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Regulatory T cells and myeloid-derived suppressor cells in metastatic renal cell carcinoma: Modulation by sorafenib and sunitinib

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Introduction: Regulatory T cells (Treg) and myeloid-derived suppressor cells (MDSC) play a pivotal role in tumor escape from immunological recognition. High levels of Treg have been shown in renal cell cancer (RCC) patients and seem to correlate with an adverse outcome. A reduction of Treg has been reported for RCC patients under sunitinib therapy. The aim of our study was to analyze the influence of multikinase inhibitors such as sorafenib and sunitinib on the frequency of Treg and MDSC in patients with metastatic RCC (mRCC). **Methods:**The number of Treg, MDSC and lymphocyte subpopulations was analyzed by flowcytometry in peripheral blood (pb) of patients with mRCC (n=19) under treatment with either sunitinib (50mg/d, n=11) or sorafenib (800 mg/d, n=8). After informed consent blood samples were taken before and during the 1st, 2nd, and 3rd month of therapy. Flowcytometric analysis was performed using fluororochrome labeled antibodies against CD3, CD4, CD25, CD127, FOXp3, CD33, CD14, CD11b, and HLA-DR. Treg were identified by expression of FOXp3 in CD3+CD4+CD25+ T cells, MDSC were characterized as CD33+CD11b+HLA-DR- PBMC.
Results: The baseline level of Treg in our RCC patients did not differ from healthy controls. However, in the group of patients treated with sorafenib there was a significant increase of CD3+CD4+CD25+FOXp3+ Treg (13,5% vs. 36,3% of gated cells, p=0,02) and the ratio FOXp3+/ FOXp3- within the CD3+CD4+CD25+ T cell population (0,16 vs. 0,56 in the gate, p=0,02). In contrast, these parameters remained unchanged in the group of patients under sunitinib treatment. Sorafenib- and sunitinib-induced changes in the numbers of Treg and MDSC were confirmed by an intragroup analysis. Analysis of CD33+/ CD11b+/HLA-DR- MDSC did not reveal any change under treatment with either sorafenib or sunitinib. Conclusions :Sorafenib but not sunitinib leads to an early and sustained increase of Treg in pb of mRCC patients. So far, there have been conflicting data on the influence of sorafenib on primary immune responses including modulation of dendritic cell (DC) function. Whether sorafenib exerts a direct influence on T cells or whether an indirect modulation via DC is responsible for the observed changes, will have to be investigated in further studies. In immunoresponsive tumors such as RCC, immunological effects of kinase inhibitors may be particularly relevant for the design of combination trials with immunotherapeutic agents.

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Westermann,J.: No conflict of interest disclosed.

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Post-hoc analysis on basis of a systematic review of DC-based immunotherapy in patients with urogenital tumors

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So far, more than 200 – mostly small phase I/II - clinical trials using dendritic cells (DC) as cellular adjuvant have been published. Nevertheless, heterogeneity of vaccination strategies, non standardized cellular products and the lack of standard criteria for clinical and immunological responses make it difficult to draw solid conclusions from single trials. Urogenital tumors, prostate and renal cell cancer (RCC), are regularly infiltrated by antigen-specific immune cells and are considered susceptible to immunotherapy. They have therefore been studied intensively as targets of DC-based interventions. Thus, we chose these entities for a systematic review of DC based tumor vaccines using single patient data to perform more comprehensive statistic analyses. We searched the Medline database from January 2000 to December 2007. We excluded articles published with mixed entities, follow-up studies and trials using allogeneic DC. Twenty four studies in prostate cancer (15) and RCC (9) were analyzed in detail comprising a total of 445 patients. Available individual patient data of 349 patients were used for post hoc analyses. DC types used for the vaccination strategies were mature monocyte derived DC (moDC), immature moDC, density grade enriched DC fractions in 11, 9 and 4 studies, respectively. For antigen delivery peptides, proteins and tumor lysates were utilized in 33%, 21% and 25% of the patients, respectively. Comparison of quality control standards and DC dose revealed striking differences between the studies. Pooled analyses (chisquare test) revealed induction of cellular immune response, dose of DC vaccine and route of vaccination to have a significant influence on the clinical benefit rate (CR, PR, MR, SD). Analyses stratified by studies confirmed a significant odds ratio for the association of cellular immune response and clinical benefit rate both for prostate cancer and RCC. Taken together, this systematic review disclosed a strong heterogeneity regarding vaccine dose, DC type, antigen delivery, application mode and quality controls. However, as a 'proof of principle' post-hoc analyses on individual patient levels revealed an association between the induction of cellular immune response and clinical benefit rate both for prostate cancer and RCC. To our knowledge this is the first systematic review demonstrating statistically significant effects of DC based vaccines further underlining the potential of this treatment strategy.

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Correlation of generation of specific T-cells against HM1.24, NY-ESO and MAGE-Antigens and the expression of these antigens in malignant plasma cells.

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Introduction: Multiple Myeloma (MM) remains despite advances in chemotherapy and hematopoietic stem cell transplantation still an incurable malignancy, new therapeutical options like an immunotherapy with a dentritic cell (DC) based vaccination could be a promising approach. Aim of this project is to analyze the development of immune tolerance against HM1.24, NY-ESO and MAGE-antigens depending on their expression on MM-cells in patients with early (EPD) and advanced plasmacell-dyscrasia (APD). **Methods:** We analyzed the expression of HM1.24, NY-ESO, MAGE A2 and MAGE A3 in autologous $CD138^+$ plasmacells of 26 newly diagnosed patients with plasmacell-dyscrasias (9 patients with EPD; 17 patients with APD) by RT-PCR. Specific $CD8^+$ T-cells against HM1.24, NY-ESO, MAGE A2 and MAGE A3 were expanded by autologous DC from fresh blood (PBMCs) or bone marrow (BMMCs) of 50 newly diagnosed patients with plasmacell-dyscrasias (23 patients with EPD; 27 patients with APD). Antigen-specific T-cell activation was analyzed by interferon-gamma secretion in ELIspot-assays. **Results:** The generation of MAGE A3 specific $CD8^+$ T-cells is decreased in patients with EPD, while generation of HM1.24 specific $CD8^+$ T-cells is impaired in APD. Regarding the results of the RT-PCR, most of patients with EPD (89%) do not express MAGE A3, while in APD we found an expression in 41% of the patients. HM1.24 is expressed in EPD (100%) and APD in 100% of the patients. **Conclusion:** The data show that the generation of activated cytotoxic T-cells against MAGE A3 correlates with the expression of this antigen on $CD138^+$ plasmacells, while generation of HM1.24 specific T-cells correlates with the stage of disease.

Disclosure: Fichtner,S.: Anstellungsverhältnis oder Führungsposition:wissenschaftlich Angestellte der Uniklinik Heidelberg
Hundemer,M.: Anstellungsverhältnis oder Führungsposition:Laborleiter des Labors Zelltherapie aus der Inneren Med. V Uniklinik Heidelberg

V331

Peptide vaccination effectively mounts cytotoxic T-cell responses with potential clinical relevance in patients with chronic lymphocytic leukemia

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Earlier, we characterized the receptor for hyaluronic acid mediated motility (RHAMM) as antigen associated with proliferation and negative prognosis in chronic lymphocytic leukemia (CLL). We also demonstrated that RHAMM-derived HLA-A2-restricted epitope(R3)-primed T cells were able to lyse $RHAMM^+$ target CLL cells. Therefore, we initiated phase I/II clinical trial with R3 peptide vaccination for patients with CLL. Six CLL patients were vaccinated four times at a biweekly interval with R3 peptide (ILSLELMKL, 300µg/dose) emulsified in incomplete Freund's adjuvant (IFA) with concomitant administration of GM-CSF (100µg/dose). $CD4^+CD25^{hi}CD127^{lo}FOXP3^+$ Tregs, Th17, $CD8^+CD137^+$ and $CD8^+CD103^+$ T cell were assessed by flow cytometry. IL-2, IL-10, TNF and TGF-ß serum levels were evaluated by ELISA. No severe adverse events greater than CTC I° skin toxicity were observed. Four patients showed a reduction of WBC during vaccination. The immune responses were found in 5/6 patients as assessed by tetramer-staining and confirmed in 4/5 in ELISPOT assay. Vaccination induced Tregs in 4 patients (2 non-responders and 2 responders). We observed an association between the frequency of Tregs and activated $CD8^+CD69^+$ T cells as well as IL-2 serum levels. In conclusion, peptide vaccination in CLL patients is safe and feasible to mount immune responses against the tumor antigen RHAMM

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CCL21 (SLC) as an adjuvant for DNA vaccination: enhancement of tumor protection and induction of a TH1-polarized immune response in a Her2/neu mouse tumor model

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Introduction: Her-2/neu is an epidermal growth factor receptor which is over-expressed in many cases of breast, ovarian, stomach and lung cancer. Her2/neu peptide vaccines have already demonstrated promising clinical results in breast cancer. In DNA vaccination, coexpression of tumor antigens together with immunomodulatory molecules is a strategy aiming at an amplification of the anti-tumor immune response. The Secondary Lymphoid-tissue Chemokine (SLC/CCL21) is a CC chemokine that is constitutively expressed in various lymphoid tissues. CCL21 (SLC) binds to the chemokine receptor CCR7 on mature dendritic cells (DC) and distinct T-and B cell subpopulations. In vivo CCL21 is able to increase the encounters between DC and T cells in regional lymph nodes. We asked whether CCL21 is able to augment the immunogenicity of a DNA-based vaccine against Her2/neu in a Balb/c mouse model with syngeneic Her2/neu+ tumor cells (D2F2E2). **Methods:** Mice were vaccinated twice intramuscularly on days 1 and 15 and tumor challenge was performed subcutaneously on day 25. Coadministration of plasmid DNA (pDNA) (Her-2/neu) plus pDNA (CCL21) was compared with pDNA (Her-2/neu), pDNA (CCL21) and mock vector. **Results:** Coexpression of CCL21 and Her-2/neu resulted in a substantial improvement of the protective effect of the DNA vaccine against Her2/neu+ tumor cells. Furthermore, it could be demonstrated by ELISpot and FACS-ELISA assays that coexpression of CCL21 is able to amplify both a Th1-polarized T cell response and a humoral anti Her2/neu immune response. In the group of mice immunized with pDNA(Her-2/neu) plus pDNA (CCL21) 38% of the animals remained tumor-free after 35 days compared with 8% with pDNA(Her-2/neu), 15% with pDNA (CCL21) and 8% with mock vector. Additional coexpression of pDNA(GM-CSF) together with pDNA(Her-2/neu) and pDNA (CCL21) lead to a further increase of the tumor-protective effect of the vaccine (70% tumor-free mice on day 35, 38% with pDNA(Her2/neu) plus pDNA(GM-CSF), 8% with pDNA(GM-CSF) alone). Our data do not allow to determine whether tumor protection was mainly mediated by a T-cellular or a humoral immune response. **Conclusion:** Our results show that coadministration of DNA encoding CCL21 can amplify the tumor-protective effect of an anti-Her2/neu DNA vaccine. Clinical use of a pDNA(Her2/neu-CCL21) vaccine might be particulary promising in Her2/neu+ breast cancer in the clinical situation of minimal residual disease after surgery and/or systemic therapy.

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Wissenschaftliches Symposium
Optimizing disease control in Multiple Myeloma

V333 – Best Abstract

Bortezomib, iv cyclophosphamide and dexamethasone (VCD) as induction therapy in newly diagnosed multiple myeloma: Update on the interim results with 300 patients of the German DSMM XIa trial

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Introduction: Cytoreductive induction followed by HD-MEL/auto-SCT is considered standard of care for younger pts with multiple myeloma (MM). The success of this combined procedure partially depends on the efficacy of induction treatment. Bortezomib-containing induction regimens have proven superior to anthracyline/dex combinations. In order to further improve the depth of response prior to SCT we combined Bortezomib (V) with intravenous (IV) cyclophosphamide (C) and dexamethasone (D). **Methods:** This trial is an open, prospective, multicenter, uncontrolled phase II/III study with a recruitment of 400 pts. The first 30 pts were included in the dose finding study to determine the optimum dose of IV C in conjunction with V and D. The subsequent 370 pts up to 60 years of age with untreated MM were enrolled to receive 3 cycles of induction

with V 1.3 mg/m2 IV d1,4,8,11; D 40 mg/d d1,2,4,5,8,9,11,12; and C 900mg/m2 d1. Primary study objective is response rate (= PR) to VCD according to EBMT criteria. **Results:** Data from the first completed 300 pts from 36 German centers analyzed as ITT population will be presented. 89% of pts completed 3 cycles of the VCD combination in an ambulatory setting. Assessment by investigators based on 200 pts shows excellent response rates (ORR 84%, CR 12.5%) with only 2% progressing. ORR was > 80% in all FISH subgroups except deletion 17p where it was 70%. SAEs occurred in 24.5% of the pts and were related to V, C or D in 16%, 14.5% or 9.5% respectively. The mortality rate of 1% is low, 51% of the pts experienced grade 3/4 adverse events (11% grade 4, 39.5% grade 3). Leukopenia was the most frequently reported AE (21.5% grade 3, 9.5% grade 4) followed by thrombocytopenia and anemia (4.5% grade 3, 0.5% grade 4). Infections of grade 3 and 4 occurred in 2%, polyneuropathy of maximal grade 3 was reported in 3.5%. **Conclusions:** This interim analysis demonstrates Bortezomib combined with dexamethasone and intravenous cyclophosphamide (VCD) to be among the most effective and feasible induction regimens for pts = 60 years with newly diagnosed MM.

Disclosure: Knop,S.: Honorare:Janssen-Cilag, Celgene
Einsele,H.: Beratungstätigkeit:Janssen-Cilag, Celgene
Honorare: Janssen-Cilag, Celgene

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The Arkansas approach in the treatment of multiple myeloma: 20 years of experience

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Data will be presented on the evolution of Total Therapy protocols since the inception of the Arkansas Myeloma program in 1989. Total Therapy 1 (TT1) was the first tandem transplant trial for myeloma, applying 2 cycles of melphalan 200mg/m2. Almost 20 years later, 51 patients of 231 enrolled are alive, including 24 without recurrence, and 12 of 87 achieving complete response (CR) have not relapsed. Thus, at 15 years, ~30% are alive and ~15% event-free. Total Therapy 2 (TT2) examined whether the up-front addition of thalidomide would increase the frequency of CR and thus prolong event-free and overall survival. After enrolling 668 patients, 10-yr estimates of survival and relapse-free status are 50% and 35%, while more than 40% of the ~50% achieving CR sustained this response at 10 years. Relevant to thalidomide randomization, CR rate was 60% v 40% on the control arm, and both event-free and overall survival segregated with significant p-values in favor of the thalidomide arm after 7 and 10 years, respectively. Gene expression profiling (GEP) generated a prognostic model with unprecedented separation power between risk groups: ~15% of patients with high-risk myeloma had a median survival of 2 years versus 10+ years for those with low-risk disease. Total Therapy 3 (TT3) incorporated bortezomib and thalidomide into the front-line management of myeloma. Among 480 patients enrolled, almost 80% achieved CR. The 85% with GEP-defined low-risk myeloma have 5-year overall and event-free survival estimates of 90% and 85% versus 45% and 35%, respectively; of those achieving CR, 90% are estimated in continuous CR at 4 years, as opposed to 40% in the high-risk setting. The adverse implications of FGFR3-type myeloma and of TP53 deletion no longer held among low-risk myeloma. Total Therapy 4 and 5 (TT4, TT5) have since been initiated based on GEP assignment to low- or high-risk myeloma, respectively. TT4 randomizes patients between standard TT3 and a lighter version that reduces induction and consolidation cycles from 2 in TT3 to just 1 each and adds VTD to 4-day-fractionated melphalan 200mg/m2, in an effort to reduce side effects and shorten the length of treatment while sustaining efficacy. TT5 emphasizes more dose-dense and less dose-intense therapies to sustain high CR rate by avoiding treatment-free intervals required for patients to recover from toxicities associated with high treatment intensities in TT3. Almost 70 patients have been accrued to TT4 and 10 to TT5.

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Freie Vorträge
Hodgkin Lymphom

V336

Treatment with 2 cycles of BEACOPPesc followed by 2 cycles of ABVD and IF-RT is superior to 4 cycles of ABVD and IF-RT in patients with early unfavourable Hodgkin lymphoma (HL): An analysis of the German Hodgkin Study Group (GHSG) HD14 trial

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Purpose: Combined modality treatment consisting of 4 cycles of chemotherapy and IF-RT is the standard treatment for early unfavourable HL. Overall survival (OS) and freedom from treatment failure (FFTF) in this group of patients was 91% and 83%, respectively, at 5 years in our prior HD8 study. Thus, the rationale for HD14 was to improve on these results by increasing dose intensity using BEACOPP escalated. **Methods:** Between January 2003 and January 2007, 1.216 patients aged 16–60 with untreated early unfavourable stage HL (CS I, IIA with one of the following risk factors: large mediastinal mass (a), extranodal disease (b), elevated ESR (c), or = 3 nodal areas (d); IIB with risk factors c and d) were randomized to either 4 cycles of ABVD (arm A) or 2x BEACOPP escalated followed by 2x ABVD (arm B). All patients received 30Gy IF-RT after chemotherapy. Primary objective was the improvement of the FFTF. Here we present the results of the predefined 3rd interim analysis within the prespecified group sequential analysis. **Results:** Of the 1.216 patients included, 1.010 were evaluable for this analysis. Patient characteristics were well balanced between both arms. At 3 years, the FFTF for arm A is 90% (95% CI: 87%-93%), and for arm B 96% (95% CI: 94%-98%). Since the observed inverse normal test statistic exceeds the critical level, the null hypothesis of equal FFTF in each arm can already be rejected. The improved FFTF is mainly due to differences in progression and early relapses (arm A 5.9% versus arm B 1.8%). Protocol adherence for chemotherapy was high and not different in both arms (arm A 98.8%, am B 97.3%). Though the chemotherapy-intensity was higher in the experimental arm, safety was comparable to the standard treatment. Secondary neoplasias occurred in 8 patients in each arm so far. **Conclusion:** Based on the significantly superior FFTF of the intensified therapy (2x BEACOPP escalated + 2x ABVD + IF-RT) compared to the prior standard (4x ABVD + IF-RT), this more aggressive treatment strategy will become the new standard for early unfavourable HL patients within the GHSG. Whether the improved FFTF translates into an improved overall survival must be awaited. Future strategies should aim at identification of those patient subgroups that profit most from this approach.

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Final Results of the HDR2 Study – A european multicenter Trial in Patients with relapsed Hodgkin Lymphoma

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Background: In patients with relapsed Hodgkin lymphoma (HL), high dose chemotherapy (HDCT) followed by autologous stem cell transplantation is being regarded as standard of care. However, the optimal regimen and intensity of chemotherapy is unclear. Therefore, the European intergroup study (HDR2) performed by the GHSG, EORTC and EBMT evaluated the impact of additional sequential high dose chemotherapy (sHDCT) after 2 cycles of DHAP and before BEAM to improve the efficacy of the standard regimen without sHDCT. **Methods:** Patients with histologically confirmed relapsed HL in first relapse with CR > 3 months or second relapse without prior HDCT were included. Responding patients after two cycles of DHAP were randomized between BEAM or sequential high dose (CTX, MTX, VP-16) followed by BEAM. Freedom-from-treatment-failure (FFTF) was the primary end point and progression-free survival (PFS) and overall survival (OS) were secondary end points. Kaplan-Meier estimates were used for the evaluation of survival and follow-up time. A prognostic score based on stage of disease, relapse type and presence of anemia before treatment (Josting et al, JCO 2002) was used to predict PFS. **Results:** A total of 284 patients were included in this trial. The median follow-up time was 42 months. 240 patients were randomized after DHAP and first restaging. There were no major differences in patient characteristics between the two arms with most of the patients in late first relapse (CR > 12 months). The intensified experimental arm showed significantly longer mean treatment duration, more frequent WHO-Grade IV toxicity before BEAM and more frequent protocol violations (p<.05). Although there was slightly lower mortality in the intensified arm (16% vs. 20%), there were no differences in terms of FFTF, PFS and OS. The respective 3-year-rates for the standard arm vs. the intensified arm were: FFTF: 71% vs. 67%, PFS: 72% vs. 69%, and OS: 87% vs. 83%. Patients with Ann-Arbor stage IV, early or multiple relapse and anemia had a significantly higher risk of recurrence of HL (all single bivariate p <.05, combined p < .001). **Conclusions:** Both regimens tested showed equally favorable results in outcome and survival. Since further intensification did not improve results, 2 cycles of DHAP followed by BEAM are the standard of care for patients with relapsed HL in our hands.

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Josting,A.: No conflict of interest disclosed..

V338

The Newly Developed Modified BEACOPP-Regimen (BACOPP) is Active and Feasible in Elderly Patientswith Hodgkin Lymphoma: Results of a Phase II Study of the German Hodgkin StudyGroup (GHSG)

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Background: Approximately 20% of patients diagnosed with Hodgkin lymphoma (HL) are more than 60 years of age. These patients still have a poor prognosis, especially when presenting with advanced stages and higher age. The main reason is underdosing of treatment, which is due to reduced tolerability of chemotherapy and age-related comorbidities. In the GHSG experience, elderly patients in the HD9 trial did not profit from the BEACOPP regimen in terms of overall survival, though a better HL specific freedom from treatment failure was achieved as compared to COPP/ABVD. Thus, the GHSG has developed the BACOPP regimen in which etoposide was omitted to improve tolerability. Further modifications were: a 1 week pretreatment phase with vincristine and prednisone, a limitation of vincristine in patients older than 65 years and an increase of the anthracyclines dose. Here we report on the final analysis of this multi-center phase II study for elderly patients. **Methods:** Between 2004 and 2005 a total of 65 patients with HL in intermediate or advanced stages aged between 60 and 75 years were recruited. Treatment consisted of 6 cycles BACOPP in patients achieving a complete remission (CR) after 4 cycles or 8 cycles BACOPP in case of PR (partial remission) after 4 cycles. The primary endpoints were protocol adherence and response rates. Secondary endpoints included WHO grade III/IV toxicities, Kaplan Meier estimates of progression free survival (PFS), freedom from treatment failure (FFTF), and overall survival (OS). **Results:** Sixty patients (92%) were eligible for the final analysis. The majority of treatment courses (75%) was administered according to protocol. However, there was a tendency towards reduced dosing in cycles 5 to 8, especially for patients who had reached a CR after 4 cycles of BACOPP. In total, 51 patients showed CR/CRu (85%), 2 PR (3%) and 4 progression of disease (7%). Survival estimates are shown in table 1.

Table 1. Kaplan-Meier rates and 95% confidence intervals (CI) for FFTF, PFS and OS.

	time point	rate (%)	CI (95%)
FFTF	12 months	73	61-84
	24 months	67	55-79
PFS	12 months	75	64-86
	24 months	68	56-80
OS	12 months	85	76-94
	24 months	76	65-87

WHO grade III-IV toxicities were documented in 52 patients (87%). With a median observation time of 33 months, 18 deaths (30%) have been observed. Seven therapy associated fatal outcomes were documented. **Conclusion:** The new BACOPP regimen shows a high CR rate (85%). The FFTF rate at 2 years is within the range known from other schedules in this patient cohort. Overall, the regimen is feasible, but the therapy associated death rate was high in our patient cohort. Thus, further studies and new approaches are still needed to substantially improve the outcome of elderly patients with early unfavourable or advanced stage HL.

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V339

Eight Cycles of BEACOPPesc compared with 4 Cycles of BEACOPPesc followed by 4 cycles of BEACOPP baseline with or without RT in Advanced Stage Hodgkin Lymphoma (HL): Final Analysis of the HD12 trial of the German Hodgkin Study Group (GHSG)

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Purpose: The GHSG HD9 trial had established BEACOPP escalated (BE) as new standard of care for advanced-stage HL patients by showing significant superiority in terms of failure-free survival (FFTF) and overall survival (OS) over COPP/ABVD and BEACOPP baseline (BB) (each 8 cycles). The successor study, HD12, evaluated a possible reduction in toxicity by comparing 8 cycles of BE with 4 cycles BE followed by 4 cycles BB. The second question in this trial related to the need of additional radiotherapy (RT) to initial bulk and residual disease. **Patients** and **methods:** HL patients in stage IIB with large mediastinal mass and/or E-lesions or stage III/IV were randomised according to a 2x2-factorial design between: 8BE + RT, 8BE no RT, 4BE+4BB + RT, 4BE+4BB no RT. Reviewing CT-images before and after chemotherapy treatment, fields for RT were centrally planned by a multidisciplinary diagnostic panel blinded for the randomisation arm. Primary endpoint of the trial was FFTF. Between 9/1999 and 1/2003, a total of 1,670 patients aged 16-65 were randomized. For this final analysis at a median follow up of 78 months, 96 patients were excluded (42 HL not confirmed, 20 revision of stage, 17 no study treatment or documentation, 17 others) resulting in 1,574 eligible patients. **Results:** Patient characteristics in the 4 groups were comparable with 49% of patients in stage III, 35% in stage IV, 68% reporting B-symptoms and 28% having a large mediastinal tumor. An IPS of 3 or greater was reported for 38% of patients, predominant histology was nodular sclerosis with 57% of cases. Treatment-related toxicity of WHO grade III/IV was observed in 97% of patients. Most prominent differences between pooled chemotherapy arms were anemia (65% 8BE vs 50% 4BE+4BB) and thrombopenia (65% vs 51%). Treatment outcome: complete remission 92.1%; early progression 2.2%; progression/relapse 7.8% (6.7% and 8.9%). A total of 156 (9.9%) deaths (73 vs 83) have been observed (22 vs 32 acute or salvage treatment toxicity; 15 vs 23 HL; 22 vs 13 secondary neoplasia). Most treatment related deaths occurred in the =60 years age group, the first 4 cycles and the IPS> 3 RF groups. Secondary neoplasias were observed in 76 patients (4.8%): AML/MDS 1.5% vs 1.4%, NHL 1.5% vs 0.6% and solid tumors 2.3% vs 2.3%. At 5 years, OS was 91% and FFTF 85.4% (Kaplan-Meier estimates). Estimates for the difference at 5 years are 1.7% (95% CI [-1.2%, 4.6%]) for OS and 1.6% [-1.9%, 5.2%] for FFTF favoring BE. However, there was no statistical difference between 8x BE and 4BE+4BB in all outcome parameters (p>0.25, log rank test). There is also no significant difference between the RT or no-RT arms in this study with the caveat that a number of high-risk patients receiving RT based on the blinded panel decision. **Conclusion:** The adoption of 4BE+4BB as a new standard in the future GHSG studies will depend on a refined analysis of the total data set and will be presented.

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V340

Comprehensive Geriatric Assessment (CGA) in patients with malignant lymphoma

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Introduction: The number of elderly patients (pts) with malignant lymphoma (ML) will increase within the coming decades. Elderly pts are a heterogeneous population. CGA is established in geriatric medicine. Its prognostic value has not been analysed in pts with ML so far. We therefore analysed in the subgroup of pts with ML included in the Geriatric Oncology Program at our institution whether deficits in CGA are associated with decreased survival. **Methods:** Newly diagnosed pts with cancer and indication for chemotherapeutical treatment were prospectively recruited in an observational trial. In addition to usual diagnostic work up including Karnofsky-Performance-Status (KPS) and standard treatment pts´, activities of daily living (ADL), instrumental activities of daily living (IADL), and comorbidities (Cumulative Illness Rating Scale – CIRS) were assessed. Association of pts´ characteristics and results of CGA with survival were calculated according to Kaplan-Meier method (Log-Rank-test). In addition a multivariate Cox-regression analysis was performed. **Results:** 143 (34%) out of 425 pts had a ML, median age was 63 (range 18 - 88). 63 pts (44.1%) were female. Diagnosis were Non-Hodgkin-Lymphoma n=87, Multiple Myeloma n=29, Hodgkin´s Disease n=16, chronic lymphocytic leukaemia n=8, others n=2. Treatment approach was curative in 65 pts (45.5%). KPS was < 80 in 16.1%, ADL < 100 in 18.2%, IADL < 8 in 21.0%, severe comorbidity with CIRS score 3-4 was present in 55.9%. Median follow up of surviving pts was 62 months. 66 pts (46.2%) died within this time. The following factors were significantly associated with shorter survival time in univariate analysis: age (< 60 vs. >= 60 yrs: 74 vs. 54 months; p=0.004), KPS (80-100 vs. < 80: 69 vs. 38 months; p<0.001), ADL (100 vs. < 100: 71 vs. 42 months; p=0.021); IADL (8 vs. < 8; 69 vs. 47 months; p=0.004), comorbidities (severe comorbidity not present vs. present: 80 vs. 48 months; p<0.001). Gender (male vs. female) and treatment approach (curative vs. non-curative) were not significantly associated with survival time. In a Cox-regression analysis, IADL (HR 2.1 – 95% CI 1.1 – 3.9) and comorbidity (HR 1.9 – 95% CI 0.9 – 3.9) were independent and strongest associated with survival time. **Conclusion:** Results of CGA, such as IADL and comorbidities, are important prognostic variables for survival in patients with ML. Results should be validated and if confirmed included in diagnostic and therapeutic algorithm.

Disclosure: No conflict of interest disclosed..

Freie Vorträge
ALL klinisch

V341

Blinatumomab (Anti-CD19 BiTE®) for targeted therapy of minimal residual disease (MRD) in patients with B precursor acute lymphoblastic leukemia (ALL): Update of an ongoing phase II study

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Background: Blinatumomab (MT103/MEDI-538) is targeting the CD19 and CD3 antigens, and is a member of a novel class of bispecific BiTE antibodies that redirect T cells for lysis of target cells. In patients with B-precursor ALL, MRD positivity after induction therapy or at any time point later predicts a hematological relapse, despite undergoing further intense chemotherapy. A phase II study is being conducted by the Ger-

man Multicenter Study Group on Adult Lymphoblastic Leukemia (GMALL) in patients with MRD-positive B precursor ALL. **Methods:** B–precursor ALL patients in complete hematological remission with molecular failure or molecular relapse (defined as MRD $>10^{-4}$) starting at any time after consolidation I of front-line therapy were included. MRD is assessed by quantitative PCR of either individual rearrangements of immunoglobulin/TCR-genes or specific genetic aberrations such as bcr/abl or ALL1-AF4, defined as $< 10^{-4}$. One treatment cycle of blinatumomab is a 4-week continuous i.v. infusion, which can be followed by allogeneic SCT or repeated cycles of blinatumomab with 2-week treatment-free intervals. The dose level is 15 microgram/m^2/24 hr. Based on clinical activity and lack of safety concerns, the dose may be increased in this trial. **Results:** At the date of submission, 10 patients have been enrolled and 7 patients are evaluable for response. Most common adverse events included pyrexia, chills, hypoimmunoglobulinemia, and lymphopenia. Except for 1 lymphopenia grade 4, 1 hypoimmunoglobulinaemia grade 3, and 1 port infection grade 3 (unrelated), these AEs had an intensity of grade 1 or 2. Most AEs resolved during treatment. Overall, treatment demonstrated no unexpected safety concerns and no permanent treatment discontinuation was required. Five of seven evaluable patients went into molecular remission (mCR) after one cycle of blinatumomab treatment. One of these responding patients became negative for both individual rearrangements and bcr/abl. Another patient with bcr-abl positive B-ALL, who has been treated with 4 cycles of blinatumomab (first three cycles in combination with dasatinib) remained at stable MRD level as did one patient with ALL1-AF4 after one cycle. Of the 5 responding patients one patient had an extramedullar testicular relapse followed by hematological relapse one month after discontinuation of treatment. **Conclusions:** Blinatumomab as a single agent has induced molecular remissions in 5 out of 7 patients with MRD-positive ALL. Treatment has exhibited a safety profile that supports further study and recruitment is ongoing.

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V342

A prospective,randomized multicenter trial of Imatinib following allogeneic stem celltransplantation (SCT) for Philadelphia-positive acute lymphoblastic leukemia(Ph+ALL)

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Introduction: Reappearance of bcr-abl transcripts after allogeneic SCT for Ph+ALL is indicative of evolving relapse. We hypothesized that initiation of imatinib therapy in the setting of a low leukemic cell burden. i.e. early after SCT, may prevent relapse and improve outcome. The optimal timepoint for starting imatinib is not known. In this randomized multicenter clinical trial, we compared interventional administration of imatinib in response to PCR positivity after transplant with and MRD independent initiation of IM as early after SCT as possible, with respect to tolerability and duration of molecular and hematologic remission: **Methods:** We report on the results of a protocol-specified interim analysis of the first 40 enrolled patients, who were randomly assigned, within 6 weeks after SCT, to receive IM up-front (cohort 1) (n=20) or following detection of bcr-abl transcripts by real time quantitative and/or nested rtPCR (cohort 2)(n=20). Bcr-abl transcripts were assessed in peripheral blood and bone marrow samples every 3 and 6 weeks, respectively. The target dose of IM was 600 mg, 400 mg was permitted if deemed necessary by the investigator for reasons of tolerability. Administration of imatinib was planned for one year of PCR negativity. Informed written consent was obtained from all patients prior to enrolment. **Results:** SCT was performed in CR1 in 17 pts. of cohort 1 and in 18 pts. of cohort 2, 2 each were transplanted in CR2 and 1 with active disease (cohort 2). To date, imatinib was started in 17/20 patients receiving imatinib up-front and in 10/20 patients treated with imatinub after PCR positivity. Median time from SCT to start of IM in these two cohorts was 45 days and 89 days, respectively. The most frequently used imatinib dose was 400 mg IM (19/27 pts.). There were three deaths in CR, all in cohort 1; howver, only one of these pts. had actually received imatinib. After a median follow-up of 438 days (131-1329d) and 577 d (196-1316d) in cohorts 1 and 2, respectively, none of the 35 pts. transplanted in CR 1 and 2 of 5 with SCT in CR2 or active disease have relapsed. Premature discontinuation of imatinib was more frequent among patients in the IM up-front cohort (10/17 pts.) than among pts. in the MRD-triggered cohort (3/10 pts.). Most frequent reasons were gastrointestinal toxicity (n=5) and GvHD (n=3). **Conclusions:** Post-transplant administration of imatinib is associated with a low relapse rate and no evidence of increased non-relapse mortality, although imatinib appears to be less well tolerated when started very early after SCT. Administration of imatinib after allogeneic SCT is a promising strategy to improve outcome of patients with Ph+ALL.

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V343

Prognostic significance of *BAALC* expression in adult B-precursor acute lymphoblastic leukemia

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Introduction: Overexpression of the gene BAALC (brain and acute leukemia, cytoplasmic) has been identified as an adverse prognostic factor in adult patients with cytogenetically normal acute myeloid leukemia (CN-AML) and acute T-lymphoblastic leukemia (T-ALL). This lineage-independent prognostic relevance of BAALC in acute leukemias prompted us to analyse its expression pattern and prognostic implication in adult B-precursor ALL. The identification of novel predictive molecular markers in B-precursor ALL is particularly important for patients lacking the two main molecular high-risk factors BCR-ABL and MLL-AF4. **Methods:** BAALC expression was determined by quantitative real-time PCR in pretreatment bone marrow samples of 368 adults with newly diagnosed B-precursor ALL enrolled between 1999-2006 on the 06/99 (n=199) and 07/03 (n=169) GMALL multicenter trials. For statistical analyses, patients were grouped into terziles according to BAALC expression levels [T1: low BAALC (n=123), T2: intermediate BAALC (n=123), T3: high BAALC (n=122)]. The median follow-up for living patients was 4 years. Patients who received autologous (n=5) or allogeneic (n=122) stem cell transplantation were excluded for survival analyses. Multivariate analyses were performed according to the Cox proportional hazards model for overall survival (OS) and with logistic regression for primary therapy resistance, both including the following variables in the full model: white blood cell (WBC) count (=30/nl v <30/nl), age (10-year increase), CD20 (positive v negative), and immunophenotype (pre-B v common/pro-B). **Results:** Higher BAALC expression (T3 v T2 v T1) was associated with a higher age (P< 0.001), higher WBC (P=0.008), CD34 positivity (P=0.001), BCR-ABL (P<0.001), and MLL-AF4 (P<0.001). Higher BAALC expression was significantly correlated with primary resistant disease in univariate (BAALC T3 15%, BAALC T2 8%, BAALC T1 3%; P=0.004) and multivariate models [OR 2.4 (95% CI 1.4-4.3); P=0.002]. Within the subgroup of BCR-ABL- and MLL-AF4-negative ALL, patients with higher BAALC expression had a significantly shorter OS [5-year OS: BAALC T3: 36% (95%-CI: 20-52), BAALC T2: 49% (95%-CI: 34-64), BAALC T1: 67% (95%-CI: 55-78); P=0.01]. In multivariate analysis, BAALC was of independent prognostic significance for OS in BCR-ABL- and MLL-AF4-negative patients [HR 1.4 (95%-CI 1.0-1.9); P=0.05]. **Conclusions:** High BAALC expression identifies patients with an unfavourable response to induction chemotherapy and inferior OS. Thus, determination of BAALC might contribute to a more detailed risk assessment of molecularly yet undefined subgroups of adult

B-precursor ALL. The lineage-independent association of BAALC with an immature, more aggressive leukemic subtype highlights its possible involvement in leukemogenesis and potential role in chemotherapy resistance.

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V344

Allogeneic Stem Cell Transplantation (SCT) beyond First Remission in Adult Patients with Acute Lymphocytic Leukemia (ALL)

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70 adult ALL patients (pts) were transplanted after 1. relapse (leukemic blasts >5% in bone marrow or central nervous system involvement) in a single center between 1995 and 2007. 2 pts were excluded from the analysis because diagnosis of ALL was made in childhood and they were transplanted in adult age. Median age of the analyzed 68 pts was 28 years (16–54). 43 pts were male, 25 female. 32 pts were transplanted in 1. relapse, 24 pts in CR2 and 12 pts beyond CR2 (>CR2). Stem cell donors were HLA identical sibling (n=24), identical twin (n=1), HLA identical child (n=1) and unrelated donors (n=42). Standard conditioning was 12GyTBI and cyclophosphamide (CY) or VP16 (myeloablative conditioning = MAC). Pts with comorbidities and contraindications against MAC received a dose reduced regimen (RIC) with FLUD, BU and ATG (n=3). 18/68 pts (26%) are alive in CCR, 50/68 pts (74%) are dead. Causes of death were leukemia in 30/50 and transplant related morbidity, TRM in 20/50 pts (GvHD and/or infection). For the whole patient group, probability of survival (OS) at 7 years is 0.21 as is disease free survival. TRM is 0.43. OS regarding stage of disease at SCT was 0.26 for pts transplanted in 1. relapse, 0.23 for pts transplanted in CR2 and 0.08 for pts transplanted beyond CR2. OS after unrelated donor or family donor transplantation was not different (0.20 vs 0.22). Influence of leukemic risk factors on the OS was analyzed. Risk group at diagnosis and during chemotherapy (standard vs high risk group, criteria according to the GMALL Studies) had no significant influence on the OS: 0.18 vs 0.25. Pts with B-lineage ALL had a OS of 0.25 vs 0.08 in pts with T-lineage (n. s.). The median duration of 1. remission for all pts was 9 months (1–72). OS for pts with a remission duration =9 months or >9 months was 0.24 and 0.17, resp. In conclusion, allogeneic SCT can cure ALL pts after 1. clinical relapse. Our data show a high relapse rate even after standard conditioning with 12GyTBI. Since conditioning can not be intensified further because of high TRM, another transplant strategy should be discussed. Analysis of minimal residual disease (MRD) should be done regularly during therapy and follow up of ALL pts. If molecular relapse is detected, allogeneic SCT should be performed immediately to avoid therapy refractory clinical relapse. In this setting 5 pts with MRD positivity were transplanted in CR1 and 4/5 pts are alive in CCR.

Disclosure: No conflict of interest disclosed..

V345

Imatinib-based maintenance Therapy in Elderly Patients with Philadelphia-Positive Acute Lymphoblastic Leukaemia (Ph+ALL) Ineligible for Allogeneic Stem Cell Transplantation (SCT)

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Introduction: The tyrosine kinase inhibitor imatinib (IM), alone or in combination with chemotherapy followed by allogeneic SCT has become the mainstay of front-line treatment for Ph+ ALL. However, allo-SCT is not feasible in many elderly or comorbid patients. In a prospective, randomized clinical trial of elderly patients with de novo Ph+ ALL, we recently demonstrated that IM induction followed by IM in combination with intensive consolidation chemotherapy of ca. one year duration was feasible in the majority of pts. but associated with a high relapse rate. To assess the impact of IM-based maintenance, we here provide an analysis of maintenance therapy with IM either alone, in combination with low dose interferon- (LD-IFN), or with zoledronic acid. In addition, we examined whether determination of minimal residual disease (MRD) and/or BCR-ABL mutation status prior to and during pre-maintenance therapy were predictive of freedom from relapse and remission duration. **Methods:** Remission induction and consolidation therapy have been reported previously (Ottmann et al., Cancer 109:2068-76, 2007). As the present analysis focuses entirely on the maintenance phase, pts. who failed to achieve a CR, relapsed or died during the consolidation cycles are not included. Following a median of 6 cycles of chemotherapy given concurrently with IM, 32 CR pts. (n=32; median age 66 yrs; [57-75 yrs.]) were enrolled either in a clinical trial of IM in combination with LD-IFN (n=19), or with zoledronic acid (n=3), or received IM as a single agent (n=10). MRD was serially assessed by quantitative RT-PCR and mutational analyses was performed by D-HPLC and direct sequencing. **Results:** With a median duration of maintenance of 18.2 mos. (range 1-90 mos.), of 10 of 32 pts. (31 %) are in ongoing CR, with a median maintenance duration of 58 mos. (37-90 mos.). Median overall survival of all pts. is 42 mos. (range: 9-99 mos.). Remission was independent of the MRD response during induction and consolidation: 13 mos. and 20 mos. in pts. who did or did not achieve MRD negativity at any time. In this pts. the MRD level was often below the quantitative range. Similarly, detection of MRD at the start of maintenance had no impact on time to progression. **Conclusions:** Among elderly Ph+ALL pts. who did not undergo SCT, treatment outcome with IM-based maintenance therapy is encouraging, but remissions are not sustained in the majority of patients. Surprisingly, relapses occurred even in patients who had achieved prolonged MRD negativity.

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Freie Vorträge
Sonstige Supportiv

V346

A Therapeutic Platelet Transfusion Strategy without Routine Prophylactic Transfusion Is Feasible and Reduces Platelet Transfusion Numbers Significantly: Analysis of a Randomized Study after Autologous Stem Cell Transplantation

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We performed a multicenter randomized trial comparing the traditional *prophylactic* platelet transfusion strategy -arm P- (trigger: morning platelet count = 10/nL) with an experimental *therapeutic* transfusion strategy (arm T) where patients (pts) received platelet transfusions only if they experienced clinically relevant bleeding (more than petechias or minimal mucosal bleeding). For safety reasons prophylactic transfusion was recommended, however, for pts with invasive aspergillosis, sepsis syndrome and unexpected headache. Randomisation was stratified according to age, sex and center. 185 consecutive pts with a median of 56 years (19-68) were included in the study. Primary objective was a reduction of platelet transfusions of 15-25%; secondary objectives were safety, duration of leuko- and thrombocytopenia, hospitalisation, and numbers of red blood cell transfusion. **Results:** Platelet transfusions could be reduced significantly by 29% in arm T compared with arm P (p 0.002). In arm T 47% of pts did not need any platelet transfusion and this was more than the double compared to arm P (0.001). Between younger and older pts there was no difference in numbers of platelet transfusions needed. Overall, adherence to the protocol was good. Since clinically relevant

bleeding (more than petechias and minimal mucosal bleeding) was the trigger for platelet transfusion in arm T consequently more such hemorrhages occured in arm T (28.7% vs 9.5%). No life threatening or fatal bleeding was registered. Hemorrhages were mainly (21.8%) epistaxis or mucosal. One pt with sudden headache had a minor cerebral hemorrhage (subarachnoid) documented by ct-scan without any clinical sequelae. Days with hemorrhage overall were rare but significantly increased in arm T (0.69 vs 0.17 days per pt). Age was no risk factor for bleeding. As already expected by our former experience (Wandt, H et al. Bone Marrow Transplant 2006; 37:387-392) we could show that fever and infection were no additional risk factor for bleeding in arm T compared with arm P. In pts with multiple myeloma bleeding events were very rare compared to other diagnoses (p <0.0001). Duration of leukocytopenia and hospitalisation were not different. In contrast duration of thrombocytopenia <20/nL was significantly longer in arm T (median 5 vs 3 days; p 0.004) as expected. We conclude that our therapeutic platelet transfusion strategy is feasible, cost effective and safe in pts after autologous stem cell transplantation. Despite more minor hemorrhages in the experimental arm compared with the traditional prophylactic strategy all bleeding events could be safely controlled by consecutive platelet transfusion. Major bleeding could be prevented by the therapeutic transfusion strategy according to our protocol.

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Massive Platelet Transfusion in Patients with Refractory Autoimmune Thrombocytopenia

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Introduction: Patients with refractory autoimmune thrombocytopenia (ITP) may develop life-threatening bleeding that cannot be immediately controlled by drug administration. To date, there have been no studies conducted to evaluate the efficacy of platelet transfusion alone in such cases. Methods Ten patients with refractory ITP and bleeding or a high bleeding risk were consecutively transfused (one unit/30 min) apheresis platelet concentrates (APC) without the administration of new drugs. The used APCs (average 3 - 7 units) contained 2.7 x 1011 (medium) platelets and were leukodepleted (= 1 x 106 leukocytes/unit). Platelet serology was performed using standard techniques. Results Platelet transfusion resulted in an increase in the platelet count to 84 - 157 x 103/µl, and the cessation of bleeding in all patients without any serious adverse effects. Although platelet counts gradually decreased within a few days post-transfusion, bleeding was sustained in all cases. Conclusion These findings indicate that consecutive platelet transfusion using APCs is the most effective means of emergency treatment in patients with refractory ITP.

Disclosure: No conflict of interest disclosed..

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Epoetin theta shows efficacy and safety in a placebo-controlled, randomized phase III study in cancer patients receiving non-platinum chemotherapy

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Background: Epoetin theta is a recombinant human erythropoietin developed for the treatment of symptomatic chemotherapy-induced anaemia in cancer patients and anaemia in chronic renal failure patients. **Objective:** To investigate the efficacy and safety of Epoetin theta in comparison to placebo in cancer patients receiving non-platinum chemotherapy. The primary objective was to demonstrate superiority of Epoetin theta versus placebo for efficacy. The primary endpoint was the number of patients with a complete haemoglobin response, defined as an increase in haemoglobin of = 2 g/dL from baseline, without the benefit of a transfusion within the previous 4 weeks. **Methods:** This 12-week, multicentre, randomized, double-blind, placebo-controlled study included 186 anaemic (Hb level of = 11 g/dL at baseline) cancer patients receiving non-platinum chemotherapy. A total of 95 patients were treated with Epoetin theta and 91 patients with placebo. Epoetin theta patients received a starting dose of 20,000 IU once a week as a subcutaneous injection with potential increases to 40,000 IU/week and 60,000 IU/week in case of insufficient response. **Results:** The demographic and baseline characteristics were comparable across both treatment groups. The mean (± SD) age was 56.3 (± 14.5) years. The primary malignant disease were haematological malignancies in 102 (54.8%) patients. The most common solid tumour types were breast cancer (33 patients) and gastric cancer (9 patients). 72.6% of patients in the Epoetin theta group and 25.3% in the placebo group had a complete haemoglobin response without blood transfusion (p < 0.0001). A higher proportion of patients in the placebo group than in the Epoetin theta group received blood transfusions after randomisation (25.3 vs. 13.7%; p=0.0277). The overall frequencies of adverse events were similar in both treatment groups: 80.0% (76/95) in the Epoetin theta group and 78.0% (71/91) in the placebo group. Eleven of the patients in this study (5 placebo, 6 Epoetin theta) died during the study period. None of the deaths was assessed as being related to the study medication. **Conclusion:** Epoetin theta is a safe and effective treatment for anaemia due to non-platinum chemotherapy in patients with solid tumours or non-myeloid haematological malignancies.

Disclosure: Buchner,A.: Anstellungsverhältnis oder Führungsposition:Senior Manager Clinical Research, Merckle GmbH, ratiopharm group
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Do erythropoiesis-stimulating agents increase mortality in cancer patients? Results of an individual patient data meta-analysis

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Introduction: Within the framework of an international collaboration we conducted a patient-level meta-analysis to assess the effects of Erythropoiesis-stimulating agents (ESAs) on mortality in cancer patients. **Methods:** We performed a meta-analysis of cancer patients enrolled in randomized controlled trials comparing epoetin alfa, epoetin beta or darbepoetin alfa plus red blood cell transfusions as needed versus transfusion alone, for the prophylaxis or treatment of anemia during or after receiving anticancer therapy. Patient-level data were obtained and analyzed independently using the intention to treat principle by statisticians at two academic departments. Primary outcomes were on-study mortality and total mortality in patients receiving chemotherapy and in all cancer patients regardless of anticancer therapy. On-study mortality was defined as death from any cause between randomization and 28 days after the end of the active study phase. Total mortality was defined as death from any cause between randomization and longest follow up available. Hazard ratios (HR) and 95% confidence intervals (CIs) were calculated per study and meta-analyzed with fixed-effects and random-effects models. Tests for interactions were used to identify differences in effect across pre-specified subgroups. An independent steering committee of clinicians and methodologists agreed on all analyses and interpretations. **Results:** Data from 13,933 cancer patients enrolled in 53 studies were included in the analysis; 38 trials including 10,441 patients used mainly chemotherapy. For all cancer patients combined, ESAs increased on-study mortality by factor 1.17 (HR 1.17; 95% CI 1.06-1.30), with little

evidence for a difference between trials with and without chemotherapy (p for interaction=0.42). Total mortality was increased by factor 1.06 (HR 1.06; 95% CI 1.00-1.12). In the chemotherapy population, on-study mortality was increased by factor 1.10 (HR 1.10, 95% CI 0.98-1.24) and total mortality was increased by factor 1.04 (HR 1.04; 95% CI 0.97-1.11). There was no conclusive evidence for effect modification by patient or study level characteristics (reference: Bohlius, Schmidlin, Brillant et al, Lancet, 373, 2009, pages 1532-42). **Conclusions:** ESA treatment in cancer patients increased on-study mortality by 1.17 and total mortality by 1.06. For patients undergoing chemotherapy the increase was less pronounced. In clinical practice, risks of ESAs must be balanced against benefits of ESAs, which may vary with the clinical circumstances of the individual patient.

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PARP-inhibition as a means of protecting the inner ear from cisplatin-mediated ototoxicity without affecting anti-tumor efficacy in vitro

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Background: Cisplatin is used in the treatment of many solid tumors, but its ototoxicity can be dose limiting. However, in some scenarios like treatment of germ cell tumors (GCT) or adjuvant therapy of non-small cell lung cancer (NSCLC), cisplatin cannot be replaced or omitted without undue loss of efficacy. Inhibition of Polyadenosine diphosphate-ribose polymerase (PARP), an enzyme which contributes to the base excision repair pathway, is presently being evaluated as a novel anti-neoplastic principle. Of note, PARP-activation has been identified to induce cell death following inner ear trauma, and PARP-inhibition may thus exert a protective effect on the inner ear. We here evaluated PARP-inhibition as a means to protect the inner ear from cisplatin-mediated ototoxicity. Material and method: Heterocygous and homocygous PARP-knock out mice were derived from Prof. Wang, Jena. Inner ears from these mice and wild type controls were isolated and treated continuously with cisplatin at a dose of 1.4µM. The number of preserved hair cell stereocilia bundles was counted by fluorescence microscopy after labeling with phalloidin. Cytotoxicity of escalating doses of cisplatin and the effect of the PARP-inhibitors DPQ, JP34, and 3-AB were assessed by MTS-assays and FACS using the cell lines 2102EP, NT2 (both embryonal GCT) and NCI-H460 (NSCLC). **Results:** In heterocygous PARP-knock out mice, 90% of the inner hair cells and 80% of the outer hair cells were preserved following cisplatin treatment compared to untreated controls. 45% and 30% preservation, respectively, was observed in wild type animals, while the homocygous knock out mice displayed 70 and 50% preservation, respectively. The protective effect was statistically signigicant ($p<0.05$). Protection of 60-80% hair cells could be achieved by PARP-inhibition in wild type mice. The administration of PARP-inhibitors alone did not affect cell growth of the three analyzed cell lines. Cytotoxicity of cisplatin as measured by IC50 determination was not affected by DPQ, 3-AB, and JP34 in NCI-H460, NT2, or 2102EP cells. The MTS-results were confirmed by flow-cytometric assessment of apoptotic and necrotic cells. **Discussion:** PARP-activation seems to play a relevant role in cisplatin-induced ototoxicity. Thus, PARP-inhibition appears to be a promising means to protect hearing under cisplatin therapy, especially since PARP-inhibition did not diminish sensitivity of GCT and NSCLC cell lines to cisplatin, even though no synergistic effect could be observed.

