line EM-3 vs. ED50 = 25 ± 2 µM for L-744,832 after 48 hrs of treatment and assessment by MTT assay. The growth of BCR-ABL+ K562 and LAMA84 cells was not measurably inhibited by L-744,832 doses up to 25 µM. Similarly, the growth of K562 was not affected by 25 µM of LB42918. However, for LB42918 in LAMA84 an ED50 value of 30 ± 11 µM could be determined. In the FTT-sensitive cell line EM-3, combination index values (CI) obtained using the method of Chow and Talalay indicated synergistic effects following simultaneous treatment with imatinib and FTI (CI = 0.5 ± 0.2 and 0.7 ± 0.2 at ED75 for imatinib + L-744,832 and imatinib + LB42918 respectively). Annexin V / propidium showed a strong increase of the apoptotic cell fraction in EM-3 cells treated for 24 hrs by the combination imatinib + L-744,832 as compared to treatment with each drug alone. Growth inhibition of CFU-GM colonies of primary CML cells obtained from 4 patients is stronger after treatment with different concentrations of the combination of both drugs than after monotherapy with either imatinib or L-744,832. In the cell lines K562 and LAMA84 a trend to lower ED50 values of imatinib was determined when up to 5 µM of L-744,832 or LB42918 were added, indicating potentiation of imatinib.

On the basis of the observed potentiation effects FTIs may find their place as supplement for CML patients on imatinib treatment.
Dörken B., Telomere shortening.

Telomeres and which must be dissected from telomerase suppressed overall exerts a direct cytotoxic effect on malignant cells of the hematopoietic system. We conclude that using this class of telomerase inhibitor at higher concentrations loss of TRF2 and increased phosphorylation of p53.

Cord blood and leukapheresis samples was not affected by treatment with BIBR1532 cells from patients with AML and CLL.

Telomerase enzyme maintains the telomeres of eukaryotic chromosomes, and Telomere maintenance has been shown to be one hallmark of cancer and has been attributed to unlimited growth potential of human cells. Telomerase enzyme maintains the telomeres of eukaryotic chromosomes, and is active in most human cancers but, with few exceptions, not in normal human somatic tissues Therefore, telomerase has become an attractive target for development of new cancer therapeutics.

Selective Telomere Decapping: a Novel Therapeutic Modality for Leukemia Treatment?

Purpose: Telomere maintenance has been shown to be one hallmark of cancer and has been attributed to unlimited growth potential of human cells. Telomerase enzyme maintains the telomeres of eukaryotic chromosomes, and is active in most human cancers but, with few exceptions, not in normal human somatic tissues Therefore, telomerase has become an attractive target for development of new cancer therapeutics.

Methods: Here we examined the effects of BIBR1532 in different leukemia cell lines as well as in primary cells from patients with AML and CLL. Results: We observed a dose dependent direct cytotoxicity in concentrations ranging from 30 to 80 µM. Importantly, the in vitro proliferative capacity of normal CD34+ cells from cord blood and leukapheresis samples was not affected by treatment with BIBR1532. Q-FISH analysis revealed that high dose BIBR1532 induced a time-dependent individual telomere decapping which was associated with loss of TRF2 and increased phosphorylation of p53. Conclusions: We conclude that using this class of telomerase inhibitor at higher concentrations exerts a direct cytotoxic effect on malignant cells of the hematopoietic system which appears to derive from direct damage of the structure of individual telomeres and which must be dissected from telomerase suppressed overall telomere shortening.

The immunosuppressive macrolide RAD (INN: everolimus), a chemical derivative of rapamycin, has previously been shown to potentely inhibit tumor cell growth of Epstein-Barr virus transformed B lymphocytes of posttransplant lymphoproliferative disorders in vitro and in vivo. In this study, we provide evidence that RAD has profound anti-proliferative activity against tumor cell lines of B-cell-derived Hodgkin lymphoma (HL) and morphologically related T-cell-derived anaplastic large cell lymphoma (ALCL), that appear to be resistant to conventional chemotherapy. In addition, we show that RAD blocks cell-cycle progression in G1 phase. Moreover, we established SCID mouse models with xenotransplanted HL and ALCL cells where we measured the inhibitory effect on tumor cell growth in vivo. While daily treatment (5 mg/kg) with RAD was nontoxic, it markedly suppressed tumor growth, as determined by decreased tumor volume and survival. Our results demonstrate that RAD has potent direct anti-tumor effects in vitro and in vivo on B- and T-cell derived lymphoma cells and might therefore be considered as a new drug used in combination chemotherapy regimens for the treatment of HL and ALCL.

The immunosuppressive derivative of rapamycin, RAD (Everolimus), Promotes Growth Arrest in Vitro and has Profound Anti-Tumor Activity in SCID Mouse Models of Hodgkin and Anaplastic Large Cell Lymphoma

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Profound Anti-Tumor Activity in SCID Mouse Models of Hodgkin and Anaplastic Large Cell Lymphoma

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