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Expertenseminar
Adenokarzinom des Magens und des gastro-ösophagealen Übergangs

V351

Innovative Approaches in the Multimodal Treatment of theOesophago-Gastric Junction (OGJ) Tumours

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Background: Multimodal treatment has become the standard of care for locally advanced cancer of the OGJ. Neoadjuvant chemotherapy and chemoradiation improves overall survival after 5 years up to 15%. However, relapse rates within 5 years are usually > 50%. Therefore, further improvements are urgently needed. **Methods:** A review of the current literature, of recent congress contributions and of planned and currently performed studies has been undertaken to illustrate new approaches to the treatment of gastro-oesophageal cancer in curative intention. **Results:** According to randomised trials and meta-analyses preoperative chemotherapy and chemoradiation is effective in treating adenocarcinoma of the distal oesophagus. For stomach cancer, neoadjuvant chemotherapy as well as postoperative adjuvant chemoradiation has proved to be effective. Which of these approaches is superior in OGJ tumours is not entirely clear. The chemosensitivity of OGJ tumours is comparable to that of other adenocarcinomas of the oesophagus and stomach. Therefore, the inclusion of taxanes (e.g. docetaxel) into the neoadjuvant treatment may be advantageous. Several phase II studies are currently being performed to show the feasibility of this approach. As shown recently, about 30% of the OGJ tumours display an overexpression of the Her2 protein. In the metastatic setting, trastuzumab, a humanized monoclonal antibody directed against Her2, was shown to improve the response rate of chemotherapy by >10% and has led to an improved efficacy of chemotherapy. The incorporation of Her2 targeting agents into neoadjuvant treatment protocols could certainly by an interesting option for Her2 overexpressing OGJ tumours. Radiation can probably improve the local response rate and can possibly reduce the local relapse rate. Whether chemoradiation is more efficacious than chemotherapy alone has not yet been shown. In contrast, the addition of radiation to chemotherapy may increase the postoperative morbidity or mortality. As not all tumours display the same sensitivity to preoperative treatment, an early sensitivity test would be intriguing in order to tailor treatment early to the individual biology of each tumour. Sequential PET-CT investigations may open a new access to studying the chemosensitivity of tumours early during the course of neoadjuvant treatment. Studies that incorporate early PET-CT testing are underway. **Conclusions:** Although neoadjuvant treatment of locally advanced OGJ tumours is a standard of care, the results are not yet entirely satisfying. The incorporation of new drugs, the individualisation of the choice of drugs on biologic grounds and early response detection by PET-CT are the current approaches that may eventually lead to better outcomes.

Disclosure: Lordick,F.: Anstellungsverhältnis oder Führungsposition: (Chefarzt) Beratungstätigkeit:Ganymed, Fresenius Biotech; Honorare:Roche, Sanofi-Aventis, Merck, Amgen, Österreich; Finanzierung wissenschaftlicher Untersuchung:Merck, GSK
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Expertenseminar
Aggressive B-Zell-Lymphome

V352

Treatment of aggressive B-cell-lymphoma in the elderly, old and very old

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Standard first line therapy of aggressive B-cell-lymphoma consists of R-CHOP-chemoimmunotherapy, which offers cure for the majority of patients. In the relapsed situation other chemoimmunotherapies

like R-DHAP followed by high dose chemotherapy with autologous stem cell transplantation are applied successfully with curative intent in some patients. Prerequisite for R-CHOP and especially for R-DHAP followed by high dose chemotherapy with autologous stem cell transplantation is a normal performance score and normal organ function with no or little comorbidities. Due to improved life expectation in western societies elderly, old and very old patients are diagnosed increasingly with aggressive B-cell-lymphoma. These patients frequently present with an impaired performance score and impaired organ function due to significant comorbidities. The focus of this seminar will be to discuss some questions concerning the optimal treatment of these patients: Should age be the only discriminator for therapy decision? How important is the performance status of the patient? How should therapy be altered due to comorbidities? How should therapy be altered due to comedication? Which therapy is the optimal therapy for the individual patient? Where should therapy be delivered (inpatient or outpatient)? How should the benefits and risks of therapy be communicated to patients and relatives to achieve shared decision making?

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Expertenseminar
MPS

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Expert Tutorial Ph$^-$ CMPDs

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Chronic myeloproliferative diseases (CMPDs) are clonal neoplastic disorders of the bone marrow stem cell. Polycythemia vera (PV), essential thrombocytosis (ET), primary myelofibrosis (PMF; also called osteomyelofibrosis, OMF; osteomyelosclerosis, OMS) and chronic myelogenous leukemia (CML) were summarized under the term "chronic myeloproliferative diseases". These diseases have in common clinical features like thrombosis, haemorrhage and leukemic transformations. With the detection of the Philadelphia translocation in 1960 in CML and subsequently the BCR-ABL translocation as molecular hallmark of CML, CML became a distinct entity. According to the WHO classification the other chronic myeloproliferative diseases like polycythemia vera, essential thrombocytosis and osteomyelofibrosis are summarized as Philadelphia Chromosome negative chronic myeloproliferative diseases (Ph$^-$ CMPD). In 2005, several investigators succeeded in identifying a common point mutation in exon 14 of the Janus Kinase 2 (JAK2) gene in >90% of PV patients and >50% of ET and PMF patients, respectively. This mutation leads to a substitution of valin to phenylalanine at position 617 (V617F) in the protein. The definitive role of this mutation concerning the pathogenesis of CMPD is, however, still unclear. The somatic mutation of the JAK2 Gene (V617F) leads to a constitutive tyrosine kinase activation with consequent increased sensitivity to various growth factors up to factor independence of the bone marrow stem and progenitor cells. Inhibition of activated JAK2 therefore offers potentially new therapeutic options in the treatment of these diseases. First results of JAK2 inhibitors will be presented. Current diagnostic algorithms, the current WHO classification of Ph$^-$ CMPDs as well as available therapeutic options will be discussed.

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Freie Vorträge
Pankreas-Tumoren klinisch

V354
THE PROGNOSTIC VALUE OF CA 19-9 KINETICS FOR TIME-TO-PROGRESSION AND OVERALL SURVIVAL IN ADVANCED PANCREATIC CANCER: IMPLEMENTATION OF A TIME-VARYING-COVARIATE MODEL

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Introduction: The role of baseline CA 19-9 or CA 19-9 kinetics during chemotherapy in patients (pts) with pancreatic cancer (PC) turned out to be contradictory in previous studies. **Methods:** For inclusion into this retrospective multicenter analysis a histologically confirmed diagnosis of PC, treatment with first-line therapy for advanced disease and a pre-treatment CA 19-9 level of > 5.2 U/ml were required. The use of a unique assay (Elecsys®, Roche Diagnostics) assured the comparability of CA 19-9 measurements. For the analysis of CA 19-9 kinetics, at least three measurements during first-line therapy had to be available. The effect of the pre-treatment CA 19-9 level on time-to-progression (TTP) and overall survival (OS) was analyzed by Cox proportional hazards regression. In a further step the effect of CA 19-9 kinetics was modelled by the application of CA 19-9 into a Cox proportional hazards regression as a time-varying covariate. Univariate and multivariate Cox models were developed where we selected additional predictors (e.g. performance status, stage) using backward elimination performing likelihood ratio tests on a significance level of 0.05. **Results:** One-hundred and fifteen pts from 5 German centers were included. Median age was 63 years, 12% had locally advanced and 88% metastatic disease; 73 % of the pts were treated within prospective clinical trials. Median baseline CA 19-9 was 1059 U/ml (range 9.5-100000), median pre-treatment bilirubin 0.6 mg/dl. The median TTP in the study population was 4.4 months, median OS 9.4 months. Univariate analysis showed that the pre-treatment CA 19-9 level (as continuous variable, log [CA 19-9]) was significantly associated with TTP (HR 1.24, 95% CI 1.12-1.37, p<0.001) and OS (HR 1.16, 95% CI 1.06-1.28, p=0.002). These associations remained significant also within a multivariate analysis. For CA 19-9 kinetics during chemotherapy, data from 69 pts (TTP) and 84 pts (OS) were available, respectively; log [CA 19-9] kinetics were found to be a significant predictor for TTP in univariate (HR 1.44, 95% CI 1.25-1.67, p<0.001) and multivariate (HR 1.39, 95% CI 1.19-1.62, p<0.001) analyses, and also for OS (univariate: HR 1.34, 95% CI 1.20-1.49, p<0.001; multivariate: HR 1.39, 95% CI 1.23-1.57, p<0.001). **Conclusion:** The implementation of CA 19-9 as a time-varying covariate showed encouraging results for the use of this biomarker as a predictive tool in PC.

Disclosure: No conflict of interest disclosed..

V355
Gemcitabine/cisplatin-based vs. 5-fluorouracil (5-FU)-based chemoradiotherapy in the treatment of patients with locally advanced pancreatic cancer: a multicenter, randomized phase II trial

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Introduction: No standard treatment for patients with locally advanced pancreatic cancer (LAPC) is defined to date. The efficacy and safety of modern chemoradiotherapy (CRT) regimens in LAPC remains unclear.

Methods: In this multicenter, prospective phase II study untreated patients with LAPC and adequate organ function were randomly assigned to three different CRT regimens: all patients received a conventionally fractionated radiotherapy of 50 Gy (with a daily dose of 2.0 Gy) and were randomized to either concurrent 5-FU as a 24h-infusion (350 mg/m²/d on each day of radiotherapy, RT-5FU arm), concurrent low-dose gemcitabine 300 mg/m² and cisplatin 30 mg/m² on days 1, 8, 22, and 29 (RT-GC arm), or the same concurrent treatment followed by a sequential chemotherapy with full-dose gemcitabine (1000 mg/m²) and cisplatin (50 mg/m²) every two weeks (RT-GC+GC arm). Treatment duration in the RT-GC+GC arm was upon disease progression or unacceptable toxicity. The primary endpoint of this phase II study was the overall survival (OS) rate after 9 months (mo); secondary endpoints included response rate (WHO criteria), progression-free survival (PFS), resectability and toxicity. **Results:** Between February 2002 and July 2005 95 patients (median age 64 years, 50% with a KPS of 90-100%) were recruited from 12 German centers. Seventy patients were evaluable for objective response: the intent-to-treat response rate (CR+PR) was 19% in the RT-5FU arm, 22% in the RT-GC arm and 13% in the RT-GC+GC arm, respectively. Overall, 18 patients (19%) underwent surgical resection after initial CRT (R0 in 8 patients). With a median follow-up of 8.6 mo, median PFS was estimated with 4 mo (RT-5FU), 5.6 mo (RT-GC) and 6 mo (RT-GC+GC), respectively (p=0.21); corresponding median OS times were 9.6 mo, 9.3 mo and 7.3 mo (p=0.61). Hematological grade 3/4 toxicities were higher in the two gemcitabine/cisplatin-containing arms, but no grade 3/4 febrile neutopenia was observed. Regarding non-hematological toxicity, nausea/vomiting were more frequently in the RT-GC and RT-GC+GC arm, whereas diarrhea was more frequent in the RT-5FU arm. **Conclusion:** Based on data from this randomized phase II study, gemcitabine/cisplatin-based CRT does not achieve a higher clinical efficacy compared to RT-5FU, and is associated with increased hematological toxicity.

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V356

HIF1 gene expression is an independent prognostic parameter in pancreatic cancer

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Introduction: We have previously described an independent association between the gene expression of angiogenesis-regulating factors and outcome in pancreatic ductal adenocarcinomas (PDA). The present study aimed to validate this hypothesis with two independent patient sets. Moreover, it was unclear whether these alterations in gene expression are restricted to cancer tissues. **Methods:** Paraffin-embedded tissue samples were obtained from 92 patients (31 female, 61 male; mean age 65 years, range 26-85 years) with PDA (72/92, 87.3 %) or chronic pancreatitis (20/92, 21.7 %) undergoing primary surgical resection. Following laser capture microdissection, PDGF-a, bFGF and HIF1a gene expression levels were determined by direct quantitative real-time reverse transcriptase PCR (RT-PCR, TaqMan™). To assess the association of HIF1a gene expression and other clinicopathologic parameters with prognosis, we applied multivariate Cox proportional hazards regression analysis with backward selection after adjustment for potential confounders. **Results:** The expression of HIF1a was significantly correlated with bFGF (p<0.001) and PDGF-a (p=0.009) expression. The overall model fit (Cox) revealed a significance level of p=0.002, and HIF1a, PDGF-a, and bFGF had stronger independent associations with overall survival than established clinicopathological parameters. HIF1a expression exhibited the strongest correlation for all 72 patients with PDA (relative risk 3.26 Exp(b), p=0.004). Moreover, we were able to confirm an independent association of HIF1a expression with overall survival in the PDA validation subgroup. Mann-Whitney-U-Test and Receiver Operating Characteristic curve analysis demonstrated the feasibility of each of these 3 genes to distinguish between cancerous and non-cancerous tissues. Combining HIF1a, PDGF-a, and bFGF gene expression into one factor we developed an algorithm to discriminate between PDA and inflammatory pancreastic tissue with a true positive rate of 42% and a specificity of 100% (p=0.0006). **Conclusions:** HIF1a gene expression could be confirmed as an independent progonostic parameter in patients with PDA. Furthermore, gene expression of PDGF-a, bFGF and HIF1a is highly specific for malignant pancreatic tissue. These results suggest a high potential for HIF1a gene expression as a prognostic parameter in pancreatic cancer.

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V357

First-line treatment of patients with metastatic pancreatic cancer: results of a phase II trial of S-1 (CESAR-Study group)

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Introduction: This study evaluated the antitumor effect and safety of S-1, an oral fluoropyrimidine derivative, in patients (pts) with metastatic pancreatic cancer.**Methods:**Chemo-naive pts with measurable metastatic lesions of pancreatic adenocarcinoma were enrolled and received S-1 orally after meals at a dose of 30 mg/m² twice daily (BID) for 14 days followed by a 1-week recovery period, repeated every 3 weeks. The trial had a two-stage design with 22 patients evaluable for efficacy in stage 1 and an additional 18 patients in stage 2, if = 3/22 pts (=13.6%) have achieved a confirmed response at the first stage.**Results:**Twenty-seven pts with 23(85%) Karnofsky Performance Status 90-100% received a median of 5 cycles (1-19 cycles). A total of 22 pts were evaluable for response (RECIST). Three pts showed confirmed PR of the target lesions. However, detection of clinically asymptomatic brain metastases in one of these pts upon confirmation of PR prevented this study proceeding to the second stage. Overall, 15 pts (68,2%) had SD and 4 pts (18,18%) PD. The median TTP for all evaluable pts was 3.5 months (95% CI, 2.5-5.3 months). The OS for all pts (n=27) was 9.1 months (95% CI, 4.7-11.2 months). The median duration of disease control for pts with SD or PR (n=17) was 4.3 months (95% CI, 2.8-7.2 months). S1 was well tolerated. The most frequent grade 3/4 toxicities (occurring in >5 % of patients) was fatigue (7,4%). Two pts.had to be withdrawn from study due to drug-related AEs (erythrodysaesthesia (n=1) and sepsis (n=1)).**Conclusions:**Although this study did not meet the tumor response predefined targets to proceed to the second stage, preliminary efficacy data in TTP and OS are at least comparable to gemcitabine as monotherapy, the current standard of care. Therefore, S-1 seems an active and well-tolerated drug in metastatic pancreatic cancer and effectiveness of this drug should be confirmed further in a larger study.

Disclosure: No conflict of interest disclosed.

V358

Multicenter phase II trial of Trastuzumab and Capecitabine in patients with HER2 expressing metastasized pancreatic cancer

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Introduction: Patients (pts) with metastasized pancreatic cancer (PaCa) have a dismal prognosis with systemic chemotherapy being of little benefit. New therapeutic options are therefore urgently needed. In PaCa overexpression of the epidermal growth factor receptor 2 (HER2) has been reported in up to 82% of pts, suggesting its use as a therapeutic target. The anti-HER2 antibody trastuzumab is already used in clinical practice for gene amplified HER2 expressing breast cancer patients. Therefore, this phase II study was conducted to determine the efficacy and toxicity of capecitabine (CAP) and trastuzumab (TRAS) in pts with metastatic PaCa. **Methods:** Eligible patients had histologically confirmed metastatic pancreatic adenocarcinoma. The primary endpoint was progression free survival (PFS) after a treatment period of 12 weeks. Pts with PaCa immunohistochemically overexpressing HER2 grade 3 or grade 2 with gene amplification (FISH) received TRAS 4 mg/kg at first infusion followed by weekly 2 mg/kg combined with CAP 1250 mg/m² bid day 1-14, q21. The study was prematurely closed due to unexpected low HER2 expression. **Results:** Between May 2004 and February 2008 a total of 212 pts, 97 women (46%), 115 men (54%); median age 64 years (38-86) were screened for HER2 expression at 9 institutions. In 207 pts the tumor specimens could be assessed for HER2 expression and gene amplification. In IHC 83 (40%) were grade 0, 71 (34%) grade 1, 31 (15%) grade 2, and 22 (11%) grade 3, respectively. One IHC grade 2 and all grade 3 specimens showed gene amplification. From the 23 pts with HER2 gene amplification 17 could be assessed for response to treatment and toxicity in an intention-to treat analysis. Reported grade 3/4 toxicities in 88 cycles of chemotherapy were: leucopenia 6%, anemia 0%, thrombocytopenia 0%, diarrhea 6%, nausea 6%, hand-foot syndrome 6%. There had been no TRAS-attributable cardiac toxicity. The PFS after 12 weeks had been 23.5% and the median overall survival 211 days. **Conclusion:** In conctrast to previous findings, this multi-center study demonstrates HER2 overexpression and gene amplification in only 11% of patients with metastatic PaCa. This discrepancy can be explained by the use of standardized test methods and the examination of a large unselected cohort. Due to the low incidence of HER2 overexpression only 17 pts could be treated with CAP and trastuzumab. Although the therapy was well tolerated, PFS and OS did not perform favourably compared to standard gemcitabine chemotherapy. Due to the low HER-2 overexpression found in this study we do not recommend further evaluation of anti-HER2 treatment in patients with metastatic PaCa.

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Wissenschaftliches Symposium
CUP

V363

Cancer of Unknown Primary Site: Missing Primary or Missing Biology?

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Metastatic adenocarcinoma of unknown primary origin (ACUP) is a major problem in cancer management, because this concerns often relatively young patients and optimal treatment resulting in significant tumor response and survival is considered to depend on treatment that is best focused to the true nature of the primary tumor. With currently generally available approaches, including standardized blood and biochemistry survey, radiological examinations and histological and extensive immunohistochemical evaluation of biopsy samples, the success rate of the diagnostic work-up is only 20% to 40%. Several platforms have been developed to study the histogenesis of ACUP at a molecular level. A gene-expression platform based on oligonucleotide microarray (CupPrint®) has been designed to allow differentiation of a wide range of tumor types. In a retrospective study, we could show a performance of 85-90% in metastatic carcinoma samples of known histogenetic background and in cases with a limited differential diagnosis based on immunohistochemical results. CupPrint® was very good at recognizing breast, colon, serous ovary, prostate and thyroid carcinomas (all 100%) and moderately good at recognizing renal and gastric cancer (88% and 60% respectively). The assay performed rather poorly for primary lung cancer, however (64% incorrectly classified). A similar performance may be extrapolated to "true ACUP" cases tested (n=22). Preliminary results of an evaluation of all ACUP patients in the Netherlands Cancer Institute between 2005 and 2009 in whom CupPrint® results were obtained as part of the workup (n=37) showed that the biopsy material was of sufficient quality to obtain results in 93% of the cases. In 69%, results of CupPrint® provided a significant contribution to the medical decision process and supported the choice for a specific treatment. In 24% of the cases, however, the results did not make any sense in the clinical context and were completely non-contributory. Alternative methods by others based on different gene-expression platforms, RT-PCR-based methods, microRNA-arrays and proteomic screens, seem to have similar overall performances with different patterns of strong and weaker performance in specific tumor types. All methods are hampered to some extent by the level of differentiation of tumor cells that may cause loss of histiogenetic characteristics. At this stage, a step-wise approach in the diagnostic workup of ACUP patients based on medical common sense seems advisable, starting with a classical approach with the collection of clinical, basic laboratory and radiodiagnostic information followed by histological and immunohistochemical information. In the next line, micro-array-based gene expression profiling may be introduced as a promising additional tool for the determination of the origin of ACUPs.

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V364

Evolving understanding and therapy for patients with Unknown Primary Carcinoma

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Unknown Primary carcinoma is an extremely diverse and heterogeneous syndrome and continues to represent a clinical and pathologic enigma. Over the past 30 years several subsets of more treatable patients have been recognized with rather specific clinical and/or pathologic features. Most of the other patients have unfavorable prognostic features and have a poor overall survival. Empiric chemotherapy, particularly with newer broad spectrum cytotoxic drugs, have proven to be useful for a number of patients with unfavorable prognostic features, particularly those with good performance status. Most data concerning clinical trials since the late 1990's have included relatively small patient numbers in phase II trials. Nonetheless, their survival appears to clearly shifted to the right with these therapies; a minority are surviving at two or three year time points. Previous autopsy studies have documented small clinically occult carcinomas in many patients who present with carcinoma of unknown primary site. The majority arise from in the lung, pancreas, colon, rectum, liver and biliary tract, although there are several other sites that have been documented. The survival of patients with known advanced carcinomas of the colon, rectum, lung, pancreas, and many others has improved in recent years by the use of chemotherapy alone or combined with targeted drugs (erlotinib, bevacizumab). Since about 60% of patients with unknown primary adenocarcinomas have occult lesions of the colon, rectum, lung or pancreas it would be expected that site specific treatment would improve the prognosis of these patients. Immunohistochemical staining and the evolving field of molecular profiling is clearly changing our

approach to patients with unknown primary cancer. Studies are ongoing to determine whether a site specific or tailored approach to therapy, based upon immunohistochemical and molecular classification, will be superior to the empiric approach where all patients initially receive the same chemotherapy regimen. Validation of the clincal usefulness of molecular profiling is currently incomplete, however preliminary data is quite promising illustrating that a number of the patients with unknown primary cancer can be more appropriately classified by clinical features, immunohistochemical stains and molecular profiling. Progress in the area of unknown primary cancer is difficult because of the heterogeneity of the patient population, difficulty in performing phase III trials and the paucity of research support. Nonetheless, the challenges are being met and as the biology of advanced cancer is better understood, patients with unknown primary cancer will continue to be treated more successfully.

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V365
CUP syndrome: The rationale for molecular based treatment options

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The term carcinoma of unknown primary site (CUP) syndrome is used to describe malignancies in which a complete diagnostic work-up detects metastases in the absence of an identifiable primary tumor. The presentation of the CUP syndrome is histopathologically and clinically heterogenous, with several common biological characteristics. The clinical course is characterized by a short medical history with nonspecific symptoms and atypical pattern of metastasis. CUP is a relatively common clinical entity, accounting for approximately 3% of all cancer diagnoses. Although several clinicopathologic subsets with favorable prognosis have been identified, most patients do not fit into any of these subsets and have an unfavorable prognosis, with a life expectancy of less than 12 months. Empiric chemotherapy with newer regimens (primarily taxane/platinum or gemcitabine/platinum) have produced response rates between 30% and 40%, with 1- and 2-year survivals of approximately 50% and 25%, respectively. However, complete remissions with these regimens are unusual, and the remission durations are usually brief. In addition, several clinical features (liver metastases, multiple metastatic sites, high lactic dehydrogenase levels) have consistently predicted a poor outcome with empiric chemotherapy. Empiric second-line treatment is usually ineffective. To make additional improvements in the treatment of patients with CUP, agents with novel mechanisms of action are needed. Two of the most promising new classes of agents are epidermal growth factor receptor (EGFR) inhibitors and antiangiogenic agents. Overexpression of EGFR is common in a variety of adult solid tumors, including adenocarcinomas. Similarly, vascular epithelial growth factor (VEGF) is overexpressed in many solid tumors, and is known to be vital for the development of tumor vasculature. Analysis of a limited number of samples suggests that both VEGF and EGFR are expressed in the majority of CUP cases. Also, most of these carcinomas probably arise from organs in which angiogenesis or EGFR signaling are known to contribute to cancer progression or survival. The clinical utility of inhibiting these critical cellular pathways has now been demonstrated in a variety of cancer types. Preliminary evidence suggests that inhibition of VEGF and EGFR has activity in the treatment of patients with CUP as well. Patients with CUP syndrome, like patients with other malignancies, need to be treated within controlled clinical studies using novel targeted treatment approaches, accompanied by a scientific program to improve our understanding of the pathophysiology of this disease at a molecular level.

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Fortbildung
Chronische myeloische Leukämie

V367
Current role of allogeneic transplantation in CML

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The use of allogeneic haematopoietic stem cell transplantation for the treatment of chronic myeloid leukaemia patients has changed dramatically during the past decade. It was standard of care for all younger CML patients with a compatible donor before the introduction of imatinib. It is used know as a rescue treatment for patients who failed tyrosine kinase inhibitors. Both treatments, tyrosine kinase inhibitors and allogeneic transplantation, have been shown to be are very powerful and able to control the disease long term. After almost 10 years of experience with TKI treatment in CML its power, but also its limitations have become apparent. Most recent results of allogeneic transplantation in CML patients are encouraging with a 4 year probability of survival of 90% in first chronic phase patients. In order to integrate allogeneic haematopoietic stem cell transplantation into the treatment plan of each individual patient we have to know the strength and limitations of each therapy. Factors known to influence the outcome of allogeneic transplantation in CML are the age of the patient, the disease stage, time interval from diagnosis to transplant, donor type and donor recipient gender combination which are the variables to calculate the EBMT transplantation risk score. Next to the characteristics of the disease at diagnosis response to TKI-therapy has become a strong prognostic factor as well. Monitoring of the disease is therefore essential. Transplant techniques like the stem cell source and the conditioning regimen (reduced intensity versus myeloablative treatment) have an impact on transplantation outcome and relapse incidence post-transplant. Progress has been made in the detection of post-transplant relapse, its characterization and monitoring. DLI and/or TKI's are very efficient in the treatment of molecular relapse. Prophylactic use of those treatments is proposed by some in case of high risk CML/acute Ph+ leukemias. Expert guidelines agree on the recommendation that every CML patient should be treated with imatinib initially. In case of imatinib failure or intolerance to imatinib or in patients with advanced phases of the disease a second line treatment is needed. The new ELN guidelines recommend allogeneic HSCT for patients in first chronic phase who failed second line TKI therapy and for all patients in case of accelerated phase, blastic transformation or T315I mutation. Allogeneic HSCT is considered a significant option in patients who have a suboptimal response to dasatinib or nilotinib as second line therapy. It is recommended if this patient had prior haematological resistance to imatinib, has developed mutations or has a low EBMT risk score donor.

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V369
Molecular Monitoring in CML

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CML is characterized by the *BCR-ABL* rearrangement that is mostly due to a translocation between the long arm of chromosome 9 and the long arm of chromosome 22, the so-called Philadelphia-translocation. In the progress of the disease additional clonal chromosomal aberrations can emerge. Therapeutic strategies for CML have changed fundamentally in the last decade. Since the approval of the first tyrosine kinase inhibitors allogenic stem cell transplantation is now just a recommendation for patients with insufficient response to those pharmaceuticals or patients with unfavourable prognostic markers. These new therapeutic options need a sophisticated evaluation of the course of the disease to allow a change of treatment in time, if the patient does either not respond to the drug or the disease progresses. A combination of different techniques - cytomorphology, cytogenetics and molecular genetics - with different sensitivities and different levels of remission is well-established in clinical routine. The "European Leukemia Network" has established recommendations for the application of the different methods within the

diagnostic work-up. Chromosome analysis of bone marrow cells is recommended to be performed before treatment, at least every 6 months until a complete cytogenetic remission has been achieved and confirmed. Then an evaluation every 12 months is sufficient. Molecular diagnostics, including a quantification of the *BCR-ABL*-transcripts is proposed to be performed every 3 months. In patients not responding or showing an increase of the *BCR-ABL*-transcript level while treated with a tyrosine kinase inhibitor a screening for mutations which cause imatinib-resistance is recommended. Goals to be reached with tyrosine kinase inhibitor treatment were defined as well: a complete cytogenetic remission should be reached after 12 months and in addition a "major molecular remission" with a clear reduction of the *BCR-ABL*-transcripts (BCR-ABL/ABL 0.1)Additionally research indicates that higher imatinib serum levels correlate with higher cytogenetic and molecular remission rates as well as superior overall survival - which is evaluated in clinical trials at present. The developments in the therapeutic and diagnostic setting reveal the increasing complexity of follow-up diagnostics in CML which is necessary to ensure the optimal care for each individual patient.

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Freie Vorträge
MDS

V371
Cytogenetic Risk Features in MDS – Update and Present State

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Introduction: The IPSS-Score, published by Greenberg et al. (1997), defines the gold standard in risk stratification of patients suffering from MDS. Nevertheless, the assessment of cytogenetic findings within the IPSS since its implementation has revealed several shortcomings: 1) the number of abnormal karyotypes was too small to calculate prognosis for rare abnormalities which all were incorporated into the intermediate prognostic subgroup; 2) combinations of abnormalities were not considered; and 3) the weighting of poor risk cytogenetic findings vs. blast counts appears to be underestimated. Patients and **methods:** In an international collaboration, 3 large, well-characterized international databases (German-Austrian (GA), Spanish MDS-registry, and IMRAW) were merged. Colleagues from the ICWG of the MDS foundation (MLS and KO) contributed data from pts. with rare abnormalities. Inclusion criteria were defined as follows: Primary MDS, age >=16, no treatment except supportive care and bone marrow blasts <30%. In total, 2650 pts. were included. Univariate and multivariate analysis concerning overall survival and AML-transformation was performed. **Results:** A total of 22 cytogenetic subgroups as proposed by the GA-study group (ASH 2008) were examined and classified into 4 prognostic subgroups: Favorable (5q-, 12p-, 20q-, +21, -Y, 11q-, t(11)(q23), normal, 2 abnormalities including 5q-), intermediate-1 (+1q, 3q21/q26-abnormalities, +8, t(7q), +19, -21, any other single, any other double), int-2 (-X, -7/7q-, 2 abnormalities incl. -7/7q-, complex 3 abnormalities) and poor (complex >3 abnormalities). Median survival was 50.3 months (favorable), 29.7 months (int-1), 15.6 months (int-2) and 5.9 months (poor). Time from first diagnosis to 25% AML-transformation was 71.9 months (favorable), 16.0 (int-1), 6.0 (int-2) and 2.8 months (poor). Differences were highly significant (p<0.0001). The Hazard ratio concerning overall survival (favorable subgroup as baseline) was 1.6 (int-1), 2.5 (int-2) and 4.8 (poor), respectively. Additionally, the relative risk (RR) for death and AML-transformation, compared to the normal karyotype (RR=0), was calculated for distinct abnormalities. For instance, RR for death/AML was -0.02/0.13 in 5q-, 1.19/1.63 in -7 and 1.71/2.05 in complex>3 abnormalities. **Conclusions:** Recapitulatory, we could generate a solid database for a revised cytogenetic scoring system which can serve as the cytogenetic module for the upcoming revision of the IPSS.

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V372
Clinical utility of multiparameter flow cytometry in the diagnosis of 1013 patients with suspected myelodysplastic syndrome: Correlation to cytomorphology, cytogenetic, and clinical data

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Introduction: Diagnosis and classification of myelodysplastic syndromes (MDS) is based on cytomorpholgy (CM) and cytogenetics (CG). Multiparameter flow cytometry (MFC) may add important diagnostic information. **Methods:** We analyzed 1013 cases with suspected MDS by CM, CG, and MFC in parallel. **Results:** Concordance between CM and MFC was 82.0% for diagnostic results in 788 cases with unequivocal CM. Additional 225 cases showed only minor dysplastic features by CM, 51 (22.7%) of which showed clear evidence of MDS by MFC. In 6/12 (50.0%) cases with no indication of MDS by CM and MDS-typical cytogenetic aberrations MFC revealed MDS characteristics. In another 11/23 (47.8%) cases with minor dysplastic features by CM and MDS-typical cytogenetic aberrations MFC revealed MDS characteristics. Percentages of blasts as determined by CM and MFC strongly correlated (p<0.001). Frequencies of aberrantly expressed antigens significantly differed between cases rated by CM as MDS (highest frequencies), suspected MDS, and no MDS (lowest frequencies). In various cases MFC identified MDS-typical aberrant antigen expression in cell compartments not rated dysplastic by CM. Numbers of aberrantly expressed antigens correlated with IPSS and overall survival. **Conclusions:** The present analysis clearly demonstrates a diagnostic yield of MFC in addition to cytomorphology and cytogenetics in cases with suspected MDS.

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Prognostic Factors and their Relevance in Different Subgroups of Myelodysplastic Syndromes – An Analysis of 2553 Patients from the Düsseldorf MDS Registry

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Introduction: The IPSS is gold standard for MDS prognostication. However, with the heterogeneity of MDS in mind, we asked ourselves, if it is legitimate to use one score for all subtypes just because all of them exhibit dysplastic features? **Methods:** We compared the relevance of different variables in primary, untreated patients with a blast count of < or =5% (table), =10%, and =20% (not shown), including 181 RA, 169 RARS, 649 RCMD, 322 RSCMD, 79 5q-Syndrome, 290 RAEBI, 324 RAEBII, 266 CMMLI, 64 CMMLII, and 209 RAEB-T. **Results:** Strong prognostic factors in all subgroups were hemoglobin, transfusion dependency, increased WBC, age and LDH. However, all variables became less important in

patients with RAEB-T and increased WBC was rare. Platelet count and IPSS cytogenetic risk-groups were strong prognostic factors in patients with <5%, =5%, and =10% marrow blasts, but not in RAEB-T. Likewise fibrosis was only important in patients with <5% or =5% blasts. Sex and ANC<1000/µl were only significant for survival in patients with normal blast count. Furthermore we looked for the effect of the IPSS-karyotypes (-Y, del5q, del20q, others, del7q/-7, complex) and found a comparable influence on survival irrelevant if patients had <or=5% marrow blasts. Finally we performed a multivariate analysis in the different WHO-subgroups including hemoglobin, platelet count, ANC, cytogenetic risk-group, transfusion dependency, sex, and age. In patients with RA, RARS, and 5q-Syndrome LDH, transfusion, and age were independent prognostic parameters. For RCMD+RSCMD karyotype, age, transfusion, and platelets were the relevant factors. In RAEBI+II, analyzed together, the order was hemoglobin, karyotype, age, and platelets while in CMMLI+II only hemoglobin had independent influence. In RAEB-T patients none of the factors examined was of independent significance. **Conclusion:** In univariate analysis we could show, the prognostic factors included in IPSS and WPSS, except ANC, are relevant in most subgroups. However, multivariate analysis reveals heterogeneity between WHO-groups. Especially CMML and RAEB-T differ from the other MDS subgroups.

	<5% blasts		=5% blasts	
	Log-Rank	p	Log-Rank	p
Hemoglobin </=10g/dl	76	<0,0001	90	<0,0001
Sex m/w	14	0,0002	0,4	0,52
Age </=60years	64	<0,0001	10,5	0,001
LDH </=200U/l	55	<0,0001	33	<0,0001
Platelets </=50000/µl	29	<0,0001	35	<0,0001
ANC </=1000/µl	4	0,04	3	0,08
WBC </=20000/µl	21	<0,0001	3,8	0,051
Transfusion y/n	72	<0,0001	68	<0,0001
Fibrosis y/n	10	0,001	10,2	0,001
Karyotype -risk	51	<0,0001	45	<0,0001

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Minimal diagnostic criteria for myelodysplastic syndromes (MDS), dissection from IDUS and ICUS, and evolution from pre-MDS: IDUS + ICUS = MDS

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Myelodysplastic syndromes (MDS) are hematopoietic neoplasms characterized by a maturation defect in myelopoietic cells, peripheral cytopenia, and clonal instability with enhanced risk to transform to secondary acute myeloid leukemia (AML). Although a classification for MDS has been proposed by the FAB and WHO, with criteria useful to discriminate between disease-variants, the important issue of minimal diagnostic criteria have only recently been discussed. Minimal diagnostic criteria of MDS include i) marked and constant (>6 months) cytopenia in at least one major hematopoietic lineage, ii) MDS-specific bone marrow features (dysplasia =10%, ring sideroblasts >15%, myeloblasts 5-19%, or an MDS-related karyotype), and iii) exclusion of all other hematopoietic and non-hematopoietic disorders as primary reason for dysplasia and/or cytopenia. Based on these definitions, all MDS patients must have a bone marrow biopsy in order to exclude other underlying disorders including AML. A diagnostic challenge are patients who do not fulfil minimal diagnostic criteria for MDS but are suffering from constant (> 6 months) or even progressive cytopenia or unexplained dysplasia. In these patients repeated investigations of the bone marrow may be required to exclude or reveal an underlying hematologic or non-hematologic disease. If this is not the case, a provisional diagnosis should be established: in those with marked constant cytopenia (haemoglobin <11 g/dL and/or neutrophils <1,500/µL and/or platelets <100,000/µL) but no dysplasia, the diagnosis Idiopathic Cytopenia of Undetermined Significance (ICUS) should be established, and in those with marked dysplasia but no or only mild dysplasia, the term Idiopathic Dysplasia of Undetermined Significance (IDUS) should be applied. Both conditions may progress to MDS over time. Therefore, once diagnosed, these patients should have a hematologic follow up. Patients with IDUS usually are young patients with a good erythropoietin (EPO) production, whereas ICUS patients usually are older patients with low EPO levels. When IDUS patients become older and thus their EPO levels decrease, frank MDS may develop (IDUS+ICUS=MDS). These patients are low risk MDS patients who often respond to EPO therapy. The biochemical basis of EPO deficiency in elderly patients (ICUS) remains unknown. All in all, IDUS and ICUS are important diagnostic checkpoints in hematology and indicative of a potential prephase of MDS. The diagnosis MDS, on the other hand, needs to be based on robust criteria and exclusion of all other causes of cytopenia and dysplasia, which requires detailed and sometimes extensive investigations, including a bone marrow biopsy, cytogenetic analyses, molecular studies, and flow cytometry.

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Azacytidine impairs NK cell anti-tumor reactivity while decitabine enhances their effector functions by sensitization of NK cells towards stimulation

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The cytosine analogues 5-azacytosine (azacytidine) and 2'-deoxy-5-azacytidine (decitabine) display substantial therapeutic potential in patients with AML and MDS. Clinical responses are caused by both epigenetic alterations and direct apoptosis-induction in the tumor cells. However, the molecular alterations caused by these drugs are yet not fully understood and may also affect immune effector cells. NK cells as components of the innate immunity play an important role in tumor immunosurveillance by confining development and progression of hematopoietic malignancies and contribute to the success of therapeutic strategies like e.g. haploidentical stem cell transplantation. Here we studied the effect of pharmacological concentrations of azacytidine and decitabine on NK cell effector functions. After preincubation with the cytosine analogues in the absence of target cells, NK cell cytotoxicity was found to be largely impaired by azacytidine, while decitabine, in contrast, significantly enhanced NK cell lysis of tumor cells (mean 60% reduction (n=5, range 37% to 88%) and mean 74% increase (n=4, range 32 % to 135%), respectively). In line, NK cell IFN- production after pretreatment with the compounds was inhibited by azacytidine but enhanced by decitabine (mean 69% reduction (n=7, range 19% to 89%) and mean 98% increase (n=7, range 34% to 230%), respectively). Of note, NK cell effector functions were not affected by deoxycytidine and cytidine, the physiological counterparts of the azanucleosides. While azacytidine treatment substantially induced NK cell apoptosis (mean 59% after 24h (n=3, range 54% to 62%) which may explain its inhibitory effect, no apoptosis was observed after decitabine treatment. Decitabine did not directly enhance NK cell cytotoxicity and cytokine production but rather increased NK cell responsiveness to activating stimuli including cytokines and tumor target cells. This "sensitizing" effect of decitabine was blocked by actinomycin D, an inhibitor of DNA transcription and is thus due to induction of synthesis of yet unknown factors in NK cells. Thus, the azanucleosides azacytidine and decitabine differentially affect NK cell anti-tumor reactivity. While azacytidine causes NK cell apoptosis and thereby impairs antitumor immunity, decitabine enhances NK cell responsiveness via a yet unknown transcription-dependent mechanism which is presently under study.

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FAMILIAL MDS-RELATED AML - CAN THESE FAMILIES SERVE AS A MULTISTEP MODEL FOR LEUKAEMIC TRANSFORMATION IN MDS AND AML?

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Introduction: Heterozygous germline mutations in *RUNX1* are causative genetic alterations in familial platelet disorder with a propensity to myeloid malignancy (FPD/MM). To discuss the potential use of FPD/MM as a multistep model for leukaemic transformation in MDS/AML, we report here on a father and daughter with familial MDS-related AML (MDR-AML) and differing clinical course caused by a heterozygous germline mutation in *RUNX1* and different secondary chromosomal aberrations detected by routine chromosome analyses and array-based comparative genomic hybridization (aCGH). Subjects: Following a brief history of anaemia, the female index patient presented with MDS-related AML (MDR-AML) at age 13. While her twin brother, her one younger and one older brother, and her mother are clinically healthy, MDR-AML was diagnosed in her 47-year-old father six months earlier. No history of thrombocytopenia or platelet defects was reported in the family. **Results:** In both patients, a heterozygous mutation was detected in *RUNX1* (NM_001001890.2) by direct sequencing. The identified nonsense mutation c.520C>T (p.Arg174X) causes a premature truncation at the end of the runt homology domain (RHD). Chromosome analysis of bone marrow cells from the index patient showed a deletion of 5q (del(5q)) and a structural aberration of 2q. In addition, aCGH confirmed the del(5q) and pointed to an unbalanced translocation t(2;6)(q36;q23), finally confirmed by fluorescence in situ hybridization using a specific probe for *MYB*. In contrast, the only aberration identified in bone marrow cells of the diseased father was a loss of the Y chromosome (-Y). **Conclusion:** Currently less than 30 pedigrees with FPD/MM are known. While most *RUNX1* mutations in FPD/MM cluster in RHD and are unique to individual pedigrees, a broad range of intra and interfamilial clinical variability characterizes FPD/MM. Whereas -Y is a typical chromosome aberration of adult MDS associated with a good prognosis, del(5q) is rarely seen in childhood MDS and usually occurs within complex clones associated with a more unfavourable prognosis. Notably, del(5q) is frequently accompanied by aberrations of chromosome 6. In the index patient, the gain of 6q led to an additional copy of the proto oncogene *MYB*, an essential transcription factor in haematopoietic cells. While heterozygous mutations in *RUNX1* are not sufficient for leukaemogenesis, somatically acquired secondary events may promote transformation leading to overt MDS and AML. The recruitment of different secondary alterations may partially explain the variable penetrance and clinical heterogeneity seen in FPD/MM. Consequently, rare FPD/MM-related myeloid malignancies may serve as a model for multistep leukaemogenesis in MDS/AML and, as illustrated here, aCGH may lead to the identification of candidate genes involved in malignant transformation in familial and sporadic myeloid malignancies.

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Freie Vorträge
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V377

Under the Fungiscope - Risk factors, Treatment and Outcome of 26 Oncological Patients with Invasive Zygomycosis

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Introduction: Invasive fungal infections (IFI) with Zygomycetes account for a significant proportion of all IFI in patients with hematological malignancies or those undergoing hematopoietic stem cell transplantation (HSCT). Data on their clinical course and response to treatment is limited. **Methods:** Fungiscope™ – A Global Rare Fungal Infection Registry, collects data of patients with rare IFI, using a web-based electronic case report form (www.fungiscope.net). Inclusion criteria: Cultural, histopathological, antigen, or DNA/RNA evidence of IFI. Exclusion criteria: Infection with *Aspergillus* spp., *Candida* spp., *Cryptococcus neoformans, Pneumocystis jiroveci* or any endemic fungal infections, as well as colonization or other non-invasive infections. A search for infections with zygomycetes in oncological patients was performed. **Results:** *Demography:* 26 hematological and oncological patients with invasive zygomycosis were registered (23 adult, 3 pediatric). The mean age was 49.4 years (range: 15-88 years). 17 subjects (65.4%) were male. *Infection*: Main sites of infection were the lung (n=22; 84.6%), the sinonasal tract (n=4; 15.4%) and the spleen (n=4; 15.4%). Disseminated infection (>2 non-contiguous sites) was seen in four patients (15.4%). A chest CT was performed in 22 patients showing a nodule with or without a halo in 17 cases (62.9%). diagnosis was made by culture and histology in 16 (61.5%) patients each. Three diagnoses were made post mortem. *Therapy and Outcome:* 13 patients (50%) were under continuous antifungal prophylaxis with anidulafungin (n=1; 3.8%), fluconazole (n=3; 11.5%), posaconazole (n=4; 15.4%) or voriconazole (n=5; 19.2%) when the diagnosis of IFI was established. Empiric treatment with an antifungal showing activity against zygomycetes was given in seven patients (25.9%). Five of these patients (18.5%) had a positive outcome. Table 1 shows information on the antifungals used for initial targeted treatment and the associated outcomes.

Initial Treatment	n (%)	Positive response (%)	P	Overall Survival	P
None‡	3 (11.5)	0 (0)	NS	0 (0)	NS
Caspofungin	1 (3.8)	0 (0)	NS	0 (0)	NS
L-AmB alone	11 (42.3)	9 (81.8)	0.021	8 (72,7)	0,015
Posaconazole alone	4 (15.4)	2 (50.0)	NS	1 (25.0)	NS
L-AmB+Posaconazole	7 (26.9)	3 (42.9)	NS	2 (28.6)	NS

Table 1: Initial treatment, response and survival; ‡Diagnosis was made post mortem; PR=partial response, CR=complete response, NA=not applicable, NS=not significant, L-Amb=liposomal amphotericin B. A positive response, defined as complete or partial response, was observed in 14 patients (53.8%). Surgical treatment was performed in 10 patients and not associated with significant improvement of response or survival.

Conclusion: Invasive zygomycosis is associated with low response rates to surgical and antifungal therapy. Using L-AmB as initial therapy was associated with improved outcome and survival.

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V378

Therapeutic and prophylactic use of systemic antifungal agents in cancer patients – a prospective analysis of clinical parameters, side effects, drug interactions and costs

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Invasive mycoses (IM) show high morbidity and mortality rates among immunocompromised patients (pts). Especially allogeneic stem cell transplant (allo-SCT) recipients and pts under induction chemotherapy

are at risk and benefit from antifungal prophylaxis and early therapeutic interventions when IM occur. Nevertheless, the increasing use of systemic antifungal drugs (SAD) has led to considerable costs and represents a substantial burden for public health systems. Available SAD differ in their antifungal properties, side effects (SE), potential for drug interactions (DI) and costs. Clinical trials have assessed their efficacy, but do not reflect 'everyday clinics' due to tight inclusion criteria. The aim of our analysis was to prevent application errors in consecutive SAD pts and to assure economically appropriate SAD use. Since 11/2006, we prospectively analyzed SAD on a general hematology/oncology ward (H&O, n=42) and SCT-unit (n=158). Pt characteristics, organ function, SE, DI, treatment outcome and costs were assessed. SAD were given according to EORTC-adapted guidelines, with use of fluconazole as primary prophylaxis in allo-SCT and posaconazole (pos) for AML/MDS pts under induction chemotherapy. Empirical therapy was implemented with liposomal amphotericin B (amb) or caspofungin (cas), preemptive therapy and therapy of aspergillus infections with voriconazole (vor), amb or cas. 200 consecutive pts showed a median age of 56 years with leukemia (n=125) and lymphoma (n=51) as predominant diseases. Most pts received allogeneic (n=147) and/or autologous SCT (n=41). SCT-recipients were treated earlier (higher rates of prophylaxis and empirical therapy) and more frequently with intravenous SAD and two or more consecutively applied SAD compared with H&O-ward pts. Due to more complex co-medications, DI were detected more often among SCT-pts and SAD use was considerably longer and therefore more expensive on the SCT-unit. In contrast to cas, amb and vor were commonly used as 1-line therapy with the polyene mainly applied as empirical and the azole as preemptive therapy. Amb showed highest rate of SE (nephrotoxicity and elevated bilirubin levels) and potential DI occurred more frequently (mean 2.93) than with vor (1.19) or cas (1.05). Pos showed more hepatotoxicity in 5/9 pts than previously described. Due to the detection of frequent SE and DI with SAD, this and our ongoing analyses are of importance to avoid application errors and should eventually impact on pts safety.

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V379

A Randomized Trial of Caspofungin or Itraconazol for the Empiric Antifungal Treatment of Patients with Acute Leukemia or Patients after Stem Cell Transplantation Failing First-Line Therapy with c-Amphotericin B

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Introduction: Invasive fungal infections are a major cause of morbidity and mortality in patients with hematological malignancies. Frequent isolates include Aspergillus spp. and Candida spp. As those infections are often times hard to diagnose, patients with persistent fever failing antibiotic treatment, are usually started on an empiric antifungal therapy with the historic standard being conventional (c-) amphotericin B. Patients and **Methods:** Here we report on the results of our randomized trial using caspofungin (arm A) or itraconazol (arm B) for the empiric antifungal treatment of patients failing first-line therapy with c-amphotericin B. From 08/2004 to 05/2007 a total number of n=27 patients were randomized. Underlying hematological malignancies included acute leukemia (24), lymphoma (1), multiple myeloma (1) and sarcoma (1). Three subjects underwent auto-TPX and 1 subject allo-TPX. Using EORTC criteria, 15 patients had a "possible" and 12 a "probable" invasive fungal infection at screening. Thirteen patients were allocated to arm A and 14 to arm B. Gender was well distributed between the two treatment arms (female=16, male=11). Average age was 46 years in arm A and 52 years in arm B (p=.21). Caspofungin was administered 70 mg i.v. once daily on day 1 and 50 mg i.v. once daily on the subsequent days for up to 4 weeks. Itraconazol was dosed 200 mg i.v. twice daily on days 1 and 2 and 200 mg i.v. once daily on days 3 to 14. At the investigator´s choice, dosing from day 15 up to day 28 was switched to p.o. **Results:** All patients received at least one dose of the allocated treatment. Median duration of therapy was 14 days in arm A and 8 days in arm B. An overall response rate of 46% (6/13) was observed for arm A and 36% (5/14) for arm B (p=1.0), respectively. Toxicity was assessed by comparing changes in WBC, haemoglobin, ALAT, total serum bilirubin and creatinine on days 1 and 7 of treatment. No statistically significant changes were found within the two treatment arms. 14 patients (6 in arm A and 8 in arm B) experienced clinically significant changes in liver and/or kidney function which lead to discontinuation of treatment in 1 subject in arm A and 3 in arm B. Overall, 6 subjects died due to detoriation of their infection and 1 subject because of progression of the underlying hematological malignancy. This resulted in an overall survival of 78% at 2 month. **Conclusions:** Outcome for patients failing the historic standard c-amphotericin B is grim. In our study, neither response rates to caspofungin or itraconazol, nor differences regarding toxicity proofed to be statistically significant in both treatment arms. The evolving use of new prophylactic and therapeutic antifungal agents with a more favourable spectrum of side effects will certainly help to improve future outcomes compared to the historically used c-amphotericin B.

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V380

Antibiotics or GranulocyteColony Stimulating Factors for the Prevention of Infection in Cancer Patients: A Network Analysis

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Introduction: Febrile neutropenia (FN) and other infectious complications are among the most serious treatment-related toxicities in chemotherapy for cancer, with a mortality rate ranging between 2 and 21 percent. Two different types of prophylactic regimens exist: granulocyte- or granulocyte-macrophage colony stimulating factors (CSF) and antibiotics, frequently quinolones or cotrimoxazole. Many trials have shown CSFs and antibiotics to be superior to placebo or no prophylaxis. However, a comprehensive review of these prophylaxis options including their combination is lacking. **Methods:** We searched MEDLINE, CENTRAL and EMBASE. A Bayesian network analysis of RCTs with four treatment options (antibiotics, CSF, the antibiotics plus CSFs and no prophylaxis) was performed. Trials not examining infection incidence, overall mortality or infection-related mortality were excluded. Subgroup analyses for the type of cancer, type of treatment (bone marrow or stem cell transplantation versus chemotherapy), age of patients and trial quality were performed. **Results:** The search retrieved 125 trials, of which 75 were included in the network analysis. The other trials did not specify the type of infection prophylaxis given in both arms of the trial. Odds ratios and 95% credibility intervals for infections in the network were: CSF versus none 0.63 (0.44-0.84); CSFs plus antibiotics versus antibiotics 0.61(0.46-0.76); antibiotics verus CSFs (only 2 direct trials) 1.0 (0.69-1.50); antibiotics versus none 0.63 (0.47-0.84); CSFs plus antibiotics verus CSFs alone 0.61 (0.36-0.93); antibiotics plus CSFs versus none (no direct evidence) 0.40 (0.26-0.58). There was no evidence for heterogeneity or inconsistency in the network. In subgroup analyses, high risk patients (e.g. with acute leukemia or SCT) profited from the combination of both antibiotics and CSFs. **Conclusions:** Both, antibiotics and CSFs prevent infections when used alone. The combination of both is more effective. This strategy may be especially useful in patients at high risk of infections, such as those with acute leukemia or patients undergoing hematopoietic stem cell transplantation.

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V381

Randomized Comparison of Safety, Tolerance and Pharmacokinetics of Caspofungin, liposomal Amphotericin B and the Combination of Both in Allogeneic Hematopoietic Stem Cell Recipients (CASLAMB Trial)

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Background: The combination of liposomal amphotericin B (LAMB) and caspofungin (CAS) holds promise to improve the outcome of invasive aspergillosis. Little is known, however, about the safety and pharmacokinetics of this combination in a target population. **Methods:** The safety, tolerance and pharmacokinetics of the combination of LAMB and CAS were investigated in a risk-stratified, randomized, multicenter phase II trial in 55 adult allogeneic hematopoietic stem cell transplant (aHSCT) recipients with granulocytopenia and refractory fever. Patients received either LAMB (3mg/kg/d), CAS (50mg/d; d1:70mg) or the combination of both (CASLAMB) until defervescence and granulocyte recovery. Safety, tolerance, development of fungal infections and survival were assessed through day 14 post end of therapy (EOT). PK sampling was performed on days 1 and 4 and drug concentrations determined in plasma by HPLC. **Results:** All 3 regimens were well-tolerated. Premature study drug discontinuations due to grade III/IV AEs occurred in 2/20, 1/18 and 0/17 pts. randomized to LAMB, CAS and CASLAMB, respectively. AEs not leading to study drug discontinuation were frequent but similar across cohorts except for a higher frequency of hypokalemia in CASLAMB ($p<0.05$). Drug exposures were similar for pts. receiving combination as compared to monotherapy. Treatment success (no discontinuation for toxicity, absence of proven/probable invasive fungal infection and survival through day 14 post EOT) was observed in 15/20, 14/18 and 16/17 pts. receiving LAMB, CAS and CASLAMB. **Conclusion:** CASLAMB combination therapy in severely immunocompromised aHSCT patients was as safe as monotherapy with CAS or LAMB and had similar plasma pharmacokinetics, lending support to further investigations in patients with invasive aspergillosis.

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Silling,G.: Honorare:Vortragstätigkeit; Finanzierung wissenschaftlicher Untersuchung:Vorgestellte Studie wurde gemeinsam von MSD Sharp & Dohme bzw. Gilead Sciences finanziert; Sponsor: KKS UKM Münster

V382

Comparison of antibiotic prophylaxis with trimethoprim-sulfamethoxazole/colistin (TMP-SMZ/COL) versus ciprofloxacin in patients with acute myeloid leukaemia and melosuppressive chemotherapy

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Introduction: A meta-analysis by Gafter-Gvilli et al. 2005 showed a significant reduction of febrile neutropenia and mortality by antibiotic prophylaxis with fluoroquinolones. Accordingly, we changed our antibiotic prophylaxis from TMP-SMZ/COL to the fluoroquinolone ciprofloxacin (CIP) in April 2008. The aim of the present study was to compare efficacy and duration of neutropenia during the two prophylaxis regimes. *Methods:* Myelosuppressive chemotherapy courses given for AML during the antibiotic prophylaxis period with TMP-SMZ/COL (01/2006 - 04/2008) and the period with CIP (04/2008 – 04/2009) were retrospectively analyzed with a standard questionnaire. *Results:* In total, 215 chemotherapy courses were given. Importantly, throughout the entire duration of study the same treatment protocols were used (AMLSG). 160 courses were applied with TMP-SMZ/COL prophylaxis and 55 courses with CIP. Median age was 59 years (range 18-85). There was no significant difference between both groups regarding status of disease before chemotherapy, intensity of chemotherapy and patient age. The incidence of fever was significantly lower during CIP: 32/55 (58%, 95%CI 44-71% vs 125/160 (78%, 95%CI 71-84%) during TMP-SMZ/COL, p=0.008. There was also a trend to a decrease in the number of microbiologically documented infections: 11/55 courses (20%) in the CIP group vs 54/160 courses (33.8%) in the TMP-SMZ/COL group, p=0.062. Whereas there was no change in incidence of gram(+) and mixed infections, the incidence of gram(-) infections was significantly lower in the CIP group (0% vs 9.4%, p=0.014). The mortality rate between the two groups was not significantly different: 7.3% during CIP vs 10% during TMP-SMZ/COL, p=0.788. Interestingly, we found a significant reduction in the duration of neutropenia in the CIP group, 11.5 days (interquartile range, IQR 3-19 days) vs 18 days (IQR 12-23 days), p=0.002. *Conclusion:* Up to now antibiotic prophylaxis with CIP compared to TMP-SMZ/COL in neutropenic patients with AML had no influence on the mortality rate in our centre, but we observed a significant decrease in the incidence of fever, the incidence of gram (-)-infections and the duration of neutropenia. This observational study will be continued.

Disclosure: No conflict of interest disclosed..

Expertenseminar
Mammakarzinom und Bisphosphonate

V385

Treatment induced bone loss in breast cancer patients

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Bisphosphonates are potent inhibitors of bone resorption through inhibition of osteoclastic activity. They are selectively adsorbed to the mineral surface in the bone and stay there for a long time. In cancer patients with bone metastases bisphosphonates are currently standard of care. However women with non metastatic breast cancer have a substantial risk of treatment induced bone loss leading to osteopenia or osteoporosis. Several studies proved that bisphosphonates are preventing bone loss mainly in women receiving aromatase inhibitors or women having premature treatment induced menopause. Denosumab is a promising new agent to reduce the degradation of bone in breast cancer patients with treatment induced bone loss. The risk of treatment induced bone loss in breast cancer patients and the therapeutic options will be discussed.

Disclosure: Huober,J.: Beratungstätigkeit:Novartis; Honorare:Novartis, Roche

Expertenseminar
ZNS-Lymphome

V387

Primary and secondary central nervous system lymphoma: Recent aspects on pathogenesis and therapy

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Recent evidence suggests a specific pathogenesis for primary CNS lymphoma (PCNSL) which may explain its particular clinical behavior compared to systemic aggressive lymphoma. The optimal treatment for patients with PCNSL has not been established thus far due to the lack of published randomized clinical trials of this rare tumour. The addition of high-dose methotrexate (HDMTX) chemotherapy to whole-brain radiotherapy (WBRT) has considerably improved the prognosis, leading to a threefold longer median survival time as compared to WBRT alone. However, the prolonged survival is overshadowed, especially in older patients, by delayed neurotoxicity. Recent attempts have thus been focused on the role of WBRT in primary therapy and optimising multi-drug chemotherapy regimens. A promising area of research is the incorporation of novel targeted drugs and high-dose chemotherapy with autologous stem cell transplantation into treatments schedules. In CNS involvement of systemic lymphoma an intensive systemic chemotherapy including HDMTX and high-dose chemotherapy may offer a prolonged survival and probably cure. In the future, cooperation between research groups will hopefully lead to further therapeutic advances in both PCNSL and secondary CNS lymphoma..

Disclosure: No conflict of interest disclosed..

Freie Vorträge
ALL/AML experimentell I

V388

Leukemia induced by altered TRK-signaling is sensitive to treatment with mTOR inhibitors in a preclinical model

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Neurotrophins (NTs) and their receptors play a key role in neurogenesis and survival. The TRK (tropomyosin-related kinase) receptor protein tyrosine kinases (TRKA, TRKB, TRKC) are high affinity NT-receptors that are expressed in a variety of human tissues. Their role in normal and malignant hematopoiesis is poorly understood. Recently, we and others have obtained evidence for potential involvement of this receptor system in leukemia. We demonstrated for the first time cell surface expression of the three TRKs and constitutive activation in blasts from patients with *de novo* or secondary acute leukemia. At least one TRK receptor was expressed in 55% of the analyzed cases (Li Z et al., Blood 2009). Altered TRK signaling efficiently transformed murine hematopoietic stem/progenitor cells (Meyer J et al., Leukemia 2007; Li Z et al., Blood 2009). We observed constitutive activation of mammalian target of rapamycin (mTOR) both in murine and human leukemic cells. Murine leukemic cells induced by altered TRK signaling were very sensitive to rapamycin or RAD001 treatment *in vitro*. We next tested the therapeutic effect of rapamycin on altered TRK-induced leukemia in a mouse model (C57Bl/6J). Leukemic cells isolated from #483 mouse transplanted with primary hematopoietic stem/progenitor cells modified with dTrkA, an active mutant of TRKA isolated from a patient with acute myeloid leukemia, grew factor-independently. Treatment of #483 cells with rapamycin or RAD001 (10-50nM) induced apoptosis and induced a dose-dependent growth inhibition (up to 100%) in colony forming assays. Consistently, mTOR was strongly dephosphorylated. In pilot studies, we found that i.v. injection of 10^6 #483 cells into recipients conditioned with sublethal irradiation (7.5Gy) induced leukemia in all animals after a latency of 8 weeks. Furthermore, daily i.p. injection of 2mg/kg rapamycin or 1mg/kg RAD001 in healthy animals mediated a high drug level in whole blood (around 50ng/ml and 175ng/ml, respectively). Thus, 20 sublethally irradiated animals were i.v. injected with 10^6 #483 cells and randomized in two groups. One group was treated daily with rapamycin 2mg/kg i.p., the other received only carrier (placebo). The treatment begun 3 weeks after cell injection and continued until the last animal succumbed to leukemia. Rapamycin treatment significantly prolonged the survival of animals compared with control group (mean survival 48.5 and 32 days, respectively, P=0.0087). In a separate experiment RAD001 treatment (1mg/kg) had a similar effect. Two RAD001-treated animals were even free of leukemia upon termination of the experiment (11 weeks). Concentration of rapamycin and RAD001 in whole blood at the time of end point analysis ranged 26-151ng/ml and 53-347ng/ml, respectively. Our findings suggest that mTOR plays an important role in leukemogenesis induced by altered TRK signaling, and might serve as a therapeutic target.

Disclosure:

V389

High density SNP array allelokaryotyping of human acute lymphoblastic leukemia (ALL) xenografts in immunodeficient mice reveals genomic changes upon in vivo induction of chemoresistance

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Introduction: Xenograft models of human leukemia in immunodeficient mice are valuable tools to perform preclinical testing of anti-cancer therapies. Use of high density SNP arrays allows a detailed detection of genomic copy number alterations and uniparental disomy (UPD) in genomic DNA. Combining these two methods could be a powerful approach to identify genomic alterations and genes governing chemoresistance in vivo. **Methods:** Genomic DNA of 19 xenografted childhood ALL samples from the pediatric preclinical testing panel of the Children's Cancer Institute, Australia was analyzed with 250K Nsp SNP arrays. From two patients, samples of different xenograft passages (passage 1 to 4) and the original patient sample were analyzed. Additionally, xenografts from 2 patients were studied, which had been made chemoresistant against either Vincristine, Dexamethasone, Cytosine Arabinoside (ARA-C) or Methotrexate by in vivo treatment. **Results:** In 19 different ALL samples analyzed in xenograft passage 3, we detected 90 hemizygous and homozygous deletions, 31 duplications and 7 regions of UPD resulting in a mean 6.73 copy number alterations (CNAs) per patient. This number is comparable to the number of CNAs detected in recent studies using SNP arrays to detect CNAs in primary ALL samples (5.6 – 7.6) (Kawamata et al. Blood 2008) or (6.46) (Mullighan et al. Nature 2007). Also the pattern of genomic alterations was characteristic for ALL as described previously, showing frequent deletions of CDNK2A (11/19), Pax5 (3/19), ETV6 (6/19), BTG1 (1/19), LEF1 (1/19) and IKZF1 (2/19). Assessment of genomic changes during serial passage of the xenografts showed that the samples widely reproduced the genomic alterations found in the primary patient samples. During passaging, new genomic alterations emerged gradually on chromosomes 4, 5, 6, 9 and 17 in the cells of one patient. This could indicate the outgrowth of clones already present in the original patient sample.One vincristine-resistant sample displayed a new deletion of *CDK6* and a new trisomy of chromosome 18 as compared to the untreated ALL cells. An ARA-C resistant sample displayed new duplications and a new region of UPD on chromosome 20. **Conclusions:** Analysis of human ALL xenografts with high density SNP arrays offer the possibility to study clonal evolution of ALL cell populations and also to discover genomic lesions associated with resistance against specific chemotherapeutic drugs or drug combinations.

Disclosure: Nowak,D.: No conflict of interest disclosed..

V390

Aberrant CDX2 Expression is a Frequent and Prognostically Relevant Event in Human ALL

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Introduction: We previously could show that the homeobox gene CDX2 is aberrantly expressed in 79% of acute myeloid leukemia (AML) patients with especially high expression levels in patients with normal karyotypes (median $?C_T$ 7.58). In AML, expression levels of CDX2 were highly correlated with deregulated expression of HOX genes. **Methods:** To test if this aberrant expression of CDX2 is restricted to AML or is a more common event which can also be observed in other kinds of leukemia, we analyzed the expression of CDX2 in 57 adult patients with acute lymphoblastic leukemia (ALL) by TaqMan real-time qRT-PCR. **Results:** Of these patients, the majority of 81% was positive for CDX2 expression. With highest median expression in pre-T ALL ($?C_T$ 4.1, n=7) followed by cALL ($?C_T$ 5.4, n=10) and pro-B ALL ($?C_T$ 5.78, n=9) those groups showed higher expression of CDX2 than AML patients with normal karyotype. The proportion of patients aberrantly expressing CDX2 differed between ALL subtypes with 100% positivity in patients with pro-B ALL, c-ALL and Ph+ ALL versus 40% positivity in B-ALL/Burkitt lymphoma, 70% in thymic T-ALL and 71% in pre-T ALL. In contrast to AML, where CDX2 expression correlated with HOX gene deregulation, in ALL no correlation between CDX2 and HOXA7 or HOXA9, respectively, could be detected, and only a weak correlation between CDX2 and HOXB6 was observed (p= 0.048, Mann–Whitney U-test). As these results suggest that in ALL CDX2 is exerting its leukemogenic effect not by deregulation of HOX genes but via different pathways, we tested by TaqMan LDA for differential regulation of genes involved in lymphopoiesis. Hereby we found that Cdx2 is able to significantly upregulate lymphoid genes such as Lef1 (3.9-fold, p=0.001),

Tcf3 (13.3-fold, p=0.0004), and Id3 (10.2-fold, p=0.0001). Promoter hypomethylation could be excluded as a possible cause for the aberrant CDX2 expression as no methylation differences between CDX2 positive and negative ALL patients in a methylation region surrounding the transcription start site of CDX2 could be detected (n=9). Expression of CDX2 was highest in high risk and very high risk patients. Despite the small number of only 30 patients included in statistical analysis, CDX2 turned out to significantly correlate to poor overall survival (p=0.019, log rank test), and remained a significant risk factor also after adjusting for the other risk factors age or presence of molecular markers by bivariate analysis. **Conclusions:** Here we show that CDX2 does not perturb HOX expression in the same extent as in AML, but is able to upregulate also lymphopoietic genes. Furthermore, we could demonstrate for the first time that aberrant CDX2 expression is a frequent event in adult ALL, that high expression levels of this protooncogene predict poor treatment outcome in these patients and CDX2 expression therefore has prognostic impact in adult patients with ALL.

Disclosure: No conflict of interest disclosed..

V391

XIAP inhibitors prime acute leukemia cells for TRAIL-induced apoptosis, bypass Bcl-2-imposed resistance and exert anti-leukemic activity in a NOD/SCID mouse model of pediatric acute leukemia

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Introduction: Children with high risk acute lymphoblastic leukemia (ALL) do not respond well to current treatments. This failure is, at least in part, due to defects in apoptosis programs. Therefore, new strategies are required that counter apoptosis resistance in order to improve the poor prognosis of high risk pediatric acute leukemia. Since XIAP, a member of "Inhibitor of Apoptosis" (IAP) proteins, is expressed at high levels in acute leukemia and blocks apoptosis at a central point of the apoptotic machinery, XIAP may present a suitable molecular target for therapeutic intervention. **Methods:** We investigated the effect of small molecule XIAP inhibitors alone and in combination with the death receptor ligand TRAIL or chemotherapeutic drugs on apoptosis induction in ALL cell lines, primary leukemic blasts from children with ALL, normal peripheral blood lymphocytes and in a mouse model of pediatric ALL engrafted in NOD/SCID mice. **Results:** XIAP inhibitors at subtoxic concentrations, but not a structurally related control compound, synergize with TRAIL to induce apoptosis in several ALL cell lines. Also, XIAP inhibitors act in concert with TRAIL to reduce clonogenic growth of ALL cells demonstrating that they suppress longterm survival. Analysis of signaling pathways reveals that XIAP inhibitors enhance TRAIL-induced activation of caspases, loss of mitochondrial membrane potential and cytochrome c release in a caspase-dependent manner, indicating that they promote a caspase-dependent feedback mitochondrial amplification loop. Intriguingly, XIAP inhibitors overcome Bcl-2-mediated resistance to TRAIL by enhancing Bcl-2 cleavage and Bak conformational change. Thus, XIAP inhibitors combined with TRAIL even break Bcl-2-imposed resistance, a defect in the apoptotic pathway that is common in acute leukemia and associated with poor prognosis. Further, XIAP inhibitors prime ALL cells for apoptosis induced by various anti-leukemic drugs, e.g. cytarabine, doxorubicin, etoposide and 6-mercaptopurine, as well as for CD95-triggered apoptosis. Notably, XIAP inhibitors alone and combined with TRAIL induce apoptosis in leukemic blasts from children with ALL ex vivo. In contrast to malignant cells, XIAP inhibitors and TRAIL at equimolar concentrations are non-toxic to normal peripheral blood lymphocytes despite expression of the apoptosis-inducing TRAIL receptors on the cell surface, pointing to a therapeutic window. Most importantly, XIAP inhibitors significantly reduce leukemic burden in vivo in a mouse model of pediatric ALL engrafted in NOD/SCID mice. **Conclusions:** Thus, small molecule XIAP inhibitors present a novel and effective approach to sensitize childhood acute leukemia cells for TRAIL- or chemotherapy-induced apoptosis.

Disclosure: No conflict of interest disclosed..

V392

BAALC associated gene expression signature pinpoints to a role in T-ALL

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The gene *BAALC* (Brain And Acute Leukemia, Cytoplasmic) is a molecular marker of hematopoietic progenitors. *BAALC* is aberrantly expressed in a subset of acute myeloid (AML) and lymphoblastic (ALL) leukemias with high mRNA expression levels predicting an inferior outcome in newly diagnosed AML patients with normal cytogenetics (CN-AML) and T-lymphoblastic leukemia (T-ALL). The aim of this study was to identify genes specifically correlated with *BAALC* to gain insight into its functional role in T-ALL. Gene expression profiles (GEP) of 86 T-ALL patients were generated from the MILE Stage I study (HG-U133 Plus 2.0, Affymetrix). Patients samples were divided into quartile (Q) groups according to *BAALC* expression and were defined as low *BAALC* with expression levels in Q1 to Q3 (n=64) and as high *BAALC* with expression levels in Q4 (n=37). Differentially expressed genes between low and high *BAALC* groups were defined with a minimum expression change of threefold. A gene-expression signature was identified composing of 102 differently expressed probe sets corresponding to 83 unique genes, hypothetical genes/proteins, and open reading frames. Of the 83 genes, 56 were up-regulated and 27 down-regulated in the high *BAALC* group. Several genes associated with hematopoietic stem cells were up-regulated in high *BAALC* group and were previously found to be highly expressed in CD133+ progenitors: *CD34, C5orf23, JUP, NPR3, SPINK2, HOPX, DEPDC6, LAPTM4B, KIAA0125, TFPI, ANGP1, MYCN, ANKRD6, HHEX*, and *IGFBP7*. In addition, the *HOX* genes *HOXA9*, *HOXA5*, and *HOXA3* involved in hematopoietic stem cell regulation were highly up-regulated. Moreover, the role of *BAALC* in myeloid as well as T-lymphoblastic leukemogenesis was strengthened by an overlap of genes that were also present in *BAALC* AML signature reported by Langer et al (Blood 2008) including: *CD34, C5orf23, JUP, NPR3, PROM1, MN1, FAM69B*, and *IGFBP7*. Consistent with the predicted adverse outcome associated with high *BAALC*, this common gene signature included up-regulation of the *MN1* gene, whose over expression was associated with inferior outcome and a higher risk of relapse in CN-AML. In conclusion, high *BAALC* expression is associated with a specific GEP in T-ALL that underscores its role as a marker of an undifferentiated hematopoietic cell. Co-expression of genes common in T-ALL and AML, including the *MN1* gene, may contribute to the unfavourable outcome observed in high *BAALC* expressers in both subtypes of leukemia.

Disclosure: No conflict of interest disclosed..

V393

THE ACETYLTRANSFERASE MYST2/ HBO1 IS ASSOCIATED WITH HEMATOPOIETIC CELL GROWTH AND IS REPRESSED IN LEUKEMOGENESIS

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The histone acetyltransferase Myst2/ Hbo1 is a member of the MYST family of acetyltransferases. Several members of this family, e.g. Tip60, Moz and Morf play important roles in leukemia development. Myst2 is the main acetyltransferase for histone H4 and plays an important role in replication licensing. Its involvement in leukemogenesis is unknown. The aim of the current study was to elucidate its potential role in leukemic cells and leukemia development. To further validate the role of Myst2 in Leukemia we analyzed Myst2/ Hbo1 expression in primary patient samples and in myeloid cell lines. We used overexpression of Myst2 in murine bone marrow cells as well as shRNA mediated repression of Myst2 in a cell line model to further show the role of this protein in the growth regulation of leukemic cells. As identified by ChIP on Chip experiments, the Myst2 promoter is a direct target of PML-RARalpha. The mRNA expression of Myst2 was significantly reduced in APL as well as other

AML patients compared to normal bone marrow and CD34+ progenitor cells. Myst2 was induced during monocytic differentiation in U937 cells and repressed during granulocytic differentiation in HL60 cells. Total histone H4 acetylation levels at lysines 5 and 8 were altered accordingly. PML-RARalpha repressed the differentiation-dependent induction of Myst2 with a concomitant decrease in histone H4 lysine 5 and lysine 8 acetylation. In functional experiments, Myst2 expression in Lineage negative murine bone marrow cells resulted in decreased colony formation and proliferation. On the other hand, shRNA mediated repression of Myst2 in the leukemic cell line Kcl22 enhanced growth and colony formation compared to cells containing scrambled shRNA sequences. These data establish that Myst2 is a direct target of PML-RARalpha. Its widespread repression in AML suggests other regulatory mechanisms as well. Its regulation and growth suppressive functions suggest a potential role in leukemogenesis.

Disclosure: No conflict of interest disclosed..

Freie Vorträge
Stammzellbiologie I

V394

Hematopoietic activity of human short term repopulating cells in mobilized peripheral blood cell transplants is restricted to the first 20 weeks after transplantation

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Two classes of human short-term repopulating cells (STRC) dominate early hematopoiesis in NOD.Cg-Prkdc^{scid}B2m^{tm1Unc}/J (B2m$^{-/-}$) mice: myeloid-restricted STRC-M and lympho-myeloid STRC-ML. STRC are largely enriched in human mobilized peripheral blood (mPB) compared to bone marrow (BM) and cord blood (CB). However, the kinetics of STRC contribution to post-transplant hematopoiesis remain unclear. To assess STRC activity over time, we compared the engraftment kinetics of human transplants from mPB to CB after xenotransplantation into long-living NOD.Cg-Prkdc^{scid}Il2rg^{tm1Wjl}/SzJ (IL2RG$^{-/-}$) mice. For this, CD34$^+$ CB (low content of STRC) and mPB cells (high content of STRC) were transplanted into B2m$^{-/-}$ and IL2RG$^{-/-}$ mice and the engraftment and differentiation of human cells in the murine peripheral blood (PB) and BM was monitored. Lineage distribution and levels of human cells in both mouse strains were similar with the exception of a 4-fold larger proportion of erythroid and CD34$^+$ cells 3 weeks and 3-fold larger proportion of myeloid cells 8 weeks after mPB transplantation in IL2RG$^{-/-}$ compared to B2m$^{-/-}$ mice, indicating efficient engraftment of human STRC in both strains. Serial analysis of BM aspirates of IL2RG$^{-/-}$ mice revealed different engraftment kinetics of mPB compared to CB transplants. The levels of human cells generated from mPB reached a maximum at 3 weeks, remained constant for up to 8 weeks and then gradually declined to threshold levels 28 weeks after transplantation, indicating quantitative hematopoietic contribution of STRC-ML from mPB only for up to 20 weeks after transplantation. In contrast, maximum engraftment of CB transplants was detected 8 weeks post transplantation and remained stable for the whole observation period, demonstrating a larger contribution of long-term repopulating cells (LTRC) to the overall engraftment in CB than in mPB transplants. Comparison of the peripherilization of CB and mPB transplants revealed further differences. Human mPB transplants did not reliably peripheralize. The full differentiation potential of human cells was only detected in murine BM. In summary, we provide direct evidence that the hematopoietic activity of human STRC is restricted to the first 20 weeks after transplantation and that the ratio of STRC to long-term repopulating cells dramatically changes during ontogeny. Our results emphasize that strategies able to selectively amplify STRC are needed to overcome extended cytopenia after clinical CB transplantation.

Disclosure: Zavidij,O.: Anstellungsverhältnis oder Führungsposition:PhD student
Glimm,H.: Anstellungsverhältnis oder Führungsposition:AG Leiter

V395

Extrinsic regulation of early hematopoiesis and hematopoietic engraftment by the Wnt modulator Secreted frizzled-related protein 1

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Hematopoietic stem cells (HSC) reside in a regulating microenvironment, the niche. Secreted frizzled-related protein 1 (Sfrp1) is highly expressed by stromal cell lines which maintain HSC in long-term culture. Sfrp1 is known as a secreted negative regulator of canonical, ß-catenin-dependent Wnt signaling. To study the role of Sfrp1 in hematopoiesis, we knocked down Sfrp1 in these cell lines and found that the lack of Sfrp1 promoted progenitor proliferation. This increase in proliferation was abrogated by a conditioned medium from a cell line in which Sfrp1 was not knocked down, suggesting that an Sfrp1-dependent soluble factor was responsible. To study the role of Sfrp1 in hematopoiesis in vivo, we studied Sfrp1-/- mice. These mice show increased blood cell numbers and an increase of non-committed CD34- CD48- CD150+ Flk2- Lin- Sca1+ Kit+ (LSK) cells, but not in lineage-committed cells (multipotent MPP, CMP, GMP, CLP). Also, BrdU uptake was decreased in LSK and multipotent progenitors (MPP), suggesting that a larger proportion of HSC in Sfrp1-/- reside in G0/G1 phase of the cell cycle. To study the underlying mechanism we looked more closely to expression of activated ß-catenin. Total catenin levels were decreased and its localisation was also confined to the nucleus in LSK cells of Sfrp1-/- mice. Concomittantly, expression of catenin-dependent targets (cyclin D1 and Dkk1) were also decreased. However, in MPP, we observed an increase of Pparg, Hes1 and Runx1 suggesting a differential effect of the lack of Sfrp1 on LSK and MPP. To find out whether the observed effects were related to intrinsic or extrinsic defec ts, we performed transplantation experiments into lethally irradiated recipients. These experiments showed, that there showed no intrinsic effect of Sfrp1 loss in HSC numbers or their ability to engraft irradiated wild-type recipients. In contrast, serial transplantations of wild-type HSC into Sfrp1-/- mice show a clear decrease of both LSK and MPP numbers in secondary mice. Our results demonstrate that microenvironmental Sfrp1 is required to maintain HSC homeostasis through regulation of ß-catenin-dependent signals. Furthermore, our studies show the value of studying stromal cells to identify novel extrinsic regulators of hematopoiesis.

Disclosure: Istvanffy,R.: Anstellungsverhältnis oder Führungsposition:Angestellte des Klinikums rechts der Isars
Oostendorp,R.: Anstellungsverhältnis oder Führungsposition:Angestellter des Klinikums rechts der Isars

V396

The cell cycle protein Cks1 regulates steady state and stress-induced hematopoiesis

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The Cyclin-dependent kinase inhibitor (CKI) p27^{Kip1} is a key regulator of cell cycle progression and controls G1-S transition. Loss of p27^{Kip1} results in increased cell proliferation in various tissues and knockout mice exhibit enlarged organs. Furthermore, p27^{Kip1} has been shown to control hematopoietic stem cell repopulation efficiency. p27^{Kip1} expression is mainly controlled by post-translational mechanisms. These involve phosphorylation of p27^{Kip1}, which allows recognition by and binding to the SCF^{Skp2} ubiquitin ligase. Cyclin-dependent kinase subunit 1 (Cks1) has heretofore been recognized as a rate limiting component of the SCF^{Skp2} complex that allows binding of phosphorylated p27^{Kip1}, which targets it for proteasomal degradation. Mice lacking Cks1 are abnormally small and primary fibroblasts derived from *Cks1*$^{-/-}$ embryos proliferate slower than wild type cells. Based on the role of CKIs in hematopoiesis and Cks1 function in regulating CKI levels we investigated hematopoiesis in *Cks1*$^{-/-}$ mice. We found that the number of bone marrow hematopoietic cells is decreased in *Cks1*$^{-/-}$ mice, and at the same time, there is a concomitant decrease in the number of Lin$^-$ Sca1$^+$ Kit$^+$ cells (LSK cells), as well as the primitive CD34$^-$ subset of LSK cells. In slightly later stages of hematopoiesis we observed a significant increase in the relative number of common myeloid progenitors (CMP),

whereas the relative numbers of more lineage-restricted progenitors (megakaryocyte/erythroid progenitors [MEP] and granulocytic progenitors [GMP]) is unaffected. We next studied whether the observed changes might be due to deregulated cell cycle progression. However, during steady state hematopoiesis the relative number of BrdU-positive cells is unchanged in *Cks1*$^{-/-}$ mice as compared to wild type mice. In contrast, when the mice were challenged by administration of the hematotoxin 5-FU, hematopoietic regeneration was severely disturbed in *Cks1*$^{-/-}$ mice and only a minority of early hematopoietic cells (LSK and multipotent progenitors [MPP]) showed incorporation of BrdU. As during steady-state hematopoiesis, the changes were most prominent in the later stages within the hematopoietic hierarchy (MPP>LSK), suggesting that the MPP were more affected by the absence of Cks1 than the earlier LSK cells. Our findings demonstrate that Cks1 is important in early hematopoiesis and that Cks1 differentially regulates proliferation and hematopoietic differentiation.

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V397

Pleiotrophin is a novel extrinsic regulator of progenitor proliferation and hematopoietic stem cell engraftment

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Hematopoietic stem cells (HSC) reside in specialized niches comprising stromal cells. In order to investigate how stromal cells regulate HSC, we recently established two stromal cell lines (UG26-1B6 and EL08-1D2), both able to maintain HSC in non-contact cultures. A comparison of gene expression between these cells lines and non-supportive stromal cell lines showed an overrepresentation of the small cytokine Pleiotrophin (Ptn, HB-GAM). To examine the role of Ptn for the maintenance of HSC, we introduced a stable knockdown of Pleiotrophin in UG26-1B6 and EL08-1D2. Cocultures with lineage negative cells revealed an increase of colony-forming cells in short-term cultures lacking Ptn, followed by a decline of colony-forming ability in long-term cultures. Thus, the lack of Ptn, accelerates progenitor expansion, but may deplete early hematopoietic activity. To investigate the role of Ptn in early hematopoiesis further, we studied Ptn knockout (PtnKO) mice. We found that these mice show no significant phenotype in steady-state hematopoiesis: There was no difference in earliest stem cells (Cd34- Flk2- LSK) or lineage commited progenitors (MPP, CMP, MEP, GMP) in the marrow of PtnKO mice. Since steady-state hematopoiesis and hematopoietic regeneration after stress (culture, transplantation, chemostatic treatment) are regulated through different mechanisms, we hypothesized that Ptn might be involved in the regulation of hematopoietic regeneration. We first showed that HSC lacking Ptn behave the same as wild-type HSC in primary and secondary irradiated recipients. Thus, the lack of Ptn does not cause an intrinsic engraftment defect in HSC. However, after transplantation of wildtype cells into Ptn knockout mice, early hematopoiesis is dysregulated. Interestingly, we observed that HSC which engrafted in primary Ptn-knockout mice show a small but reprodicible increase in engraftment in secondary recipients, with an increase of CD34-LSK as well as committed MPP. Since the HSC were not altered in the mice, the increase in engraftment is caused by the extrinsic effects of the lack of Ptn. In conclusion, our results show that Ptn is a novel regulator of hematopoietic regeneration after environmental stress.

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V398

The CysLT1 ligand LTD4 supports 4 1 and 5 1-mediated adhesion and proliferation of CD34+ hematopoietic progenitor cells

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Cytokines and chemokines control hematopoietic stem- and progenitor cell (HPC) proliferation and trafficking. However, the role of non-peptide mediators in the bone marrow microenvironment has remained elusive. Particularly $CysLT_1$, a G protein-coupled receptor (GPCR) recognizing inflammatory mediators of the cysteinyl leukotriene family, is highly expressed in HPC. We therefore analyzed the effects of its ligands on human $CD34^+$ HPC. The most potent $CysLT_1$ ligand LTD_4 rapidly and significantly upregulated a4ß1 and a5ß1 integrin-dependent adhesion of both, primitive and committed HPC. LTD_4-triggered adhesion was inhibited by specific $CysLT_1$ antagonists. The effects of other $CysLT_1$ ligands were weak (LTC_4) or absent (LTE_4). In serum-free liquid cultures supplemented with various hematopoietic cytokines including interleukin-3, only LTD_4 significantly augmented expansion of HPC in a dose-dependent manner comparable to peptide growth factors. LTC_4 and LTE_4 were less effective. In $CD34^+$ cell lines and primary HPC, LTD_4 induced phosphorylation of p44/42 ERK/MAP-kinase and focal adhesion kinase-related tyrosine kinase Pyk2, which is linked to integrin activation. BM stromal cells produced biologically significant amounts of cysteinyl leukotrienes only when hematopoietic cells were absent, suggesting a regulatory feed-back mechanism in the hematopoietic microenvironment. In contrast to antagonists of the homing-related GPCR CXCR4, administration of a $CysLT_1$ antagonist failed to induce human $CD34^+$ HPC mobilization in vivo. Our results suggest that cysteinyl leukotrienes may contribute to HPC retention and proliferation only when cysteinyl leukotriene levels are increased either systemically during inflammation or locally during marrow aplasia.

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V399

G-CSF induced mobilisation, expansion, and repopulation ability of hematopoietic stem cells is altered by the morphogen Osteopontin

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Introduction: In an adult mammalian organism hematopoietic stem cells (HSC) reside in the bone marrow and are in part regulated by the cues of the bone marrow microenvironment, called the stem cell niche. Although there is some controversy about the cellular identity of the niche cells, there is no doubt that changes in the number of osteoblastic cells influence the number and function of hematopoietic stem cells in vivo. We have previously identified the bone marrow morphogene Osteopontin, which is almost exclusively expressed by the osteoblasts, as a negative regulator of HSC pool size under physiological conditions. **Methods:** Here we analyzed the impact of Osteopontin on hematopoietic stem cell function under conditions of G-SCF stimulation using Osteopontin knockout mouse model. We analysed the impact of short term G-CSF stimulation in vivo on the frequency of osteoblastic cells using the CFU-F assay. Further we analyzed changes in hematopoietic stem cell number und function under stimulation of wild type and Osteopontin mutant mice in vivo. **Results:** We found a pronounced increase in the abondance of osteoblasts in Osteopontin null mice after stimmulation with G-CSF compared with wildtype control. Although there was no difference in the increase of peripheral blood leukocytes between the genotypes after stimulation with G-CSF we observed a significant higher proportion of primitive hematopoietic cells in the peripheral blood in the mutant mice compared with wildtype controls. When wildtype HSC were transplanted into Osteopontin deficient recipients and subsequently stimulated with G-CSF similar effects were observed, indicating that the difference in mobilization was due to the Osteopontin expression in the stem cell niche and not a stem cell intrinsic mechanism. To further investigate the role of Osteopontin-expression in the niche on HSC function under condition of G-CSF stimulation we have exposed wildtype HSC to WT or Osteopontin$^{-/-}$ microenvironment for 6 weeks, than we administered G-CSF to the recipients and subsequently harvested the bone marrow and transplanted into secondary recipients in a competitive setting. HSC which were exposed to Osteopontin deficient bone marrow displayed substantially better hematopoietic reconstitution than did cells that were exposed to wildtype bone marrow, indicating negative regulation of the repopulation ability of HSC by Osteopontin. In addition we detected alterations in proliferation and the rate of apoptosis in mutant mice compared with wildtype controls when G-CSF was administered. The molecular bases for these effects still remain to be determined. **Conclusions:** Our data demanstrate that Osteopontin plays an important role in sup-

pression of excess stem cell mobilization and expansion under conditions of proliferative stress. The biological relevance for this effect is most likely the prevention of exhaustion of HSC's.

Disclosure: No conflict of interest disclosed.

Posterdiskussion
Allogene Transplantation III

P400

Comparative analysis of peripheral blood CD34+ progenitor cell count and hematopoietic progenitor cell count (HPC) regarding stem cell yield in healthy donors

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Leukapheresis is a standard procedure to separate CD34+ peripheral progenitor cells for allogeneic and autologous stem cell transplantation. Here, we evaluated whether determination of CD34+ counts by flow cytometry or estimation of immature cells referred to as hematopoietic progenitor cells (HPC) by Sysmex SE-9000 prior to apheresis can serve to predict the subsequent total CD34+ cell harvest. A sequential analysis of 308 leukapheresis runs of healthy donors at a single institution during January 2008 and December 2008 was performed. Donors received lenograstim 7.5–10µg/kg s.c. for stem cell mobilisation on day 1 to 5 with apheresis starting on day 5. Peripheral CD34+ cell counts and HPC counts were correlated with the subsequently achieved actual stem cell yield. Within the 308 apheresis runs, we observed a mean CD34+ harvest of 5.08+/-0.17 x 10^6 CD34+ cells/kg donor weight (mean+/-SEM). 71.5% of donors achieved a yield of >4 x 10^6 CD34+ cells/kg recipient weight on day 1. Comparison of CD34+ cell count in peripheral blood prior to apheresis with final progenitor cell yield revealed a significant correlation allowing early prediction of sufficient CD34+ progenitor cell harvest. 246 pre-apheresis CD34 analyses with a mean of 66.87+/-2.78 CD34+ cells/µl were available. Statistical analysis revealed a correlation coefficient of r=0.84 (p<0.0001). Regarding the 246 pre-apheresis CD34 concentrations and using the cutoff of 4 x 10^6 CD34+ cells/kg KG recipient weight, linear equation allows the prediction of successful harvest with a positive predictive value of 85% (137 successful harvests/162 successful harvests predicted). With regard to HPC counts we also observed a significant correlation, though on a lower level (r=0.54, p<0.0001). Using linear equation, a considerably lower positive predictive value of 60.9% (98 successful harvests/161 successful harvests predicted) was obtained. Our results demonstrate that determination of CD34+ count in peripheral blood prior to apheresis provides an easy and sufficient parameter for predicting absolute CD34+ progenitor cell numbers in the apheresis product by linear equation. HPC number in peripheral blood showed a considerably lower correlation and may – despite its use in the autologous setting – not serve well for prediction of stem cell yield in the healthy donor situation.

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P401

Impact of CD52-expression in reconstituting T cells after alemtuzumab-mediated in vivo T-cell depleted allo-transplantation

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Targeting CD52 by the monoclonal antibody alemtuzumab is a common means for in vivo depletion of T cells in the context of allogeneic hematopoietic stem cells transplantation (HSCT). The CD52 molecule is a glycosylphosphatidylinositol (GPI)-anchored surface protein expressed on granulocytes, B cells, T cells, dendritic cells and monocytes. However, functional properties and regulation of CD52 remains largely unknown. We have demonstrated earlier that alemtuzumab-mediated T-cell depletion leads to the reconstitution of CD52-negative ($CD52^{neg}$) T cells. CD8-depleted DLI are able to restore CD52-positive ($CD52^{pos}$) T cell-populations. In the absence of DLI, however, these T cells can be detected beyond the second year after HSCT. Here we analyzed T cells of patients in the third year after HSCT with persisting $CD52^{neg}$ T cell ex vivo and after in vitro culture. We sorted $CD52^{pos}$ and $CD52^{neg}$ CD8 as well as CD4 T cells and cultured them over more than 8 weeks by non-specific stimulation. CD52-expression of sorted T cells remained stable throughout the culture. In contrast, after in vitro application of alemtuzumab to T cells isolated from the peripheral blood of healthy donors, we found only a transient loss of CD52-expression with a minimum expression after 3-7 days. The sorted and cultured $CD52^{neg}$ and $CD52^{pos}$ T cells generated from our patients did not differ in phenotype, maturation status and growth kinetics. In contrast, first experiments of T cell function indicated, that CMV-specific T cell response might be impaired in $CD52^{neg}$ T-cells as determined by IFN-g ELISPOT assay. We also looked for CD52 mRNA-expression and found no difference in $CD52^{pos}$ compared to $CD52^{neg}$ T cells. Since CD52-expression might also be regulated by their GPI-anchors, we stained for further GPI-linked surface proteins on T cells isolated from our patients. Thereby, we saw that $CD52^{neg}$ T cells also had lost the expression of CD55 and CD59. These data were confirmed on ex vivo as well as on cultured T cells from our patients. In summary, $CD52^{neg}$ T cells persist long-term after alemtuzumab-mediated T-cell depletion in allo HSCT. The loss of CD52-expression is most likely regulated via GPI anchors. $CD52^{neg}$ T cells remain negative even when T cells are taken into culture. Functional analyses point toward a potential difference of antigen-specific T cell responses between $CD52^{pos}$ and $CD52^{neg}$ T cells.

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P402

MHC class II independent lethal graft versus host disease can be modulated by $CD4^+FoxP3^+$ regulatory T cells

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Allogenic bone marrow transplantations are the only curative treatment options for patients with relapsed or high risk leukemias or lymphomas. The recognition of host alloantigens by donor cells is most likely the basis of graft-versus-tumor effects, but is oftentimes also the cause of graft-versus-host disease (GVHD), a key contributor to the high morbidity and mortality rates in the context of bone marrow transplantation.
In our present study we analyzed potential mechanisms to prevent or attenuate acute graft versus host reactions in an allogenic MHC mismatched transplantation model. We were interested whether priming of $CD4^+$ T cells is necessary for the induction of acute GVHD. Therefore we transferred T cells from BALB/c mice into MHC class II deficient hosts (C57BL/6 background) and observed the mice during the following two weeks with regard to the development of a graft versus host reaction. In comparison to wild type hosts, we observed no difference in clinical signs of acute GvHD, such as weight loss, hunched posture and death after transplantation. CFSE-labeling of the transplanted T- cells prior to transplantation showed marked proliferation of the donor $CD8^+$ and $CD4^+$ T cells in the blood of wild type mice already 5 days past transplantation. In MHC class II deficient host animals we observed a strong proliferation of donor $CD8^+$ in the absence of $CD4^+$ T cells. Regulatory $FoxP3^+$ $CD4^+$ T cells (Tregs) are known to suppress the activation of conventional T cells (e.g. CD8+ or $CD4^+$ T cells). To study whether preactivated Tregs (preTregs) can inhibit the activation of allogenic $CD8^+$ T cells in this setting we initially performed an allogenic mixed lymphocyte reaction in vitro. A 3H-thymidine assay demonstrated a decreased proliferation of T cells which had been stimulated with allogenic MHC class II deficient dendritic cells as well as of T cells that had been stimulated with wildtype DCs in the presence of preTreg cells indicating that preTregs can suppress $CD8^+$ T cell responses in a MHC class II independent manner. In conclusion, our results demonstrate that the activation of allogenic $CD8^+$ T cells alone is sufficient to induce lethal graft versus host disease and that this immune response can be modulated by Treg cells. These data provide the basis for future concepts to manipulate allogenic T cell resonses to prevent GvHD.

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P403

Health status of unrelatedallogeneic stem cell donors affects CD34+ progenitor mobilization and donorsafety issues

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Introduction: Allogeneic unrelated transplantation success is dependent on the number of progenitors infused and therefore on the yield of G-CSF mobilization in healthy donors. The most frighten complication of mobilization is spleenic rupture due to spleen enlargement (SpEnl) during G-CSF administration. Although several factors influencing CD34+ progenitor yield (CD34) have been investigated, health status (eg.metabolic syndrome (MeSy)) have not been associated with either CD34 or SpEnl. **Methods:** 129 healthy unrelated PBPC donors (70% male) were enrolled in this study consecutively after clearance for donation. At time of medical examination (ME) donors were checked for health history, WHO-MeSy parameters, spleen size (SpSi), and others. SpEnl was also checked after collection and after one month by ultra sonic investigation. After mobilizing with G-CSF (lenograstim) at 10µg/kg BW d the duration of the apheresis on d5 was adapted to peripheral CD34 and, if necessary, repeated on d6. After filtering factors which may influence either CD34 per fold processed blood volume or SpEnl by ANOVA linear regression (LR) was applied. **Results:** LR revealed that males mobilize more CD34 ($p < 0.031$). Postive correlation with CD34 was also seen for donors with large ME-SpSi ($p < 0.032$), high ME-WBC counts ($p < 0.044$), high ME alcalic phosphatase levels ($p < 0.012$), high ME monocyte counts ($p < 0.047$). Protective medication for gastritis had had a negative impact on CD34 ($p < 0.01$). WHO metabolic syndrome score as well as e.g. fold spleen enlargement had had no influence on the yield. The model explained 42.2% of the observed variance. SpEnl after 5d of G-CSF (mean 1.36, range 0.87 to 2.09 fold) was lower for females ($p < 0.01$), older donors ($p < 0.01$), donors with high blood pressure ($p < 0.049$), and donors with high pre mobilization SpSi ($p < 0.01$). These factors explain 54.5% of the observed variance, with pre mobilization SpSi as most important one. No direct correlation between SpEnl and CD34 was seen. Same results were seen for SpEnl after one month after (mean 1.15, 0.66 - 1.7 fold) **Conclusions:** Mobilization efficacy of G-CSF is dependent on pre mobilization factors. Metabolic syndrom as well as SpEnl failed to show an impact on CD34 in this study. Remarkably donors with a small pre mobilisation spleen had had a huge SpEnl during mobilization and thus may be on a higher risk for rupture.

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P404

ALLOGENEIC NON ADHERENT BONE MARROW CELLS FACILITATE HEMATOPOIETIC RECOVERY BUT DO NOT LEAD TO ALLOGENEIC ENGRAFTMENT

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Introduction: Non adherent bone marrow derived stem cells have been recently described to give rise to multiple mesenchymal phenotypes and have an impact in tissue regeneration. Therefore, the effects of murine bone marrow derived non adherent cells compared to murine bone marrow cells were investigated with regard to engraftment capacities in allogeneic and syngeneic stem cell transplantation using transgenic, human $CD4^+$, murine $CD4^{-/-}$, $HLA\text{-}DR3^+$ mice. **Methods:** Bone marrow cells were harvested from C57Bl/6 and Balb/c wild-type mice, expanded to non adherent cells for 4 days and characterized by flow cytometry, PCR, and CFU-f before transplantation in lethally irradiated recipient mice. Chimerism was detected at day 0, 21, 33, 40, and 50 using flow cytometry (MHC-I (H-2D[b], H-2K[d]), human CD4, HLA-DR), quantitative PCR, and immunohistology of the gut. **Results:** Culturing of bone marrow cells in a dexamethasone containing DMEM medium induced expansion of non adherent cells expressing CD11b, CD45, and CD90. Analysis of the $CD45^+$ cells showed depletion of $CD4^+$, $CD8^+$, $CD19^+$, and $CD117^+$ cells. Expanded syngeneic and allogeneic non adherent cells were transplanted into triple transgenic mice. The recovery of white blood cell count after transplantation of syngeneic non adherent cells was significantly earlier at day 35, and 50 (day 35 *P = .023, day 50 **P = .006), of allogeneic non adherent cells at day 28 (day 28 **P = .019) compared to bone marrow controls. After transplantation of non adherent cells, granulocytes and monocytes recovered earlier than lymphocytes [day 21]. Syngeneic non adherent cells protected 83% of mice from death (n=8, hematopoietic $CD4^+$ donor chimerism of 5.8±2.4% [day 40], P<.001). Allogeneic non adherent cells preserved 62.5% (n=8) of mice from death without detectable hematopoietic donor chimerism. Co-transplantation of allogeneic murine spleen cells and allogeneic bone marrow cells induced engraftment of donor cells in surviving mice in this model. **Conclusions:** Non adherent cells triggered endogenous hematopoiesis and induced faster recovery compared to bone marrow controls. These findings might be of relevance in the refinement of strategies in the treatment of hematological malignancies.

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P405

Clinical Outcome and Immune Reconstitution after Haploidentical Hematopoietic Cell Transplantation in Adults Using Reduced Intensity Conditioning and CD3/CD19-Depleted Grafts: A Single Center Experience

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Haploidentical hematopoietic cell transplantation (HHCT) using reduced intensity conditioning (RIC) and CD3/CD19 graft depletion enables faster engraftment and immune reconstitution than CD34 selected grafts. 28 adults with a median age of 45 years (range, 19-59) received HHCT with CD3/CD19-depleted grafts after RIC (fludarabine, thiothepa, melphalan and OKT-3) and were evaluated for clinical outcome and immune reconstitution. Diagnoses were AML (n=21), ALL (n=3), NHL (n=1), myeloma (n=2), and CML (n=1). Patients were "high risk" because of refractory disease (n=13) or relapse after prior HCT (n=15). At HHCT, 20 patients were in CR and 8 in PR. Grafts contained a median of $8.6x10^6$ (range 4.3-17) CD34+ cells/kg, $2.9x10^4$ (range 0.9-9.2) CD3+ T cells/kg and $2.9x10^7$ (range 0.02-23.3) CD56+ cells/kg. Engraftment was rapid with a median of 12 days to >500 granulocytes/µL (range 9-22) and 11 days to >20000 platelets/µL (range 7-23). Full chimerism was reached after median 14 days (range 11-28). Incidence of grade II-III acute and chronic GVHD was 54% and 14%, respectively. TRM at day 100 was 2 of 28 (21%) and in total 10 of 28 (36%). Overall survival is 9 of 28 patients (32%) with median follow-up of patients alive of 338 days (range 196-1384), resulting in a Kaplan-Meier estimate 1-year survival of 52%. A KIR-mismatched donor had no positive impact on survival. NK-cell engraftment was fast, reaching normal levels at day 20 (median 248 CD16+56+CD3- cells/µl, range 1-886). T-cells regenerated delayed with median of 205 CD3+ cells/µl (range 59-799) on day 100. CD8+ T-cells increased early after HHCT compared to CD4+ T-cells with a median of 41 (range 0-1277) versus 33 (range 0-301) and 213 (range, 0-558) versus 171 (range 0-284) cells on day 80 and 250, respectively. We further observed rapid regeneration of memory T-cells compared to late reconstitution of naïve T-cells with a median of 11 CD4+45RA+ (range 0-109) versus 47 CD4+45R0+ cells/µl (range 0 to 255) and 44 (range 0-193) versus164 (range 73-323) on days 80 and 250, respectively. T-cell repertoire was skewed with oligoclonal T-cell expansions to day 100 and normalization after day 200. B-cell reconstitution was slow with 6 (range 0-867) and 194

(range 0-371) CD19+20+ cells/µl on days 80 and 250, respectively. Seven patients died because of infections, mainly late pneumonias. Thus, HHCT using RIC and CD3/CD19 depleted grafts has low toxicity and allows fast engraftment and immune reconstitution.

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P406

Impact of Donor on Outcome of Allogeneic Hematopoietic Cell Transplantation in AML: Comparable Results with Matched or Mismatched Unrelated versus Related Donors in CR-Patients and Benefit of Matched Unrelated Donors in PR-Patients

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Hematopoietic stem cell transplantation (HCT) is a curative treatment option for patients with intermediate/high-risk acute myeloid leukemia (AML). Current treatment algorithms employ allogeneic HCT from a matched related donor (MRD) for these patients in first complete remission (CR). However, the role of HCT from a matched or mismatched unrelated donor (MUD/MMUD) remains unclear. We retrospectively analyzed a cohort of 208 consecutive adult patients with AML who received HCT in 2000-2008 at our institution. The patients were transplanted after either myeloablative (MA, n=147) or dose-reduced-conditioning regimens (RIC, n=61). All patients with a MMUD received ATG in the conditioning regimen. Median age of patients was 49 years (range, 18-76). 75 patients were transplanted from MRD, 78 patients from MUD and 53 patients from MMUD. Age, risk profile and pretreatment were evenly distributed among the three cohorts of patients. 20 (MRD), 18 (MUD) and 23 (MMUD) patients were not in CR at time of transplant. Kaplan-Meier-estimated 3-year overall survival (OS) was non-significantly different with 52% after MRD-, 62% after MUD- and 46% after MMUD-HCT with 1278 (range, 147-3090), 967 (range, 201-3075) and 572 (range, 87-1471) days median follow-up of patients alive, respectively. In patients transplanted in CR (63% MRD vs. 59% MUD vs. 51% MMUD), 3-year estimated OS was also non-significantly different. However, in patients transplanted in PR we observed a significant advantage for patients receiving a MUD graft (29% MRD vs. 69% MUD vs. 45% MMUD, p=0.027 and 0.3510). In the subgroup receiving MA conditioning we observed a non-significantly better OS compared to RIC with an estimated 3-year OS of 60% vs. 44%, mainly due to a lower incidence of relapse. There was no significant difference in the incidence of acute GvHD >II with 24% (MRD), 13% (MUD) and 35% (MMUD) or chronic GvHD with 36% (MRD), 44% (MUD) and 27% (MMUD), respectively. A significantly better survival of patients with limited cGvHD vs. extensive or without cGvHD (estimated 3-year OS 76% vs. 26% vs. 51%, p=0.0001/0.0009) was observed. Regarding the influence of HLA-mismatch there was no advantage of antigenic vs. allelic MM but a trend towards better survival with A locus MM. In conclusion, HCT from MUD or MMUD in AML may result in a similar outcome compared to MRD. The use of MUD and limited cGVHD may lead to improved survival due to an enhanced graft-versus-leukemia-effect, particularly in patients in PR.

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Bethge,W. A.: Anstellungsverhältnis oder Führungsposition:Anstellungsverhältnis

P407

Chronic GVHD is associated with a deficiency of CD27+IgD+IgM+ B cells

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Background: The pathophysiology of chronic graft-versus-host-disease (cGVHD) and involved cellular subsets remain poorly understood. In this study, we analysed B and T cell subsets in the peripheral blood of 69 patients (pts) after allogeneic haematopoietic stem cell transplantation (alloHSCT). **Methods:** Peripheral blood samples from 16 pts who never experienced cGVHD (group 1), 12 pts with resolved (group 2) and 41 pts with active cGVHD (group 3) were analysed for lymphocyte subsets by FACS. Chronic GVHD was evaluated using criteria and guidelines of the National Institute of Health. **Results:** The absolute CD19+ B cell count (in x109/l) in cGVHD pts was subnormal in group 2 (median 0.14, range 0.008-0.69), low in group 3 (median 0.04, range 0.001-2.59) compared to group 1 (median 0.237, range 0.03-0.55) (normal range: 0.2-0.4). Furthermore, absolute numbers of the CD27- B cell compartment, which includes immature and transitional B cells, were lower in cGVHD pts (group 2: median 0.12, range 0-0.66; group 3: median 0.003, range 0 -2.53) compared to pts of group 1 (median 0.20, range 0.03-0.52). The absolute number of cells of the CD 27+ memory B cell compartment was lower in the active cGVHD group (median 0.002, range 0-0.315) compared to pts. of group 1 (median 0.01, range 0.004-0.65) and group 2 (median: 0.01, range 0-0.08). CD 27+IgD+IgM+ B cells (in 106/l) could not be detected in pts with active cGVHD (median 0, range 0-1,35) (except two patients with DLI induced cGVHD: 66,64 and 119,51) in contrast to pts of group 1 (median 1.26, range 0-6.26) and group 2 (median 1.23, range 0-10.53). No significant differences in absolute CD4+ (median (range) 0.29 (0.11-0.89)/ 0.43 (0.05-0.76) / 0.29 (0.02-1.2)) and CD8+ (median (range) 0.44 (0.11-1.61) /0.47 (0.17-1.96) /0.31 (0.01-1.76)) as well as regulatory (median (range) 9.33 (0.68-27.43) / 3.82 (1.19-17.16) / 7.9 (0.1-37.2)) T cell counts were observed between groups 1-3 respectively. **Conclusion:** This small series confirms a close association of diminished B cell counts with cGVHD while no direct association was detected for T cells. Furthermore, the loss of CD 27+IgD+IgM+ B cells in pts with active cGVHD indicates functional asplenia in these pts. Analysis of B cell subsets can provide a diagnostic tool for monitoring cGVHD activity but requires prospective evaluation.

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P408

Sequential use of the MFAC regimen and reduced-intensity conditioning for allogeneic hematopoietic stem cell transplantation in relapsed or refractory CD33+ AML

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Introduction: Gemutzumab ozogamicin (GO) has been used as a single agent, as well as in combination with conventional chemotherapy in CD33+ acute myeloid leukemia (AML). **methods:** We report 26 patients with CD33+ primary refractory or relapsed AML receiving the MFAC regimen (GO, fludarabine, ara-C and ciclosporin) directly followed in aplasia by reduced intensity conditioning (RIC) for allogeneic hematopoetic stem cell transplantation (HSCT). **Results:** Early treatment assessment revealed an overall response (OR) of 65.4%. Grade III/IV hepatic toxicity after MFAC occured in 30.8% of the patients. The median time to allograft was 22.5 days. 77% of all patients proceeded to allogeneic HSCT. Grade III/IV hepatic toxicity after allografting was observed in 25%, including one patient with veno-occlusive disease (VOD). When summarizing the sequential regimens, CTC grade III/IV hepatic toxicity (including hyperbilirubinemia and transaminitis) was seen in 16 patients (= 61.5 %). Median follow up was 8.2 months (range 0.3 – 39.7 months). Estimated twelve-month overall survival (OS) was 52.4%. **Conclusions:** MFAC directly followed by RIC for allogeneic HSCT seems to be active and feasible, but with an increase in hepatic toxicity.

Disclosure: No conflict of interest disclosed.

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Extracorporeal Photophoresis for chronic GvHD: results from a phase II clinical trial demonstrating evidence of immunodulation.

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Introduction: Graft versus Host disease (GvHD) is the major limitation to successful allogeneic haematopoietic stem cell transplantation and contributes significantly to transplant related mortality and morbidity. Steroid refractory or steroid dependent GvHD in particular is linked to poor survival and poor quality of life. Conventional immunosuppression has limited success in these conditions and increases susceptibility to infection and relapse. Extracorporeal Photopheresis (ECP) is a promising therapy for acute and chronic GvHD in patients not responding to conventional immunosuppression. ECP mechanisms are still poorly understood and although a number of studies using ECP treatment for conditions including acute and chronic GvHD have been published, there is limited data on the variations in blood cell subsets post ECP therapy. **Methods:** In a phase II clinical trial, ten patients with steroid refractory chronic GvHD were treated with ECP. Monocyte, T cell, NK cell and dendritic cell subsets were analysed by flow cytometry prior to ECP and, at cycle two, six and three months after the end of ECP treatment. The overall response rate to ECP in this study was 70% (3 Complete responders, 4 partial responders and 3 non-responders). **Results:** A statistically significant increase ($p<0.05$, Mann-Whitney test) during ECP treatment and follow-up could be detected for total numbers of CD4+ T cells, regulatory T cells (CD4+ CD25bright foxP3+), CCR4+ CD4+ (Th2) cells and dendritic cells (lin- HLA-DR+). Interestingly, the percentage of CD16+ CD14dim monocytes was significantly increased only in patients who responded to ECP treatment. There were no significant changes in numbers or percentages of NK/NK-T cell and CD8+/CD4+ effector/memory/naïve subsets. **Conclusion:** In summary, the results suggest that ECP treatment alters the composition of immune cell subsets and switches the blood cell compartment towards a regulatory immune repertoire. The role of CD16+ monocytes, which were only found to be increased in patients responding to ECP, needs further investigation. An immune monitoring platform has been established in Cologne to identify cellular subsets of importance for immune responses after allogeneic haematopoietic stem cell transplantation.

Disclosure: No conflict of interest disclosed.

P410

Allogeneic stem cell transplantation after a treosulfan and fludarabine preparative regimen in otherwise refractory chronic lymphocytic leukemia, multiple myeloma, or malignant lymphoma

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Introduction: The combination of treosulfan (3 x 14 g/qm) and fludarabine (5 x 30 mg/qm) (Treo/Flud) is an emerging preparative regimen for allogeneic stem cell transplantation (alloSCT), which so far has been preferably tested in myeloid malignancies. One ongoing trial supports that Treo/Flud is well tolerable and effective in patients (pts) with advanced multiple myeloma (Bone Marrow Transplant 39:389-396, 2007). **Methods:** In a single centre retrospective analysis, we evaluated the clinical results using the Treo/Flud regimen in a total of 52 pts (median age 47 [24 – 70] years [yrs.]) with chronic lymphocytic leukemia (CLL, n=11), multiple myeloma (MM, n=22), and malignant lymphoma (NHL, n=19), who underwent alloSCT from matched related (MRD, n=13 [25%]) or unrelated donors (MUD, n=39 [75%]) between 12/04 and 12/08. All 52 pts. had been extensively pretreated and were deemed refractory to conventional therapy. Forty-seven pts (90%) had relapsed after previous autologous (n=41) or allogeneic (n=6) SCT. Median disease duration before alloSCT was 39 (5 – 138) months (mo.) and median follow-up is 12 (5 – 53) mo. after alloSCT. **Results:** The overall survival estimate (OS) for all pts. at 2 yrs. is 53% (95% confidence limits [CI]: 38% - 67%) and is significantly different between the 3 disease categories with 90% (95%-CI: 47% - 99%) for CLL pts, 52% (95%-CI: 27% - 73%) for MM pts, and 25% (95%-CI: 7% - 48%) for NHL ($p < 0.008$). Transplant-related mortality (TRM) is not influenced by the disease category and 13 pts (25%) have died from transplant-related causes (infections associated with graft-versus-host disease, n=11) accounting for a TRM estimate at 2 yrs. of 31% (95%-CI: 19% - 49%). In contrast, the relapse risk (RR) at 2 yrs. is significantly lower for CLL pts with 12% (95%-CI: 2% - 61%) when compared to MM pts (50% [95%-CI: 27% - 78%]) and to NHL pts (57% [95%-CI: 26% - 91%]) ($p<0.04$). The OS after MUD transplants is 52% (95%-CI: 33% - 69%) and 39% (95%-CI: 12% - 66%) after MRD transplants and there is no indication for an adverse impact of MUD alloSCT on either RR or TRM in this cohort. **Conclusions:** The present analysis demonstrates that alloSCT after the Treo/Flud preparative regimen can induce prolonged remissions with acceptable TRM in a large proportion of otherwise refractory pts with CLL, MM, or NHL. The clinical results appear particularly promising in refractory CLL and MM pts, which should be further evaluated in prospective clinical trials.

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P411

Characterisation of taste disturbances following myeloablative or nonmyeloablative chemotherapy and stem cell transplantation

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Background: Hematopoeitic stem cell transplantation (SCT) after myeloablative (MA) or nonmyeloablative chemotherapy (NMA) is a successful treatment option for a variety of diseases. Although alterations of taste and smell are frequent after these therapeutic modalities, no systematic evaluation is available so far. **Methods:** A questionnaire covering aspects of the dietary pattern and changes in the perception of taste and smell was developed. Clinical data were gathered from patient's (pts) charts, and the study was approved by the institutional review board. Data from 181 pts are presented. **Results:** The pts were surveyed after a median of 25 months from SCT (range 1-292), their age ranged from 19-79 years. Indications for SCT included acute leukemia (n=72), myeloproliferative disease (n=32), lymphoma (n=29), and others (n=48). Pts received an allogeneic graft after MA (n=86) or NMA (n=55) conditioning, and 33 pts received an autologous SCT. 71% of pts reported moderate to severe changes in taste perception on a semiquantitative visual analogue scale during the acute phase of SCT with limited differences between the three groups (79%, 65%, 72%). A complete regression at the time of the survey was reported by 27% of the pts, 26% still suffered from ongoing moderate to severe alterations. 67% of the patients perceiving changes in taste during the acute phase reported improvements of the symptoms after a median of 90 days (range 3-600 days). Changes in taste perception correlate with loss of body weight. Severe alterations were more prevalent after allogeneic SCT (29% after MA conditioning, 31% after NMA) compared to pts with autologous grafts (8%). Whereas pts without persisting changes in taste perception lost a median of 1 kg of body weight, pts suffering from severe changes lost median 7 kg. **Conclusions:** Taste disturbances are a common event in the early course of a SCT. The symptoms persist in one quarter of the pts receiving allogeneic grafts. In the allogeneic setting, no differences are observed between MA and NMA conditioning. Following autologous transplantation, a lasting impairment is encountered only in 8% of pts. A subjective improvement of symptoms is encountered after a median of three months.

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SECOND ALLOGENEIC STEM CELL TRANSPLANTATION AS SALVAGE THERAPY FOR PATIENTS WITH RELAPSED ACUTE MYELOID LEUKEMIA AFTER FIRST ALLOGENEIC STEM CELL TRANSPLANTATION

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Introduction: Allogeneic stem cell transplantation (alloSCT) represents a highly curative treatment for patients (pts.) with high-risk acute myeloid leukemia (AML) if a suitable donor is available. However, not all patients are ultimately cured and those who succumb to relapse after alloSCT have a dismal prognosis due to the highly limited set of therapeutic options one of which may be re-transplantation from an (alternative) donor. **Patients and Methods:** Here, we report the outcome of 13 pts. with high-risk AML who received a second alloSCT as salvage therapy for relapsed AML following first alloSCT at our institution between 1994 and 2008. Initial alloSCT was performed in CR1/CR2 (5/13 pts.) or active disease (8/13 pts.) using either bone marrow (4/13 pts.) or peripheral blood stem cells (PBSCs) (9/13 pts.) from a matched related donor (mrd: 8/13 pts.), a matched unrelated donor (murd: 3/13 pts.), or a mismatched unrelated donor (mmurd: 2/13 pts.). 10/13 pts. received standard myeloablative conditioning (MAC) (12 Gy total body irradiation, 2 x 60 mg/kg cyclophosphamide), whereas in 3/13 pts. reduced intensity conditioning (RIC) (Flu 6x30 mg/m^2, Bu 2x4 mg/kg, ATG 4x10 mg/kg) was used as a preparative regimen. Median time to relapse was 5 (2-138) months. Median age at 2nd alloSCT was 49 (20-56) years. In 3/13 pts. CR2/CR3 was achieved by reinducton chemotherapy, 10/13 had active/refractory disease at the time of 2nd alloSCT. In all pts. PBSCs from an alternative donor (murd: 7/13 pts.; mmurd: 5/13 pts.; haplo-identical family donor: 1/13 pts.) were used. 12 pts. received RIC, 1/13 pts. was conditioned using FLAMSA-RIC (Schmid et al., Blood 2006 & JCO 2005). **Results and Conclusions:** Only 2/13 pts. are alive and in remission (day +60 or day +184) after the 2nd alloSCT. Median leukemia-free survival (LFS) or overall survival (OS) of all pts. is 2 (0-15) months or 2 (0-12) months. In turn, 11/13 pts. died from relapse (8/13 pts.) or infections (3/13 pts.). These results indicate re-transplantation from an alternative donor as a salvage therapy for pts. with relapsed AML after allogeneic stem cell transplantation is feasible. However, the curative potential of this procedure is very low and OS may not be superior as compared to that achieved by best supportive care or palliative chemotherapy. Therefore, further efforts should be made to avoid relapse after 1st alloSCT, e.g. by risk-adapted adoptive immunotherapy or administration of targeted therapeutics.

Disclosure: No conflict of interest disclosed.

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KIR2DL2/3 - HLA-C-Group1 and KIR2DL1 - HLA-C-Group2 mismatches improve the clinical outcome in patients with acute myelogenous leukaemia after HLA-matched allogeneic stem cell transplantation

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Introduction: HLA class I antigens are ligands for killer cell immunoglobulin-like receptors (KIRs). These receptors are expressed by NK and T-cells and thus modulate innate and adaptive immunity. KIR mismatches have been suggested to modify the outcome after stem cell transplantation. The clinical impact of killer cell receptors may vary between disease entities and pre- and post transplant therapeutic regiments. We have therefore performed a retrospective study with patients transplanted in our centre between 1998 and 2007. **Aim:** To analyze the impact of KIR / HLA mismatches on eventfree survival (EFS), overall survival (OAS), non-relapse mortality (NRM) and posttransplant relapse in AML-patients receiving an allogeneic transplant from an HLA-matched donor. Patients and **Methods:** Samples from 54 donor/patients pairs were availabe for retrospective KIR-ligand matching out of a consecutive single center cohort of 147 AML patients. The median patient age was 47 (25-66) years. Patients were transplanted with G-CSF-stimulated PBSC (n=51) or bone marrow (n=3) from HLA matched unrelated (n=31) donors or family related donors (n=23). KIR typing was performed as previously described. Patients were categorised according to their HLA inhibitory KIR ligand group C1, C2, Bw4, A3/A11 and presence or absence of KIR. **Results:** Twentynine of 54 patients are alive with a median follow up of 3.5 years (range 1.6 – 7.1 years), while 9 patients died in remission, mostly due to GvHD/infection and 16 patients died due to relapse. At 5 years, EFS and OAS of all 54 evaluable patients were 42% and 52%, respectively, with a NRM of 23% and a relapse rate of 42%. Patients with KIR-ligand mismatches (KIR-mm) had a lower actuarial NRM (13% vs. 39%, p=0.04) and a trend towards a lower relapse rate (37% vs 45%, p=0.16). EFS and OAS were significantly superior in patients with KIR-mm compared to the group of patients without mismatches (EFS: 55% vs. 25%, log rank p= 0.009; and OAS: 68% vs. 35%, log rank p= 0.004). Patients with 2 KIR mismatches (C1 and/or C2) had even better survival compared to patients with single KIR mismatches. **Conclusions:** Patients with AML receiving an HLA-matched allogeneic transplantation benefit significantly from KIR-ligand mismatches. Allografted AML-patients with KIR mismatch have a significantly superior survival based on a reduced mortality due to both relapse as well as transplant-related causes. KIR typing would be a useful tool to be included to define optimal histocompatibility of donor patient pairs.

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Posterdiskussion
AML I

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THE PROGNOSTIC VALUE OF WT1–OVEREXPRESSION IN ADULT AML PATIENTS IN 1st COMPLETE REMISSION (CR1)

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Introduction: The overexpression of the Wilm's Tumor 1 (WT1) gene can be detected in AML patients and is an established marker for monitoring minimal residual disease. Since up to 90% of patients overexpress WT1 at diagnosis, its prognostic relevance at that time-point is limited. A good response to induction chemotherapy (CR1) is generally accepted as a hallmark of successful therapy. In this study, we therefore compared the outcome between AML patients in 1st CR who achieved normal levels of WT1-expression (normalised WT1) and those with persistently WT1-overexpression (high WT1). **Methods:** Bone-marrow samples from 42 AML patients with WT1 overexpression at diagnosis of disease (14 male, 28 female, median age 54 years [17 – 84]) were taken at the end of induction chemotherapy (22 FLAG-like, 20 HIDAC 7-3-7) and WT1 mRNA expression was determined via a TaqMan RT-PCR assay (Ogawa H et al: Blood 101 [2003], 1698ff). **Results:** Nine out of 33 patients (27 %) in CR1 did not achieve normalisation of WT1 expression. These patients had a significant shorter time to relapse than patients with normalised WT1 after induction chemotherapy (median time to relapse 7 vs. 26 months, Grey P < .001). More importantly, all patients with high WT1 relapsed within 17 months, while the cumulative incidence of relapse after 5 years was only 56% for patients with normalised WT1 (median survival: 11 months vs. 58 months, Log Rank P < .001). Compared to patients with normalised WT1, the relative risk of death was 2.8 (95%CI 1.3-6.3) in patients with high WT1 even after adjustment for age, karyotype, NPM1 mutation, Flt3/ITD, WBC and therapy regimen. **Conclusions:** Despite successful induction chemotherapy, AML patients with persistently high WT1 expression have a high risk of relapse. Whether this patients might benefit from allogeneic stem-cell transplantation needs to be determined.

Disclosure: No conflict of interest disclosed.

P415

Cbl mutants interact with c-Kit independent of its kinase activity and lead to factor independent growth and transformation of a myeloid cell line 32D

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Purpose: The Cbl proto-oncogene products have emerged as important components of the signal transduction cascades downstream of both non-receptor and receptor tyrosine kinases (RTKs). By regulation of receptor trafficking and degradation, they have been shown to tightly regulate the intensity and amplitude of RTK activation. c-Kit belongs to the family of the class-III RTKs and plays an important role in the pathogenesis of acute myeloid leukemia (AML). So far, very little is known about the role of c-Cbl mutants in the role of c-Kit signaling. **Results:** We analyzed the interaction of c-Cbl with c-Kit and the functional relevance of this interaction in the IL-3-dependent murine myeloid progenitor cell line 32Dcl3. We recently identified the first c-Cbl mutation in human disease in an AML patient, named Cbl-R420Q. Co-expression of two different dominant negative mutants of c-Cbl (Cbl-R420Q or Cbl-70Z) with Kit induced cytokine-independent proliferation, survival and clonogenic growth. Overexpression of these mutants inhibited SCF-induced ubiquitination and internalization of the activated Kit receptor. Both Cbl mutants enhanced the basal activation of Akt and prolonged the ligand-dependent activation. Importantly, transformation was observed also with kinase-dead forms of Kit and Flt3 in the presence of Cbl-70Z, but not in the absence of Kit or Flt3, suggesting a mechanism dependent on RTKs, but independent of their kinase activity. Instead, transformation appeared to depend on Src family kinases (SFKs), as c-Cbl co-immunoprecipitated with SFKs and SFK inhibition abolished transformation. **Conclusion:** Our results indicate that c-Cbl has an important role in c-Kit signal mitigation. They demonstrate that disturbed mechanisms of c-Kit internalization have important implications for its transforming potential, possibly in the development of AML. Furthermore, these findings may explain primary resistance to tyrosine kinase inhibitors targeted at RTKs.

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ONCOGENIC POTENTIAL OF CBL MUTANTS IN CYTOKINE RECEPTORS AND RECEPTORTYROSINE KINASES EXPRESSING Ba/F3 CELLS

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Introduction: Growth factor receptors and receptor tyrosine kinases (RTKs) play an important role in initiation and propagation of many types of cancer. Aberrant activation of receptor tyrosine kinases by overexpression and activating mutations is a well investigated topic in oncology research but impaired receptor downregulation might contribute to the malignant phenotype as well. Cbl is a known negative regulator of several receptors and is responsible for their ubiquitination. Recent findings have addressed the aberrant downregulation of FLT3 by Cbl in acute myeloid leukemia (AML). Besides FLT3 CBL is shown to interact with other RTKs including EGFR and hematopoietic receptors including EpoR.We want to examine the oncogenic potential of Cbl deletion mutants in cytokine receptors and RTKs expressing cells. Thus, to proof if the malignant transformation by CBL is a potentially general mechanism in human cancer. Methods Transduction of IL-3-dependent Ba/F3 murine pro-B cells and cell proliferation assays. FLT3, EGFR and EPOR cDNA was transduced in Ba/F3 cells via a retroviral expression vector. Stable receptor expression after transduction and fluorescence-activated cell sorting (FACS) was confirmed by western blotting and cellsurface-marker expression by flow cytometry. Proliferation and apoptosis assays were done in presence and absence of IL-3 or receptor-ligands and selective PTK inhibitors. Results Coexpression of FLT3-WT and CBL deletion mutants causes IL-3 independent and FLT3-ligand (FL) dependent growth of Ba/F3 cells. The FLT3 Inhibitor PKC412 abrogates this proliferation. FLT3-WT/CBL?exon8 cells show a 12,24 fold hyperproliferation compared to FLT3-WT/CBL-WT cells in presence of 100ng FL. Ba/F3 cells expressing EGFR or coexpressing EGFR and CBL-WT or CBL mutants show IL-3 independent growth and EGF dependent growth. EGFR-WT/CBL?exon8 cells show a 1,6 fold proliferation compared to EGFR-WT/CBL-WT cells in presence of 100ng EGF. Coexpression of EPOR-WT and CBL?exon8 causes IL-3 independent growth and EPO dependent growth. Ba/F3 cells coexpressing EPOR-WT and CBL?exon8 have a 1,1 fold hyperproliferation rate compared to cells expressing EPOR-WT/CBL-WT in presence of 0,25U/ml EPO. Conclusions An alternative mechanism for the constitutive activation of RTKs in tumors occurs through inactivation of a negative regulator. CBL seems to be a selective negative regulator of the RTK Flt3 and shows only weak interaction with EPOR or EGFR.

Disclosure: No conflict of interest disclosed.

P417

KIT inhibition may upregulate heat shock proteins resulting in resistance to KIT tyrosine kinase inhibitors – evidence for beneficial effects of additional heat shock protein inhibition

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Activating mutations of the KIT class III receptor tyrosine kinase are associated with core binding factor leukemias (CBF AML), systemic mastocytosis (SM), gastrointestinal stromal tumors (GIST), melanomas, seminoma/dysgerminoma and sinonasal natural killer/T-cell non-Hodgkin lymphoma. Despite initial efficacy with KIT-tyrosine kinase inhibitors (TKI), resistance develops in many patients, particularly after TKI monotherapy, leading to disease progression. We hypothesized that alternative signaling pathways might be activated to override TKI inhibition and promote resistance to TKI therapy. To identify cellular pathways that are activated downstream of the signal transduction of class III receptor tyrosine kinases, we performed an unbiased phoshoproteomic analysis of leukemia and mastocytosis cell lines before and after TK-inhibition. Interestingly, after KIT-inhibition with imatinib mesylate at IC90, we detected a significant increase in peptides that were identified (using immunoaffinity purification of phosphopeptides followed by tandem mass spectrometry) as members of the heat shock protein (HSP) family. This finding was confirmed by Western blot analysis of a variety of mutant-KIT hematopoietic, leukemia and mastocytosis cell line models that had been treated with the TKI imatinib mesylate or dasatinib.Next, we tested the antiproliferative and proapoptotic effect of IPI-504, an HSP90 inhibitor and 17-AGG derivative, in cells expressing mutant-KIT. Depending on the KIT isoform, IPI-504 was demonstrated to potently inhibit proliferation and induce apoptosis with IC50s of 0.5 and 7.5 micromolar, respectively. Importantly, the combination of IPI-504 with TKI clearly potentiated the antiproliferative and proapoptotic effects achieved by either drug alone. Cytotoxicity in combination therapy was observed even at IPI-504 concentrations that did have only moderate antitumor activity when given alone. In conclusion, our model suggests that inhibition of KIT upregulates expression of heat shock proteins, putatively to stabilize the functionality of the targeted receptor tyrosine kinase. This mechanism could contribute to the development of resistance to TKI therapy. Importantly, these finidings provide a rationale for combining TKI with (low-dose) HSP90 inhibitors, such as IPI-504, to optimize TKI therapy.

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Leukemia-driving mutant FLT3 and KIT isoforms activate PI3K/AKT signaling which is insufficiently inhibited by tyrosine kinase inhibitors - but potently targeted by the dual PI3K/MTOR inhibitors NVP-BEZ235 and NVP-BGT226

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In ~40% of de novo cases of acute myeloid leukemia (AML), autoactivating mutations in the class III receptor tyrosine kinases (RTK) KIT and FLT3 are observed and are closely linked to leukemogenesis. We previously demonstrated that leukemia-driving gain-of-function mutations of KIT and FLT3 consecutively activate AKT, MAPK1/2 (ERK1/2) and STAT signalling. Although, RTK-inhibition only partially blocks PI3K/AKT/p70S6K signaling, which suggests the existence of an escape mechanism that is not yet understood. Combination of tyrosine kinase inhibitors (TKI) with rapamycin, a specific MTOR inhibitor acting upstream of p70S6K, potentiates the antitumor activity of TKI. However, upregulation of AKT-phophorylation – possibly by feedback loops – is observed in western blot analysis. In an attempt to globally block AKT signaling we tested the dual PI3K/MTOR inhibitors NVP-BEZ235 and NVP-BGT226 with regard to their antiproliferative and proapoptotic potential in a variety of mutant-KIT/FLT3 leukemia cell lines. Both inhibitors displayed antitumor activity - with NVP-BGT226 the more potent agent with IC50s in the lower micromolar range. Of note, we also tested a blast crisis CML cell line –which also revealed sensitivity towards PI3K/MTOR inhibition in the same dose range. To study the influence of different mutant isoforms of KIT/FLT3 on sensitivity to TKI, we created FLT3, KIT and BCR/ABL transfectants in a Ba/F3 cell line background. Both inhibitors displayed the most profound activity in cells transfected with an autoactivating FLT3 or KIT isoform with IC50s between 250-500 nanomolar. In contrast, the FLT3 wildtype isoform displayed decreased sensitivity towards both inhibitors. No sensitivity to NVP-BEZ235 and NVP-BGT226 was observed in BCR/ABL transfectants and the Ba/F3 parental cell line up to 5-10 micromolar. As expected, combination of the dual PI3K/MTOR inhibitors with TKI potentiated the antiproliferative and proapoptotic effects observed for the single agents. We conclude that the PI3K/AKT pathway is activated by mutant FLT3 and KIT isoforms, but not by BCR/ABL. Thus, the PI3K/AKT pathway provides an escape mechanism from TKI therapy contributing to the moderate/shorter response rates in acute forms of leukemia (including CML blast crisis) compared to TKI therapy in CML. NVP-BEZ235 and NVP-BGT226 display potent antiproliferative as well as proapoptotic effects in leukemia in vitro models alone and in combination with TKI and warrant clinical evaluation.

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P419

CBL mutations in acute myeloid leukemia (AML) and myeloproliferative neoplasms (MPN)

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Introduction: Deregulation of tyrosine kinase (TK) activity is one hallmark in the pathogenesis of acute myeloid leukemia (AML) and myeloproliferative neoplasms (MPN). The proto-oncogene *Casitas B-lineage lymphoma* gene (*CBL)* functions as E3 ligase that ubiquitinates and regulates activated RTKs, i.e. FLT3. First studies on *CBL* mutations in AML and MPN showed that these mutations occur with a low frequency, but they are highly associated with 11q acquired uniparental disomy (UPD) in MPN. Aims: To evaluate the frequencies of *CBL* mutations in myeloid leukemias. **Methods:** A large cohort of adult AML [n=321; normal karyotype (CN), n=119; various, n=5; core-binding factor (CBF) leukemia, n=196; karyotype not evaluable, n=1] and MPN patients (pts) [n=95; essential thrombocythemia (ET), n=29; polycythemia vera (PV), n=37; primary myelofibrosis (MF), n=17; post-ET-MF, n=2; post-PV-MF, n=7; MPN not classified, n=3] were analyzed by using a DNA-based PCR assay for amplification of exons 8 (including introns 7 and 8) and 9 (including intron 9) followed by direct sequencing. **Results:** Mutation analysis revealed 7 *CBL* mutations (exon 8, n=6; exon 9, n=1) in 7 AML cases and 3 mutations in the 95 MPN pts [exon 9, n=3). In AML, *CBL* mutations affected exon 8 in six cases and exon 9 in one case. All three MPN associated mutations were located in exon 9. Most of the mutations have already been described consisting of substitutions as well as frameshift mutations. Of note, 6 of the 7 *CBL* mutations in AML were detected in CBF leukemias [t(8;21), n=1; inv(16), n=5)] with two of them also exhibiting *FLT3*-TKD mutations and one having a mutation affecting *cKIT*; one mutation occurred in CN-AML. With regard to the MPN cohort, all 3 *CBL*-mutated pts (one ET, two PV) also had a *JAK2V617F* mutation. In addition, SNP array data were available in 26/119 CN-AML cases as well as in 40/95 MPN cases revealing one case with 11qUPD in each group showing *CBL* wildtype. **Conclusions:** In this large cohort of myeloid leukemias, *CBL* mutations occurred at low frequencies and were associated with AML CBF leukemias as previously described. Therefore, it will be of interest to further investigate the potential interaction of mutant *CBL* with the CBF-related fusion proteins CBFB-MYH11 and RUNX1-RUNX1T1 as well as with other CBF-associated gene mutations (e.g. *FLT3, KIT*).

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P420

Interpretation of mixed chimerism in patients with acute myeloid leukemia after allogeneic stem cell transplantation

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Introduction: Chimerism is routine for follow-up in patients with acute myeloid leukemia (AML) after allogeneic stem cell transplantation (SCT), but techniques and interpretation of results are highly individualized between different transplantation centres. **Methods:** We retrospectively analyzed the results of molecular chimerism analysis from bone marrow or peripheral blood in 77 adult and pediatric patients at diagnosis or relapse of *de novo* or secondary AML (39 males, 38 females; median age 48 years; 3-67). Reduced conditioning (RIC) was performed in 43, myeloablative (MAC) in 34 patients from related or unrelated donors. Chimerism was done with highly sensitive ($=10^{-4}$) quantitative real-time PCR (Taqman®) for short insertions or deletions (Alizadeh et al., 2002) or Y-specific sequences in case of sex-mismatched SCT (Fehse et al., 2001) on days +30, +60, +90, +120, and at 2 years from SCT. Complete donor chimerism (DC) was defined as =99.0% donor cells; mixed chimerism (MC) as <99.0% donor cells. Mixed chimerism was subdivided into „increasing MC" (increasing proportions of recipient cells) and „decreasing MC" (increasing proportions of donor cells) according to Barrios et al., 2003. Comparison of values was done with Mann-Whitney-U test, of categories with chi square. **Results:** Following SCT, a total of 74 of patients achieved complete remission (CR), but 30/77 patients (38.9%) developed relapse within the 2 years of study. Reduced conditioned patients had lower mean chimerism at the 1st control after SCT (RIC: 98.9%; 0-100%; MAC: 99.9%; 77.8-100%; p=0.004) and at 12 months from SCT (p=0.037). Failure to achieve full donor chimerism at at least 1 time point was seen in 5/77 patients: in 4 patients after RIC, and in one patient after MAC. From 48 patients with mixed chimerism at =1 time point within the 2 years of study, only 19 patients developed relapse, while 29 did not (n.s.). Thus, the observation of MC *per se* did not correlate with the relapse risk, while dynamics of MC was relevant: 19/21 patients with an increase of recipient cells at follow-up developed relapse within the next 2 years. In contrast, 27 patients who failed to develop stable donor chimerism but showed continuous decrease of recipient cells remained in CR (p<0.001). Thus, „decreasing MC" was associated with stable remission, while patients with "increasing MC" had a relapse risk of 90.5%. The median interval between decrease of donor chimerism and relapse was 21 days (0-133). **Conclusions:** The dynamics of mixed chimerism is highly relevant for the relapse risk in AML, whereas single results are difficult to interpret. Considering the short

intervals between decrease of donor chimerism and the manifestation of relapse in AML even when highly sensitive methods are being performed, frequent monitoring of chimerism is required in this entity following SCT.

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Bacher,U.: Anstellungsverhältnis oder Führungsposition:U. Bacher ist Oberärztin in der Klinik für Stammzelltransplantation (UKE Hamburg)

P421

Molecular Determinants and Functional Characteristics of Leukemic Stem Cell and Their Niche

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Introduction: Leukemic stem cells (LSC) and normal hematopoietic stem cells (HSC) share many features, including the ability to self-renew and to generate a multitude of daughter cells. We have recently described a subset of leukemic blasts with a high activity of aldehyde dehydrogenase ($ALDH^{br}$) that is enriched in LSC candidates. These cells, among other features, are characterized by an increased affinity to the stem cell niche. In the current study, we have further examined the interaction of LSC with the niche, have identified molecules involved in this interaction, and have correlated those features to functional characteristics like chemotherapy resistance. **Methods:** Mononuclear cells from the bone marrow or peripheral blood of patients with AML were stained with Aldefluor and sorted by flow cytometry according to their side scatter characteristics and ALDH activity ($ALDH^{br}$ vs. $ALDH^{dim}$). The expression of adhesion molecules like CXCR4, Cdh2, or CD44 was measured by qPCR and flow cytometry. The influence of the niche interaction on adhesion molecule expression was evaluated by co-cultures with mesenchymal stromal cells (MSCs) which served as surrogate niche model. Additionally, leukemic blast subsets were treated with chemotherapeutic agents, with and without MSC co-cultures, and vital cells were quantified by PI-/AxV-staining followed by flow cytometry. Co-incubation with novel CXCR4-antagonists and functional in vivo assays are currently underway. **Results:** Adhesion molecules, such as CD44s, Cdh2, and CXCR4, were consistently increased in the $ALDH^{br}$ subset - both on mRNA and protein level - as compared to the $ALDH^{dim}$ subset. Their expression was further upregulated upon niche interaction in a time-dependent manner. For instance, the CXCR4 Expression in $ALDH^{br}$ cells was elevated up to 6-fold within 24 hours of co-cultivation, then decreased and was even downregulated lateron. After chemotherapy treatment, significantly more vital cells were found in the $ALDH^{br}$ subset as compared to $ALDH^{dim}$ cells. Moreover, co-culture with MSC further increased the frequency of blasts that survived chemotherapy treatment. **Conclusion:** Our current data confirmed that a LSC subset can be isolated based on a high ALDH activity. The increased expression of adhesion molecules in this subset mediates a close affinity to the marrow niche, which, in turn, protects LSC against proapoptotic stimuli and helps them maintain their "stemness". Our present study and our ongoing research will help in developing novel therapeutic methods.

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P422

Leukemia antigen-specific donor lymphocyte infusions made possible through streptamer-based selection

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Introduction: Administration of donor lymphocyte infusions (DLIs) after allogeneic hematopoietic stem cell transplantation may result in a desirable graft-versus-leukemia (GVL) effect, but might also elicit a noxious graft-versus-host disease (GVHD). To render DLIs more specific towards an anti-leukemic effect, a positive selection of leukemia (antigen)-specific T cells would be highly desirable. In this study we focused on the leukemia-antigen Wilms Tumor gene 1 (WT1). For this particular antigen, a good correlation of WT1 expression and the number of leukemia blasts could be demonstrated in patients with myelodysplastic syndrome (MDS) and acute myeloid leukemia (AML). Several groups described immunogenic T cell epitopes derived from the WT1 protein and restricted to certain HLA types such as HLA-A2 and HLA-A24. **Methods:** The novel technology of streptamers available on a GMP level was employed to detect the frequency of HLA-A2 restricted CD8+ T cells in the naïve peripheral blood (PB) from both healthy donors (HDs) and AML patients. Such WT1-specific CD8+ T cells were further characterized for the expression of CD27, CD28, CD45RA, CCR7, CD69, CD107a and CD137. In the next step, WT1-specific cells were positive selected by MACS columns after labeling with streptamers and thereafter immunophenotyped. Moreover, mixed lymphocyte peptide cultures (MLPCs) were preformed to enrich WT1 specific T cells derived from the PB of HDs. At last, WT1 specific T cells were evaluated in a cytotoxicity assay. **Results:** 21 of 40 HDs showed naïve WT1 specific T cell frequencies of 0.5 to 1.4% of all CD8+ T cells. In four of ten AML patients in complete remission, also 0.6 to 6.0% of WT1-specific T cells could be detected. These cells revealed to be CD8+WT1_tetramer+CD45RA+CCR7- effector T cells in flow cytometry. After positive selection by MACS columns a purity of 20-90% could be achieved for CD8+WT1_tetramer+CD27-CD28+CD45RA+CD107a+CD137-CCR7- WT1-specific T cells. Through streptamer-powered column separation a higher activation of WT1-specific T cells could be achieved than with tetramer selection. After a maximum of three rounds of MLPC, only a frequency of 2-10% WT1-specific CD8+ T cells could be achieved, thus demonstrating the power of the streptamer technology. In cytotoxicity assays, WT1-specific CD8+ T cells were able to lyse 60-100% of HLA-A2+WT1+ AML cells at an effector/target ratio of 20:1. **Conclusions:** In summary, the streptamer technology allows to select a highly pure fraction of WT1-specific effector T cells with cytotoxic properties. In analogy to DLIs specific for viral antigens, production of leukemia specific DLIs is feasible on a Good Manufacturing Practice (GMP) level. Further leukemia antigens such as the receptor for hyaluronic acid mediated motility (RHAMM) are currently evaluated by our group.

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Schmitt,M.: Anstellungsverhältnis oder Führungsposition:OA Universitätsklinikum Rostock

P423

micro-RNA let7e is a critical mediator of monocytic differentiation

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Introduction: The differentiation block which is common to the pathogenesis of Acute Myeloid Leukemias can be due to numerous molecular and epigenetic abnormalities. Seldom are those overcome by conventional therapeutics. Thus, AML still has a high lethality and further general insights into its pathogenesis are urgently warranted. **Methods.** We used U937 cells stimulated with the phorbol ester TPA to globally assess changes in micro-RNAs during monocytic differentiation, and further characterized those. **Results:** Amongst other micro-RNAs, we identified an increased expression of the micro-RNA let 7e after 24 hours of stimulation with TPA using microarrays. This result was confirmed by quantitative PCR. The increase in the expression of micro-RNA let 7e persisted after 48 and 72 hours of stimulation with TPA. We transiently overexpressed locked nucleic acids (LNA) against micro-RNA let 7e to assess whether the micro-RNA let 7e is necessary to induce monocytic differentiation. This reduced the expression of the MCSF-receptor on mRNA level and the expression of the panmyeloid surface marker CD11b after stimulation with TPA. **Conclusion:** We thus conclude that the expression of the micro-RNA let 7e is critical and necessary to induce monocytic differentiation in this system. Future studies will include an assessment whether the micro-RNA let 7e is sufficient to induce monocytic differentiation. This work is supported by grants of the German Federal Ministry for Research (BMBF, NBL3 Rouxprogramm FK15/34 and FK19/37) to MC and by a joint stipend of the Brazilian Government and the DAAD (CAPES) to CYN.

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P424

The glycogensynthase kinase 3b (GSK-3ß) is involved in leukemic transformation

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Introduction: Signal transduction pathways, such as the PI3K/AKT cascade, are frequently activated in acute myeloid leukemia and stimulate the proliferation and survival of leukemic cells. Mutations in upstream genes such as Class III receptor tyrosine kinases are frequent but not exclusive causes of that activation. An important downstream target of PI3K/AKT is the key regulator GSK-3ß. It controls anti-apoptotic genes such as NF-kappaB and Cyclin D1, is involved in wnt-pathway activation and drug resistance of leukemic cells. Deregulated signaling by GSK-3ß occurs through inhibition by AKT-mediated phosphorylation. We have observed that constitutive phosphorylation of GSK-3ß occurred in hematopoietic cells with pro-leukemogenic PI3K-mutations. We wanted to evaluate the relevance of GSK-3ß inactivation in the transformation process of hematopoietic cells. Methods and **Results:** We used an in vitro factor-independent growth assay, GSK-3b inhibitors (Lithium, BIO) and established a second hit model using retroviral gene transfer of the weak oncogene Bcl X_L. Signaling cascades were analysed by western blot.We demonstrate that inactivation of GSK-3b alone was not sufficient to induce factor-independent growth in IL-3 dependent early hematopoietic cells (Ba/F3). Induction of apoptosis upon growth factor withdrawal was reduced , but not prevented, in the presence of GSK-3b inhibitors, leading to a delayed Caspase 3 activation, PARP cleavage and DNA fragmentation. Overexpression of Bcl-X_L also did not result in a prevention of apoptosis. GSK-3b inhibition in synergy with Bcl-X_L overexpression resulted in the establishement of several growth factor-independent cell lines, which were characterized by the activation of multiple signaling cascades including AKT, MAPK, STAT5, but not STAT3. Also Cyclin D1 was overexpressed in contrast to other cyclines (D2, D3) which are no substrates of GSK-3b. **Conclusions:** Our data show that GSK-3ß is part of the apoptotic response to growth factor withdrawal and suggest that GSK-3ß is causaly involved in the transformation process of hematopoietic cells and seems to have an synergistic role in addition to other pro-leukemic mutations

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P425

Impact ofcomorbidity on Outcome in AML: Comparison of two comorbidity scores

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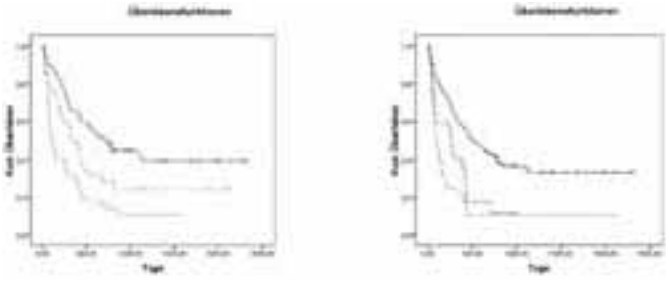
Objective: Death is the worst outcome of acute myeloid leukemia (AML). Thus, prognostic factors are used to predict outcome and allocate treatment. Besides two well known factors, namely karyotype and age, comorbidity may play a crucial role in the outcome of AML. To test this hypothesis we applied two comorbidity scores at the time of first admission. **Methods:** 198 consecutive patients with AML were analysed, retrospectively. Karyotype risk group was 11.6 % good, 68.0 intermediate and 20.4 % poor risk. The median age was 62 years (range 20 – 81), 95 were male, 103 were female. The median survival was 382 days. The criteria of the Charlson Comorbidity Index (CCI) and the Hematopoietic Cell Transplantation-Specific Comorbidity Index (HCT-CI) were applied. However, the cut-offs were set lower than in the original paper in order to detect the possible impact of minor comorbidity. **Results:** The HCT-CI (left figure) separated clearly between patients with a score of zero (continous line), one (dashed line) and more than one (dotted line) (median survival: 718 vs 328 vs 100 days; log rank test p<0.0001). Similarly, the CCI separated the patients with none (continous line), one (dashed line) and more than one (dotted line) (median survival: 515 vs 293 vs 94 days; log rank test p<0.0001). Multivariate analysis showed karyotype (p < 0.001), HCT-CI (p = 0.003) and age (cut-off 60 years; p = 0.006) as independent risk factors. The CCI (right figure) separated only between patients with a score of zero (continous line) and those with at least one score point. Patients with one (dashed line) and patients more than one (dotted line) were not separated. **Conclusions:** Our findings show that HCT-CI is an additional prognostic factor in AML. We extend previous findings severalfold. Firstly, we directly compared two comorbidity indices, showing that the HCT-CI separates our patient population in three risk groups. Secondly, we showed that comorbidity is an independent predictor for survival. Thirdly, our analysis was not restricted to patients older than 60 years but rather includes 90 patients younger than 60 years. However, one limitation of our retrospective study is that the cohort is not treated uniformly. In summary we conclude that comorbidity should be studied prospectively to clarify its use for stratification in future studies.



HCT-CI CCI

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P426

CNS manifestation in patients with acute myeloid leukemia scheduled for allogeneic hematopoietic stem cell transplantation

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Introduction: Transplantation centres in Europe use heterogenic strategies concerning intrathecal (IT) prophylaxis as part of the conditioning regimen before allogeneic stem cell transplantation. It remains unclear whether regular evaluations of the cerebrospinal fluid (CSF) are performed in patients with acute myeloid leukaemia, who are scheduled for allogeneic transplantation at all and how the incidence of CNS involvement prior to allo-HSCT can be rated. This might be particularly important, because nowadays more AML patients suffering from refractory disease are treated and the more frequent use of fludarabine based reduced-intensity conditioning regimens leads to a reduced penetration of active cytotoxic drugs into the CNS. Here, we report on our prospective analysis evaluating the CSFs of 199 AML patients receiving an allogeneic HSCT at our centre in the years from 1997 to 2008. Patients and **Methods:** Between 1996 and 2009, 250 adult patients with AML received an allo-HSCT at our institution. The median age was 46 years at the time of transplant. Within this group, cerebrospinal fluids of 204 adult AML patients were examined for cell counts and if abnormal results were seen cytological thereafter. A small fraction of patients did not get a CNS evaluation due to a high number of blasts in the peripheral blood, acquired coagulation disorders or technical problems. We did not use a routine flow-cytometry. All patients gave written informed consent. **Results:** In total, we identified 17/204 (8%) patients with cytological proven CSF involvement with the half of the patients CNS expressing an AML FAB M4 or AML FABM5 phenotype. The cell counts at the time point of diagnosis ranged from normal to 1497/3 cells per 1 CSF. Normal cell counts did not exclude CNS disease. In 10/17 of the patients the CNS disease was not expected, since they were clinically asymptomatic, and CNS involvement would not have been detected without a routinely scheduled examination. In a subgroup analysis, patients with refractory disease (12/61) had a significant higher risk for CNS disease when compared to patients responding to prior systemic therapy (19% versus 3.4 %; p=0.0008, two sided Fisher`s exact test). All patients received a prophylactic dose of 12 mg methotrexate during the diagnostic lumbar puncture. Patients with CNS involvement underwent a compartment directed approach with repeated courses of intrathecal chemotherapy or radiation of the neuro-axis after engraftment. **Conclusion:** In refractory AML patients scheduled for allogeneic hematopoietic stem cell transplantation, we found an unexpected high proportion of CNS involve-

ment (19%). Since there were patients with CNS disease who were asymptomatic and had normal CSF cell counts, we emphasize on routine morphological CSF evaluations before starting conditioning in order to optimize post-transplant treatment strategies.

Disclosure: Bommer,M.: Anstellungsverhältnis oder Führungsposition:Oberarzt Ringhoffer,M.: Anstellungsverhältnis oder Führungsposition:Wissenschaftlicher Mitarbeiter

P427

Rapid flow cytometric detection of cytoplasmic NPM1 expression (NPMc) adds to the distinct diagnostic features of HLA-DR^{neg} patients without acute promyelocytic leukemia (nonAPL) compared to APL

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Loss of HLA-DR and CD34 is a well known immunophenotypic characteristic of malignant promyelocytes in APL. However, it is not exclusive for APL. The purpose of this study was to investigate whether further biological characterization of the HLA-DR^{neg} AML patients would allow to more clearly define criteria to separate APL from nonAPL patients. Immunophenotyping, cytogenetics, molecular analyses, and cytomorphology were prospectively performed within routine diagnostics of 800 patients included in different prospective AML multicenter trials of the Study Alliance Leukemia (SAL). Moreover, cytoplasmic accumulation of NPM1 (NPMc) was measured flow cytometrically in immunophenotyped blasts using a monoclonal NPM1 antibody combined with Zenon labeling technique and subsequent intracellular staining. Beside 60 APL, an additional 62 HLA-DR^{neg} nonAPL patients were identified. The main differential characteristics between HLA-DR^{neg} nonAPL and APL included the high CXCR-4 expression in most patients (97% vs. 82%, p<0.001) and almost all leukemic cells (89% vs. 36%, p<0.001), as well as the significant association with cup like nuclear morphology (29% vs. 4%, p<0.01). The biological distinctness of both leukemia subtypes was further supported by the complete absence of aberrant CD2 expression (0 vs. 33%, p<0.001) and increased leukocyte and platelet counts (25 vs. 2.5 GPT/L, p<0.001; 56 vs. 23 GPT/L, p<0.001) in HLA-DR^{neg} nonAPL patients. Even in the CD-34^{pos} subgroup of HLA-DR^{neg} nonAPL these features contributed in at least the same way to the separation from APL. Moreover, a high proportion of HLA-DR^{neg} nonAPL patients carried a *NPM1* mutation in exon 12 (79%, p<0.001). Since *NPM1* mutations and the t(15;17) are mutually exclusive, we further optimized the diagnostic panel incorporating a recently developed flow cytometric approach for the detection of NPMc expression. In an ongoing study we could confirm the molecular results in 94% (44/47 patients) of cases with a complete concordance for APL patients. Summarizing, the present study shows that an immunophenotypic, molecular, and cytomorphologic separation of both HLA-DR^{neg} leukemia subgroups is possible. Moreover, the simple and reliable flow cytometric NPMc detection as surrogate for the presence of *NPM1* mutations in conjunction with the absence of HLA-DR expression already provides a rapid diagnostic method with high accuracy, which could further guide the important differentiation of HLA-DR^{neg} nonAPL from APL.

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P428

Sequential conditioning with melphalan, fludarabine and 8 Gy total body irradiation (TBI) for allogeneic hematopoietic stem cell transplantation (HSCT) in patients with refractory acute myeloid leukemia (AML)

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Introduction: We have previously shown that dose adapted conditioning with fractionated 8 Gy TBI and fludarabine is feasible with low non-relapse mortality (NRM) and preserved antileukemic activity in patients with AML in complete remission. The prognosis of patients with refractory AML remained poor. The sequential regimen with intensive chemotherapy (fludarabine, cytarabine, and amsacrine) for cytoreduction followed by reduced intensity conditioning (FLAMSA-regimen) is a promising approach to the treatment of patients with unfavorable prognosis. To further improve the outcome of patients with refractory AML we employed a sequential regimen consisting of high dose melphalan followed after 5 days by 8 Gy TBI and fludarabine (Mel/TBI/Flu-regimen). With this retrospective analysis, we compared the outcome of refractory AML patients treated with FLAMSA to patients conditioned with sequential Mel/TBI/Flu at our center. **Methods:** From 2005–2008, 29 patients (median age 52 y, range 23-68) with primary refractory (failure to induction therapy, 15 patients) or secondary refractory AML (14) received a conditioning with FLAMSA. After October 2007, 21 patients (median age 50 y, range 27-66) with primary (9) or secondary (12) refractory AML received a sequential conditioning therapy with melphalan (140 - 150mg/m^2), followed by fludarabine (120mg/m^2) and 8 Gy TBI. Patients in both groups were transplanted with peripheral blood stem cells from matched related (18), matched unrelated (31) or mismatched related donors (1). GvHD prophylaxis consisted of antithymocyte globulin, ciclosporin and mycofenolate mofetil. **Results:** Three patients conditioned with FLAMSA and 3 patients treated with MEL/TBI/Flu died within the first 30 days after transplantation due to infections. Engraftment with neutrophils (>500/microL) in both groups was at a median of 17 days (range 6-29 days). With a median follow-up of 564 days of surviving patients after FLAMSA conditioning and 282 days in patients with MEL/TBI/Flu conditioning therapy, the estimated relapse free survival (RFS) and overall survival rates at 1 year were 38% (95% C.I., 20-56%) and 66% (95% C.I., 46-86%), and 44% (95% C.I., 26-62%) and 66% (95% C.I., 46-86%), respectively. Cumulative incidences of NRM were 29% (95% C.I., 19-39%) and 30% (95% C.I., 21-39%), respectively. Ten patients (35%) relapsed after FLAMSA conditioning therapy at a median of 126 days after transplantation (range 30-258 days). In contrast, only 3 patients (14%) relapsed between 36-231 days after MEL/TBI/Flu conditioning. **Conclusions:** These preliminary data demonstrate that sequential conditioning is feasible and effective in patients with refractory AML. With a trend to superior RFS and lower relapse rate in patients receiving MEL/TBI/Flu conditioning, our data suggest that melphalan / TBI based sequential conditioning is a promising option in patients with refractory AML.

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P429

PRAME mRNA transfection into AML cell lines induces ATRA resistance and an inhibition of cell differentiation

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PRAME (preferentially expressed antigen of melanoma) is frequently expressed in several solid tumors and different acute and chronic leukemias. We and other groups already described that expression of the leukemia-associated antigen PRAME provided a favorable prognostic effect in AML patients. In this work, we detected that cell proliferation and differentiation of several AML cell lines was dependent on PRAME expression in the presence of retinoic acid. PRAME negative cell lines treated with all-trans retinoic acid (ATRA) in cell culture showed a significant reduction of cell proliferation in cell counts and FACS analysis for BrdU, but an increase of cell differentiation detected by FACS analysis for CD66b in contrast with PRAME positive leukemia cell lines. PRAME seems to be responsible for the resistance of PRAME-positive AML cells to ATRA treatment. We detected no differences for the induction of apoptosis in PRAME-positive or PRAME-negative cell lines treated with ATRA in FACS analysis for annexin. For PRAME mRNA transfection of PRAME negative AML cell lines we used the method of nucleofection. Cell viability was not markedly reduced by PRAME mRNA or mock transfection. In cell culture, PRAME negative AML cell lines develop a reduction of differentiation and increase of cell proliferation after PRAME mRNA transfection. Moreover, PRAME expression was correlated to the

clinical outcome of AML patients treated with ATRA in two randomized clinical trials. Taken together, PRAME is involved in a crucial mechanism for cell growth of leukemic cells and sensibility of AML cell lines to ATRA treatment depend on PRAME expression. Therefore, PRAME is an appropriate target structure for targeted therapies in AML.

Disclosure: No conflict of interest disclosed.

P430

Azacitidine In Newly-Diagnosed and Refractory/Relapsed AML Not Eligible For Or Resistant To Chemotherapy: A Multi-Center Phase I/II-Study Of The East German Haematology And Oncology Study Group (OSHO)

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In a multi-center phase I/II-study the safety and efficacy of azacitidine (aza) in patients (pts) with newly diagnosed and refractory/relapsed AML not eligible for intensive chemotherapy were studied. In this analysis, the safety and correlation between marrow blasts day 15 of the first cycle and later response to aza are presented. **Patients and methods:** From April-October, 2008, 40 pts with AML [19m/21f, median age 72 (range 32-84) years] were included. Median WBC was 3.6 (range 0.7-187)x10^9/L. Median marrow blasts were 42%. High-risk cytogenetics were found in 12/38 (32%), FLT3mut in 9/34 (26%) and NPM1mut in 7/34 (21%) pts. All pts [newly-diagnosed AML (group A, n=20) and refractory/relapsed AML (groupB, n=20) received aza 75 mg/m^2/day sc for 5 days every 4 weeks. **Results:** To date, a total of 164 treatment-cycles with a median of 3 cycles/patient were applied. 29 (73%) pts received at least 2 treatment-cycles. Neutropenia ≥grade III occurred in 26 (65%) patients on day 8 (median) and lasted for a median of 15 (range 8-22) days. Thrombocytopenia ≥grade III occurred in 29 (73%) pts on day 15 (median) and lasted for a median of 14 (range 7-21) days. Non-hematologic events ≥grade III were reported 39 times (infections n=21, hepatotoxicity n=5, bleeding n=5, nephrotoxicity n=3, myocardial infarction n=1, and hyperkalemia.n=1, tumorlysis n=1, diarrhea n=1, hypoglycaemia n=1). Overall response was 68% [CR, PR, hematologic improvement (HI) n=11 (28%) and stable disease (SD) n=16 (40%)]. Response occurred after a median of 2 months. The probability of response in group A was 69% compared to 15% for group B (p=0.07). Both high-risk cytogenetics and FLT3mut had no negative impact on response. Marrow blasts on day 15 of the first aza course correlated with later response (p=0.01). For the entire cohort, pts achieving CR, PR or HI after 2 courses had a median of 13% marrow blasts day 15 compared to 52% blasts for pts achieving SD only. In group A, pts achieving SD had a median of 66% (range 45%-80%) marrow blasts day 15 compared to only a median of 13% (range 2%-44%) blasts day 15 for pts achieving CR, PR, and HI. **Conclusions:** Azacitidine applied for 5 days every 4 weeks is well tolerated in pts with AML. It could induce remarkable hematologic responses especially in patients with newly-diagnosed AML. Bone marrow blasts on day 15 of the first cycle strongly correlate with later response to aza. Whether this correlates with hypomethylation is currently analysed.

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Posterdiskussion
Aggressive B-Zell-Lymphome I

P431

An individual Patient data meta-analysis for evaluating high dose chemotherapy with autologues stem cell support in first line treatment of aggressive non Hodgkin lymphoma

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Background: Randomised controlled trials (RCTs) reported conflicting results on the impact of high-dose chemotherapy (HDCT) and autologous stem cell transplantation in the first-line treatment of patients with aggressive non-Hodgkin Lymphoma (NHL). **Aims/Methods:** We performed a meta-analysis based on individual patient data (IPD) to assess the efficacy of HDCT compared to conventional chemotherapy in aggressive NHL patients with regard to overall survival (OS) and progression-free survival (PFS). Furthermore, we wanted to determine the efficacy on the intervention in specific subgroups of patients. Particularly we analysed the impact of the age-adjusted International Prognostic Index (aaIPI). We searched the Cochrane Library, MEDLINE and other databases (1/1990 to 12/2007). The RCTs were conducted mainly without Rituximab. Hazard ratios (HR) with 95% confidence intervals (CIs) were calculated using the Cox proportional hazards model stratified by study. The conventional chemotherapy-arm is taken as reference in the analysis. **Results:** Individual patient data were available from 11 RCTs including 2,132 randomised patients. Information on patient characteristics, treatment, events and survival was collected. Overall, there was no evidence for HDCT to improve OS (HR 1.09; 95% CI 0.95-1.24) or PFS (HR 1.04; 95% CI 0.92-1.17) when compared with conventional chemotherapy. In subgroup analysis hazard ratios for OS was 1.34 (95% CI 0.98-1.82) for good risk patients and 1.01 (95% CI 0.87-1.17) for poor risk patients (p-value for interaction = 0.10). Subgroup analysis did not indicate differences in terms of PFS between good (HR 1.07, 95% CI 0.84-1.36) and poor risk (HR 0.99, 95% CI 0.87-1.14) patients (p value for interaction = 0.61). **Conclusion:** Preliminary analyses suggest that there is no evidence for HDCT to improve OS or PFS in NHL patients compared to conventional chemotherapy. There was no evidence for different treatment outcomes in patients with good or poor IPI risk group. Further results will be presented. Stichwort: Experimentelle Untersuchungen

Disclosure: Brillant,C.: Anstellungsverhältnis oder Führungsposition: Anstellungsverhältnis bei der Uni Köln
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P432

Development of a semi-quantitative mathematical model to optimize methotrexate- based chemotherapy

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Introduction: Optimization of doses and administration schedules for anticancer drugs is crucial for effective and safe treatment. Since chemotherapy (CT) remains based predominately on empiric data, the creation

of a mathematical model describing the complex interplay between dosing, tumor-response, agents' clearance, and toxicity, presents the opportunity to optimize treatment strategies of established but also new drugs. Primary central nervous system lymphoma (PCNSL) is a rare disease whose incidence is rising. Standard treatment of methotrexat (MTX) based CT has been approved in several studies. Here we report the most recent development of a real-life mathematical model describing the relationship between MTX serum levels and myelosuppression to optimize current treatment protocols. **Materials and Methods:** We retrospectively analyzed 38 patients (pts) diagnosed and treated for PCNSL from 2005-2009. Pts <65 yrs of age were treated according to the high-dose MTX induction protocol (MTX 8g/m^2/4hrs followed by leucovorin rescue), whereas treatment of pts >65 yrs of age followed the elderly protocol (MTX 3g/m^2/4hrs followed by leucovorin rescue). Leukocyte and MTX serum levels were documented. We used an extended version of the mathematical model by Friberg et al. (J Clin Oncol 2002) as basis for our computations. The model was fitted to the data by using a least-squares-error approach. **Results:** First order elimination proves sufficient for describing the pharmacokinetics of MTX. The average elimination rate is 0.25 h^{-1} with similar inter-patient and inter-therapy variation: the STD is 0.03 h^{-1} among patients and 0.02 h^{-1} among treatments. Similar analysis of all present parameters highlights the overall robustness of our approach. **Conclusion:** Our model makes it possible to predict myelosuppression after treatment with MTX following leucovorin rescue based on clinical data. The parameters estimated are in accordance with those provided by Friberg et al. and thus confirm the models general relevance. Extensions of the model describing tumor size reduction by MTX will be implemented to describe the complex interplay between MTX serum levels, clearance and anti-tumor effect. Additionally, mathematical optimal control theory methods will be employed to compute optimized schedules, i.e. doses and timing for the drugs that minimize tumor size and do not violate toxicity constraints such as leukocyte count.

Disclosure: Kasenda,B.: Anstellungsverhältnis oder Führungsposition: Assistenzarzt Illerhaus,G.: Anstellungsverhältnis oder Führungsposition: Oberarzt, Arbeitsgruppenleiter

P433

Cetuximab in Refractory or Relapsed Multiple Myeloma: Preliminary Results of a Phase II Clinical Trial

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Introduction: Cetuximab is an antibody targeting the epidermal growth factor receptor (EGFR) which is expressed on multiple myeloma (MM) plasma and bone marrow stromal cells (BMSC). Recently, the inhibition of EGFR has been shown to induce apoptosis in myeloma cells revealing a synergistic effect with dexamethasone. Therefore, the anti-EGFR antibody cetuximab might be beneficial in the treatment of MM, especially in combination with dexamethasone. Here we show preliminary data of the first clinical trial with an anti-EGFR antibody in MM. **METHODS:** Cetuximab once weekly was administered to patients with refractory or relapsed MM who had previously received at least one line of treatment. In case of tumor progression with cetuximab alone dexamethasone 20 mg on day 1-3 of each cycle was added starting week 5 or week 9 if no partial response (PR) or complete response (CR) was achieved. Planned treatment duration was 16 weeks (primary endpoint). Patients achieving a response or stable disease (SD) after 16 weeks of treatment could continue study medication. To assess possible predictive markers for a response to cetuximab we collected patient plasma cells at different time points to perform toxicity assays. Furthermore, we analyzed BMSCs of patients for IL-6 secretion after treatment with cetuximab in vitro. **RESULTS:** Thirteen patients have been enrolled so far. Thrombocytopenia and hyponatremia were the most common CTC grade 3 or 4 side effects. Acneiform rash CTC grade 1 occurred in all patients and 1 patient suffered from acneiform rash CTC grade 2. Two serious adverse events (SAEs) with a possible relationship to the study medication were observed: Fever and shivering requiring hospitalization in 1 patient and dyspnea in 1 patient who suffered from chronic obstructive pulmonary disease. After 16 weeks (primary endpoint) cetuximab in combination with dexamethasone induced 3 responses (2 minimal responses (MR) and 1 PR) and led to SD in 3 patients, cetuximab as single agent led to SD in 1 patient. Five of the 13 patients included did not receive the planned 16 weeks of treatment due to progressive disease (PD). Six patients were treated more than 16 weeks: 1 patient still receives cetuximab as single agent and is in SD more than one year; 5 patients continued treatment with cetuximab and dexamethasone in combination. There was 1 PD after 21 weeks and 2 SDs and 2 MRs after 28 weeks in this cohort. In viability assays with patient plasma cells we could demonstrate that cetuximab is cytotoxic in some of the patient samples. Furthermore, cetuximab suppressed the production of growth stimulating IL-6 in BMSCs of the patient who still receives cetuximab and remains in SD. **CONCLUSIONS:** Cetuximab seems to be safe and effective in MM patients. Because of its favourable side effect profile it should be evaluated in clinical trials in combination with other compounds.

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P434

Cnicin, a sesquiterpene lactone, reveals a role for Pim-2 in multiple myeloma

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Introduction: Despite advances in therapy, multiple myeloma, a plasma cell tumor, remains incurable. In an attempt to find new anti-tumor agents we tested novel natural compounds for their apoptosis-inducing property and further analysed the effects ofthe most promising compound, cnicin. Methods: We analyzed apoptosis, proliferation, and cell cycle effects of cnicin on 6 myeloma cell lines. In order to identify the invovled mechanisms we performed microarray analysis and phosphoprotein analysis. In vitro, siRNA knock down of Pim-2 was performed. In order to assess possible in vivo correlation, immunohistochemistry was applied to bone marrow samples of myeloma patients. Results: We found that cnicin, a sesquiterpene lactone, efficiently decreased proliferation and induced apoptosis in myeloma cells and identified Pim-2 as a target of cnicin. In siRNA experiments we could confirm an essential role of Pim-2 in myeloma apoptosis. Analysis of bone marrow from controls, MGUS patients, and myeloma patients revealed correlation of Pim-2 expression with the establishment of disease. Furthermore, nuclear expression of Pim-2 positively correlated with Ki67 expression and with plasmablastic tumor cell morphology. Combining cnicin with other conventional and novel drugs led to additive apoptotic effects. Conclusion: Pim-2 represents an underestimated survival protein of malignant plasma cells and targeting this pathway by cnicin or related compounds has the potential to improve therapeutic outcome of myeloma patients.

Disclosure: No conflict of interest disclosed.

P435

Functional impact of p53 hotspot mutants on tumor suppressor mechanisms in myc-driven lymphoma

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The tumor suppressor p53 is mutated in the majority of human cancers, and inactivation of p53 correlates with more aggressive clinical courses and resistance to therapy in human hematological malignancies. While loss of p53 may result in pleiotropic effects such as cell-cycle deregulation, increased survival and gross aneuploidy, we identified apoptosis as the prime tumor suppressor function of p53 that is selected against during Myc-driven lymphoma development. By contrast, increasing evidence supports premature senescence as another tumor suppressor program in which p53 plays also an important role. In addition, lymphomas

exposed to anticancer treatment selected against p53 genes even if apoptosis was efficiently blocked, because p53 not only controls apoptosis but premature senescence in response to DNA damage. Most of the naturally occurring p53 mutations in hematological malignancies, preferentially at some 'hotspot' residues, result in expressed mutant proteins. Moreover, different entities show distinct hotspot preferences, implying that individual p53 mutants may have different impact on p53 controlled effector programs, or may even acquire novel, oncogenic functions. Despite numerous studies including individual mutant knock-in mouse models, no comprehensive approach to elucidate the specific role of the most relevant cancer-derived mutations in tumor formation and treatment responses has been undertaken yet. Our study focuses on the functions of p53 hotspot mutants in a physiological mouse lymhoma model. Mutant p53 constructs were introduced into myc-transgenic hematopoietic stem cells and lymphoma cells in order to generate primary malignancies that express individual mutants under different status of endogenous wild-type p53. Tumor development under different wild-type/mutant p53 combinations were compared and the biology of the resulting lymphomas were assayed with particular emphasis on apoptosis and senescence as primary tumor suppressor mechanism. Most mutants supported rapid tumor development in the presence of wild-type p53, while only some promoted lymphoma formation in p53+/- and p53-null backgrounds. How distinct p53 mutants impact on apoptotic and senescent functions will be discussed in greater detail at the meeting.

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P436

Karyotyping and Molecular Karyotyping can give more information than array-CGH in a case with mantle cell lymphoma

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Introduction: Up to now array-CGH (aCGH) is used in research, but not in the diagnostics of hematological neoplasias. Nevertheless there are a few reasons for adopting this method in the diagnostic field of these diseases such as the use of genomic DNA (no cultivation artefacts and problems), high resolution and the possibility to identify complex chromosomal aberrations. The disadvantages are the disability to detect balanced aberrations and small mosaicisms, high costs and the interpretation of aberrations with unknown prognostical relevance. Here we present a 87 year-old woman with suspicion of chronic lymphocytic leukemia (CLL) who has a complex karyotype identified by chromosomal and FISH-analysis but not by aCGH. **Methods and Results:** Cytogenetic analysis on bone marrow (BM) revealed in 7/47 metaphases a complex karyotype with 47,XX,der(2)t(2;8)(p2?3;q2?2),+3,t(11;14)(q13;q32),del(17)(p13). These metaphases were only found in the DSP30-stimulated 120h-culture. Fluorescence in situ hybridisation (FISH) on interphases from an unstimulated 24h-culture using the LSI IGH Dual Color, Break Apart Rearrangement probe (Fa. Abbott, Wiesbaden) and the TP53 Deletion probe (Fa. Cytocell, Cambridge) showed a split in the IGH-region in 27,7% and a deletion of TP53 in 12,8% of analyzed interphases. M-FISH on metaphases from the DSP30-stimulated 120h-culture confirmed the trisomy 3, the translocation t(11;14) and identified the der(2) as der(2)t(2;8)(p2?3;q2?2). ACGH with genomic DNA from BM and peripheral blood (PB) was performed to further characterize the aberrations. Surprisingly only the trisomy 3 and loss of material in the breakpoint region of 14q32 were detected. **Conclusions:** Cultivation of BM with stimulation of the affected cell-line and different cultivation times are very important to get all relevant information with prognostic significance. The translocation t(11;14) is a diagnostic criterion for mantle cell lymphoma. Gains in 3q and 8q and loss in 17p are also described with MCL which is an aggressive lymphoma with median survival times of 3-5 years. ACGH with genomic DNA from BM and PB only detected the trisomy 3 and loss of chromosomal material in the breakpoint region of 14q32, but not the unbalanced translocation t(2;8) and the deletion at 17p13. Maybe this aberrations are in small mosaicisms and only can be detected by aCGH after 96h/120h-cultivation with DSP30. Chromosomal and FISH-analysis can not be replaced by aCGH in the diagnostics of hematological neoplasia.

Disclosure: Hinrichsen,T.: Anstellungsverhältnis oder Führungsposition: Abteilungsleiterin der Abteilung Molekulare Onkologie im Zentrum für Humangenetik und Laboratoriumsmedizin Dr. Klein und Dr. Rost
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P437

Simultaneous IGH/BCL-2 and MYC Rearrangements In a Grad I Follicular Lymphoma Resulting In A Clinically Secondary Transformation To A Very Aggressive Leucaemic Lymphoma Phenotype & Clinical Course

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Introduction: The IGH/bcl-2 translocation is generally regarded as the molecular hallmark defining follicular lymphoma (FL) , while the detection of c-myc translocations [especially t(8; 14)] is a definitive prerequisite for the diagnosis of Burkitt´s lymphoma (BL). Usually this disease are not interrelated and represent opposite ends of the malignancy spectrum of lymphatic neoplastic diseases. CASE: We report on the case of a 59 year old female diagnosed with grade I Ann Arbor stage III A FLIPI score 1 follicular NHL, first diagnosed in 07/08. No treatment indication was seen while the pt. was under an uneventful routine surveillance for 9 months. In FEB 09 the pt. presented himself with an acute massive clinical deterioration (ECOG 3), confusion, thrombopenia , very high LDH and a leucocytosis with 42% L3 blasts. Bone marrow as well as liquor were 100% infiltrated by typical L3 blasts. The immunophenotype was: CD19+, CD5-, CD23-, kappa-, lambda+, CD20+/-, CD11c-, FMC7-, CD38+, CD79a-, CD34-, CD79b+, CD103-, CD10+. Intracytoplasmatic analysis showed: cytCD22-, cytCD79a+, cytMPO-, cytTdT- , cytIgM-. KI 67 positivity was 100%. Furthermore bcl-2 was strongly expressed in contrast to negative bcl-6 and EBER expression. Cytogenetic analysis showed t(14; 18) as well as a a c-myc rearrangement. By refined multi-color FISH analysis these two aberrations could be proven to be localized in the same cell clone. Best response to a treatment according to the GMALL B-ALL protocol resulted in PR, after which the pt. was switched to FLAG and a MUD search is going on. **Results:** These morphologic, histologic, molecular and cytogenetic findings prove the secondary transformation of grade I FL to very aggressive typical BL which to our knowledge has never been described before. An update of the clinical course as well as further results of the ongoing molecular analysis of this case will be presented.

Disclosure: No conflict of interest disclosed.

P438

Successful treatment of a hepatic DLBCL in a patient with chronic hepatitis c and decompensated liver cirrhosis CHILD C

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Introduction: Chemotherapy in patients with decompensated liver cirrhosis is limited especially in hepatic encephalopathy and massive ascites due to toxicity. We describe a patient receiving 6 cycles R-CHOP treatment after successful recompensation of ascites by means of an intrahepatic stent-shunt (TIPS). **Results:** The 38 years old patient presented with new ascites and edemas. He suffered from chronic hepatitis C with liver cirrhosis Child C (13p). An Interferon therapy was´t successful 1,5 years ago. He reported a history of intravenos drug abuse, methadon substitution since years without current substance abuse and a negative HIV ELISA. The ascites was steril and did not show malignant cells, but diuretic therapy showed no effect. CT-scans showed 7 hepatic leasions up to 8,5cm, clinical presentation included mediastinal, inguinal (9x7cm) and iliacal(2,5cm) lymphomas. DLBCL was diagnosed via liver biopsy and treatment with prophase followed by R-CHOP was initiated. After one cycle of R-CHOP lymphomas decreased but ascites, anasacrca and

stasis eczema worsened. The weight increased from 100 to a maximum of 140 kg. A transient hepatic encephalopathy grade II occurred.
After ascites drainage of 5 litres and application of a transjugular intrahepatic shunt with 3 overlapping stents (TIPS) the portovenous pressure could be diminished (minus 10cm H2O). The patient improved to a weight of 106 kg and therapy was initiated again. After 6 cycles of R-CHOP (dose-adapted, 10/2007-2/2008) the patient reached a good remission with continuous improvement. The histology of the visible intrahepatic lesions at the last follow up 3/2009, was not verified but differential diagnosis include regenerative nodules. **Conclusion:** Special treatment options (TIPS) might help to give therapy to initial "untreatable" patients with liver cirrhosis improving prior quality of life.

Disclosure: No conflict of interest disclosed.

P439

Intravascular large B-cell lymphoma (IVLBCL) presenting as hemiparesis, dysarthria and epileptic seizures: a case report

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Introduction: Intravascular large B-cell lymphoma (IVLBCL) is a rare subtype of extranodal large B-cell lymphoma which occurs in adults (median: 67 years). It is an aggressive systemic disease and histologically characterized by massive proliferation and accumulation of large tumor cells within the lumina of small to medium-sized blood vessels. Intravascular lymphomas are predominantly of B-cell lineage. Occlusion of the vessels by neoplastic cells results in clinical symptoms which are highly variable and depend on the involved organs. Cutaneous and neurological involvement is frequent and was first described as a specific manifestation of IVLBCL. IVLBCL responds poorly to chemotherapy and in most cases death occurs within a short time after diagnosis. **Case presentation:** Here we report a case of a 69-year-old female patient who presented with right sided hemiparesis, dysarthria and epileptic seizures. The progressive clinical symptoms developed within a few weeks. Magnet resonance imaging of the brain and the spine showed multiple disseminated lesions with hemorrhage and perifocal edema in the brain. In addition, an intramedullar focus in the height of the twelfth thoracic vertebra was detected. The diagnosis was confirmed by an intracerebral biopsy of the left occipital lobe. Immunohistochemistry revealed large CD20 positive B lymphocytes filling the lumina of small blood vessels. Moreover CD3 positive T-cells could be detected in the perivascular tissue. Diffuse nerve cell degeneration in combination with ß-amyloid plaques characteristic for Alzheimer's disease was seen as well. Bone marrow was not infiltrated by lymphoma. The patient was treated with one cycle of chemotherapy consisting of high dose methotrexate and ifosfamide. Unfortunately, the patient's condition worsened rapidly and did not allow continuation of high-dose chemotherapy and palliative therapy was initiated. This case demonstrates that the onset of IVLBCL can be insidious and that therapeutic improvement of this rare aggressive lymphoma subtype is needed.

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Posterdiskussion
CLL I

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The role of the VLA-4 integrin in adhesion and survival of chronic lymphocytic leukemia cells

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Introduction: Growth and survival of chronic lymphocytic leukemia (CLL) B cells are favored by microenvironmental interactions between CLL and nontumoral accessory cells. The integrin VLA-4 has a key role in the retention of hematopoietic stem and leukaemia cells in supportive bone marrow niches. Here, we aimed to dissect the role of VLA-4 for cell adhesion and cell adhesion-mediated drug resistance of CLL cells within the microenvironmental niche. **Methods:** Peripheral blood mononuclear cells (PBMCs) of CLL patients were cultivated with or without stromal cells (M2-10B4 or HS-5 cell lines) and treated with 5 µM fludarabine and blocking anti-VLA-4 antibody (clone HP2.1, 0.3 µg/ml) where indicated. After 48 h, non-adherent CLL cells were carefully washed off from the stromal layer and the number of adherent CLL cells was cytometrically determined using anti-CD5, anti-CD19. CLL cell viability was determined by flow cytometry using Annexin V-FITC, DNA-staining with fluorescent dye 7-AAD, anti-CD5 and anti-CD19 antibodies. Transwell assays were used to dissect the contribution of soluble factors vs. direct cell-cell contact in stroma-mediated survival. **Results:** VLA-4 expression on CLL cells was strongly associated with CD38 expression and with unmutated immunoglobulin variable heavy chain (IGVH) gene status. CLL cells that were co-cultured with bone marrow stromal cells were protected against spontaneous and fludarabine-induced apoptosis and this protection was dependent on the direct cell-cell contact. VLA-4 positive CLL cells had a significant higher potential to adhere to bone marrow-derived stromal cells than VLA-4 negative cells. Concordantly, VLA-4 positive cells that adhered to the stromal cells were more resistant towards fludarabine. Targeting the CLL-stromal cell interactions with an inhibitory anti-VLA-4 antibody reduced the adhesion of high risk CLL cells to the stroma and antagonized cell adhesion mediated drug resistance. **Conclusion:** Our study demonstrates that VLA-4 expression in CLL cells is strongly associated with molecular markers for poor clinical outcome. Furthermore, VLA-4 is functionally implicated in cell adhesion mediated drug resistance phenomena in CLL. Our results highlight the possibility that VLA-4 detection could be a useful prognostic marker of CLL severity. Additionally, it could aid in evaluating the suseptibility of CLL cells to VLA-4 blocking therapy. VLA-4 antagonists may be further explored as anti-adhesive drugs to target CLL cells within their microenvironment.

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Vascular endothelial growth factor (VEGF) promotes chronic lymphocytic leukemia (CLL) cell survival via upregulation of the oncogene STAT3 and downregulation of the tumor supressor RB1 and E2F1

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VEGF has been demonstrated to be involved in the pathogenesis of CLL. Most of these observations are descriptive correlating advanced disease stage or increased microvessel density in the bone marrow with VEGF plasma concentration, whereas functional data are missing. We found a positive autocrine VEGF feedback loop in CLL, but not healthy B-cells. VEGF stimulation went along with increased expression of the antiapoptotic proteins Mcl1 and XIAP. In addition, these proteins were downregulated upon inhibition of VEGF receptor signaling, which subsequently lead to apoptosis. Coculture of CLL cells with the bone marrow derived stromal cell line HS5 induced a paracrine VEGF loop, which was accompanied by an enhanced survival of CLL cells in coculture. After 72 hours cocultivated CLL cells survived 30% better when compared to monoculture, whereas healthy B-cells did not profit from coculture. Neutralization of VEGF using a monoclonal antibody, reduced this survival advantage by 60%. A siRNA-mediated reduction of VEGF in HS5 even completely diminished the coculture-mediated survival support. Looking into expression changes after VEGF stimulation by PCR array we found STAT3 to be upregulated 8.8-fold. STAT3 was constitutively activated at Ser727, which was not sufficient to induce transcriptional activity. By immunoblot we found VEGF stimulation to activate STAT3 via phosphorylation at Tyr705, which lead to expression of the STAT3 target genes cyclin D1 and Bclxl. Inhibition of VEGF signaling by blockage of the VEGFR reduced both, Tyr705 phosphorylation and expression of STAT3 target genes. In addition PCR array revealed a down-regulation of the tumor supressor Rb1 and E2F1 by 5.8-fold and 3.7-fold, respectively. Out of the E2F family exclusively E2F1 was identified as potential tumor supressor as it can act as strong apoptosis inducer. Its activity can be modified by RB1 de-

pendent on phosphorylation status, cell type and differentiation state. In conclusion, VEGF might carry out its pro-survival function through a dual mechanism by upregulating the potent oncogene STAT3 and down-regulating RB1/E2F1, subsequently protecting the CLL cell against apoptosis. This novel connection describes a potential mechanism of the apoptotic block in CLL cells, therefore allows the development of new selective therapeutic strategies for treatment of this disease

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Circulating B-cell chronic lymphocytic leukemia cells display impaired migration to lymph nodes and bone marrow

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Introduction: Homing to secondary lymphoid organs and bone marrow (BM) is a central aspect of leukemic pathophysiology. Abnormalities in the expression and function of cell adhesion molecules such as integrins may account for the patterns of intranodal growth and hematogenous spread of the malignant cells. The role of the two major lymphocyte integrins LFA-1 and VLA-4 in the migration of B-cell chronic lymphocytic leukemia (CLL) cells is largely unknown. We investigated expression and function of these integrins in extravasation and homing processes of CLL cells *in vitro* and *in vivo*. **Methods:** Integrin expression on peripheral blood and BM derived CLL and normal B lymphocytes was determined by flow cytometry and quantitative RT-PCR. Rapid chemokine-induced integrin activation and transendothelial migration was studied *in vitro* under conditions that simulate the blood flow and was combined with short term *in vivo* homing experiments of CLL and normal human B lymphocytes to BM, spleen and LNs of immunodeficient mice. **Results:** We found that the majority of CLL cells expressed significantly reduced LFA-1 due to low beta2 integrin transcripts. VLA-4 expression was heterogenous but underwent rapid activation by the BM chemokine CXCL12. CLL cells failed to transmigrate across VCAM-1, ICAM-1 and CXCL12 expressing endothelium whereas when LFA-1 expression was regained in subsets of CLL cells, these lymphocytes rapidly transmigrated the endothelium. Furthermore, when injected into tail veins of immunodeficient mice, normal B cells rapidly homed to lymph nodes (LNs) in an LFA-1 dependent manner whereas CLL cells did not. Nevertheless, only residual CLL subsets could re-enter BM whereas, both, normal and CLL cells homed to the mice spleen in an LFA-1- and VLA-4-independent manner. **Conclusion:** Our results suggest that CLL cells have a reduced capacity to adhere and transmigrate through multiple vascular endothelial beds and poorly home to lymphoid organs other than spleen. Integrin blocking could thus be an efficient strategy to prevent circulating CLL cells from reaching prosurvival niches in LNs and BM but not in spleen.

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Experimental targeting of lymphoid enhancerfactor-1 (LEF-1) in chronic lymphocytic leukemia (CLL) using the small molecule inhibitors CGP049090 and PKF115-584.

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Introduction: We and others have shown that LEF-1 is excessively upregulated in CLL cells when compared to normal B cells. Here we report about the role of LEF-1 in CLL cell survival and characterize response of two small molecules (CGP049090 and PKF115-584) which specifically inhibit LEF-1/ß-catenin interaction. **Methods:** JVM-3 cells and primary CLL cells were investigated by siRNA mediated knock down of LEF-1 and viability was assayed after 16h of incubation by FACS. In vitro cytotoxicity and LC_{50} of the two compounds was enumerated using ATP based cell viability assay. Apoptotic response was investigated in time course experiments. Specificity of the small molecules was demonstrated by co-immune precipitation experiments for the LEF-1/ßcatenin interaction in primary CLL cells. *In vivo* efficacy of the small molecules inhibitors were studied using a JVM-3 subcutaneous xenograft model in nu/nu mice. **Results:** Knocking down of LEF-1 led to increased apoptosis in CLL cells *in vitro* indicating that LEF-1 has a vital role in extended survival of CLL cells. The viability was correlated to the LEF-1 knock down as assessed by western blot. This observation was extended by using two small molecule inhibitors of LEF-1/ß-catenin signaling (CGP049090 and PKF115-584) which induced apoptosis of the CLL cell lines and primary CLL cells (LC50: ranging from 0.7 µM to 0.9 µM). Healthy B cells were not significantly affected, as ascertained by the fact that LC_{50} values could not be reached due to lacking total cell death. Co-immuneprecipitation showed a selective disruption of LEF-1/ß-catenin interaction. Further, a decrease of the LEF-1/ß-catenin target genes C-MYC, cyclin D1 and LEF-1/ß-catenin itself could be demonstrated. We tested the in vivo efficacy of both inhibitors in a JVM-3 xenograft model. Mice treated with 25 mg/kg for 14 days exhibited tumor inhibition of 70% with CGP049090 and 57% with PKF115-584 and the intervention was well tolerated. Kaplan-Meier survival analysis of this treatment significantly improved median survival by 12.5 days with CGP049090 and 15.5 days with PKF115-584 ($p < 0.003$). **Conclusion:** Summing up, LEF-1 appears to play a pivotal role for CLL cell survival and therefore qualifies for an attractive therapeutic target in this disease. As the LEF-1/ß-catenin inhibitors CGP049090 and PKF115-584 potently induce apoptosis in CLL cells and produce no fatalities in mice further studies are needed to investigate the applicability of these compounds in humans.

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P444

Lack of LRP6, a co-receptor of the WNT pathway, as a possible cause for the resistance of chronic lymphocytic leukemia (CLL) cells to the physiological WNT inhibitor Dickkopf-1 (DKK-1)

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Background: WNT signaling is speculated to be responsible for the abnormal long survival of neoplastic cells in CLL. The canonical WNT pathway is initiated through proteins of the WNT ligand family, which bind to a receptor complex composed of the Frizzled proteins (FZD and low-density lipoprotein receptor-related proteins (LRP5/6). DKK-1 is known to antagonize WNT/ß-catenin signaling by direct high-affinity binding to the WNT coreceptor LRP5/6 and inhibiting interaction of LRP5/6 with the WNT/FZD complex. Therefore, inactivation of the WNT pathway by DKK-1 could lead to an increased apoptosis rate in CLL cells. The purpose of this study was to investigate the effect of DKK-1 on CLL cells. **Methods:** B cells from 10 different CLL patients were incubated in presence or absence of DKK-1 (0.3 µg/ml) for 24 and 48 hours. Survival was measured by flow cytometry and caspase 3/7 activation. Western blot and real-time PCR were used to estimate expression of Wnt-signalling components ß-catenin, LEF-1, C-MYC and LRP6 on protein and mRNA levels, respectively. **Results:** Flow cytometry analysis showed that incubation with DKK-1 resulted in increase of overall survival of CLL cells (DKK-1 91.1 ± 5.3%; vehicle control 71.3 ±19.7%) after 24 hours and (DKK-1 80.2 ± 8.2%; vehicle control 65.8 ± 13.5%) after 48 hours. Accordingly, the activity of caspases 3 and 7 in these cultures was decreased. Compared to vehicle control, ß-catenin and C-MYC expression were increased, indicating a higher activity of WNT pathway, which might explain increased cell survival upon DKK1 treatment. Expression of LRP6 mRNA, acquired by means of real-time PCR, was very low in CLL cells compared to healthy B-cells. Since LRP6 is essential for

DKK-1 perform its functions, low expression of this coreceptor could be a possible reason for lack of response of CLL-cells to DKK1. **Conclusion:** Summing up, unlike in similar tumors, addition of DKK-1 to the culture of CLL cells does not lead to inactivation of the WNT pathway. This effect might be explained by the lack of the WNT coreceptor LRP6 on CLL cells. Further investigations on WNT signaling in CLL cells are needed.

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In vivo application of the lipase inhibitor orlistat suppresses chronic lymphocytic leukemia (CLL) in an Eμ-TCL1 transgenic mouse model

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Introduction: We recently identified increased lipase activity as a novel drug target in chronic lymphocytic leukemia (CLL). Targeting lipoprotein lipase as other lipases and phospholipases by the pan-lipase inhibitor orlistat we could specifically induce apoptosis in primary CLL patient samples while not inducing apoptosis in healthy donor controls. Orlistat is widely used in obesity treatment by oral application. However intestinal mucosal catabolism of orlistat prevents high systemic drug levels by oral application. **Methods:** We addressed in vivo application of orlistat in Eμ-TCL1 transgenic mice based on systemic application by intraperitoneal injection. Orlistat serum concentrations were assessed by mass spectrometry. Treatment response was assessed by blood count and flow cytometry for CD5/CD19/IgM co-expressing cells. **Results:** Injection of orlistat up to 1000 mg/kg/d to BL6 control mice was well tolerated, no hematological or other toxicities could be observed. A pharmakokinetic approach showed 1.66 h serum half life of orlistat, applying 500 mg/kg orlistat revealed 4.3 μM maximum serum concentration reaching the *in vitro* predicted half-maximal concentration for induction of apoptosis. Based on pharmakokinetic results a continous 3-week daily application schedule of 500 mg/kg/d i.p. was chosen for systemic treatment of orlistat in Eμ-TCL1 transgenic mice harboring a leukemic phenotype of elevated CD5+/CD19+/IgM+ in the peripheral blood. Again, application of orlistat was well tolerated without hematological toxicities. The amount of peripheral leukemic cells was assessed by multicolor flow cytometry and used for follow-up of orlistat treatment. Orlistat treatment significantly reduced CD5+/CD19+/IgM+ leukemic cells after treatment onset by 75% on day 8 related to day 0 pre-treatment values. The total white blood cell count was reduced from 73.100/μl to 51.950/μl. Particularly the subset of leukemic cells were significantly reduced from 56.4% at d0 to 14.7 % at d8 (n=8; p=0.002). However, from the nadir at day 8 the amount of malignant cells in the peripheral blood showed a significant increase at day 21. **Conclusions:** We could demonstrate orlistat as an effective drug in an *in vivo* model of CLL. Future strategies will aim to combine cytotoxic drugs with orlistat to overcome treatment resistance in this still incurable leukemic disorder.

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Akt inhibition preferentially leads to apoptotic cell death in B-CLL cells with unfavorableclinical markers and can overcome the protective effect of the tumour microenvironment

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Akt is a key factor in cell survival and is implicated in the progression of many tumour types. In CLL, pharmacological interference with the PI3K/Akt/mTOR signalling pathway has demonstrated a central role for this axis in CLL cell survival, however direct targeting of Akt has not been extensively addressed so far. Cell viability was assessed by an annexin V/7-AAD staining and flow cytometry, PARP cleavage and chromatin fragmentation. For simulation of the tumour microenvironment CLL cells were cocultured with stromal cell lines or bone marrow-derived stromal cells. The effect of Akt inhibition was further monitored by immunoblotting of phosphorylated Akt and MDM as well as p53 expression. We studied the efficacy of the novel Akt inhibitor in inducing cell death *in vitro* in cell lines with constitutive Akt activity and primary human CLL cells. Akt-inhibition in these cell lines as well as primary CLL Cells induced apoptotic cell death in a dose-response manner, but interestingly exhibited a preference for high-risk CLL samples with an unmutated immunoglobulin status or high CD38 expression. This preference may be due to higher constitutive Akt activation in unmutated CLL cells, and increased p53 signalling upon Akt inhibition. Moreover, B-CLL cells cocultured with stromal cells showed higher Akt activation, which correlated with stromal protection (r2=0.81, p=0.037). Treatment with Akt inhibitor was able to overcome the protective effects mediated by coculture with primary stromal cells without interfering with the stromal cell survival. Taken together, Akt inhibtion preferentially leads to apoptotic cell death in B-CLL cells with unfavorable clinical markers and can overcome te protective effect of the tumour microenvironment. Pan-Akt inhibitors therefore should be further evaluated for the treatment of high risk B-CLL.

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Small molecule XIAP inhibitors act in concert with TRAIL to overcome apoptosis resistance in chronic lymphocytic leukemia

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Introduction: CLL is characterized by the abnormal accumulation of malignant monoclonal B cells, which has been largely attributed to defective apoptosis programs rather than aberrant proliferation. This calls for new strategies to re-activate apoptosis in CLL. "Inhibitor of Apoptosis Proteins" (IAPs) such as XIAP are aberrantly expressed in many human cancers and block apoptosis at a key node by inhibiting activation of caspases. In the present study, we explored whether targeting XIAP is a suitable strategy to overcome apoptosis resistance of CLL. **Methods:** We investigated the effect of small molecule XIAP inhibitors alone and in combination with the death receptor ligand TRAIL on apoptosis induction in CLL cells. **Results:** Subtoxic concentrations of XIAP inhibitors significantly enhance TRAIL-induced apoptosis in CLL cells. Molecular studies reveal that XIAP inhibitors promote TRAIL-induced caspase-3 activation and caspase-dependent apoptosis. Also, XIAP inhibitors sensitize CLL cells for CD95-triggered apoptosis, whereas they no not alter the susceptibility to Fludarabine or Chlorambucil. Most importantly, XIAP inhibitor acts in concert with TRAIL to trigger caspase-3 activation and caspase-dependent apoptosis in 18 of 27 (67%) primary CLL samples. This cooperative interaction of XIAP inhibitor and TRAIL is also evident in patients with poor prognostic features, i.e. in all four investigated patients with 17p deletion as well as in cases with TP53 mutation, chemotherapy-refractory disease or unmutated VH genes. Interestingly, patients with unmutated VH genes were significantly more sensitive to XIAP inhibitor- and TRAIL-induced apoptosis compared to those with VH gene mutation, pointing to a role of B-cell receptor signaling in apoptosis regulation. **Conclusions:** By demonstrating that XIAP inhibitors enhance the lethality of TRAIL in CLL, our findings provide first evidence that this combination presents a novel strategy to trigger apoptosis even in resistant forms of CLL.

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Association of genetic polymorphisms with cytogenetic subgroups in B-cell chronic lymphocytic leukemia

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Introduction: Genetic polymorphisms in DNA repair genes and in metabolizing enzymes may influence the susceptibility to different forms of cancer. We investigated the association of seven single nucleotide polymorphisms (SNPs) in five DNA repair genes and of 17 polymorphisms in 13 metabolizing enzymes with the incidence of chronic lymphocytic leukemia (CLL). We also evaluated the frequency of genetic polymorphisms in CLL patients with high- and low risk cytogenetic abnormalities as chromosomal aberrations have been shown to be important prognostic markers in CLL. **Methods:** We analyzed 461 CLL patients and an equal number of sex and age matched controls using PCR followed by digestion with restriction enzymes. Odds ratios (OR) and p-values were calculated by logistic regression analysis. The 133 patients with the favorable cytogenetic aberration del(13q) as a sole aberration and the 69 patients with the unfavorable cytogenetic aberrations del(17p) and del(11q) were evaluated separately. **Results:** The rare genotypes of rs13181 in the gene *Excision-repair, complementing defective, in Chinese hamster, 2* (*ERCC2*), of rs25487 in the base excision repair gene *X-ray repair complementing defective repair in Chinese hamster cells 1* (*XRCC1*), and of rs1056836 in the Phase I enzyme *CYP1B1* occurred significantly more frequently in patients with unfavorable cytogenetic aberrations compared to controls. The genotypes of rs13181 and rs1056836 were differently distributed under the co-dominant model (rs13181: A/A vs. G/G: OR=2.66, p=0.024; rs1056836: G/G vs. C/C: OR=2.62, p (unadjusted)=0.004) and those of rs25487 under the dominant model of inheritance (A/C and C/C vs. A/A: OR=2.44, p=0.01). Furthermore, differences in the genotype distribution were found between all CLL patients and controls for rs1048943 in the Phase I enzyme *CYP1A1* (A/G and G/G vs. A/A: OR=0.40, p (unadjusted)=0.004) and for rs1695 in the Phase II enzyme *Glutathione S-Transferase PI* (*GSTP1*) (A/G vs. A/A: OR=1.46, p (unadjusted)=0.009). Preliminary results show that there is also an association with time to first treatment. **Conclusions:** Our results indicate that inborn polymorphisms in DNA repair genes and in genes encoding metabolizing enzymes may help to predict the outcome of CLL. We now test their potential as surrogate markers by correlating them with established prognostic markers.

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Spleen Tyrosine Kinase (SYK) Inhibition prevents CLL-Stroma Interactions

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B cell chronic lymphocytic leukemia (CLL), the most prevalent leukemia in adults, is characterized by the expansion of monoclonal, mature B lymphocytes. Despite treatment advances, the disease remains incurable warranting further efforts to identify novel molecular targets in CLL. Spleen tyrosine kinase (SYK) is a key component of the BCR signaling pathway but is also involved in the signaling of various other extracellular stimulations, such as integrins and chemokines. Microenvironmental signals contribute to apoptosis resistance in CLL limiting the efficacy of therapeutic approaches. *In vitro* coculture with stromal cells mimicks this environment and protects CLL cells from spontaneous and chemotherapy induced apoptosis. In this study, we evaluated the expression and phosphorylation status of SYK and its downstream pathway in CLL, examined the effects of pharmacological SYK inhibition, and analyzed a potential role of SYK in CLL-stroma interaction. Immunoblotting revealed an approximately 2-fold higher SYK protein level in CLL compared to healthy B cells. *Per se*, the SYK phosphorylation status was significantly higher in CLL (calculated by % phospho-SYK per total-SYK: 70%±8 in CLL, n=10 vs. 28%±10 in healthy B cells, n=8; p<0.05). This hyperactivation was also reflected in the phosphorylation status of downstream targets, including $PLCy_2$, STAT3, and ERK1/2. SYK inhibitors reduced phosphorylation of SYK downstream targets and induced Caspase 3 dependent apoptosis in primary CLL cells. By *in vitro* coculture with SDF1 producing stromal cells$SYK phosphorylation was enhanced in CLL cells along with an induction of F-actin. Concomitant addition of a SYK inhibitor significantly reduced this induced F-actin formation. The antiapoptotic molecule Mcl-1 was upregulated by stromal cell contact, causing at least in parts the protective effect on CLL cells. SYK inhibition prevented Mcl-1 upregulation induced by stroma. In line with this, no significant change in cytotoxic effects, as shown for standard chemotherapy, was observed by incubation of CLL cells with a SYK inhibitor in the presence and absence of stromal cells. In conclusion, this work establishes SYK inhibition as a rational and promising therapeutic principle in CLL. In addition to the predicted effects of disruption of tonic B cell receptor signaling, SYK inhibition prevented proapoptotic stimuli from the microenvironment and might therefore also target CLL cells in protective microenvironmental niches.

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P450

Non-toxic concentrations of para nitricoxide-donating acetylic salicylic acid (p-NO-ASA) effectively induce apoptosisof chronic lymphocytic leukemia (CLL) cells in vitro and in vivo.

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Introduction: Non-steroidal anti-inflammatory drugs (NSAIDs) can induce cell death in WNT driven cancers through inhibition of ß-catenin (CTNNB1) stability. Furthermore, it is known that in CLL, WNT/CTNNB1/LEF-1 signalling is aberrantly activated. So far, clinical studies with NSAIDs revealed that therapeutic plasma concentrations could not be reached without producing significant toxicities. Therefore, NO-ASA, which achieves high plasma levels in doses not leading to major side effects in humans, has been developed. NO-ASA has been shown to disrupt the CTNNB1/TCF-4 complex in vitro, whereas the latter belongs to the transcription factors having a central function in mediating WNT signalling. The aim of our study was to evaluate the effect of the para- and meta-isomer of NO-ASA in CLL. **Methods:** Primary CLL cells, as well as healthy cells, were treated with varying concentrations of both isomers. Cytotoxicity was assessed by microscopic cell viability testing and an ATP assay. Induction of apoptosis was investigated by Annexin V-FITC/PI staining and immunoblotting of PARP, caspases 3 and 9. CTNNB1 protein amount was measured by immunoblotting and expression of WNT effector proteins like cyclin D1 (CCND1), C-MYC and LEF-1 was evaluated with immunoblot analysis as well. *In vivo* activity was evaluated by treating irradiated CD1 nu/nu female mice, carrying a JVM-3 cell line xenograft, with 50 mg/kg/day of p-NO-ASA or vehicle control *p.o.* for sixteen days. **Results:** The meta-isoform of NO-ASA did not have any effect on CLL cells whereas the para-isomer showed a selective cytotoxic effect. Mean lethal concentrations (LC50) values were 4.83 µM and 4.64 µM in CLL cells, respectively, whereas the effect on healthy cells was not significant. Annexin V-FITC/PI staining revealed that the induced cell death is mediated by apoptosis and by immunoblot analysis we observed that p-NO-ASA cleaves PARP, caspase 3 and caspase 9, decreases CTNNB1 protein levels and downregulates WNT target genes in a concentration dependent manner. *In vivo* results revealed that p-NO-ASA shows an antitumor efficacy with a maximum inhibitory rate (IRmax) of 65.8%. **Conclusions:** Summing up, our findings show that p-NO-ASA induces apoptosis *in vitro* and *in vivo* in CLL cells and shows good tolerability. Therefore p-NO-ASA might be a valuable compound for the treatment of CLL. More investigations of the exact mechanism of action and the specific difference between the positional isomers are indicated.

Disclosure: No conflict of interest disclosed.

P451

VEGF receptor inhibitors pazopanib and vatalanib induce apoptosis in chronic lymphocytic leukemia (CLL) cells in vitro and significantly inhibit growth of human CLL like tumor xenografts in mice

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Introduction: We and others have shown that vascular endothelial growth factor (VEGF) plays a pivotal role in growth, survival and migration of chronic lymphocytic leukemia (CLL) cells. However, it is still unknown if selective inhibition of VEGF leads to apoptosis in these cells and if this is tolerated by normal peripheral blood cells. Vatalanib (PTK787/ZK 222584) and pazopanib (GW786034) are potent orally available VEGF tyrosine kinase inhibitors. The aim of the present investigation was to study the efficacy and selectivity of both inhibitors in CLL cells, to simulate potential combination with conventional cytostatics in vitro and to test the effect on CLL like tumor xenografts in a mouse model. **Methods:** Primary CLL as well as normal cells were incubated with varying concentrations of both inhibitors for different time periods. Cells were then treated with combinations of each inhibitor and fludarabine, vincristin and doxorubicin, respectively. Apoptosis induction was analysed by flow cytometry (annexin V-FITC/PI staining) and cell survival was additionally investigated by an ATP depended fluorescence assay. For in vivo experiments, four-week old BALB/c nu/nu mice were grafted with cells of a human chronic B cell leukemia cell line (JVM3). After tumors reached a mean volume of 100 mm^3 per group (10 mice), drugs were administered once daily by oral gavage at 100 mg/kg bodyweight. Tumor volume was measured every second day by calliper. **Results:** Vatalanib and pazopanib effectively induced apoptosis in CLL cells in a dose and time dependent fashion. After 24h of incubation with the two VEGF Inhibitors CLL cells showed a 50% lethal concentration (LC50) of 32.0 µM for vatalanib and 32.6 µM for pazopanib (flow cytometry). In contrast, normal PBMCs were significantly less affected as indicated by LC50 values of 169.4 µM (vatalanib) and >1,000 µM (pazopanib). Combination of the low dosed drugs with conventional cytostatics significantly increased apoptosis rate in vitro. After 4 weeks of treatment the mean xenograft volume was 795 mm^3 with pazopanib, 1,483 mm^3 with vatalanib and 3,306 mm^3 in the vehicle treated group. This translated into a mean tumor inhibition rate of 75.9% for pazopanib and 55.1% for vatalanib. **Conclusions:** Specific inhibition of VEGF by vatalanib or pazopanib might be a promising new therapeutic approach in CLL. As both compounds possess acceptable in vivo toxicities in humans they are attractive candidates for further development in CLL.

Disclosure: No conflict of interest disclosed.

P452

T cell leukemia/lymphoma 1 (TCL1) expression is correlated with clinical progression in chronic lymphocytic leukemia

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Introduction: Expression of the human oncogene TCL1 in transgenic mice produces B-cell tumors that resemble chronic lymphocytic leukemia (CLL) suggesting its role in B-cell tumorigenesis. Recent data in a small study group (n=51) suggests that the TCL1 expression could predict prognosis in CLL. Therefore, we investigated TCL1 mRNA expression in a large cohort with 107 patients along with the most commonly used biological markers in order to assess its role in risk prediction in B-CLL. Methods and **Results:** Evaluation of several disease characteristics in association with the TCL1 expression status of the patients' B-CLL cells showed no significant differences for ZAP-70 expression (p=0.70), CD38 expression (p=0.28) and Binet stage (p=0.65) suggesting no correlation of TCL1 expression with these already established adverse prognostic factors. However, high TCL1 expression was associated with unmutated IgVH status (p=0.037). Patients with high TCL1 expression (according to ROC-analysis) had a significantly shorter treatment-free survival (TFS) than patients with high TCL1 expression (median TFS: 52 versus 119 months, p=0.042). In multivariate analysis high TCL1 expression was an independent prognostic factor (hazard ratio 1.84; 95% CI 1.0-3.3; p=0.047) next to CD38 status (2.9; 95% CI 1.6-5.5; p=0.001). Further, we could show that the combination of TCL1 expression with the already established prognosticator CD38 or ZAP-70 could further refine the prognostic information provided by either of the factors alone (TCL1 and CD38: p<0.0001; TCL1 and ZAP-70: p=0.005). To further analyse the regulation of TCL1 expression we investigated the influence of a validated singular nucleotide polymorphism TCL1 -957 A>T with mRNA expression. We could not find any association of the TCL1 -957 A>T genotypes and the TCL1 mRNA expression suggesting that this polymorphism has no impact on the TCL1 expression in contrast to the recently described regulation by miR-29 and miR-181. **Conclusion:** Here we demonstrate for the first time in a large cohort that the level of TCL1 expression is correlated with prognosis in B-CLL. Additionally, efforts to target TCL1, through down-regulation or interference with TCL-1/AKT interactions might represent a novel therapeutic approach.

Disclosure: No conflict of interest disclosed.

P453

IgVH hypermutation reverses p53 mutation associated poor prognosis but is not related to drug or g-irradiation sensitivity in B-CLL

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Background: The presence or absence of immunglobulin heavy gene (IgVH) mutational status is an independent prognostic factor in chronic lymphocytic leukemia of B cell type. Nevertheless, little is known about the impact of IgVH status on apoptosis induction by various cytotoxic stimuli. We therefore analysed the in vitro chemosensitivity and radiosensitivity of primary B-Cll cells and clinical data including the p53 gene mutational status in relation to the IgVH mutational status. **Methods:** 138 B-CLL samples were analysed for Ig VH mutations and for p53 mutations. The in vitro cytotoxicity assay (DiSC-assay) was performed with 6 drugs (chlorambucil, mafosfamide, fludarabine phosphate, methylprednisolone, doxorubicin, vincristine) and g-irradiation. **Results:** Of the 138 B-CLL samples, 113 were informative for the Ig VH mutational analysis. Of these, 73 (64,6%) were unmutated, 40 (35,4%) were mutated. No differences were observed for the cell death induction by chlorambucil, mafosfamide, fludarabine phosphate, doxorubicin, vincristine or g-irradiation. Only methylprednisolone was significantly less effective in the IgVH mutated samples (p=0.018), this resulted in survival advantage for low LD90 in the IgVH umutated group only (p=0.005). There was no correlation in the distribution of IgVH and p53 gene mutational status. Nevertheless, patients with a p53 mutated, IgVH unmutated B-CLL have a dismal prognosis (median survival 10.1±0.8 months (n=11) versus 37.2±3.4 months (p53 mutated, IgVH hypermutated, n=7), p=0.015). **Conclusions:** The IgVH mutational status has no impact on the cytotoxic effect of chemotherapy or irradiation in B-CLL. Steroids are less effective in IgVH mutated samples. The poor prognosis effect of IgVH unmutated disease adds to the poor prognosis conferred by p53 gene mutation.

Disclosure: No conflict of interest disclosed.

P454

p53 and HistoneDeacetylase Inhibition in CLL

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Introduction: Histone deacetylase inhibitors (HDACi) are a promising new class of chemotherapeutics currently in clinical trials. These compounds exhibit promising anti-cancer activity (e.g. tumor cell growth and survival inhibition and low toxicity in healthy cells), but their mechanism of action is not fully understood. There exists a discourse as to whether p53 plays a major role in HDACi-mediated cell death and few studies have examined this question in CLL. In this study, we examine HDAC-induced

cell death with regards to TP53-status. Materials and **Methods:** The HDACi Trichostatin A (TSA) was tested on three CLL cell lines (Mec1, Mec2, and EHEB) with 24h, 48h, and 72h incubation times and 19 primary CLL cell samples (8 of which with 17p deletion) using a chemiluminescence-based cell viability assay (CellTiterGlo, Promega) with 24 h and 48 h incubation times. These results were confirmed on 6 patients (3 of which with 17p deletion) using FACS. HDACi with purportedly similar mechanisms, SAHA (Vorinostat) and CHAHA, were tested on 8 primary CLL cell samples (4 of which with 17p deletion) using CellTiterGlo. **Results:** Significant cell death was observed in all cell lines tested after 24 h incubation with 100 nM TSA. Near absolute cell death was observed after 48 h incubation with 1 µM TSA. Interestingly, Mec1 and Mec2 (both of which have TP53 mutations) exhibited a higher sensitivity to TSA than EHEB (wild-type TP53). Primary CLL cell samples exhibited sensitivity to 100 nM and 10 nM TSA after 24 h and 48 h, respectively. Similar to the cell lines, near absolute cell death was observed after 48 h with 100 nM TSA. Cases with 17p deletion (6/8 of which also had TP53 mutations) showed a distinctly (>two-fold) higher response to TSA than those without. SAHA and CHAHA were observed to induce less cell death in primary CLL cell samples than TSA at equimolar concentrations: sensitivity was observed after 48 h with 1 µM and 10 µM SAHA and CHAHA, respectively. Unlike TSA, neither SAHA nor CHAHA induced cell death more prominently in cells with 17p deletion. **Conclusions:** SAHA and CHAHA induced cell death in primary CLL cells irrespective of 17p-status suggesting a p53-independent mechanism; however, TSA exhibited a more prominent effect in cell lines with TP53 mutation and primary cells with 17p deletion which suggests a possible role of p53. Further study is required to address the mechanisms of action of TSA in cells with TP53 mutation. The high sensitivity of primary CLL cells to HDACi at minute concentrations and their activity regardless of 17p-status are promising.

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Posterdiskussion
Hämostaseologie/sonstige Hämatologie

P455
NIPA phosphorylation and inactivation at G2/M is mediated by ERK2

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Regulated oscillation of protein expression is an essential mechanism of cell cycle control. The SCF class of E3 ubiquitin ligases is involved in this process by targeting cell cycle regulatory proteins for degradation by the proteasome. We previously reported cloning of NIPA (Nuclear Interaction Partner of ALK) in complex with constitutively active oncogenic fusions of ALK, contributing to the development of lymphomas and sarcomas. Subsequently we characterized NIPA as a F-Box protein that defines an oscillating ubiquitin E3-ligase. The SCFNIPA complex targets nuclear cyclin B1 for ubiquitination in interphase while phosphorylation of NIPA in late G2 phase and mitosis inactivates the complex to allow for accumulation of cyclin B1. Thus, SCFNIPA executes an important G2/M checkpoint control. We recently specified three serine residues Ser 354, 359 and 395 implicated in NIPA phosphorylation at G2/M. These data suggest a sequential NIPA phosphorylation, where initial Ser 354 and 359 phosphorylation is most crucial for SCFNIPA inactivation by dissociating the SCFNIPA complex. Here we aimed to find the kinase responsible in this initial most important phosphorylation step. Using in vitro kinase assays we identified both ERK1 and ERK2 to phosphorylate NIPA with high efficiency. Mutation of either Ser 354 or Ser 359 abolished ERK-dependent NIPA phosphorylation. Inhibition of ERK1/2 activity in cell lines by specific inhibitors resulted in decreased NIPA phosphorylation at G2/M. To differentiate between phosphorylation by ERK1 and ERK2, we combined cell cycle analysis with stable expression of microRNA's targeting both isoforms. To this end NIH/3T3 cells were retrovirally transduced with microRNAs targeting ERK1 and 2 and cell cycle progression was analysed by BRDU/PI labeling. Using this approach, we are able to show that ERK2 but not ERK1 mediates NIPA phosphorylation at G2/M. Furthermore, ERK2 silencing leads to a distinct phenotype in cell cycle progression with a delay of ERK2 knockdown cells at the G2/M transition. Thus, our data indicate, that the recently described divergent functions of ERK1 and ERK2 in cell cycle regulation could be in part due to the differential ability of these kinases to phosphorylate and inactivate NIPA at G2/M. Since checkpoint proteins such as NIPA are constitutively inactivated in tumor cells ERK2 might represent an interesting target to reconstitute important cell cycle checkpoint controls in malignant cells.

Disclosure: No conflict of interest disclosed.

P456
Inhibition of mTOR Activity by Novel Therapeutic Drugs – Consequences for NK Cell Anti-Tumor Functions

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Introduction: Protein kinases (PK) are responsible for most of the signal transduction in eukaryotic cells and control various cellular mechanisms including transcription, cell cycle progression and apoptosis. Abnormally activated signaling pathways like the phosphatidylinositol 3-kinase – protein kinase B – mammalian target of rapamycin (PI3K – AKT – mTOR) pathway are found in many malignancies. This led to the development of PK inhibitors (PKI) targeting different signaling molecules of this pathway and their clinical evaluation e.g. in sarcoma, myeloma or renal cell cancer. However, PKI may also influence anti-tumor immune responses of NK cells, which play an important role in tumor immunesurveillance. Here we employed PKI targeting PI3K, AKT, and mTOR to elucidate how signaling via this pathway is involved in the regulation of NK cell functions and whether interference with the respective signaling events affects NK cell reactivity. **Methods:** NK cells were cultured with tumor cells in the presence or absence of the PI3K inhibitors Wortmannin and BEZ235, the AKT inhibitor Triciribine as well as the mTOR inhibitors Sirolimus, Temsirolimus, and Everolimus. Cytotoxicity and IFN- production were determined by chromium release assays and ELISA, respectively. **Results:** Presence of Wortmannin and BEZ235 significantly inhibited IFN-? production and cytotoxicity of NK cells, which is in line with available data defining PI3K as a central regulator of target cell recognition by NK cells. In contrast, the AKT inhibitor Triciribine did not influence cytotoxicity and, tantalizingly, even enhanced NK cell IFN- production. The mTOR inhibitors Sirolimus, Temsirolimus, and Everolimus did not alter cytotoxicity but impaired NK cell IFN- production. **Conclusion:** After target cell recognition and the activation of proximal PK like PI3K, different signaling events are involved in the regulation of NK cell cytokine production and cellular cytotoxicity. While the activity of PI3K followed by the activation of mitogen-activated PK is known to be crucial for NK cell cytotoxicity, we identified the AKT – mTOR pathway as a yet unknown central component in the regulation of NK cell IFN- production. Our data demonstrate that, in PKI therapy of malignancies, the choice of the most suitable targeted kinase should take into account its role in the regulation of immune responses, especially with regard to the importance of NK cells in the immunesurveillance of residual tumor cells.

Disclosure: No conflict of interest disclosed.

P457
Treatment of Shulman syndrome (eosinophilic fasciitis) with rituximab: Case report and review of the literature

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Eosinophilic fasciitis (EF) is a rare and potentially debilitating scleroderma-like connective tissue disorder. We report a case of successful treatment with rituximab in a 25 year old man suffering from EF. Clinical presentation included progressive edematous swelling and induration of the skin associated with aching in the lower extremities and back as well as a reduced general condition. MRI showed fascial enhancement in fat

suppressed post gadolinium T1 weighted images. Diagnosis was confirmed by deep skin-to-muscle biopsy. Typically EF is associated with hyperglobulinemia, and abundance of lymphocytes and plasma cells in the fasciae is a consistent histological finding. Our hypothesis was to inhibit B cell activity through rituximab treatment and to consequently reduce disease activity as shown in other rheumatological diseases. Recent data indicate that B cells play a central role in autoimmunity, including autoantigen presentation and modulation of other immune cells. After allogeneic hematopoetic stem cell transplantation chronic graft versus host disease (GVHD) is associated with EF. It was observed that treatment with rituximab ameliorates the sclerodermoid manifestation of cGVHD. Initially our patient responded well to prednisolone but subsequently suffered from severe side effects of steroid treatment. A reduction of the steroid dose resulted in an increased disease activity. A combined treatment with azathioprine did not have any long lasting effect on EF. Only additional treatment with rituximab led to a sustained improvement of symptoms, clinical manifestation and MRI findings. It also markedly reduced the need of steroids which could be tapered off. Rituximab was well tolerated when given 4 cycles in a dose of 375mg/m² every 2 weeks, without adverse events. We conclude that B cells play an important role in the immunopathogenesis of EF and that selective depletion of B cells with rituximab could be considered in the treatment of EF.

Disclosure: No conflict of interest disclosed.

P458

Studies on cell cycle control mediated by the ubiquitin-ligase anaphase-promoting complex using live cell imaging and automated cell scanning

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Introduction: Genetic instability is a hallmark of cancer. We have recently demonstrated that deregulation of anaphase-promoting-complex (APC)-dependent proteolysis of cell cycle regulators can lead to genomic instability (Oncogene 2008; 27:907-17). The APC, with its activating subunits Cdh1 and Cdc20, is a ubiquitin ligase that plays a key role in the regulation of the metaphase to anaphase transition, mitotic exit and the establishment of a stable G1-phase. Knockdown of the activating APC-subunit Cdh1, which is active during mitotic exit and in G1, led to centrosomal aberrations, multipolar mitoses and anaphase bridges. Our work and the recently published Cdh1-knockout-mouse established Cdh1 as a tumor suppressor. Deregulated DNA replication as well as aberrant mitosis may be the underlying cause. However, further work is needed to better elucidate the exact nature of this genomic instability. APC-Cdc20 drives chromosome separation at the metaphase to anaphase transition and is the downstream target of the spindle assembly checkpoint. In yeast knockout of Cdc20 leads to a strong metaphase arrest. In contrast however, in human cells we could not detect a clear mitotic arrest, suggesting that the spindle checkpoint has additional targets in human cells (Cell Cycle 2009, 15;8:643-6). **Methods:** Live cell imaging allows the detection of more distinct phenotype variations. It is therefore a precious tool, which allows us to draw a much more detailled picture of the Cdh1- and Cdc20-knockdown phenotypes. Tagging of histones or tubulin with fluorescent proteins such as green fluorescent protein serves to visualize condensation of the chromatin, chromosome movement or remodeling of the cytoskeleton. **Results:** We generated monoclonal cell lines based on HeLa and U2OS that express tagged histones and tubulin. In these cell lines we were able to monitor the generation and fate of multipolar mitosis and anaphase bridges. Live cell imaging of the modified cell lines will allow us to better understand the phenotypes and exact nature of genetic instability after knockdown of the activating APC-subunits Cdh1 and Cdc20. Additional information will be obtained by a new technique in cell biology, known as Snap-Tagging. This technique allows the tagging and in vivo monitoring of target proteins of Cdh1 and Cdc20. Our current effort is to analyze monoclonal cell lines with inducible knockdown, which will lead to new and very precise insights of how deregulated APC-dependent proteolysis contributes to the generation of genomic instability and carcinogenesis. **Conclusion:** Using live cell imaging, we could generate more precise data concerning our knockdown phenotypes compared to classic methods in cell cycle analysis.

Disclosure: No conflict of interest disclosed.

P459

Dissecting the malignant platelet phenotype:platelet contents in cancer

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Introduction: Abundant in vivo data from animal studies indicate that platelets play a key role in tumor dissemination, metastasis, and angiogenesis. Platelets contain a large spectrum of mediators that may both inhibit and stimulate angiogenesis, immunosurveillance, plasmatic coagulation, or tumor growth. We therefore hypothesized that patients with metastatic solid tumors may display a specific „malignant platelet phenotype“, that may reflect a „pro-tumor progression“ hematopoietic environment. **Methods:** Platelet activation in percent of P-selectin positive platelets and platelet contents (i.e. plasma and platelet count-corrected serum levels of VEGF-A, CXCL12 [SDF-1], CXCL4 [pf4], and thrombospondin-1) were analyzed. Patients were eligible if they had a first diagnosis of metastatic malignancy and had not taken any medication during the last 14 days before venipuncture. EDTA blood was directly analyzed for cell counts by a SYSMEX cell counter. CPDA1 blood was prepared for flow cytometry. Serum and Citrate collection tubes were used for the preparation of serum and platelet poor plasma. Healthy age and sex-matched subjects were used as controls. **Results** (mean ± SEM):

	tumor patients (n = 43)	healthy controls (n = 40)	P (indep. t-test)
Platelet counts (Plt/µl)	301,558 ± 17,489	234,111 ± 5,946	< 0.001
CD62P-positive (%)	1.14 ± 0.20	0.24 ± 0.03	< 0.001
Tsp-1 (plasma, ng/ml)	598.40 ± 162.45	372 ± 51.35	0.202
Tsp-1 (serum, ng/ml)	1825 ± 138	2823 ± 127	< 0.001
VEGF-A (plasma, pg/ml)	44.03 ± 11.91	10.09 ± 2.27	0.009
VEGF-A (serum, pg/ml)	86.76 ± 9.71	47.34 ± 4.55	< 0.001
CXCL4 (plasma, ng/ml)	153 ± 23	83 ± 12	0.03
CXCL4 (serum, ng/ml)	702 ± 39.93	1117.59 ± 44	< 0.001
CXCL12 (plasma, pg/ml)	1965 ± 72	1847 ± 47	0.185
CXCL12 (serum, pg/ml)	301 ± 21	396 ± 16	< 0.001

Tumor patients had increased platelet counts and significantly elevated percentages of activated platelets as measured by CD62P expression. Moreover, the platelet content of VEGF-A in cancer patients was significantly increased compared to healthy controls, while thrombospondin-1, platelet factor4/CXCL4 (pf4), and CXCL12 were decreased. **Conclusion:** These results suggest that “malignant platelets” display a proangiogenic, procoagulant phenotype.

Disclosure: No conflict of interest disclosed.

P460

Platelets release TGF- upon interation with tumor cells and thereby impair tumor immunosurveillance by downregulating expression of the “danger detector” NKG2D on NK cells

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Introduction: NK cells play an important role in cancer immunosurveillance because they recognize and eliminate malignant cells and thereby prevent both local tumor progression and metastatic spread. Their reactivity is governed by various activating and inhibitory molecules expressed on target cells and by the reciprocal interaction with other hematopoietic cells like dendritic cells. We and others demonstrated previously in murine tumor models that thrombocytopenia inhibits metastasis which is reversed by additional depletion of NK cells (Jin et al., Nat Med 2006; Nieswandt et al., Cancer Res 1999). These data suggest that thrombocytopenia indirectly inhibits tumor dissemination by allowing NK cells to exert their anti-tumor effector functions and suggest that platelets are an important additional player in NK cell - tumor interaction. However, yet nothing is known regarding the mechanisms underlying tumor cell – platelet - NK cell interaction, especially in humans. **Methods:** Platelet-derived soluble factors (releasate) were ob-

tained by incubation of platelets with tumor cells or stimulation with platelet agonists including ADP, thrombin, and collagen. TGF-ß levels in releasate were determined by ELISA. Expression of NKG2D on NK cells was determined by FACS. Modulation of NK cell cytotoxicity, IFN-? production, granule mobilization and apoptosis by platelet-derived soluble factors was investigated by chromium release assays, ELISA and FACS. **Results:** Platelet releasate, secreted upon interaction and coating of tumor cells or after stimulation with classical platelet agonists, impaired NK cell anti-tumor reactivity resulting in diminished granule mobilization, cytotoxicity and IFN-? production. Impaired NK cell reactivity was not due to induction of apoptosis but mediated by downregulation of the activating immunoreceptor NKG2D on NK cells by platelet-derived TGF-ß. Neutralization of TGF-ß not only prevented NKG2D downregulation, but also restored NK cell anti-tumor reactivity. **Conclusion:** Our data provide a molecular basis for the previously described influence of platelets on NK cell tumor immunosurveillance and suggest that therapeutic intervention in tumor cell-platelet interaction and the resulting TGF-ß release by platelets may serve to enhance anti-tumor immunity.

Disclosure: No conflict of interest disclosed.

P461

Platelet Function Testing using the Impact-R in Monitoring Platelet Substitution

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Introduction: To date, platelet transfusions are usually monitored by measuring the post-transfusion platelet count and/or the corrected count increment (CCI). However, an increase in the platelet count does not necessarily correspond to an improvement in primary haemostasis. Although the response to platelet transfusions can be somewhat assessed by the physician treating the affected patient, the real effect remains frequently questionable. The application of platelet function assays might therefore be helpful in the assessment of platelet transfusions. **Methods:** We investigated 66 single-donor platelet transfusions (Median 2.5 x 1011 platelets/unit) to patients who underwent allogenic blood stem cell transplantation. Blood samples were obtained before and after transfusion. EDTA-blood samples were used to measure the platelet count and corrected count increment (CCI). Citrated blood samples were used to investigate platelet adhesion and platelet aggregation using the Impact-R device (DiaMed, Ottobrunn, Germany). **Results:** All patients were thrombocytopenic before transfusion (Median 6/nl; Range 0 – 21/nl), and platelet transfusion resulted in an increase of platelet count after 1 h (Median 24/nl; Range 1 – 53/nl). The Median 1 h CCI was 12 (Range 0 – 33). Median platelet adhesion as expressed by the surface coverage (SC) measured by the Impact-R increased from 0.3 (Range 0.1 – 2.6) to 0.7 (Range 0.1 – 8) 1 h after transfusion. The median platelet aggregation (AS by the Impact-R) was 45 (Range 10 – 114) before and 43 (Range 11 – 183) 1h after transfusion. Multi-Regression analysis showed a strong correlation of SC as measured by the Impact-R (Coef 5.46; p>0.001; 95 % Conf. Interval 2.407458 - 8.521375) to 1 h CCI. No correlation was found for AS. **Conclusions:** The Impact-R is very simple to handle and requires a very small volume of blood (0.2 ml). In our experience, the test demonstrated reproducible results, even in patients with platelet counts < 30/nl. Furthermore, the results of the Impact-R are independent of other variables (e.g. haematocrit and coagulation factors). However, the Impact-R does not provide any additional information in patients that require platelet transfusions, since we found a strong correlation of platelet adhesion (SC) and CCI after 1 h of transfusion.

Disclosure: No conflict of interest disclosed.

P462

Acquired Coagulopathy Caused by Intoxication with the Superwarfarin-Type Anticoagulant Rodenticide Flocoumafen

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A 28 year-old woman was transferred from a local hospital to our hematology department due to a two-month history of unclear coagulopathy with spontaneously occurring bruising, elevated international normalized ratio (INR), activated partial thromboplastin time (aPTT) and anemia. Similar symptoms had occurred some weeks before. Despite administration of vitamin K and a prothrombin complex concentrate, coagulation tests had only temporarily returned to normal. Upon admission to our hospital the patient presented with mild signs of ecchymosis and otherwise normal physical examination. Her hemoglobin level was 8.9 g/dl, INR 4.8 and aPTT 47 sec. Fibrinogen, platelet levels, liver and kidney function tests were normal. Further testing revealed an isolated severe deficiency in vitamin K-dependent clotting factors. Neither the patient nor family members had plausibly ever taken anticoagulant drugs (e.g. phenprocoumon). This prompted us to consider anticoagulant rodenticides as causative agents. Indeed, flocoumafen, a rodenticide belonging to the superwarfarin-family, was detectable by liquid chromatography (LC-MS-MS) at 61 ng/ml in the patient's serum. Accidental ingestion through contaminated food seemed unlikely since other family members did not display signs of bleeding nor flocoumafen in serum. Psychiatric evaluation did not provide evidence for suicidal tendencies or mental disorders leaving the mode of intoxication obscure. Treatment with vitamin K rapidly normalized clotting tests, and the patient was discharged and instructed to continue oral vitamin K substitution. During follow-up visits over a period of 10 weeks, laboratory and coagulation tests remained normal and flocoumafen levels in serum gradually fell below the detection limit (<5 ng/ml). In contrast to the reported long half-life of flocoumafen in rodents (220 days), consecutive measurements in patient's serum revealed an approximated half-life of 6.7 days. Superwarfarin poisoning has an increasing incidence (16,000/year in the US) with most cases affecting children below the age of six after accidental ingestion of rat poison. Due to long half-life and increased hepatic accumulation, supra-therapeutic doses and prolonged administration of vitamin K are needed to overcome hemorrhagic diathesis. Although severe or even fatal bleeding is rare, clinicians should consider superwarfarin poisoning when confronted with vitamin K-dependent coagulopathy resistant to standard doses of vitamin K.

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Thrombocytopenia following aortic valve replacement

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Introduction: Recently increased thrombocytopenia following implantation of biostentless aortic valves has been reported. However, incidence, duration and cause of the thrombocytopenia after aortic valve replacement using the stentless bioprosthesis are still unclear. **Materials and Methods:** In the current study we retrospectively analyzed platelet counts of 239 patients receiving aortic valve replacement between 2004 and 2008. 165 patients have received a stentless bioprosthesis (Sorin Freedom Solo stentless aortic valve; biostentless group) and 74 patients a stented Mitroflow pericardial aortic valve (stented group). Preoperative platelet counts and those of postoperative days 1 to 12 were compared. **Results:** No differences in preoperative platelet counts were observed between patient groups. Interestingly, within the biostentless group a significantly lower platelet count was observed postoperatively, with a mean platelet count below 10^5/µl on postoperative day 2. In con-

trast, the mean platelet count in the stented group did not fall below 10^5/µl at any time point. Common toxicity criteria grade 3 and 4 thrombocytopenia (< 50.000/µl and < 25.000/µl respectively) was observed in 62 patients (38 %) in the biostentless group compared to only 7 patients (9 %) in the stented group. The clinical data indicate that there was no difference in bleeding complications between the two groups. However, 17 patients with grade 3 or 4 thrombocytopenia in the biostentless group did not reach a platelet count above 50.000/µl until postoperative day 7. Follow up on these patients revealed that 8 of them died within 3 months following discharge. The cause of death is currently being clarified. **Conclusion:** Our data clearly indicate that aortic valve replacement using a stentless bioprosthesis is related with a lower postoperative platelet count, apparently not associated with an elevated bleeding rate. However, a subgroup of patients who do not recover to a platelet count of over 50.000/µl on postoperative day 7 might have a higher short term mortality.

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P464

10 year results from the ReFacto Pharmacovigilance evaluation – one decade of monitoring safety and efficacy in daily clinical practice

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Background: Non-interventional trials are generally accepted for monitoring the treatment of rare diseases like haemophilia. The pharmacovigilance evaluation (PE) of ReFacto has been ongoing in Germany and Austria for approximately 10 years. Its aim is to continuously monitor safety and efficacy of treatment of haemophilia A with Moroctocog alfa under routine clinical conditions. **Methods:** The study is a non-interventional trial. Patients with haemophilia A of any severity, treated with Moroctocog alfa could be included in the study. Safety is assessed by documentation of all (serious) adverse events during treatment with ReFacto. Special focus is on the development of inhibitors in PTPs and PUPs. Efficacy assessment is performed e.g. by evaluating the number of exposure days per bleeding episode. **Results:** Until April 2009, 287 patients were recruited in 60 centers in Germany and Austria. 24 (8.4%) were previously untreated (PUPs) and 263 (91.6%) previously treated patients (PTPs). 231 patients (80.5%) suffered from severe haemophilia A (FVIII:C <1%). 27 PTPs had a positive inhibitor history at baseline. *De novo* inhibitors developed in 4/263 (1.8%) PTPs and 3/24 (12.5%) PUPs. Treatment was effective with a median number of 1.33 exposure days per bleeding episode. **Conclusions:** The PE of ReFacto® is the first long-term analysis of a currently marketed FVIII product in Germany and Austria under routine clinical conditions. Data from ten years duration and 287 patients confirm the safety and efficacy of ReFacto® in treatment of haemophilia A in daily clinical practice.

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Westfeld,M.: Anstellungsverhältnis oder Führungsposition: Wyeth Pharma GmbH

P465

2 Years after Introducing a Mobile Electronic Patient Diary in Hemophilia – Insights from Adoption Rates and Usage Data

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Background and Objectives: Electronic Health Records are believed to improve transparency, empower patients, and enhance overall quality of care. We introduced a PDA-based mobile electronic patient diary (Haemoassist®) for haemophilia home care documentation (on-demand treatment and prophylaxis), which has been described previously. The system is adaptive and constantly adjusted to meet patients' needs. For the first time we report findings from initial adoption rates and usage data. **Methods:** Data collection and storage of Haemoassist® is carried out by an independent research organization, therefore aggregated data was available for this evaluation. We descriptively analyzed the chronological sequence of participating patients, the total number of PDAs given to patients, and the number of electronically submitted documentations (ESD) per month. **Results:** During the first 27 months of operation (Jan 2007 to Apr 2009), the number of participating patients increased from 11 to 98, who conducted a total of 9480 ESDs. The mean percentage of active (>1ESD per month) patients per month increased from 57% (1st half 2007) to 67% (2nd half 2008). The average percentage of prophylaxis patients increased from 73% (1st half 2007) to 87% (2nd half 2008). Average monthly documentation frequency increased from 5.6 (Q1/2007) to 7.9 (Q1/2009) ESDs/patient, with high month-to-month variation (average fluctuation 33.8% compared to the previous month). **Conclusions:** High month-to-month variation in Haemoassist® usage could be the result of varying numbers of bleedings and subsequent ESDs in participating on-demand patients, whereas irregular patient compliance needs to be taken into account as well. Despite an increase of prophylaxis patients in the Haemoassist® cohort, we believe that the higher frequency of ESDs per patient per month -together with an overall rising number of participants and a larger share of active patients- indicates a growing acceptance of the Haemoassist® system among Hemophiliacs.

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P466

Recurrent immunethrombocytopenic purpura (ITP) after splenectomy and successful treatment withembolization of an accessory spleen.

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Introduction: Immune thrombocytopenic purpura (ITP) is an autoimmune disorder characterized by peripheral destruction of opsonised platelets in the reticuloendothelial system, particularly the spleen. Steroids are the standard first-line therapy with a response rate > 80%. However, thrombocytopenia often recurs and splenectomy is a standard surgical treatment for chronic ITP, but it is burdened by the risk of major bleeding events. In some cases accessory spleens can cause recurrent or even refractory disease. Method: We report the case of a female ITP patient first diagnosed in 01/97 when she was 42 years old. She responded well to steroids, but two years later the disease recurred and was refractory to treatment with steroids. Splenectomy was performed and she had a complete remission. 05/03 thrombocytopenia recurred and was successful treated with rituximab. Three years later again the disease recurred, now with only a short response to rituximab and steroids. There was a transient response to immunoglobulin treatment and CT scans showed an accessory spleen. We decided to embolize the accessory spleen. **Results:** Embolization was performed by the right femoral access without any complications. The platelet counts rose to > 100.000 continuously within one year and is now in the normal range. No other treatments were required. **Conclusion:** In patients with recurrent or refractory ITP a CT scan should be performed, which may detect an accessory spleen. Embolization of the accessory spleen could be an effective treatment with a sustained response. Further investigation of this treatment option is warranted.

Posterdiskussion
Infektiologie

P467

Intracellular concentrations of antifungals in different compartments of the peripheral blood

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Peripheral blood mononuclear cells (PBMC) and polymorphonuclear leukocytes (PMN) are important components of the host defense against invasive fungal infections (IFI). However, information on the uptake and elution of antifungal drugs by these compartments of the peripheral blood is limited. Prior laboratory-based experiments showed that intracellular concentrations of fluconazole and voriconazole were increased compared to the surrounding medium. (Ballesta, S., et al., Pascual, A., et al.) Comparing posaconazole concentrations in human plasma and alveolar cells yielded a 33 fold intracellular concentration, i.e. for C_{max} as well as the area under the curve (AUC). (Conte, J.E., Jr., et al.) However, up to now no data on the intracellular concentrations of recently approved antifungals in PBMC and PMN has been published. We suspect that the efficacy of antifungals might correlate with their intracellular concentrations. Hence, we are developing a method to determine the intracellular concentrations of a number of antifungals, i.e. anidulafungin, caspofungin, isavuconazole, micafungin, posaconazole, and voriconazole by liquid chromatography tandem mass spectroscopy in the different compartments of the peripheral blood. Patient samples were collected as part of the Cologne biobank protocol on *Improving Diagnosis of Severe Infections in Immunocompromised Patients* (ISI). Whole blood, collected in EDTA-treated tubes was separated by double-discontinuous Ficoll-Hypaque density gradient centrifugation into PBMC-, PMN- and red blood cell-fractions (RBC). The washed cells were counted and fractured in methanol by sonication. The concentrations were determined by high performance liquid chromatography tandem mass spectroscopy (LC-MS/MS). The validation of the quantification method was performed with respect to linearity, intra- and inter-day accuracy and precision. The accuracies of all concentrations above the limit of quantification were within ±20%. The best fit for the calibration curves was obtained by using a weighting factor of the reciprocal concentration. The correlation coefficients of these curves were 0.99 or better. Recently analyzed peripheral blood cells of patients receiving posaconazole showed that posaconazole is increased in PBMC and PMN. To our knowledge, we are establishing the first method to determine azole and echinocandin antifungals within one sample.

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P468

Impact of Posaconazole as Prophylaxis for Invasive Fungal Infections in High-Risk Patients – a retrospective analysis on a haematological unit.

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Background: Invasive fungal infections (IFI) are major causes of death in high-risk haematological patients (pts) receiving induction therapy for acute leukaemia or intensified immunosuppression due to acute or chronic GVHD following allogeneic stem cell transplantation (SCT). Recently two randomised studies showed the efficacy of a posaconazole prophylaxis (PP) in these pts to prevent IFI resulting in strong recommendations of several national and international professional associations, to use PP. As we started PP on our leukaemia and transplantation unit in summer 2007, we retrospectively analysed the impact of PP on the incidence of possible, probable or proven IFI in this group of pts. **Methods:** Incidence of IFI according to the revised EORTC criteria, published 2008 was reviewed retrospectively in a group of high risk pts treated in our unit one year before the start of PP compared to the same group in the following year with PP. **Results:** 56 pts were analysed in the group without PP (noPP) compared to 34 pts with PP. 22 pts received double induction therapy due to AML in the noPP vs. 20 in the PP group resulting in 44 vs. 40 neutropenic episodes. 34 pts after SCT were analysed in the noPP group vs. 14 in the PP group, resulting in a total of 78 risk episodes (noPP) vs. 54 (PP). In the no PP group 62 febrile episodes (83%) were analysed compared to 45 (83%) in the PP group. Possible, probable or possible IFI were found in 22 (28%), 12 (15%) and 3 (4%) cases out of 78 episodes in the noPP group resulting in an overall incidence of 47%, compared to 13 (24%), 5 (9%) and 1 (2%) of cases of possible, probable or proven IFI out of 54 risk episodes in the PP group. The difference did not reach statistically significance, but the overall risk reduction was 12%. There was a slight tendency towards lower mortality in the PP group with overall 6/34 (18%) compared to 13/56 (23%) in the noPP group, however, this group contained a much higher portion of transplanted patients. **Conclusion:** Our data, collected in every day clinical practice add further evidence to the advantage of a PP strategy in this group of high-risk pts. However more data are urgently needed to assess the impact of PP on the incidence and pattern of fungal infections and the strategies to be used in pts with persisting fever and pulmonary infiltrates receiving PP.

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P469

A 3-day short course of Palifermin before HD-therapy is not sufficient to reduce Melphalan induced toxicity and need for supportive care in patients with multiple myeloma and renal failure

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Introduction: We have previously reported on the efficacy of a short, 3-day course of Palifermin to prevent HD-Melphalan associated mucositis in patients with multiple myeloma. In the present analysis we focus on a group of patients with impaired renal function who are known to be especially prone to increased treatment toxicity. **Methods:** Between formal approval of Palifermin in the EU in October 2005 and October 2008 a total of 67 consecutive patients with multiple myeloma received 3 intravenous doses of 60ug/kg Palifermin on consecutive days before high-dose melphalan (200mg/m^2, n=56, Group A, or 140mg/m^2 in patients with a creatinine clearance <50ml/min, n=11, Group A^f). Following transplantation of a median of 3.2x10^{6}CD34$^+$ cells/kg (range 1.8-18) patients received G-CSF to enhance granulocyte recovery. Data on haematopoietic reconstitution, toxicity and need for supportive care were analysed within the palifermin group and compared with two previously published patient groups from our institution who had received Pegfilgrastim but no Palifermin (Group B, n=21) and patients who had neither received Palifermin nor G-CSF (Group C, n=21). **Results:** In group A, Palifermin and G-CSF were very effective in reducing toxicity in terms of hospitalisation ($p<0.05$), need for parenteral narcotic analgesia (PNA, $p<0.05$) and parenteral nutrition (PEN, $p<0.05$) in comparison to groups B and C. However, patients with renal failure (Groub A^f) had a significantly higher risk for severe mucositis (64% versus 16%, $p<0.002$) and a longer duration of severe mucositis (median 9 days, range 6-43 versus median 7 days, range 4-11) despite prophylactic treatment with Palifermin and G-CSF. As a consequence, secondary parameters indicative for treatment toxicity like hospitalisation, PNA and PEN were also increased in group A^f. **Conclusion:** A short, 3-day course of palifermin is not sufficient to prevent severe mucositis in patients with impaired renal function who are at a high risk to suffer from increased toxicity of high dose Melphalan. Prolonged Palifermin dosing and/or additional strategies to reduce treatment toxicity in these patients are needed.

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P470

Aminoglycoside-free interventional antibiosis in patients undergoing haemopoietic stem cell transplantation

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Introduction: The position of aminoglycosides within interventional antibiosis in the early phase after stem cell has not been clarified so far despite their use can induce serious renal impairment. Data from conventional haematological patients suggests that they can either be omitted or replaced by other drugs. **Methods:** To investigate this question early-infection data from 152 patients undergoing 195 allogeneic and autologous stem cell transplantations were investigated. Prophylaxis and treatment of infections followed international standard, however, aminoglycosides were omitted when ever possible. **Results:** The overall-incidence of infections was 77,9% (152/195) and 67 patients suffered from more than one episode. Fever of unknown origin and bacteriaemia/septicaemia dominated the spectrum of infections. Spectrum of isolated pathogens showed no increase of Gram-negative rods. The overall-response to interventional regimen consisting of ß-lactam or carbapenem plus glycopeptides was 47,7%. Aminoglycosides were given in three patients in the late course of disease. Overall mortality was 15/195 (7,7%) and clearly related to infection in nine cases mostly due to mould infection. A comparison with previous publications gave no hint for inferiority of 'aminoglycoside-free' antibiosis. **Conclusion:** The present analysis supports the policy to omit aminoglycosides in the therapy of early infections in patients undergoing stem cell transplantation to avoid additional nephrotoxicity.
Stichwort: Klinische Fragestellungen

Disclosure: No conflict of interest disclosed.

P471

Analysis of antibiotic susceptibility profiles among bacterial isolates in patients with febrile neutropenia under piperacillin-tazobactam first line therapy on the haematology unit from 1996 – 2006 - a single centre study

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Background: Effective empiric antibiotic therapy of FN has a high potential to induce increased bacterial resistance. Under standard use of quinolones and cephalosporines as first line therapy emerging antibiotic resistance has been described in the literature. In our centre, piperacillin–tazobactam (pip-taz) is given as first-line therapy since 1996. In this retrospective analysis we evaluated, the incidence of antibiotic resistance during long-term use of pip-taz. **Methods:** Data of isolates from normally sterile body sites and the antibiotic susceptibility were collected from 1996-2006 in the department of haemato-oncology, University of Bonn. Documentation of susceptibility profile to the following antibiotic categories was performed: penicillines, cephalosporines carbapenemes, aminoglycosides, macrolides, tetracyclines, glycopeptides, and fluoroquinolones. For the analysis of the susceptibility profile organisms were categorised into two groups: good susceptibility or low susceptibility/resistant. The time period was devided into two intervals: 1996-2001 (period 1) and 2002-2006 (period 2). Data were analysed using SPSS 17. **Results:** A total of 426 isolates were identified. The most frequent organisms were CNS (105; 24.6%), E. coli (59; 13.8%), P. aeruginosa (45; 10.6 %) and Enterococcus spp (34; 16%). St. aureus was identified only in 10 patients and only three VRE isolates were found. Most pathogens were detected in blood cultures (298; 70%). Concerning the susceptibility profile E. coli showed an increase of resistance to fluoroquinolones in period 2 compared to period 1 (12/32 vs. 2/23; 37.5% % vs. 8.7%; p: 0.03).. Pseudomonas aeruginosa showed increasing resistance to acylaminopenicillines (20/22 vs. 13/23; 91% vs. 56.5%; p: 0.007) Neither an increase in MRSA nor in VRE was found. None of the organisms showed a significant increase of resistance to pip/taz, cephalosporines of 1. and 2. or 3. generation, carbapenemes, aminoglycosides, macrolides, tetracyclines or glycopeptides. **Conclusion:** The development of antibiotic resistance as shown in this analysis is probably due to the general development of resistance profile regardless of pip-taz administration. We assume that since it is concordant with current trends there were no significant increases of antibiotic resistances in FN correlated to long-term first line therapy with pip-taz.

Disclosure: No conflict of interest disclosed.

P472

Antineoplastic chemotherapy in patients with Methicillin-resistant Staphylococcus aureus (MRSA)

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Introduction: Methicillin-resistant Staphylococcus aureus (MRSA) is a major nosocomial pathogen, causing serious morbidity and mortality in immunosuppressed patients. It is well known that patients in reduced general health succumb to infections with MRSA. Antineoplastic chemotherapy causes immunosuppression and thus there is concern if such patients should proceed to treatment without delay or dose reduction. There are presently no guidelines with appropriate provisions for antineoplastic chemotherapy in cancer patients with MRSA colonization or infection. **Methods:** Of all patients treated with antineoplastic chemotherapy in our institution (Medizinische Klinik IV, UK Aachen) between 1997 and 2008, 1.3% had infection or colonization with MRSA. We retrospectively analyzed clinical outcome of these 32 patients undergoing antineoplastic chemotherapy for solid or hematological malignancies in our institution. **Results:** In our patients, MRSA was detected at multiple sites. Fourteen patients were found to be colonized with MRSA only, whereas 18 patients had colonization and/or infection. MRSA sepsis (fever and detection of MRSA in at least one blood culture) occurred in 14 cases. Interestingly, at the time of MRSA sepsis, neutrophil counts were less than 500/µl in 36% of our patients. Overall, fatal complications due to MRSA occurred in two patients. For all patients with MRSA sepsis, mortality was 14%. **Conclusions:** Our results with a very limited number of patients support the contention that antineoplastic chemotherapy may well be administered in patients with MRSA. Larger prospective trials are needed to elucidate the influence of MRSA colonization or infection on complications and outcome of antineoplastic chemotherapy and to develop guidelines for dose reduction or treatment delay.

Disclosure: No conflict of interest disclosed.

P473

Epidemiology of Aspergillus terreus at the University Hospital of Cologne

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Introduction: Environmental contamination with *Aspergillus* spp. plays a key role in infection transmission of invasive aspergillosis (IA). High environmental conidia loads have been associated with outbreaks of IA. High-efficiency particulate air (HEPA) filtration was shown to reduce incidence of the disease. Still, the relation between environmental contamination and patient colonization with these pathogenic fungi remains uncertain. In this study, we evaluate the epidemiology of *A. terreus, A. fumigatus, A. niger,* and *A. flavus* strains collected from air samples at the University Hospital of Cologne (UHC), and correlate findings with colonization of the upper respiratory tract of inpatients. **Methods:** Environmental samples are collected prospectively over a 12

month period once a week inside (hematological wards) and outside the UHC. Samples are incubated and fungal colonies identified and quantified. At the same time, patients are screened for nasal colonization with *Aspergillus* spp. via nasal swab sampling. We aim to determine frequency and distribution of these fungi and demonstrate any relationship of their epidemiology with seasonal conditions. Genotyping of *A. terreus* isolates will be performed to demonstrate whether there is a strain similarity or rather a genomic diversity of *A. terreus.* **Results:** A total of 4,919 air samples were collected over a 12 month period, 2,707 from inside and 2,212 from outside the UHC. The outdoor density of *Aspergillus* spp. ranged from 4.4 CFU/m^3 to 37.4 CFU/m^3 (mean 17.5 CFU/m^3), reaching its' peak in November. Nine *A. terreus* strains were detected; 3 strains from outside and 6 from inside the hospital. Inside the UHC, an average of 4.6 CFU/m^3 was measured. *A. fumigatus* (92.9%) was predominant both inside and outside of the hospital, followed by *A. niger* (5.3%), *A. flavus* (2.1%), and *A. terreus* (0.2%). A total of 855 nasal swabs from 240 patients have been analyzed, a single one was positive for *Aspergillus* spp. **Conclusions:** *Aspergillus* spp. are routinely detected in samples from the environment of the University Hospital of Cologne. The most frequently detected fungus was *A. fumigatus. A. terreus* was rare. These findings differ from other hospitals, possibly caused by different seasonal and environmental conditions. The average of 4.6 CFU/m^3 inside the hospital may be of concern, but was much lower than the outdoor density of *Aspergillus* spp. at all times. Nasal swabs are not helpful in identifying patients with *Aspergillus* spp. colonization of the upper respiratory tract.

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Cornely,O. A.: Anstellungsverhältnis oder Führungsposition:Leiter des Klinischen Studienzentrums Infektiologie II; Medizinischer Leiter des Zentrums für Klinische Studien der Medizinischen Fakultät Köln
Beratungstätigkeit:Astellas, Basilea, F2G, Gilead, Pfizer, Merck, Mölnlycke, Nektar, Schering-Plough, and Zeneus; Honorare:Honorare für Vorträge: Astellas, Gilead, Merck, Pfizer, Schering-Plough, SpePharm, United Medical; Finanzierung wissenschaftlicher Untersuchung:Astellas, Bayer, Basilea, Genzyme, Gilead, Pfizer, Merck, Optimer, Schering-Plough, Vicuron; Gutachtertätigkeit:Esther Initiative, Deutsche Gesellschaft fuer Technische Zusammenarbeit (GTZ)

P474

Tigecycline in febrile neutropenic patients with haematological malignancies: A retrospective case documentation in four university hospitals

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Introduction: Tigecycline (TGC) is a first-in-class glycylcycline with an expanded spectrum of activity against Gram-positive, Gram-negative, anaerobic, and atypical bacteria. Although there is little data with TGC for the treatment of febrile neutropenia (FN), we observed that occasionally critically ill neutropenic patients unresponsive to other antibiotics responded to TGC. Aim of this study was to collect data of patients treated with TGC in tertiary care centres to analyse effectiveness and toxicity of TGC in FN. **Methods:** Data of febrile neutropenic episodes treated with TGC were retrospectively collected. Baseline data of the patients, therapy of haematological malignancy, treatment of infection and adverse events were documented. Success was defervescence (>= 7d) in absence of any sign of continuing infection. Data were analysed using SPSS. 14.0.1.**Results:** Data of twenty-five patients (9 female, 16 male) were collected so far. Median age was 57 years (range 31-75 years). All patients (pts.) had haematological malignancies (11 AML, 9 ALL, 2 NHL, 2 Multiple Myeloma, 1 AL Amyloidosis) and 6 underwent allogenic SCT. All pts. were neutropenic at the onset of fever with a median duration of 28 days (d) (range 6-69 d). The type of infection was pneumonia in 17 pts., 4 Bacteriämia, 2 FUO, once osteitis and once gastrointestinal infection. Before initiation of TGC, 20 pts. received two lines or more of antibiotic therapy and five pts. received just one antibiotic regimen before TGC. TGC (100 mg/d) was given for a median of 10 d (range 1-18 d). In 10/25 pts. an invasive fungal infection was considered as possible and in three pts. a viral infection was assumed.Treatment was successful in 9/25 pts. (36 %). Excluding the pts. with probable viral infection response rate was 41 %. In pts. with prolonged neutropenia (=/> 28 days) response to TGC was significantly lower (20 % vs. 67.7 %; p= 0,04). Five pts. died due to infection. Grade 3-4 toxicity occurred in 5/25 pts. (20%). Twice the liver was affected, once nephrotoxicity was found, once diarrhea occurred and once TGC was discontinued due to nausea. **Conclusions:** Although this analysis included very ill patients, our results showed promising response rates to TGC. Since success rates of third line antibiotic therapy in FN are generally very low, we consider TGC a promising alternative for salvage antibiotic therapy in FN. This case documentation will be continued.

Disclosure: No conflict of interest disclosed.

Posterdiskussion
MDS I

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Fluorescent microscopic analysis of the subcellular distribution of the mitochondrial protein Mortalin in CD34+ cells from MDS-patients and normal G-CSF mobilized CD34+ cells from apheresis products

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Introduction: Mortalin (HspA9B) is a mitochondrial chaperone that is required to import proteins through the inner mitochondrial membrane. A mortalin gene mutation results in MDS-like impairment of hematopoiesis in zebrafish (Craven et al., Blood 2005). Chen TH et al (ASH 2007) demonstrated that knockdown of Mortalin in primary human $CD34^+$ progenitor cells results in abnormal proliferation, increased apoptosis, and altered differentiation, key features of ineffective hematopoiesis, in a dose dependent manner. Since different intracellular distributions of Mortalin in the cytoplasm of mortal versus immortalized cells have been described and mitochondrial dysfunction seem to be involved in the pathogenesis of MDS, we examined whether Mortalin shows different intracellular distribution in G-CSF mobilized $CD34^+$ cells from healthy donors as compared with primary $CD34^+$ cells from MDS patients. **Methods:** After in vitro culture of $CD34^+$ cells in the presence of TPO, SCF and FLT3L for 3 days, anti-Mortalin immunofluorescent staining was performed. To associate the anti-Mortalin staining with mitochondria, mitochondria were counterstained with MitoTracker. We studied cells that had polarized in response to the cytokines as well as round cells. **Results:** In small round $CD34^+$ cells, Mortalin showed an even intracellular distribution in MDS as well as in healthy controls. In the polarized cells, Mortalin was highly enriched at the trailing edge, i.e. the uropod. No differences were observed between healthy (n=7) and MDS (n=8) $CD34^+$ cells. We further looked at mitochondria in mitotic $CD34^+$ cells to see whether there is asymmetric distribution that might affect the cell fate of daughter cells with regard to self renewal and differentiation. However, asymmetric distribution was not observed in any of the mitotic $CD34^+$ cells from 7 healthy samples (10 to 100 mitosis in each sample, n=409). As the number of $CD34^+$ cells in bone marrow aspirates from MDS patients was small, we detected only 3 and 4 mitoses in two MDS-patients, respectively. Here, the distribution of mortalin between daughter cells was symmetrical, too. **Conclusion:** According to our observations, mitotic segregation of Mortalin, and therefore mitotic segregation of mitochondria, does not appear to be involved in determining cell fate of early hematopoietic progenitors. Furthermore, MDS bone marrow cells do not show aberrant intracellular distribution of mitochondrial Mortalin.

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Efficacy and Safety of Deferasirox (Exjade®) during 1 Year of Treatment in Transfusion-Dependent Patients with Myelodysplastic Syndromes: Overall and Austrian/German/Swiss Results from EPIC Trial

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Background: Many patients (pts) with MDS are susceptible to iron overload (IOL) from ongoing blood transfusions and increased dietary iron absorption. The efficacy and safety of deferasirox (DFX) in pts with various underlying anemias was evaluated in the large EPIC study. Here, data for MDS pts are presented. **Methods:** The EPIC study was a 1-yr, openlabel, single-arm, multicenter trial. Pts with transfusion-dependent MDS and IOL received an initial DFX dose of 10–30 mg/kg/day. Primary efficacy endpoint was the change in SF from baseline at 12 mths. Adverse events (AE) and laboratory parameters were monitored for safety. **Results:** Overall 341 MDS pts with median baseline SF of 2730 (range 951–9465) ng/mL were enrolled. Mean transfusion duration was 3.6 yrs, and pts received a mean of 116.4 mL/kg of blood in the previous yr. Almost half (48.4%) of all pts had not received any prior chelation therapy. Overall, mean actual dose of DFX over 1 yr of treatment was 19.2±5.4 mg/kg/day. At 12 mths, there was a significant reduction in median SF from baseline (by LOCF: –253.0 ng/mL; P=0.0019). Interestingly, at the German, Austrian and Swiss centers (n = 82; median baseline SF =3133 ng/mL), the significant reduction in median SF at 12 mths compared to baseline values was even more pronounced (-938.5 ng/mL). Overall, 48.7% of pts (n=166) discontinued therapy. Reasons for withdrawal included AEs [n=78, 23% (n=44, 13% for drug-related AEs)], consent withdrawal (n=33, 10%), unsatisfactory therapeutic effect (n=6, 2%), lost to follow-up (n=2, <1%), death (n=26, 8%, none treatment-related as per investigators' assessments) and other (n=21, 6%). Most common investigator-assessed drug-related AEs were diarrhea (n=110, 32%), nausea (n=45, 13%), vomiting (n=26, 8%), abdominal pain (n=26, 8%), upper abdominal pain (n=25, 7%), rash (n=23, 7%), and constipation (n=21, 6%). Most AEs were mild-to-moderate (95%) in severity. Initial rises in serum creatinine (SC) values were seen (14.7% pts had two consecutive SC values >33% above baseline (in normal range), 10.6% pts had two values above ULN), but there were no progressive increases with adequate dose modifications. 10 pts had dose interruptions due to abnormal SC. **Conclusions:** In this large cohort of MDS pts with IOL, DFX provided significant reduction in SF levels, over 1-yr treatment. The AE profile in this study is consistent with previously reported DFX data in MDS pts.

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Screening and Genetic Monitoring of Patients with MDS by Cytogenetic Analyses of Circulating CD34+ Cells: First Results of the Ongoing Diagnostic Multicentre German Study for Frequent Cytogenetic Monitoring from Peripheral Blood

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Purpose: In patients (pts) with myelodysplastic syndromes (MDS) most chromosomal aberrations detected by cytogenetic banding analyses of bone marrow (bm) metaphases are provable by fluorescence in situ hybridisation (FISH) of bone marrow as well as by enriched CD34+ cells from peripheral blood (pb). In a multicentre German diagnostic study we analyse immunomagnetically enriched circulating CD34+ cells in MDS pts by FISH using 2 different FISH probe panels for initial screening and sequential follow-up measurements to detect chromosomal aberrations in pb and follow the clone size during therapy. Here we present first results after 6 months of study duration. Aims: Screening and sequential follow-up analyses of chromosomal anomalies from pb by FISH, prospective ascertainment of karyotype evolution, correlation of measurements from pb, bm and classical cytogenetic analyses and identification of possible association of cytogenetic and haematological response. Inclusion criteria: Age >18 years, reasonable suspicion of MDS or histologically assured MDS. Running time: 24 months. **Methods:** CD34+ cells from pb (30 ml) are enriched by immunomagnetic cell sorting (MACS®) and analysed by FISH afterward using a „Superpanel" (D7/CEP7, EGR1, CEP8, CEP XY, D20, p53, IGH/BCL2, TEL/AML1, RB1, MLL, 1p36/1q25, CSF1R, all Abbott® Products) for initial screening, after 12 and 24 months and in every case of suspected disease progression and a „Standardpanel" (EGR1, D7/CEP7, CEP8, p53, D20, CEP X/Y) every 2 months during the first year and every 3 months during the second year. **Results:** After 6 months 40 pts were included by 5 German study centers, as yet: 19 male, 21 female, the average age is 69 years (range 44-85). The diagnoses are: 1 suspected MDS, 10 RA/-RS, 1 t-MDS, 12 RCMD/-RS, 4 5q-syndromes, 2 CMML, 2 RAEB-1, 5 RAEB-2, 3 sec. AML following MDS. Eleven pts had a normal karyotype, 24 showed chromosomal aberrations, in 5 cases we had no data. In 4 pts new aberrations could be detected by initial screening using the superpanel. In another 8 pts (mostly low-risk MDS) the superpanel could not be evaluated completely because of too few enriched CD34+ cells. **Conclusions:** Screening analyses from pb and frequent cytogenetic monitoring by analysing enriched CD34+ cells in MDS pts are possible and feasible, the method is less invasive than a bone marrow biopsy and may support cytogenetic diagnostics in MDS pts. In several cases CD34+ FISH can provide additional genetic information.

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Differential gene expression of CD71+ bone marrow cells in myelodysplastic syndrome

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Gene expression studies were performed to determine a specific transcription profile for myelodysplastic syndrome (MDS) including different stages of MDS during their progression to acute myeloid leukemia. Up to

date there is no "universal" gene expression signature for MDS partly due to the lack of knowledge of an appropriate hematopoietic target cell fraction. So far studies were done using granulocytes, the stem cell compartment ($CD34^+$ or $CD133^+$ cells) or unselected bone marrow cells. To account for the dysplastic features in the erythropoiesis, in particular in subgroups of RA or RARS, we examined the gene expression in $CD71^+$ erythropoietic bone marrow cells. Heparinised bone marrow samples from 17 patients with MDS and from 6 normal persons (NP) were obtained. According to the IPSS the MDS-patients could be subgrouped into low (8 pts.), intermediate-1 (4 pts.), intermediate-2 (2 pts.) and high (3 pts.) risk. After separation of mononuclear cells, $CD34^+$ and $CD71^+$-cells were purified by magnetic cell separation (MACS). Of the 17 MDS patients and 6 NP we performed high-density oligonucleotide microarray analysis using both the $CD71^+$ and the $CD34^+$ cells (Affymetrix HG- U133 plus 2.0, data analysis by GeneSpring 4.2). As expected genes known to be involved in erythropoiesis were up-regulated in the $CD71^+$ cells compared to $CD34^+$ cells, in MDS as well as in NP respectively. Moreover we could find a set of genes allowing to distinguish between MDS and NP. In the $CD71^+$ cells, 62 genes were overexpressed and six genes were underexpressed in MDS patients compared to NP as defined by a 3-fold change, p value < 0.05 and present call rate higher than 75%. Of these genes 49% were also upregulated (fold-change >2) in the CD34+-compartment in MDS patients. Differentially expressed genes including genes already described in previous studies using hematopoietic stem cells. Furthermore we detected genes that seemed to be uniquely over- or underexpressed in the erythropoietic fraction in MDS patients compared to NP, e.g. TAOK1, MPL, CNN3, CTSE. In conclusion, we have shown that dyserythropoiesis in MDS may be caused by alterations at transcription level. Therefore, analysis of gene expression of the $CD71^+$ cells may contribute to a better characterization of molecular defects in MDS subgroups, e.g. RA or RARS.

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Higher rates of immunological responses in patients with haematological malignancies received 300µg RHAMM R3 peptide in contrast to high-dose peptide vaccination

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We performed two RHAMM-R3 peptide vaccination trials using 300µg and 1000µg for patients with AML, MDS and MM overexpressing RHAMM. In the 300µg cohort we detected in 7/10 patients specific immune responses and also positive clinical effects in 5/10 patients. Some of these patients with myeloid disorders showed a reduction of blasts in the bone marrow or a reduction of reduction of free light chain serum levels in patients with multiple myeloma. One patient with MDS did not need any longer erythrocyte transfusions. In the second cohort of nine patients with AML, MDS and MM vaccinated with a higher peptide dose of 1000 µg RHAMM-R3 peptide we detected specific immune responses in a lower frequency (33%) in contrast to patients in the 300µg cohort (70%). In these patients with immune responses we found an increase of CD8+/HLA-A2/RHAMM-R3 tetramer+/CD45RA+/CCR7-/CD27-/CD28- effector T cells in flow cytometry and an increase of R3-specific CD8+ T cells in ELISpot assays. Two patients with positive immune responses showed a significant decrease of regulatory T cells. Immunological analysis were performed using ELISpot assays for Interferon gamma and Granzyme B, tetramer staining and chromium release assays. Regulatory T cells were quantified during vaccination. One patient without positive immune and clinical effects showed an impressive increase of the frequency of regulatory T cells (5.03% to 15.9%). Three patients treated with 1000µg showed positive clinical effects: One patient with MDS RAEB 2 showed a reduction of leukemic blasts in bone morrow to lower than 5%, one MDS patient an improve of peripheral blood counts and one patient with multiple myeloma a reduction of light chain in serum. Similar mild toxicity of both cohorts was found, only mild drug-related adverse events were observed such as erythema and induration of the skin. Nevertheless, the patients in the 300µg cohort showed a higher frequency of positive immunological clinical effects. Higher doses of peptide application might induce immune tolerance. Taken together, RHAMM-R3 peptide vaccination induced both immunological and clinical responses using lower and higher peptide doses. However, higher doses of peptide do not improve the frequency and intensity of immune responses in this clinical trial and might induce immune tolerance.

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Comorbidity is a Prognostic Variable in MDS: Comparison of the HCT-CI and CCI in 419 Patients of the Austrian MDS Study Group

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Introduction: The evaluation of comorbidity is of increasing importance in patients with hematologic disorders. Patients and methodes: In the present study, the influence of comorbidity on survival and AML evolution was analysed retrospectively in 419 patients with *de novo* MDS (observation period: 1985-2007). The median age was 71 years (range 24-91 years). Two different scoring systems, the hematopoietic stem cell transplantation comorbidity index (HCT-CI) and the Charlson comorbidity index (CCI) were applied. **Results:** Cardiac disorders were found to be the most frequent comorbidities in our patients followed by peripheral and cerebral arterial occlusive disease and diabetes mellitus. With regard to the HCT-CI 53.4% were in the low risk group, 27.0% in the intermediate risk group and 19.6% in the high risk group. According to the CCI 61.1% did not score any comorbidity risk points, 31.0% had 1-2 points, 6.2% had 3-4 points and 1.7% had 5 or more points. The HCT-CI was found to be a significant prognostic factor for overall survival (OS, $p<0.05$) as well as event-free survival (EFS, $p<0.05$) in our patients, whereas the CCI was of prognostic significance for OS ($p<0.05$), but not for EFS. For AML-free survival (AFS), neither the HCT-CI nor the CCI were of predictive value. A multivariate analysis including age, LDH, ferritin, karyotype, number of cytopenias, FAB-groups, and comorbidity was applied. Comorbidity was found to be an independent prognostic factor in patients with low or int-1 risk MDS ($p<0.05$) regarding OS and EFS. With regard to int-2 or high risk MDS-patients, both, HCT-CI and CCI were not of prognostic significance. **Conclusions:** Together, our data show that comorbidity is an important risk factor for OS and EFS in patients with MDS.

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Deferasirox (Exjade®) treatment of chelation-naïve patients with transfusion-dependent iron-overload in the medical practice: Results from the Extend observational study

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Background: Many anaemic patients (pts) are threatened by iron overload due to red blood cell transfusions. Deferasirox (Exjade®), an iron chelator taken orally once-daily, has been demonstrated to be efficient in several clinical trials in maintaining or reducing body iron (assessed by liver iron concentration [LIC] or serum ferritin [SF]) in pts with transfusion-dependent iron overload (IOL). Data presented here summarize results of a 1-yr observation of previously unchelated, transfusion-dependent pts with various anemias (n=226), including MDS pts (n=123). Pts were analyzed in the daily-routine-setting of office-based physicians. **Methods:** The Extend observation covers 1 yr of chelation treatment of

iron overloaded, transfusion-dependent pts. Deferasirox was prescribed in the usual manner in accordance with the terms of the marketing authorization (Fachinformation). No inclusion or exclusion criteria were applied. Hematological parameters, including SF, and AEs were collected in 2-monthly intervals. **Results:** 226 pts (118 M, 106 F; mean age 66.8, range 3.2-91.9) with a median baseline SF of 2846.0 (range 184-16500) ng/mL were observed. Mean volume of transfused blood until the first visit was 991 mL/mth. The mean prescribed daily dose of deferasirox at the first visit was 16.1 mg/kg/d. Under treatment median SF level were significantly reduced from first to final visit [-723.0 ng/ml; p<0.0001 (explorative analysis)] in the overall population. 61.1% of all pts completed the 12 mth observation period. Reasons for discontinuation included AEs (n=41) with 18.1%. Most common investigator-assessed drug-related AEs were diarrhea (n=25, 11.1%), nausea (n=17, 7.5%) and skin reactions (n=17, 7.5 %) with mild-to-moderate severity. Median creatinine clearance was stable and never below 60ml/min. **Conclusions:** In MDS pts treated with deferasirox the SF level declined significantly over the 1-yr observation. Deferasirox provided a good safety and tolerability indicated by the low incidence of drug-related AEs in comparison to previously reported study data, possibly due to longer experience of Deferasirox usage in MDS pts and improved AE management. Thus, deferasirox represents an efficient and safe iron chelator for the treatment of transfusion-dependent MDS pts in the daily-routine situation.

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Can subgroups without disease related deaths be defined in MDS, categorizing by age, sex and well known scoring systems?

Noesslinger,T.[1], Tüchler,H.[1], Makrai,A.[1], Germing,U.[2], Sperr,W.[3], Krieger,O.[4], Haase,D.[5], Lübbert,M.[6], Stauder,R.[7], Giagounidis,A.[8], Valent,P.[3], Pfeilstöcker,M.[1]

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Introduction: As the course of disease in MDS is very variable, there have been attempts to define "very low risk-subgroups" with close-to-normal life expectancy based on the standardized mortality ratio (SMR). This retrospective multicenter analysis of 897 primary MDS patients aimed to scrutinise this findings by considering also excess of mortality (ExM). **Methods:** To define possible "no risk"-subgroups in MDS, SMRs and ExM (excess mortality attributable to MDS per patient-year) were computed. Risk-groups of the IPSS, its cytogenetic subgroups, the German-Düsseldorf-score, the Spanish-Sanz-score, the French-Lille-score, the Bornemouth-score, the Lausanne-Bournemouth-score and the PI-score, subdivided by age and sex were investigated. **Results:** Median age was 68 years (16-99), median survival 47 months. The total SMR was 4.72, with 378 of 480 observed deaths attributable to MDS; ExM=.14 (additional deaths per patient-year). Males (SMR=4.83) had a slightly higher SMR than females (SMR=4.55). Younger patients had a significantly increased SMR of 10.10, but lower excess mortality (ExM=.12) than the elderly (SMR=3.52, ExM=.16) and 90% versus 72% disease related deaths. SMRs for low-risk-groups of all scores ranged between 2.03 and 3.7, ExMs between .04 and .52. Additionally splitting risk-groups by age and sex, the lowest SMRs were around 1.5 and all differed significantly from 1, except for Düsseldorf-low-risk-female-=66years (SMR=1.7) and Düsseldorf-low-risk-female->66years (SMR=1.5). **Conclusion:** We, like others found small subsamples with an non-significant SMR around 1.5, due to high general mortality in older age. Excess in mortality in older age groups is at least as high as in younger patients.

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Analysis of patients with Myelodysplastic Syndromes from 1999-2008 at 'Donauspital' Vienna

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Introduction: Myelodysplastic syndromes are a heterogeneous group of clonal disorders mainly characterised by bone marrow failure and resulting cytopenias. Patients with MDS have an increased risk of transformation to AML and a significantly reduced life expectation which ranges from 0,4 (IPSS high risk) to 8 years (IPSS low risk). For elderly patients symptom control with growth factors and transfusions was standard of care for the last decades. The availability of disease-modifying agents like Azacitidine, Decitabine or Lenalidomide may alter the natural course of disease. Here we report data from 1999 - 2008 showing treatment modalities in the routine setting of a community-based Viennese Hospital. **Methods:** All patients with suspected myelodysplastic syndromes presenting at our institution are documented in our electronic database ODS (Onkologisches Dokumentationssystem) on an ongoing basis since 1999. Statistical analyses were performed using R software package. **Results:** Data for 93 patients from 1999 to 2008 were available for analysis with a median observation time of 1987 days. The median age at time of admission was 77 (35-93) years. In 67 cases the diagnostic workup confirmed a hematological malignancy. 81% of these patients were diagnosed with MDS, 7 patients with AML and 5 with myeloproliferative diseases. Regarding MDS-subtypes (WHO) 14 patients had RARS, 18 RAEB-1 or -2, 11 RA and 2 a 5q- syndrome. Cytogenetic analyses were performed in 40 patients. 40% showed a normal karyotype, in 18% aberrations of chromosome 5 were found and in 13% of cases >2 aberrations were present. To date 41/67 patients with confirmed diagnosis have died with a median overall survival of 13,8 months (0,3 – 209,3) since diagnosis. 71/ 93 patients received any treatment.93% received erythrocyte or thrombocyte transfusions and 94% hematopoietic growth factors. In 10 cases iron chelation was deemed necessary. 27% of patients received active treatment: 13 patients chemotherapy, 7 patients Azacitidine and 2 Lenalidomide. **Conclusions:** The survival of MDS patients in our registry was only about 14 months. The old average age of this population was strongly limiting the available treatment options with the usage of transfusions and growth factors for symptom relief in the majority of patients. Therefore new treatments like demethylating agents or Lenalidomide will change the treatment landscape and hopefully contribute to a better outcome for patients with MDS.

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Posterdiskussion
Myelom I

P484

Peripheral blood stem cell transplantation in 5 patients with different types of severe aquired paraproteinemic demyelinating polyneuropathy

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Background: Immune-mediated polyneuropathies can be associated with monoclonal gammopathies in about 10% of patients. The exact pathomechanism has not been well defined yet, despite autoreactive anti-MAG Ab have been described. Classical treatment options with immune-modulating approaches can result in clinical stabilisation, however, a subset of patients remains highly refractory. We describe 5 patients with severe progressive paraproteinemic polyneuropathies despite various treatment attempts who were treated with high-dose

melphalan and autologous stem cell transplantation (ASCT). 1 patient had in addition detectable anti-MAG antibodies. **Methods:** As induction therapy one cycle of cyclophosphamid (1-3g/m^2 +/- etoposid 200mg/m^2) was given and stem cells were collected after several days of GCSF stimulation. High-dose melphalan (HD-Mel) (between 150mg and 200mg abs.) was administered on day 1 and 2 with consecutive ASCT on day 4. One patient received a second course of HD-Mel and ASCT. Responses were assessed including clinical and electrophysiological examination with additional serological measurements of immunofixation and anti-MAG titre. **Results:** All patients (4/5) with a long-term follow-up of up to 2 years experienced a remarkable objective clinical and electrophysiological response. 3 patients with previous tetraparesis and wheel-chair dependency and 1 patient with severely limiting distal prevailing hypesthesia are now able to work in their former jobs again. Post-transplantation immunofixation of the serum turned negative in 2 patients, in 1 this value is unknown, in 1 it remained positive and in 1 case the anti-MAG titre decreased below detection level (from 1:10.000 before therapy). 4 of 5 patients had uncomplicated peri-transplant periods. One patient, who was severely disabled prior to ASCT including tetraparesis, cachexia and autonomous dysregulation with intermittent hypotension, died from infectious complications after engraftment failure before a clinical or electrophysiological response after ASCT could be evaluated. As a clear response to high-dose therapy the decrease of anti-MAG antibody titre could be documented in this patient indicating effectiveness of ASCT. **Conclusion:** HD-Mel with ASCT may be a well-tolerated and efficacious therapy for patients with treatment refractory paraproteinemic immune-mediated demyelinating polyneuropathy and may be considered as an option in treatment refractory cases earlier in the disease course.

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P485

A PKC isoform is responsible for the hyperphosphorylation of paratarg-7, the first molecularly characterized antigen of paraproteins in MM/MGUS patients.

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Antigenic targets of monoclonal gammopathy of undetermined significance (MGUS) and multiple myeloma (MM) paraproteins play a role in the pathogenesis of these neoplasms. We identified paratarg-7, a ubiquitous expressed protein of unknown function, as target in 14% (35/252) of all patients analyzed. paratarg-7 is the first molecularly characterized antigen identified as a common target of the paraproteins produced in those patients. When comparing these paratarg-7-immunopositive patients with healthy donors or other MM/MGUS patients, sequence analysis of paratarg-7 revealed no mutations or deletions. However, 2D-gel-electrophoresis, isoelectric focussing and phosphatase treatment revealed that paratarg-7 was hyperphosphorylated in all patients with this paraprotein specificity. Moreover, analysis of consanguineous relatives of MM/MGUS patients with an anti-paratarg-7 specific paraprotein showed that the hyperphosphorylated modification of this protein is inherited in a dominant fashion. Here we present data showing that all paratarg-7-immunopositive patients carry an identical modified phosphorylation of this protein when compared to healthy controls or other patients with this disease. We identified a protein kinase C isoform as responsible for this phosphorylation which occurs in the 15 aa region identified as immunogenic epitope in all paratarg-7 immunopositive patients. Additional data analyzing other enzymes involved in "hyperphosphorylation" are also presented. The high frequency of hyperphosphorylated paratarg-7 as the antigenic target of paraproteins from MM/MGUS patients suggests that the modified protein is involved in the development of sporadic and familial MGUS/MM by acting as a chronic antigenic stimulus for the respective B-cell clones. The identification of paratarg-7 as a frequent antigenic target together with the enzymes involved in this process enables the more detailed analysis of tumor-host interactions in these patients and their role in the pathogenesis of MM and MGUS.

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P486

Scientific Progress in Multiple Myeloma – does it matter in daily practice? A 5 years representative overview in Germany

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Aim: To analyse therapeutic behaviour in multiple myeloma (MM) in Germany within a three months time period in 2008 with respect to previously identified prognostic markers and the availability of new effective drugs. The results were compared to similar surveys in the years 2004 and 2006. **Methods:** In this retrospective non-interventional study data from 386 patients (pts) with MM were analysed. **Results:** *Patients and disease characteristics:* Mean age at first diagnosis was 66 years. 63% of the pts had stage III disease (Salmon & Durie), 20% had stage II and 17% stage I disease. 16% of the pts suffered from impaired renal function. FISH analyses were done in 22.5%. ß2-microglobulin (B2M) was determined in 71% of all patients. *Institutions:* First line treatment was undertaken at community hospitals in 56% of pts, in 22% at university hospitals and in 22% by office-based haematologists, respectively. With increasing lines of therapy the proportion of patients treated by office-based haematologists rose (2nd line: 32%, 3rd line: 58%). *Treatment:* 48% of the pts with stage I disease received chemotherapy, whereas 95% of pts in stage II – III were immediately treated for MM. First line treatment was administered within a clinical trial in 24% of all cases. Outside high dose chemotherapy, melphalan +/- steroid was the treatment of choice in 23% of pts. Autologous transplantation (autoTx) was implemented into first line procedures in 35% of pts. The use of novel agents such as bortezomib (Vel), thalidomide (Thal), and lenalidomide (Len) depended on the line of treatment: First line Vel 14% , Thal 10%, Len 4%; second line Vel 38%, Thal 6%, Len 6%; third line Vel 40%; Thal 9%; Len 21%. Vincristine containing regimens played a substantial role in earlier lines: 25% in first line, 11% in 2nd line and 5% in 3rd line. **Discussion:** Discrepancies to international consensus recommendations become evident in this representative snap shot analysis: The high number of pts treated in stage I demands further exploration. Autologous transplantation is part of first line treatment only in a minority. The low number of pts treated within a clinical trial may be due to inhibitory effects of current regulatory issues or strict inclusion / exclusion criteria. Finally, the increasing use of newer innovative drugs illustrates the rapid implementation of clinical progress into daily clinical practice.

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P487

Extracorporal serum free light-chain elimination parallel to chemotherapy leads to a high proportion of renal functional recovery in multiple myeloma patients with dialysis-dependent acute kidney injury

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About 50% of multiple myeloma patients present with impaired renal function upon diagnosis and up to 10% are dialysis-dependent. The most common form of light-chain associated acute renal injury is cast nephropathy. While novel options in treatment of primary and relapsed myeloma disease have substantially improved patient outcome, nonetheless, renal functional recovery determines patient prognosis. Timely correc-

tion of dehydration, initiation of chemotherapy and reduction of serum free light-chain (sFLC) concentrations is paramount for renal function recovery. We and others have established extended hemodialysis using a high cut-off protein-permeable membrane (HCO1100) as an efficient means for extracorporal sFLC removal. Here we report outcome data from 16 patients with multiple myeloma and dialysis-dependent acute kidney injury who were treated with chemotherapy and concomitant extracorporal free light-chain removal. 8 patients were newly diagnosed and 8 had relapsed or refractory disease. Median age was 68 years. Median eGFR was 7.4 (range 3.3 – 10.9) ml/min/1.73m^2, median sFLC concentration was 9680 (range 1590 - 66100) mg/l. Chemotherapy mainly included new therapeutic drugs (bortezomib-based regimen, n = 10; lenalidomide-based regimen, n = 1). Two early deaths occurred due to sepsis and progressive disease such that follow-up was obtained from 14 patients. In combination with chemotherapy, a median of 8 (range 3 - 23) high cut-off dialyses were required to reduce sFLC concentrations below 500 mg/l. Sustained renal functional recovery was achieved in 12 out of 14 patients with a median time of 17 days until independency of hemodialysis. Patients who did not recover renal function suffered from refractory myeloma disease. Response to chemotherapy and duration of acute kidney injury prior to initiation of therapy were independent predictors of renal functional outcome. In summary, extracorporal elimination of serum free light-chains in combination with effective chemotherapy enables recovery of renal function in dialysis-dependent acute kidney injury in a high proportion of multiple myeloma patients. A prospective, randomized European multicenter trial (EuLITE), adressing the influence of extracorporal free light-chain elimination on renal and patient outcome in multiple myeloma and cast nephropathy is ongoing.

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P488

Risk assessment in multiple myeloma (MM) patients (pts): determination of various comorbidity factors, comparison of 4 comorbidity scores (CS) and definition of a novel MM-CS to allow improved treatment-decisions and -allocation

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Introduction: Comorbidities affect daily function, treatment tolerance and mortality of cancer pts. Their exact impact on treatment outcome is warranted, especially in times of different therapeutic options. **Methods:** We compared comorbidity factors, such as age, Karnofsky Index (KI), pain, liver-, heart-, lung-disease and estimated glomerular filtration rate (eGFR by MDRD) in 127 consecutive MM pts, receiving standard or high-dose chemotherapy at our institution. Moreover, we compared 4 well-known comorbidity indices (CI), such as Kaplan Feinstein [KF]; Charlson-Comorbidity Index [CCI]; Satariano Index [SI] and Hematopoietic Cell Transplantation-specific Comorbidity Index [HCT-CI]) and aimed to develop a novel and robust MM-specific comorbidity score. **Results:** MM pts had a median age of 60 years (y; 27-83); 91% had advanced disease (stage II/III by Durie&Salmon) and 15% stage B disease. The median PFS and OS of all pts was 69 months and 35 months, respectively. Of note, pain (p= 0.2105), liver- (p= 0.6328) and heart-disease (p= 0.9970) did not prove to be of significance on OS, in contrast to renal impairment (RI; p=0.0008): with eGFR <30 vs. eGFR >90 the OS was 15 vs. 98 months, respectively. Also age >59y (p=0.01), pulmonary disease (p <0.0001) and KI (p= 0.0003) were relevant risk factors for decreased OS (age =59y vs. >59y: 98 vs. 53 months; no/mild vs. severe lung disease: 103 vs. 25 months, and KI ≥80% vs. KI =70%: 98 vs. 41 months, respectively). The comparison of different CIs revealed that the SI and CCI had no statistical relevance in MM, with a median OS of low-- vs. high-score-pts of 77 vs. 60 months for the SI (p=0.0876) and 76 vs. 60 months for CCI (p= 0.4159), respectively. In contrast, the KF (p=0.007) and HCT-CI (p=0.002) showed striking significance. Via multivariate analyses, we are currently determining which prognostic factors are most relevant to include in a MM-specific CI. **Conclusions:** Comorbidities are frequent in MM. Age, RI, pulmonary disease and KI are relevant prognostic factors for diminished OS. Our results fit perfectly in the ongoing debate on comorbidity scoring and treatment decision-making in cancer pts. Our data should be of relevance for future prospective analyses, where more and lesser intensive treatment options are therapy options in MM pts. Whether the known CI-scores, namely KF and HCT-CI, are sufficient, or a novel MM score should be used in the future will be presented at the meeting.

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P489

The PI3-kinase/mTOR- inhibitor BGT226 induces apoptosis and delays growth and proliferation of Multiple Myeloma cells

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Introduction: Multiple myeloma (MM) is still an incurable disease. Patients die of disease progression, because Myeloma cells become resistant to common cytotoxic drugs. The PI3/AKT pathway plays a crucial role in regulation of apoptosis, cell cycle and proliferation of Myeloma cells. Therefore targeting this pathway with PI3-kinase/mTOR-inhibitors like BGT226 seems to be a promising therapeutic option. **Methods:** BGT226 was characterized by several assays. We used MM cell lines (NCI-H929, U266, OPM-2, RPMI-8226 and IM-9) and primary myeloma cells. Cell growth was measured with the WST-1 assay. After staining with annexin-V-FITC and propidium iodide induction of apoptosis was quantified by flow cytometry. Cell proliferation was shown using the BrdU assay. Modification of the cell cycle was detected by flow cytometry after staining with propidium iodide. Western blotting experiments demonstrated the modulation of intracellular signalling. **Results:** Dependent from dose and time of incubation the WST-1 assay showed inhibition of cell growth in common myeloma cell lines at nanomolar concentrations. At 50nM a significant decrease could be seen in all cell lines (NCI:-78%,U266:-44%,OPM-2:-78%,RPMI:-65%,IM-9:-59%). After 48h of incubation with BGT226 annexin-V-FITC/propidium iodide staining showed increased rates of apoptosis in all five cell lines (NCI:65%,U266:18%,OPM-2:82%,RPMI:62%,IM-9:40%) as well as in primary MM cells (56%). The BrdU cell proliferation assay indicated that inhibition of cell growth was partly due to less proliferation (NCI:-63%,U266:-59%,OPM-2:-47%,RPMI:-65%). Analysis of the cell cycle by flow cytometry demonstrated an arrest in G1-phase (U266,OPM-2,RPMI) and showed an increased amount of cells in the subG1-population (NCI,RPMI). Inhibitory effects of BGT226 on the expression of the signalling molecules p-Akt, mTOR, p-mTOR, p-4E-BP-1 and p-P70S6k were verified by western blotting experiments. **Conclusions:** We show that the PI3-kinase/mTOR pathway is essential for survival of myeloma cells and its inhibition using BGT226 induces apoptosis and leads to a decrease of cell growth and proliferation. Therefore BGT226 could be a potential new therapeutic in targeted therapy in MM.

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P490

Synergistic action of the novel HSP90 inhibitor NVP-AUY922 with melphalan and doxorubicin

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Introduction: The combination of chemotherapy with novel agents has significantly broadened treatment options for multiple myeloma (MM) in recent years. However, new therapeutic strategies are urgently needed for this disease still incurable in the majority of the patients. Inhibition of Heat shock protein 90 (HSP90), e.g. by the highly specific HSP90-inhibitor NVP-AUY922, has shown potent anti-myeloma activity in vitro and early phase clinical trials are under way. However, there are no data on the combination of HSP90 inhibitors with classical chemotherapeutic

agents in MM so far. We tested the combination of NVP-AUY922 with melphalan and doxorubicin for possible synergistic action against MM. **Methods:** The NVP-AUY922-sensitive MM cell line OPM-2, the NVP-AUY922-resistant cell line RPMI-8226 and primary MM cells from myeloma patients were incubated with combination of the HSP90 inhibitor NVP-AUY922 with either melphalan or doxorubicin. Proliferation and apoptosis were investigated. Effects of combination treatment on cell survival were tested for synergy using the method defined by Chou and Talalay. **Results:** In OPM-2 cells, combination of NVP-AUY922 with doxorubicin inhibited viability by 74% in comparison to 14% and 18% inhibition caused by single drug treatment, respectively. Combination of NVP-AUY922 with melphalan inhibited viability by 90% in comparison to 14% and 21% for either drug alone. AnnexinV analysis revealed increased apoptosis for combination treatment in comparison to single drug treatment for both doxorubicin and melphalan. In primary myeloma cells, combination of NVP-AUY922 with melphalan or doxorubicin caused significantly increased inhibition of viability (87% and 88%) in comparison to single drug treatment. Evaluation using the median effect plot method revealed synergistic effects for the combination of NVP-AUY922 with doxorubicin (CI<1) and very strong synergism (CI<0.3) for the combination of NVP-AUY922 with melphalan. **Conclusions:** Our data show significantly synergistic effects on viability and apoptosis for the combination treatment with NVP-AUY922 with either doxorubicin or melphalan in MM cell lines and in primary myeloma cells. Interestingly, the combination with melphalan resulted in very strong synergism (CI<0.3) in both the NVP-AUY922-sensitive OPM-2 as well as in the resistant cell line RPMI-8226. These data build the framework for the combination of NVP-AUY922 with melphalan or doxorubicin to be evaluated in clinical trials.

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P491

The immunotoxin HM1.24-ETA´ is a potent inducer of apoptosis in primary multiple myeloma and plasma cell leukemia cells

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Introduction: Despite new treatment modalities, the clinical outcome of at least a subgroup of patients with multiple myeloma (MM) needs improvement. Antibody-based targeted therapies are increasingly used for tumor therapy, and may represent interesting options also for MM patients. HM1.24 is a surface molecule that is over expressed on malignant plasma cells and efficiently internalized from the cell surface. It may represent a promising target for the development of myeloma-directed immunoconstructs. **Methods:** Here, a novel single-chain immunotoxin, HM1.24-ETA', is described. HM1.24-ETA' was generated by genetic fusion of an HM1.24-specific single-chain Fv (scFv) antibody and a truncated variant of Pseudomonas aeruginosa exotoxin A (ETA'). HM1.24-ETA' was expressed in E.coli and purified to homogeneity by affinity chromatography. Specific binding to antigen-positive cells was analyzed by flow cytometry. Inhibition of proliferation and induction apoptosis was analyzed by MTT assays, Annexin V / 7-AAD staining and flow cytometry as well as western blot analysis of PARP cleavage. **Results:** HM1.24-ETA' efficiently inhibited growth of IL-6 dependent (INA-6) and IL-6 independent (L363, RPMI8226, JK-6) myeloma cell lines. Half maximal growth inhibition was observed at low nanomolar concentrations. Target cell killing occurred via induction of apoptosis, as evidenced by Annexin V / 7-AAD staining and detection of PARP cleavage. The cytotoxic effect was completely blocked by adding excess of unconjugated parental antibody, demonstrating that killing was antigen-specific. Importantly, HM1.24-ETA' efficiently triggered apoptosis (>90% Annexin V positive cells) of freshly isolated plasma cell leukemia cells and multiple myeloma cells within 72 h. **Conclusions:** Thus, HM1.24-ETA' efficiently triggered apoptosis of plasmacytoma cell lines as well as freshly isolated tumor cells. These results indicate that HM1.24 may represent a promising target structure for efficient antigen-specific delivery of cytotoxic compounds in multiple myeloma.

Disclosure: No conflict of interest disclosed.

P492

Epigenetic dysregulation of the putative tumor suppressor gene disabled-2 (DAB2) in multiple myeloma

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Introduction: Multiple myeloma (MM) is a B-cell neoplasm that is characterized by the accumulation of malignant plasma cells in the bone marrow. Aberrant methylation of CpG islands near gene promoter regions is the most widely studied epigenetic abnormality in human malignancies and is associated with loss of gene function. The Wnt signaling pathway has a key function in stem cell maintenance as well as proliferation and differentiation of hematopoietic progenitors. Alterations in the Wnt pathway have been shown to contribute to the pathogenesis of various human malignancies. Disabled-2 (DAB2) is a putative tumor suppressor gene and has been shown to act as a negative regulator of Wnt signaling. **Methods:** In this study, we determined the methylation status of the promoter-associated CpG island of DAB2 in malignant plasma cell disorders by methylation-specific polymerase chain reaction (MSP) and pyrosequencing. DAB2 expression was analyzed by real-time reverse transcriptase polymerase chain reaction. **Results:** MSP analysis revealed that the DAB2 promoter region was hypermethylated in the MM cell line OPM-2 as well as in the lymphoma cell lines L540 and Raji. Aberrant methylation of DAB2 in hematopoietic tumor cell lines was associated with transcriptional silencing. Treatment of cell lines that carry a hypermethylated DAB2 gene with the demethylating agent 5-aza-2-deoxycytidine resulted in gene reexpression and partial promoter demethylation. We then analyzed the DAB2 methylation status in 95 specimens obtained from patients with malignant plasma cell disorders by MSP. The frequency of aberrant methylation among the primary patient samples was 7.4 % (7/95). Methylation patterns of the DAB2 promoter region in cell lines and selected patient samples were additionally analyzed by pyrosequencing. Aberrant DAB2 methylation in patient samples obtained at diagnosis was associated with an inferior overall survival. **Conclusions:** We conclude that promoter hypermethylation of DAB2 is a novel epigenetic event in malignant plasma cell disorders that may contribute to activation of the Wnt pathway and could serve as a prognostic biomarker. Further studies are warranted to elucidate the functional consequences of aberrant Wnt signaling by epigenetic dysregulation of DAB2 in the pathogenesis of MM. Additionally, the increasing evidence for the important role of DNA methylation changes in malignant plasma cell disorders may serve as a basis for the use of epigenetically targeted therapeutic approaches in MM.

Disclosure: No conflict of interest disclosed.

P493

N-Bisphosphonates are synergistic with farnesyltransferase inhibitors in leading to myeloma cell death

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Introduction: Activation of the Ras dependent MAPK pathway - induced by cytokines like IL-6 and IGF-1 or by Ras mutations - is essential for the growth of malignant plasma cells. Proteins of the Ras family are active only in their membrane bound form depending on posttranscriptional prenylation by farnesyltransferase (FTase). Farnesyltransferase inhibitors (FTI), designed to block prenylation of Ras and related small G proteins, have already shown activity in multiple myeloma (MM), but provided significant side effects. However, geranylgeranyltransferase may substitute for blocked FTase. Nitrogen-containing bisphosphonates (N-BP) inhibit farnesylpyrophoshate (FPP) synthase, an essential enzyme for the geranylgeranylation of small G proteins and were recently shown to block myeloma growth *in vitro* and *in vivo*. Therefore, a combination of both classes of inhibitors, N-BP and FTI, should be able to effectively block Ras as well as other G proteins by inhibiting both farnesylation and geranylgeranylation. **Methods:** The potential of such an approach was evaluated in five human myeloma cell lines, measuring

growth by MTS or [3H] thymidine assay upon treatment with FTI alone or in combination with the N-BP zoledronate (ZOL). Apoptosis was determined by propidium iodide or 7-AAD/AnnexinV staining. Ras and p53 mutation status was determined by RT-PCR and sequencing. Western blot analysis was performed to look for phosphorylated MAPK. **Results:** Both classes of inhibitors, FTI as well as N-BP were able to induce apoptosis and to inhibit growth of plasma cell lines in a dose-dependent manner. The activity of FTI and/or ZOL was not dependent on N-/K-Ras or p53 mutation status. The combination of FTI inhibitor L744.832 and ZOL showed the strongest synergistic activity in the plasmacytoma cell lines INA-6 and JK-6L (= 0.5 μM of L744.832 and = 50 μM of ZOL). However, the decrease of phosphorylation of p 44/42 MAPK required high drug concentrations (in JK-6L: 2 μM of L744.832 and 200 μM of ZOL) as shown by Western blot analysis. **Conclusions:** Essential prenylated proteins beyond Ras are likely to be important targets in multiple myeloma. Targeting prenylation of essential proteins by effectively blocking both farnesylation and geranylgeranylation should be an effective therapeutic strategy by reducing toxicity and improving anti-tumor activity of FTI and N-BP.

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P494

Antitumor activity in a human multiple myeloma xenograft model using sorafenib and bortezomib in mono- and combined therapy

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Introduction: Since novel treatment options are needed in multiple myeloma (MM), novel anti-MM agents and combinations are eagerly pursued to further improve the prognosis of this otherwise evil disease. For these novel therapeutics, functional in vivo models are highly valuable. We have established a cell line-based, disseminated MM model in NOD/SCID-IL2-receptor-gamma-chain-/- (NSG) mice. **Methods:** In the current analysis, our NSG-model was validated in various treatment groups, using bortezomib (B: 0.7mg/kg/day (d); d8, d11, d15, d18), sorafenib (S: 100mg/kg/d; d7-18), and bortezomib/sorafenib-combination (S+B: 0.7mg/kg/d; d8, d11, d15, d18 + 100mg/kg/d; d7-18), all compared to a control group without anti-MM-treatment. L363 cells were injected intratibialy into NSG mice and respective therapies were started 7 days after L363-implantation. Tumor growth was monitored with daily monitoring of MM-symptoms, flow-cytometry (FACS) and fluorescence-based in vivo imaging (FI). For FI we used an anti-human-CD138-Ab, labelled with fluorescence dye, whereby mice were anesthetised 48 hours after the injection, and pictures were taken with a Kodak in vivo imaging system. Tumor inhibition was calculated as the proportional reduction of median MM-cell-infiltration at the respective compartment of the test- compared to the control-group (in %). **Results:** L363 cells were detected by FACS and FI, not only at injection sites, but also within the bone marrow (BM) and spleen. With mono-S- or B-therapy, primary tumor development was markedly reduced by S (tumor inhibition of 34%), as well as with B, albeit with the latter to a lesser extend (tumor inhibition of 16%). BM infiltration was significantly reduced by both compounds: S induced a tumor inhibition of 99% (d35) and B of 85% (d28). Of note, S had a more pronounced and prolonged antitumoral activity in comparison to B on L363 cells. Combined therapy induced 91% tumor inhibition in the BM of S+B treated mice, which was slightly more efficient than B alone (85%) and as yet similar to S alone (99%). **Conclusions:** L363 engraftment in NSG is a valuable in vivo MM-model which exhibits high reproducibility, take- and metastases-rates, closely mimicking the clinical situation. Collection of whole-body FI data proved to be a time- and animal-saving analysis that allows to quantify MM growth. Further investigations validate the promising anti-MM agents as well as other combinations are currently also persued.

Disclosure: No conflict of interest disclosed.

P495

Lenalidomide enhances antigen-specific activity of tumor specific T-cells

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Background: We have recently shown that MART-1 specific T-cells can be triggered by the *HM1.24* myeloma antigen in healthy donors and patients with multiple myeloma (MM). Aim of this study was to investigate whether (1) unspecific T-cell response after activation with CD3/CD28 beads and (2) the specific T-cell response against the *HM1.24* antigen are enhanced by the immunomodulatory drug Lenalidomide (Revlimid®). Design and **Methods:** T-cells from healthy donors (n=22) and patients with plasmacell-dyscrasias (n=12) were incubated with CD3/CD28 beads in the presence/absence of Lenalidomide, T-cell activation was analyzed by interferon-gamma-(IFN-) secretion in EliSpot-, Elisa- assays, and by flow cytometry. Specific CD8+ T-cells against the MART-1 peptide from healthy donors (n=15) and patients with plasmacell-dyscrasias (n=12) were expanded by dendritic cells in healthy donors, activation was measured by IFN- -secretion in EliSpot-, Elisa- assays, and by flow cytometry. **Results:** (1) Activation of unspecific T-cells from healthy donors, and partly from patients with plasmacell-dyscrasias was enhanced in IFN- EliSpot, IFN- analysis by flow cytometry, and especially in IFN- Elisa- assays. (2) Activation of tumorspecific T-cells from healthy donors was enhanced in IFN- EliSpot, IFN- analysis by flow cytometry, and especially in IFN- Elisa- assays. Furthermore we found that the impact of Lenalidomide on T-cells depends on the duration of the exposition; a longer incubation time leads to an enhanced activation of the T-cells. **Conclusions:** We confirmed recent data that Lenalidomide enhances the unspecific activation of T-cells and showed for the first time, that Lenalidomide also improves the antigen-specific T-cell responses in vitro.

Disclosure: No conflict of interest disclosed.

P496

Constitutive PKB/Akt activation in primary multiple myeloma does not require oncogenic Ras

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Introduction: Cell growth, survival and drug resistance in multiple myeloma (MM) are to a large extent due to dysregulated signaling pathways. The protein kinase B (PKB)/Akt pathway is constitutively activated and essential for survival in about 50 percent of primary MM samples, thus defining Akt-dependent versus Akt-independent myeloma subsets. As oncogenic Ras has been reported to lead to the activation of various survival pathways, we analyzed the role of mutated Ras in the Akt-dependent myeloma subgroup. **Methods:** We tested 35 primary MM samples for sensitivity to Akt inhibition using a small molecule Akt-inhibitor and correlated the results to the Ras mutation status in these samples. Presence of phosphorylated Akt was shown by immunohistochemical staining. Applying siRNA knockdown and Western blotting we further investigated the consequences of Ras depletion with regard to Akt activation in a Ras wildtype (AMO-1) versus a Ras mutated (MM.1s) myeloma cell line model. **Results:** In the tested MM cell lines and primary samples Ras is expressed in a very heterogeneous manner, which is in accordance to previous studies and indicates that Ras overexpression might not be a common requirement in the propagation of MM. In the myeloma cell line model siRNA mediated depletion of K-Ras led to apoptosis in the K-Ras^{G12A} mutated cell line MM.1s, which is Akt-dependent, but did not impair survival in the K-Ras^{wt} cell line AMO-1, which is Akt-independent. K-Ras knockdown led to a moderate decrease in Akt^{Ser473} phosphorylation. While 17/35 primary MM samples showed strong sensitivity to inhibition of Akt and formed

the Akt-dependent myeloma subgroup, 14/35 samples harbored mutated Ras (either the K or N isoform). However, no correlation could be detected between the Ras mutation status and Akt sensitivity, suggesting that oncogenic Ras is not responsible for the constitutive activation of Akt in primary MM. **Conclusion:** Whereas activating Ras mutations might well contribute to MM cell survival, they do not appear to be instrumental in the constitutive activation of the PKB/Akt pathway.

Disclosure: No conflict of interest disclosed.

Posterdiskussion
Gastrointestinale Tumoren

P497

5-Fluorouracil, leucovorin and oxaliplatin with or without docetaxel in older adult patients with esophagogastric cancer: preliminary results from the FLOT65+ trial of the Arbeitsgemeinschaft Internistische Onkologie (AIO)

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Background: Older adult patients (pts) with esophagogastric cancer are under-represented in clinical trials, and most of them do not receive the effective 3-drug combinations due to tolerability concerns. This study evaluates for the first time a 2- versus 3- drug combination in older adult pts with esophagogastric cancer. **Methods:** Pts >=65 yrs of age with metastatic or locally advanced disease were stratified by ECOG PS, resectability, and pharmacogenetic risk profile and were randomized to receive infusional 5-fu 2600mg/m^2, leucovorin 200mg/m^2, and oxaliplatin 85mg/m^2 (FLO) or the same regimen plus docetaxel 50mg/m^2 (FLOT), every 2 weeks. The primary endpoint was response rate. Major secondary endpoints were toxicity, Quality of life (EORTC-C30) and survival. **Results:** 143 pts (FLOT/FLO, 72/71) were randomized between Aug 2007 and Oct 2008. Median age was 70 yrs and median ECOG was 1. 30% of pts had locally advanced potentially resectable disease (FLOT, 31%; FLO, 30%). Pts received a median of 8 cycles in both arms. 117 of 143 (82%) pts were evaluable for response at the time of the analysis. In this group, response to FLOT (55%) was significantly superior to FLO (33%; fishers exact test p=0.025), with 6% and 11% of pts having disease progression as best response to FLOT and FLO, respectively. The improved response rate was also significant in favour of FLOT when analysed in the subgroup of potentially resectable pts (fishers exact test p=0.029). Median treatment duration was 4.1 months in both arms. FLOT was associated with significantly more NCI-CTC grade 1-4 alopecia (p<.001), neutropenia (p<.001), diarrhea (p=.004), and nausea (p=.014), but there were no differences between arms for complicated neutropenia, serious adverse events (SAEs), withdrawals/discontinuations for toxicity, or toxic deaths. Survival data were immature, with only 50 of 143 (34.9%). **Conclusions:** older adult pts with gastric cancer should not be excluded from the treatment with 3-drug combinations due to tolerability concerns. In our study, they experienced an expected increase of toxicity, but no increase in SAEs, treatment discontinuations or toxic deaths. Overall the toxicity profiles did not differ from those observed in young pts. FLOT was associated with significantly higher response rates.

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Jäger,E.: No conflict of interest disclosed.

P498

Perioperative chemotherapy with epirubicin, cisplatin and 5-FU (ECF) for resectable gastro-esophageal adenocarcinoma. A retrospective analysis.

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Background: Perioperative chemotherapy with ECF is the recommended treatment in Europe (Cunningham, 2006). Here we report our single centre experience administering this treatment. **Methods:** In this retrospective analysis of all patients from 09.2004 to 09.2008 with adenocarcinoma of the distal esophagus, gastroesophageal junction (GEJ) and gastric body who received preoperative chemotherapy with ECF for initially curative intent were included. Cisplatin and 5-FU were substituted with oxaliplatin (O) or capecitabine (X), if indicated (Cunningham, 2008). Preop staging included CT-scan of chest and abdomen, endoscopy and endosonograpy. Patients had at least UICC stage II and were planned to receive 3 preop and 3 postop cycles of epirubicin 50mg/m² d1, cisplatin 60mg/m² d1, 5-FU 200mg/m² d1-21 (Cunningham, 2006). **Results:** 55 patients were analysed so far. Median age 64 (range 32-79), male 43, female 13 pts. Tumor site: distal esophagus 5, GEJ: 26, gastric 24 pts. Surgery: none: 4 pts, curative intent: 43 pts, palliative: 8 pts, esophageal resection: 16 pts, total gastrectomy: 29 pts, subtotal gastrectomy: 3pts, other: 3 pts. D2 lymph node dissection: 43 pts. Preop chemotherapy: ECF 50 pts, EOF 4 pts, ECX 1 pts. All 3 planned preop cycles administered in 80% of pts. Postop adjuvant chemotherapy initiated in 42% of pts. 3 postop cycles received in 31% of pts, postop palliative chemotherapy: 14.5% of pts. Main non hematological toxicity of curative chemotherapy (CTC grade 3 and 4, preop/postop): 48/21 pts evaluable: Nausea 0/4.8%, diarrhoea 2.1/4.8%, infection 0/4.8%, mucositis 0/0%. Hematologic toxicity CTC grade 3 and 4, preop/postop): 45/18 pts evaluable: Neutropenia 49/83%, thrombopenia 0/11%. Surgical complications of 51 pts operated: pulmonary complications 10 pts, bleeding 2, wound healing 2, anastomotic leakage 3, cardiac complications 7, pulmonary embolism 1, septicaemia 2, 30 day mortality: 3.6%. Pathological resection of 51 operated pts: R0 78.5%, R1 17.5%, R2 4%. Median follow up 17.3 mths. So far 23 pts had tumor recurrance and 22 pts died, 16 of them tumor related. The cumulative survival at 1 year is 74.8%. **Conclusion:** Our experience is concordant with the published data. Preop ECF based chemotherapy is better tolerated than postop ECF and can be administered to a higher proportion of patients. Overall ECF is a safe and well tolerated regimen, which does not seem to increase surgical complication rates.

Disclosure: No conflict of interest disclosed.

P499

Control of cancer-associated inflammation may result in survival advantage: Results from a prospective randomised phase II trial in gastric cancer

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An angiostatic approach was used to assess the impact of anti-inflammatory therapy in combination with metronomic low-dose chemotherapy. A randomized multi-institutional phase II trial was designed to select metronomic chemotherapy (arm A: capecitabine 1 g orally twice daily for 14 days with one week break until tumor progression) or combined anti-inflammatory/angiostatic treatment (arm B: capecitabine as mentioned above plus etoricoxib 60 mg orally, day 1+, and pioglitazone 60 mg orally, day 1+) for further evaluation. Patients with refractory or progressive disease following any first-line therapy except capecitabine or frail were eligible. According to the one stage design, a sample size of 64 patients was calculated for the primary objective, improvement of response rate. As similar response rates were observed (arm A/B 15/14%) after the accrual of 42 patients, the study was closed (n=20 (A), n=22 (B); median age 69 years (range 46 to 86ys); frail A/B n=9/11). Median progression-free survival for arm A/B was 3.0/2.9 months (P=0.878), and overall survival 5.0/6.1 months (P=0.778). In

both treatment arms a significant decline of serum C-reactive protein (CRP) levels was observed within the first 4 to 6 weeks on treatment, A/B P= 0.01/0.04, respectively. CRP response > 50% from baseline was associated with a significantly improved overall survival in arm A/B (3.1 versus 11.0 months, P= 0.023/ 3.3 versus 7.1 months, P= 0.078) indicating an impact of inflammation-control on survival (Reichle A, Cancer Microenvironment 2008). WHO grade 3 (no grade 4) toxicities were reported in arm A/B in 20% and 23%, respectively, mostly due to hand-foot-syndrome. Metronomic low-dose chemotherapy in gastric cancer may induce anti-inflammatory response, but the chosen additional anti-inflammatory approach neither has impact on tumor-associated inflammation nor on response or survival rate. In a historical comparison, CRP-responder have similar outcome as patients treated with combination chemotherapy in first-line.

Disclosure: Reichle,A.: Anstellungsverhältnis oder Führungsposition:Universität Regensburg
Andreesen,R.: Anstellungsverhältnis oder Führungsposition: Universität Regensburg

P500

Daily RAD001 plus mitomycin C, every three weeks in previously treated patients with advanced gastric cancer or cancer of the esophagogastric junction – preliminary results of a Phase I study

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Background: the mTOR pathway could be potential target in the treatment of gastric cancer. This study was designed to determine the maximum-tolerated-dose and preliminary safety and efficacy of the mTOR inhibitor RAD001 in combination with mitomycin C in patients with previously treated advanced gastric cancer. **Methods:** in this dose-escalation phase I trial, patients received mitomycin C at 5 mg/m^2 i.v. every 3 weeks combined with escalated doses of oral RAD001 (starting with 5 mg/day) once daily in 3-week cycles. Patients were investigated for safety every week and for efficacy every 6 weeks. **Results:** 11 patients (8 male, 3 female) have been included so far. All patients were pretreated with a platinum-based chemotherapy, and 9/11 had also received docetaxel. Treatment cohorts were: 5 mg/day, 3 patients; 7.5 mg/day, 3 patients; and 10mg/day, 5 patients. Median treatment duration was 46 days (range, 8 to 91 days). There were no dose limiting toxicities, until dose escalation of RAD001 was stopped at the 10mg/day dose. The only grade 3-4 toxicity observed was leukopenia in 9% of patients. Frequent grade 1-2 toxicities with possible relationship were mucositis 64%, leukopenia 64%, nausea 54%, thrombocytopenia 45%, fatigue 27%, and diarrhea 18%. Only mucositis and leukopenia were associated with higher dose levels. At the time of analysis, two (20%) of 10 evaluable patients experienced a major response (both liver metastases), one patient had stable disease, one patient was not evaluable for efficacy, and the rest of the patients had disease progression. Responses were independently confirmed. **Conclusions:** oral RAD001 up to 10mg once daily can be safely combined with mitomycin C at 5mg/m^2 every 3 weeks in previously treated patients with advanced gastric cancer. The achievement of major responses with the combination in this heavily pretreated population is encouraging.

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P501

Palliative chemotherapy for advanced and metastatic gastric cancer in the community

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Gastric cancer is a common malignant disease in Germany. Most patients are diagnosed with advanced or metastatic disease. Even after surgical resection many patients suffer relapse of their disease. Therefore palliative chemotherapy is regularly used in this cohort. A variety of cytotoxic regimens are administered in this setting. Only few data are available on the actual usage of these options in the community. We looked into differences of preferred cytotoxic regimens between two treatment institutions. A restrospective analysis of patients with gastric cancer treated with palliative chemotherapy in an university hospital oncology department and a private oncological practice was done. In total, 123 patients (79 male, 44 female; median age 60 years) were given palliative chemotherapy from 2000 to 2008. 79 patients were treated at the university and 44 in private practice. The median overall survival for the whole group was 9,5 months. First line treatment was platinum/5-FU-based in 70% at both institutions. Irinotecan/5-FU-based therapy was administered in 13% at the university compared to 2% in the private practice and Mitomycin C/5-FU-based in 9% versus 20%, respectively. Only 8% of cases received first line monotherapy. Median time to treatment failure or progression during first line chemotherapy was 21 weeks. Almost two thirds of patients received second line palliative chemotherapy, 67% at the university and 56% in private practice. Second line regimens differed as follows: Taxanes were given to 30% (university, U) and 48% (private practice, P)of patients, irinotecan-based therapy to 43% (U) versus 8% (P) and Mitomycin C to 13% (U) versus 32 % (P), respectively. Our data show different usage of treatment options between an university oncological department and a private oncological practice without any obvious survival differences between these two patient groups. One reason for the difference might be the importance of on and off label use, respectively. Further analysis of a larger cohort might be of interest.

Disclosure: No conflict of interest disclosed.

P502

Neoadjuvant treatment of adenocarcinomas of the distal esophagus or stomach – a feasibility trial of combining fluoropyrimidine and taxane-containing chemotherapy regimens.

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Objectives: The optimum neoadjuvant chemotherapy regimen for adenocarcinomas of the distal esophagus or stomach is still the subject of clinical trials. Here we report the combination of two different fluoropyrimidines (5-FU or capecitabine) with a taxanes and cisplatinum in terms of treatment feasibility and operability. **Methods:** 15 patients (11 male, 4 female; median age 54 years [range 28 to 67]) with histologically proven locally advanced adenocarcinomas of the distal esophagus (n = 7) or stomach (n = 8) were subjected to neoadjuvant chemotherapy containing cisplatinum and taxanes, combined with conventional 5-FU/folinic acid (n = 5) or capecitabine (n = 10). **Results:** 13 out of 15 patients could proceed to surgery, the remaining two being initially metastasized and therefore not eligible for operation, with both of them, however, showing a clinical response to therapy. From the 11 patients having undergone surgery so far (two being currently prepared for it), six (55%) showed a pathological regression (grade 1 or 2). All but one patient received a complete resection (R0) whereas one had microscopic tumour rests remaining (R1). With a median follow-up time of 174 days [range 14 – 579], no treatment-related deaths occurred. 14 out of 15 patients are still being alive, with one patient having died unrelated to surgery. Two patients relapsed and are currently undergoing palliative chemotherapy. Toxicity profiles were moderate, with 14 out of 15 patients completing the scheduled protocols. One patient stopped therapy due to a deteriorating general condition. However, in five out of ten patients treated with capecitabine, this drug had to be paused due to hand-foot syndrome (n = 3), or because of gastrointestinal symptoms (n = 1). **Conclusions:** The combination of taxanes with cisplatinum and fluoropyrimidines is a feasible chemotherapy regimen in the neoadjuvant setting. No major differences in response between the two schedules could be observed. Hand-foot-syndrome seems to be the major dose-limiting toxicity requiring dose adaptations or modifications of the application schedule for multicentre randomized trials currently being prepared.

Disclosure: No conflict of interest disclosed.

P503

Gemcitabine/erlotinib followed by capecitabine versus capecitabine/erlotinib followed by gemcitabine – interim toxicity analysis of a multicenter, randomized, phase III trial of the "AIO"

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Background: To date, only limited toxicity data are available for the combination of erlotinib (E; 150 mg/d) with either gemcitabine (G) or capecitabine (C) as first-line therapy for advanced pancreatic cancer (PC). **Methods:** In this prospective multicenter phase III trial of the "Arbeitsgemeinschaft Internistische Onkologie" (AIO), 281 untreated patients (pts) with histologically confirmed advanced exocrine PC were randomly assigned to first-line treatment with either C (2000 mg/m^2/d, d1-14 q3w) plus E (150 mg/d, arm A) or G (1000 mg/m^2 over 30 min weekly x 7, then d1, 8, 15 q4w) in combination with E (150 mg/d, arm B). In case of treatment-failure (e.g. disease progression or toxicity), pts were "crossed-over" to second-line treatment with the comparator cytostatic drug without E. The primary endpoint was time to treatment failure of second-line therapy (TTF2), treatment safety and toxicity were secondary study endpoints. **Results:** While the trial has completed recruitment, toxicity data are available from the first 127 randomized pts. Sixty pts were randomized to arm A (83% metastatic PC), 67 pts to arm B (82% metastatic PC); median age was 64 years. During first-line therapy, pts received a median number of 3 treatment cycles (range 0-13) in both arms; overall 456 treatment cycles were applied (arm A: 218, arm B: 238). Regarding chemotherapy, a treatment delay was observed in 12% of the cycles in arm A and in 22% of the cycles in arm B. Dose reductions of the cytostatic drug were performed in 18% and 27% of treatment cycles, respectively. E dose reductions were performed in 6% and 11% of all cycles. Grade 3/4 hematological toxicity was <10% in both arms; in arm A, grade 3/4 diarrhea was observed in 9% of pts (arm B: 7%), grade 3/4 skin rash in 4% (12%) and grade 3/4 hand-foot syndrome in 7% (0%), respectively. Nine pts in arm A (7 of them due to PC) and 8 pts in arm B (6 due to PC) died within 60 days after randomization. **Conclusion:** G/E and C/E were both tolerated well and toxicity was manageable. This preliminary safety analysis suggests that treatment with E 150 mg/d is feasible in combination with G or C in advanced PC.

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Böck,S.: Honorare: Roche Pharma GmbH; Finanzierung wissenschaftlicher Untersuchung:Roche Pharma GmbH

P504

KRAS mutation in metastatic pancreatic ductal adenocarcinoma: results of a multicenter phase II study evaluating efficacy of cetuximab plus gemcitabine/oxaliplatin (GEMOXCET) in 1st line therapy

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Background: Genetic alteration within the EGFR pathway including KRAS mutations have been demonstrated to be associated with response to EGFR inhibitors like cetuximab in CRC. Mutations in the KRAS gene have been found in 70-90% of pancreatic cancers. Unfortunately, the addition of cetuximab to chemotherapy did not increase response or survival for patients with advanced pancreatic cancer in phase II studies. The aim of this study was to evaluate the relationship between KRAS mutations and response or survival in patients with metastatic pancreatic cancer treated with cetuximab plus chemotherapy. **Methods:** Within a multicenter phase II trial 64 patients with metastatic pancreatic cancer were treated with cetuximab in combination with gemcitabin and oxaliplatin until disease progression. Analyses of the EGFR pathway including KRAS mutations were performed in 25 patients. Analyses were carried out following microdissection of the tumor. **Results:** 14 (56%) of the 25 patients examined harbored a point mutation in codon 12 of the KRAS gene. No differences between the groups were noted in median progression free survival (96 days in KRAS wilde-type patients versus 118 days in patients with KRAS mutations). Overall survival was longer in wilde-type patients compared to patients with KRAS mutations (243 days versus 162 days), but the difference did not reach statistical significance. **Conclusions:** KRAS mutation in codon 12 may be associated with reduced survival compared to KRAS wilde-type. The role of KRAS mutations for cetuximab therapy in pancreatic cancer warrants further investigation in larger trials.

Disclosure: Kullmann,F.: Anstellungsverhältnis oder Führungsposition: Chefarzt Klinikum Weiden; Beratungstätigkeit:Fa. Merck, Da. Sanofi, Roche, Aventis, Lilly; Honorare: Vorträge; Finanzierung wissenschaftlicher Untersuchung:Fa. Merck; Gutachtertätigkeit
Endlicher,E.: Anstellungsverhältnis oder Führungsposition: OÄ; Finanzierung wissenschaftlicher Untersuchung: Fa. Merck

P505

Decrease of CA 19-9 predicts survival time in patients with advanced pancreatic cancer (APC) undergoing chemotherapy

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Background: The prognostic value of the sialylated Lewis a blood group antigen CA 19-9 in monitoring treatment efficacy of patients with advanced pancreatic cancer (APC) remains undetermined. For patients with APC undergoing resection or radiotherapy the correlation of the tumor marker dynamic is more conclusive. However only few studies showed significant correlation between decrease of CA 19-9 level and median survival time. **Methods:** The response of CA 19-9 level to gemcitabine-based chemotherapy was retrospectively analysed in 181 consecutive patients with APC treated in our outpatient department between 12/98 and 10/05 and correlated to survival time. 150 patients with elevated baseline CA 19-9 level at time of diagnosis were seperated in two groups. Patients showing a decrease decrease of at least 20 % of CA 19-9 level after 8 weeks of therapy (group I; n=83) and patients with a stabilization or an increase of CA 19-9 level after 8 weeks of therapy (group II; n=67). **Results:** Median baseline level of CA 19-9 was 1498 U/ml (range 40-1043301 U/ml), median age was 64 years (range 33-101 yrs). Median survival of 150 patients with APC showing elevated baseline CA19-9 level treated with gemcitabine-based chemotherapy was 46 weeks (range 8-198). Patients of group I (n=83) had a median survival time of 50 weeks (range 13-198 weeks, 95% CI:38,1-61,8) versus patients of group II (n=67) with a median survival of 35 weeks (range 8-160 weeks, 95% CI:27,8-46,2; p=0.003). **Conclusions:** The decrease of CA 19-9 level of at least 20 % after 8 weeks of therapy was correlated with significant improvement in median survival in comparison with a stabilization or an increase in CA 19-9 level. Measurements of CA 19-9 should be performed in patients with APC and increased baseline level after 8 weeks of treatment to assess prognosis in addition to further clinical parameters.

Disclosure: No conflict of interest disclosed.

P506

Oncological treatment of pancreatic cancer in Germany - results from a national survey conducted on behalf of the AIO and CAO of the German Cancer Society (DKG)

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Introduction: To date, no data are available regarding the current treatment of patients (pts) with pancreatic cancer (PC) in German hospitals and medical practices. **Methods:** Between February 2007 and March 2008 we conducted a national survey regarding the current surgical and oncological treatment of PC in Germany. Standardised questionnaires were sent via mailing lists to members of the "Arbeitsgemeinschaft Internistische Onkologie" (AIO) and the "Chirurgische Arbeitsgemeinschaft Onkologie" (CAO) (n=1130). The data were analysed using SPSS software (version 16.0); pre-defined subgroup analysis was performed by grouping the results of each question with regard to the professional site of the responding physician and to the number of pts treated in their institution by year. **Results:** One-hundred and eighty-one (16%) of the oncological questionnaires were sent back. For 61% of the participating centers a histological confirmation of PC diagnosis is obligatory. Twenty-one percent of physicians offer neoadjuvant therapy to pts with potentially resectable PC. In the adjuvant treatment after curative-intent surgery, gemcitabine (Gem) is regarded as standard of care for 71% (after R0 resection) and 62% (after R1 resection), respectively. For pts with locally advanced PC, 52% of the participating centers recommend systemic chemotherapy, 17% prefer combined primary chemoradiotherapy. Most centers (59%) base their decision of combination regimens for metastatic disease on the performance status (PS) of their pts. In pts with good PS, 28% apply single-agent Gem, 3% Gem + capecitabine, 12% Gem + erlotinib, 16% Gem + oxaliplatin, and 8% Gem + cisplatin, respectively. Only 28% of the survey doctors offer second-line treatment to the majority (> 50%) of their pts with advanced PC. **Conclusion:** Not each PC patient in Germany is treated according to the present S3 guidelines. Diagnosis and treatment of PC in Germany still needs to be improved.

Disclosure: No conflict of interest disclosed.

P507

Down-regulation of Survivin/BIRC5 sensitizes human Pancreatic cancercell lines to Sorafenib induced cell death

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Study: The aim of this study was to investigate the combined effect of Survivin/BIRC5 siRNA mediated down-regulation and additional treatment with Sorafenib in human pancreatic carcinoma cell lines. Survivin/BIRC5 is a member of the inhibitor of apoptosis (IAP) family and it was reported to be expressed in fetal tissues as well as in leukemia and cancer cells, but not in normal adult tissues. The reactivation of the survivin gene occurs frequently during tumorigenesis. Moreover, Survivin expression in leukemia and cancer cells is correlated with drug resistance and clinical outcome. Therefore strategies to reduce survivin levels have been pursued for cancer therapy. **Methods:** Survivin/BIRC5 expressing human pancreatic cancer cell lines (Panc-1, AsPC-1 and BxPC-3) were incubated with siRNA targeting Survivin/BIRC5 and adjacent treatment with various concentrations of Sorafenib (0-20 µM). MTT-assay was used for determination cell proliferation. RealTime RT-PCR and Western blot were used for mRNA- and protein expression analysis. **Results:** The down-regulation of Survivin/BIRC5 with siRNA (determined with RealTime RT-PCR and western blot) resulted in morphological changes leading to expanded cells. After adjacent treatment with Sorafenib the cell proliferation was significantly inhibited (about 5-20%) compared to Sorafenib or Survivin siRNA treated cells alone. **Conclusions:** The down-regulation of Survivin/BIRC5 sensitizes tumor cells to Sorafenib. This might enhance the efficacy of Sorafenib.

Disclosure: No conflict of interest disclosed.

P508

Curcumin augments RAD001cytotoxic effect on human pancreatic cancer cell lines

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Introduction: The aim of this study was to investigate the combined effect of the mTOR inhibitor RAD001 and Curcumin in human pancreatic carcinoma cell lines with respect to cell proliferation. **Methods:** The human pancreatic cancer cell lines (Panc-1, AsPC-1 and BxPC-3) were incubated with RAD001 (1-10 µM) and Curcumin (12 µM) alone or in combination. Cell proliferation was measured over a period of 72h with MTT-assay. RealTime RT-PCR and western blot were used for mRNA- and protein expression analysis. **Results:** Curcumin as single agent had no anti-proliferative effect. The incubation with RAD001 resulted in an inhibition of cell proliferation to about 30% after 72h. The anti-proliferative effect of the mTOR inhibitor RAD001 was significantly enhanced in all cell lines tested after a combined treatment with RAD001 plus Curcumin. This effect only occured, when both compounds were given simultaneously. RealTime RT-PCR and western blot analysis revealed an increase in the expression of BCL-Xl after incubation with RAD001 alone compared to untreated control cells. This up-regulation of the anti-apoptotic protein BCL-Xl was suppressed, if the cells were treated with RAD001 and Curcumin simultaneously. **Conclusions:** Our data indicate that Curcumin may attenuate the negative feedback of the mTOR inhibitor RAD001 resulting in a decreased expression of the anti-apoptotic protein BCL-Xl. This finding may contribute to the development of more effective treatments of pancreatic carcinoma.

Disclosure: No conflict of interest disclosed.

P509

No evidence for somatic mutations within smoothened (SMO) or suppressor-of-fused (SuFu) in pancreatic cancer by direct sequencing

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Aberrant re-activation of the Hedgehog signaling pathway has recently emerged as a promising target for the development of novel treatment options for pancreatic cancer through pharmacological pathway blockade. This concept has been shown to be particularly effective in preventing metastatic spread, potentially by targeting defined subsets of cancer cells with enhanced tumor-initiating properties. However, the molecular mechanisms underlying pathway activation in pancreatic cancer are still poorly understood. The majority of studies report Hedgehog ligand overexpression in neoplastic cells and consecutive auto- or paracrine pathway activation in tumor and/or stroma cells, while others describe somatic mutations within Hedgehog pathway-related genes, the functional relevance of which is often unclear as of to date. In this study the exonic regions of the Hedgehog pathway components Smoothened (SMO) and Suppressor-of-Fused (SuFu) were sequenced, for which oncogenic mutations have been described in basal cell carcinomas and medulloblastomas. Twenty-two primer pairs were developed which were used for direct Sanger sequencing. Using this strategy, no somatic mutations were discovered in a panel of seventeen pancreatic cancer cell lines. Interestingly, however, we found a correlation between a D25G single nucleotide polymorphism rs41304185 within the N-terminal signaling peptide of SMO and basal Gli1mRNA expression levels (p=0.001). Further studies are warranted to examine possible effects of this SNP on subcellular localization of SMO and Hedgehog pathway activity

Disclosure: No conflict of interest disclosed.

P510

Capecitabine in combination with docetaxel and mitomycin C in patients with pre-treated pancreatic and cholangiocellular carcinoma

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BACKGROUND: Preclinical data suggest an enhancement of the antitumor activity of capecitabine by mitomycin C and docetaxel through upregulation of thymidine phosphorylase activity. We have previously established a safe combination regimen of these drugs (DocMitoCape). The preliminary activity was promising, especially in cholangiocellular and pancreatic carcinoma. Here we report the safety and efficacy of the DocMitoCape regimen in patients (pts) with these types of cancer treated on a compassionate use basis. **METHODS AND MATERIAL:** Pts with pre-treated cholangiocellular and pancreatic carcinoma were included in the analysis. Treatment consisted of capecitabine (2,000 mg/m^2 day 1-14) in combination with docetaxel (40 mg/m^2 day 1) and mitomycin C (4 mg/m^2 day 1). Cycles were repeated on day 22. Toxicity was graded according to NCI-CTC criteria (version 3.0) and the antitumor activity was assessed by RECIST criteria. **RESULTS:** Twenty-eight pts (m 16 / f 12) with a median age of 59 years (range 43 – 80) were included. Localisation of the primary tumor was as follows: pancreatic n=16, gallbladder n=5, intra- (IHCC) or extrahepatic (EHCC) cholangiocellular carcinoma n=5/2. Pretreatment consisted of gemcitabine n=27, capecitabine/5-FU n=10, oxaliplatin n=5, and investigational agents n=8. Eleven pts had received = 2 lines of prior chemotherapy. Pts received a median of 6 cycles (range 1 – 21). The mean dose intensity was as follows (cycles 1-2 / 3-4; %): capecitabine 100/92, docetaxel 100/100, mitomycin C 99/100. Main adverse events grades 2/3/4 were (n): leukocytopenia 3/2/2, anemia 13/4/0, thrombocytopenia 3/1/0, nausea/vomiting 1/1/0, diarrhea 5/1/0, hand-foot-syndrome 7/0/0. Two pts achieved partial and 12 pts minor remissions, while 5 pts had stable disease adding to a tumor control rate of 68%. Nine pts did either not undergo restaging or had progressive disease. The median progression-free and overall survival was 4.7 (range 1.0 – 44.9) and 6.6 months (range 1.5 – 44.9) calculated from the start of therapy. **CONCLUSION:** The DocMitoCape regimen showed a favourable safety profile and exhibited a high rate of tumor control in patients with heavily pretreated pancreatic and cholangiocellular carcinoma.

Disclosure: No conflict of interest disclosed.

Posterdiskussion
Sarkome/Hirntumoren/QM

P511

A Phase II Clinical Trial Of Neoadjuvant Trabectedin In Patients With Non Metastatic Advanced Myxoid / Round Cell Liposarcoma (MRCL)

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Background: Trabectedin (T) (ET-743, Yondelis®), a marine-derived alkaloid has demonstrated significant activity in the treatment of soft tissue sarcomas (STS) and has received EMEA approval for this indication. Myxoid /round cell liposarcoma (MRCL), a subtype that accounts for 10% of STS, displays the (12:16) (q13;p11) translocation leading to the fusion gene FUS-CHOP in 95% of all cases. Preliminary results of neoadjuvant T in advanced MRCL showed reduction in size and density of the tumor, clinical improvement, and a pathological complete response (pCR) in the resected tumor mass. A phase II multicenter study to further determine the response to T in the MRCL population is presented. **Methods:** Patients (pts) with locally advanced (stage III) or locally recurrent MRCL were treated for 3-6 cycles with T (1.5 mg/m^2 q3wk) in the neoadjuvant setting. Main endpoints were: pCR rate, objective response rate by RECIST and correlation of molecular parameters from tissue samples with clinical outcomes. **Results:** Twenty-nine pts with locally advanced MRCL were recruited, of whom 23 were evaluable. All had the translocation which causes the chimeric FUS-CHOP. Median age was 47 (23-75) and male:female ratio was 1.2;1. Nineteen pts had completed therapy and undergone curative surgery. Pathological assessment was performed in 16 pts: 2 achieved pCR, as per central pathology review, 1 pt had a very good pathological response and 7 had moderate tumor regression. Seven patients remain to be histologically evaluated. Response rate by RECIST from pts who completed therapy was: 5 partial responses (26%) and 14 disease stabilizations. Remarkably, pathological response did not entirely correlate with response by RECIST since pts with pCR still had radiological disease but no malignant component was found in the excised tumor mass (connective and reactive tissue). Three serious adverse reactions of severe rhabdomyolysis, asthenia, nausea and transaminase elevation and mucositis were reported. Most common events were liver enzyme elevation, neutropenia and thrombocytopenia. Updated results will be presented. **Conclusion:** These results in terms of objective and complete pathologic responses, strongly suggest that T may have an important role in the neoadjuvant setting in pts with MRCL.

Disclosure: No conflict of interest disclosed.

P512

Results of the trabectedin compassionate use program in advanced sarcoma failing doxorubicin containing regimens

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Background: Between 2005 and 2008, 387 patients (pts) with advanced sarcoma failing doxorubicin were treated in a compassionate use program (ATU) of trabectedin in France using the standard 1.5mg/m2/CI 24h q21d regimen. The purpose of this study was to assess the outcome of pts treated in this program. Patients and **methods:** From to December 2007, 87 centers (ctrs) included at least 1 pt in the ATU program. Inclusion criteria were those of the EORTC trial (JCO 2005;23:5276), with no restriction on the previous number of lines. A simple CRF with 22 items was used to collect pts characteristics and outcome. 189 pts files were collected as of Dec 28, 2008. Univariate and multivariate analyses of prognostic factors was performed. **Results:** 235 pts were included in the 17 ctrs in which >4 pts were treated. 44 ctrs treated only 1 pt. 52% were female; major histological subgroups were leiomyosarcoma (29%) and liposarcoma (20%). All pts had been treated with doxorubicin and ifosfamide, 3 (1.5%) in adjuvant setting only. Trabectedin was given in 1st, 2nd, 3rd or 4th line in metastatic phase in n=8, 69, 66, 42 pts respectively (median: 3rd line). The median number of courses were 3 (range 1-24). Best response reported were PR, n=15 (8%), SD, n=68 (36%) and PD, n=94 (50%), NE, n=11 (6%). With a median follow-up of 805 days (d), median PFS and OS were 91 d and 309 d respectively. 27/127 (20%) evaluable pts had to be hospitalized for treatment related side effects. PFS was superior in myxoid LPS (median 192 d vs 69 d, p=0.003), retroperitoneal sarcomas (median 104 d vs 69 d, p=0.006), and grade 1 tumors (median 141 d vs 70 d, p=0.01). In multivariate analysis (Cox model), tumor site, grade 1, histotype were the only independent prognostic factors for PFS. For OS, favourable prognostic factors in univariate analysis were histotypes (MFH, MyxLPS), grade 1 lesions, retroperitoneal site , no hospitalisation for toxicity (p<0.01 all) while Cox model identified female gender, tumor site, histotype as the only independent prognostic factors for PFS. **Conclusion:** In this compassionate use program for heavily pretreated patients with advanced soft tissue sarcomas failing doxorubicin and ifosfamide, trabectedin yielded PFS and overall survival close to those observed in phase II and III trial. PFS and OS are superior in myxoid LPS, retroperitoneal sarcomas.

Disclosure: No conflict of interest disclosed.

P513

Anti-angiogenic treatment in patients with metastatic or refractory sarcoma. A single center experience.

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Background: Angiogenesis plays a crucial role in the pathogenesis of cancer. In sarcomas the overexpression of angiogenic factors as VEGF, PDGF and Angiopoetin-2 in the tumor or blood are associated with higher tumor grade, larger tumor size and lower Overall and Progression Free Survival (OS, PFS) (Yudoh 2001, Hayes 2004, Yoon 2006). The role of anti-angiogenic treatment in sarcoma still have to be defined. There are just a few studies addressing the efficacy of drugs like thalidomide (thal), bevacizumab (bev) and sunitinib (sun) in this tumor type (Yi-Shin 2006, Keohan 2008). **Methods:** Patients with metastatic and/or refractory non-GIST-sarcomas being treated with either thal, bev or sun as single agent or in combination with chemotherapy were eligible for analysis. **Results:** 26 patients received anti-angiogenic treatment in between 2004 and 2009 for various types of sarcomas (leiomyosarcoma, osteosarcoma, Ewings sarcoma, MPNST, angiosarcoma, chondrosarcoma) in median as 3rd line therapy (range: 1st to 9th) displaying a median OS of 11 months (range: 1 to 50). Disease control rates were 66% for bev (n=21) with 6 partial responses (PR), 45% for sun (n=11) and 50% for thal (n=6) with one PR. Median PFS was 5,5 months for bev and 4,5 months for sun and thal. Bev usually was combined with ifosfamide, platinum, etoposide or anthracycline whereas thal was combined with trofosfamide and sun was administered as single therapy or in combination with radiation. Anti-angiogenic treatment generally was well tolerated. One patient had a gastrointestinal bleeding after 16 months of bev. **Conclusion:** Anti-angiogenic treatment as part of multimodal therapy in heavily pretreated non-GIST sarcoma shows considerable response and survival. The efficacy observed justifies further evaluation.

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Schmoll,H.-J.: Beratungstätigkeit:Roche, Sanofi-Aventis, Merck Sharp & Dohme; Honorare: Roche, Sanofi-Aventis, Merck Sharp & Dohme

P514

The mTOR signaling pathway as potential therapeutic target in meningiomas

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Meningiomas represent the second most frequent intracranial tumors. While most meningiomas correspond to benign WHO grade I tumors, about 5% are aggressive atypical meningiomas, and 1% of tumors are anaplastic meningiomas with poor clinical outcome. However, even the treatment of benign meningiomas might be challenging in tumors impossible to resect completely, or in recurrent tumors. So far, no chemotherapy regime has been proven to be effective; radiotherapy is used to treat aggressive meningiomas. Here, in human meningiomas we studied for the first time in detail the expression and function of different factors of the mTOR1-p70S6K signalling pathway essentially involved in the regulation of protein biosynthesis. Using phospho-specific antibodies, we found that nearly all meningiomas irrespective of their grade of malignancy did express mTOR, p70S6K, 4EB-P1, and Rheb. For p70S6K, we observed an increased phosphorylation in atypical and anaplastic meningiomas. Western blot studies confirmed high levels of phospho-p70 and phospho-mTOR in various meningiomas of WHO grade I-III. Next, using four different meningioma cell lines (two derived from benign and two derived from anaplastic meningiomas, respectively), we established rapamycin-sensitivity of these cells. Using different doses of temsirolimus, we there then able to show highly significant antiproliferative and cytotoxic effects of the drug. Temsirolimus was capable to induce apoptosis in the meningioma cells. Furthermore, the antineoplastic effect of temsirolimus could be substantially increased in cells treated concomitantly with 5Gy irradiation, independent of the cell line investigated. Taken together, our data suggest that mTOR inhibition in meningiomas might be an additional option to treat unresectable or malignant meningiomas.

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P515

Update on results of the interlaboratory tests in the quality control of leukaemia cytogenetics

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Introduction: Chromosome analyses are indispensable in leukaemia diagnostics. The findings have a major influence in leukaemia diagnosis and treatment. Because the interpretation of the results of the analyses depends both on the quality of the chromosome preparation and the experience of the investigator, an interlaboratory test system to assess the quality of cytogenetic investigations was developed in 2004 as a pilot project. We report an update of the results of a total of five tests which have been completed so far. **Methods:** A surrogate leukaemic blood sample containing distinct mixtures of cell lines carrying known chromosome aberrations was created and sent out to the participating laboratories. Analyses of the samples were performed according to the procedures of the individual laboratories. A review committee evaluated the cytogenetic reports concerning to the minimum standards according to the ISO 15189 and for formal accuracy of the determined karyotype consistent with the ISCN. Target aberrations were defined for each cell line. The analytical perfomance of the laboratories was measured on the basis of the proportion of the detected target aberrations. Altogether five different interlaboratory tests with five cell lines containing diverse chromosome aberrations were performed. **Results:** Up to 40 laboratories from Germany, Austria, Switzerland, Denmark and Turkey took part in the interlaboratory tests. Karyotype formulas were inaccurate in up to 73%. All target aberrations for each cell line were found in 8-89% of the laboratories. Low performance was associated with complex karyotypes and high performance was reached in cell lines with simple karyotypes. Delivery time did not have a negative influence on the performance. **Conclusions:** The established interlaboratory test system with viable cells revealed differences in the analytical performance between different laboratories. The differences became more evident with more complex karyotypes. Therefore, by using appropriate cell lines a detailed assessment of the quality of the complete procedure of chromosome banding analyses in leukaemias is possible. Interlaboratory tests with viable cells should be implemented in external quality assessment schemes in leukaemia cytogenetics. Supported by the BMBF, grant 01GI 9974

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P516

„FlexiDoc"– a valid database for in-house quality management and preparation of health care data"

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Introduction: The directive of 2006-01-01 issued by the Federal Joint Committee (paramount decision-making body of the medical self-governing bodies of service providers and health insurance funds) defines the requirements for an in-house quality management by SHI-authorized physicians and care centres. An indispensable tool for the documentation, evaluation, and publication of the provided quality is an adequate computer based database. We developed a software programme ("FlexiDoc"), which processes and evaluates data for quality management and certification purposes after simple standardized data input. With our tool, the data can be used for quality assurance as well as for health care research. **Objective:** The objective is the establishment of the database "FlexiDoc" in an outpatient setting in order to collect and evaluate data from patients with oncological disorders for the purpose of quality management and health care research. **Methods:** - Design of flexible and personalized documentation templates within a Firebird based database - Prompt documentation of patient data, based on standardized templates - Preparation and evaluation of input data. **Results:** The following quality assurance projects have been completed: Neofolin®, NeoTaxan®, Cisplatin NC

Period of documentation: 2006-04-01 to 2008-12-31 Number of care centres: 4. The data could be easily retrieved with standardized queries and could be displayed in graphs. **Conclusion:** "FlexiDoc" is a convenient tool for tracking and evaluating quality standards of in-house patient data. Furthermore, the software fulfils the requirements of the Federal Joint Committee concerning quality assurance and certification. The data input is easy and requires minimal expenditure of time. Moreover, it is possible to customize the documentation templates to each patient. A plausibility check avoids false input. FlexiDoc is a cheap, user-friendly, stable, and safe database, which can be used in any kind of tumour centre supporting quality management and health care research. The results of the mentioned quality management projects will be presented.

Disclosure: No conflict of interest disclosed.

Posterdiskussion
Supportive Therapie sonstige

P517

Treatment of cetuximab-induced skin rash: results of a survey among German oncologists

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Introduction: Skin reactions including akneiform rash are the main toxicities observed in patients receiving antibodies against the epidermal growth factor receptor (EGFR). The grading of these skin reactions is poorly standardized and the optimal treatment is unknown. Thus far only three small randomized trials have been published on the management of skin toxicity. **Methods:** We are currently conducting a survey using a 7-item-questionaire among German oncologists involved in the treatment with anti-EGFR antibodies. The oncologists are provided with pictures of a patient with an acneiform rash which developed after three weeks of cetuximab based therapy. By then the patient had only used skin moisturizer and sun screen. Doctors were asked to provide information on the following items : (i) affiliation, sex and age group; (ii) grading of skin rash; (iii) choice of preemptive treatment; (iv) choice of watch & wait strategy; (v) choice of local and/or systemic treatment; (vi) referral to dermatologist; (vii) use of dose reduction and/or interruption of treatment. **Results:** Thus far 101 oncologists (10 of whom dermato-oncologists) responded. The doctor´s characteristics are: 71% male, 47% age group 35-45 years, 76% affiliated at hospitals. The scoring of the skin rash was as follows (NCI-CTC grades 1/2/3; %) 11/60/29. 17% of the participants are using preemtive treatment of skin rash chiefly based on hydrocortisone (10/17) and minocycline (11/17). Only 2 doctors would have used a watch & wait strategy in the present case. 90% chose local treatment (hydrocortisone n=40, metronidazol n=30, erythromycin n=24, nadifloxacin n=9), and 63% of the doctors used systemic treatment (doxycycline n=33; minocycline n=31). 7/91 oncologists (8%) would have referred the patient to a dermatologist. 17 oncologists would have delayed cetuximab treatment, but only two participants would have reduced cetuximab dose. **Conclusions:** The results of the present analysis illustrate that the treatment of skin reactions is very heterogenous. Only a low percentage of oncologists considers referral to dermatologists or the use of preemptive treatment. Moreover, the available grading system for skin rash appears not to be suitable for the use in clinical practice. Clearly, more randomized trials on the treatment and a simple and reliable grading system of skin rash are warranted.

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Hofheinz,R.: Beratungstätigkeit: Beratungstätigkeit für die Firmen Amgen und Merck; Honorare: Beratungstätigkeit bzw Vorträge bei Advisory Boards

P518

The triple combination of the NK-1 antagonist aprepitant (APR) with granisetron and dexamethasone in high dose chemotherapy (HDC)

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Introduction: 5-HT_3 receptor antagonists (RA) plus dexamethasone (DEX) are still the standard antiemetic therapy in patients receiving HDC. However, in the few available small studies complete protection from nausea and vomiting was only achieved in a small proportion of patients. The role of aprepitant in HDC remains to be defined. **Methods:** In this study, pts. with HDC received APR orally 125 mg d1, 80 mg consecutive days, granisetron (GRAN) 1 mg i.v. daily and DEX 8 mg i.v. daily for prevention of acute chemotherapy induced nausea and vomiting (CINV) and APR 80 mg and DEX 8 mg for 2 days for delayed CINV. Endpoints were complete response (CR, no vomiting & no use of rescue therapy) in the acute (during days of HDC) and delayed (day 1 until 5 days after end of HDC) phase. Acute and delayed nausea were also evaluated. **Results:** To date 45 pts. (f/m 9/36 pts.; median age 39,4 y) with various types of cancers (testicular cancer 25 pts., sarcoma 8 pts., multiple myeloma 9 pts., thymic carcinoma 2 pts and CUP 1 pt.) were included. 30 pts. (66,7%) received High dose (HD)-PICE (paclitaxel, ifosfamide, carboplatin, etoposide; d1-3), 6 pts. (13,3%) HD-PEI (cisplatinum, etoposide, ifosfamide; d1-5) and 9 pts. (20%) HD melphalan; d1-2. The median duration of HDC was 2.8 days. Acute and delayed CR were observed in 33 pts. (73,3%) and 27 pts. (60%) respectively. Acute and delayed nausea were observed in 14 pts. (31,1%) and 18 pts. (40%). No CTC Grade 3-4 were observed within the observational period. The incidence of ifosfamide induced encephalopathy was 20,5%. **Conclusions:** The triple antiemetic combination including the NK1-antagonist aprepitant showed a favourable safety profile and good antiemetic efficacy in HDC. Compared to clinical data from the literature, aprepitant provides additional benefit in preventing CINV during HDC. The study is still ongoing.

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P519

Effect of Erythropoeitin (EPO) on the functional status (FS) in elderly patients with cancer

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Introduction: Anemia is an important prognostic factor for survival and quality of life in cancer patients. For elderly patients the ability to care for themselves is a major factor of their quality of life. Geriatric medicine provides validated tools to measure this ability, such as activities of daily living (ADL), instrumental ADL (IADL), and "Timed-up and go" test, summarized as FS. **Objectives:** Primary objective was to obtain data on the FS in elderly patients treated with chemotherapy. Secondary objectives were transfusion requirements and. **Methods:** Prospective non-interventional study. Main inclusion criteria: (1) age = 70 years, (2) solid tumor, multiple myeloma, or lymphoma, (3) ongoing chemotherapy. The choice to administer ERYPO® as well as the dosage remained at the discretion of the treating physician. FS was measured over approximately 8 months (incl. a 6-month follow-up period). Results were compared in patients with and without treatment with EPO. **Results:** 155 patients have been registered and 150 analyzed (86 with and 64 without EPO treatment = ITT and safety population). 5 patients were screening failures. Patients presented with only minor impairments in FS at baseline examination. The condition of the patients in either treatment group remained on average stable or showed only mild deteriorations without a relevant difference between the two treatment groups. Hb levels quickly

increased in the EPO treated group. The necessity for administration of blood transfusion was lower in the EPO-group (29.1%) vs. the Non-EPO group (37.5%). A total of 889 AEs were reported 81 (54%) of patients in the EPO group and 55 (37%) in the no EPO group. For 6 of 889 AEs (0.7%) in 5 of 150 patients (3.3%) a causal relationship was assessed at least as possible. None of the drug-related AEs led to premature discontinuation. 127 SAEs occurred in 39 (45.3%) patients of the EPO group and 88 SAEs occurred in 30 (46.9%) patients of the no EPO group. 24 (28.0%) patients of the EPO group and 15 (23.4%) patients of the no EPO group died during the study period. No SAE or deaths was rated as related to the study medication. **Conclusions:** (1) No significant effect of a supplemental EPO treatment on the functional status in elderly tumor patients could be demonstrated, (2) lower transfusion rates and fast Hb-responses confirmed the anti-anemic efficacy, (3) the treatment with EPO FS within the label appears to be a safe option for cancer patients undergoing chemotherapy.

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Frohn,C.: Anstellungsverhältnis oder Führungsposition: Ortho-Biotech, Division of Janssen-Cilag GmbH

P520

Use of Lenograstim in Daily Routine in German Outpatient Cancer Centres

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Introduction: Neutropenia is a common complication of myelosuppressive chemotherapy regimen, resulting in infection-related morbidity and mortality. In addition, neutropenia and febrile neutropenia cause dose-reductions and delays of the scheduled chemotherapy at a significant rate. Granulocyte-colony stimulating factors like lenograstim have been successfully used to decrease the risk and duration of neutropenia. **OBJECTIVES:** Aim of this observational study is to describe the use of lenograstim in routine daily practice, beyond clinical trials. **PATIENTS AND METHODS:** From April 2006 to December 2008 523 patients, treated with lenograstim, were enrolled by 36 outpatient cancer centres. Data with respect to patient anamnesis, tumor entity, chemotherapy and details of lenograstim application were documented. **RESULTS:** Mean age of patients treated with lenograstim was 62,1 years (+/- 12,8) with 39,5 % older then 65 years. 68,6% patients were female. Major tumor entities: breast cancer (40,9%), Non-Hodgkin`s Lymphoma (28,1%), bronchial carcinoma (7,5%) or Hodgkin-Lymphomas (4,2%). In total, 65,2% of the various tumor types were solid tumors and 33,5% had a haematological origin. Among patients with haematological tumors the most common chemotherapy substances were cyclophosphamide (70,3%), doxorubicine (65,2%) and vincristine (59,4%), often in combination with rituximab. Chemotherapy compounds applied to treat solid tumors were cyclophosphamide (43,1%), eprirubicin (29,6%), 5-FU (27,6%) and docetaxel (24,3%). The intention for the first lenograstim treatment in haematological tumors were primary prophylaxis (56%), secondary prophylaxis (12%) and as interventional treatment (31,4%). In contrast, only 33,7% of the patients with a solid tumor received the first lenograstim treatment for primary prophylaxis. An interventional treatment was the case in 43,7% of the patients and 23,3% received lenograstim as secondary prophylaxis. In general, lenograstim treatment was initiated with 263 µg/day (72,3 % of all patients) and was supplemented by antibiotics in 22,9 % of all cases. Initial lenograstim treatment was applied on day 6,5 (+/- 3,4) in haematological tumors and day 7 (+/- 3,6) in solid tumors after initiation of chemotherapy. For both settings treatment duration of lenograstim was comparable, with 4 (+/- 2,5) days in solid and 5 (+/- 3,1) days in haematological tumors.

Disclosure: Schröder, J.: Honorare: Prüfarzt – Prüfarzthonorar bis zu 250 pro Patient; Finanzierung wissenschaftlicher Untersuchung: Investigator
Heinisch,H.: Anstellungsverhältnis oder Führungsposition:Medical Director – Chugai Pharma

P521

Palatal mucormycosis in a patient with relapse of acute lymphoblastic leukemia: an infrequent and fatal diagnosis

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Introduction: Zygomycosis is an opportunistic infection caused by a series of fungi of the Mucorales group. They are ubiquitous fungi, usually harmless, characterized by causing acute rhinocerebral, pulmonary, cutaneous, gastric and disseminated manifestations. It occurs primarily in decompensated patients with diabetes and in immunocompromised patients, particulary in those with neutropenia or in transplant recipients. It involves an acute course and is a classical nosocomial condition. However, only isolated reports can be found in the literature. Case report: Here, we describe a 47 year old turkish woman with an early relapse of a common acute lymphoblastic leukemia. In neutropenia at day 14 after an induction therapy according to the FLAG-Ida regimen the patient presented a small black ulcer at the right palate level. At this time point a biopsy sample of the palatal region was taken. One day later the diagnosis of zygomyces was assumed by microscopy. Directly, we started a comprehensive treatment with liposomal amphotericin B at a dose of 3 mg/kg/day. In the following four days the dissemination converged in the nasal sinuses, the upper right lip, the bridge, the soft parts of the adjacent cheek with black dry necrotic ulcer. A few days later the species of the mucor group was confirmed by cultures. In MRT the palatal ulcer with perforation of the adjacent cheek could be demonstrated and no contrasting of the midface could be described confirming a necrosis in the region. Moreover, in MRT a dural involvement was seen. However, the patient had no neurological failures. Despite antifungal therapy, adding granulocyte colony stimulating factors in neutropenia and other stabilizing treatment the ulceration spread, and in the following days the woman died. **Conclusion:** Zygomycosis is usually an acute condition and develops in only a few days. In cases of clinical suspicion, mycological tests, such as direct examinations, are extremely effective. In most cases a resection is not possible due to defacement. The treatment of choice is still liposomal amphotericin B because of the better penetration in the soft tissue in comparison to the azoles. However, recent reports showed a great in vitro activity of a new triazole derivative, posaconazole, against Mucorales, which represents a new treatment choice against zygomycosis. Recovery from disseminated fungal infections is unlikely and in most cases fatal, however, unless the patient`s neutropenia resolves.

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P522

A 58 year old patient with black urine after chemotherapy – a hemolytic crisis caused by Clostridium perfringens.

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Introduction: A 58 year old patient was diagnosed with a granular cell tumor in the right upper and lateral abdominal wall after the initial wrong diagnosis of chronic cholecystitis, cholecystectomia and postoperative biliary stentimplantation. A chemotherapy with Epirubicine and Ifosfamide was induced, in the consecutive neutropenia the patient was hospitalised because of a severe septic event and a decrease of hemoglobin in spite of erythrocyte transfusion. Black urine and the pathological parameters of hemolysis and cholestasis were impressive and led to the diagnosis of an acute intravascular hemolysis. Differential diagnosis were a blood transfusion reaction, a infectious agent or a hemolytic-uremic syndrome. **Methods:** We will give a short review of the medical history and an introduction of granular cell tumor as a soft part sarcoma with neural derivation. Differential diagnosis of intravascular hemolysis will be discussed and Clostridium perfringens will be shown as a cause of hemolytic anemia. **Results:** Our patient presented in neutropenia a cholangitis due to cholestases after cholecystectomy and biliary stentimplantation. Clostridium perfringens was isolated in the blood culture. This ubiquitous grampositive anaerobic bacterium can cause as well the

typical gas gangrene or food intoxications as an endogenous gastrointestinal infection. The outcome of an infection with clostridium perfringens can be a pronounced intravascular hemolysis with anemia. The best therapy is the intravenous application of Penicilline G and Clindamycine. After this and a change of the biliary stent our patient recovered soon and the pathological parameters of hemolysis, cholestases and hemoglobine level normalised**. Conclusion:** Granular cell tumor is a rare soft tissue sarcoma with nerval origin. Cholestases after stentimplantation can lead to an endogenous gastrointestinal infection with Clostridium perfringens. This bacterium can cause a massive intravascular hemolysis.

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P523

Epoetin alfa maintains quality of life and improves hemoglobin in anemic cancer patients below 60 years of age

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Introduction: Malignant diseases are often associated with significant anemia due to the underlying disease itself or treatment associated. Objectives: Primary objective was to obtain data on hematologic response, defined as the proportion of patients achieving a hemoglobin (Hb) increase of 1 g/dl or to reach the upper Hb-target of 12 g/dl at end of treatment. Secondary objectives were tolerability, transfusion need, time to hematologic response and change of quality of life and physical performance status. **Methods:** Prospective non-interventional study. Main inclusion criteria were: (1) age 18-60 years (2) solid tumor (3) ongoing chemotherapy (4) Hb-value below 11 g/dl. The choice to administer ERYPO® as supportive therapy as well as the dosage remained at the discretion of the treating physician. Patients were to be documented over up to 24 weeks. **Results:** 192 patients have been documented (safety set). 19 patients had to be excluded from the full analysis set due to missing documentations. EFFICACY **RESULTS:** 49.1% of the patients achieved a hematologic response. Median time to a hematologic response was 30 days. 77.5% did not require any blood transfusion. The Karnofsky performance status scale improved significantly during treatment ($p<0.0001$). EQ-5D scores during the treatment remained stable. Linear analogue self assessment (LASA) scores of the most patients improved from baseline to last visit in each of the categories (energy 53.1%, daily activities 54.3%, overall 45.7%). The change in hemoglobin levels from baseline to last value was statistically significantly correlated ($p<0.001$) with the change in LASA scores. Safety **results:** Overall, 122 AEs were documented in 35 patients. 18.2% of patients experienced at least one AE. SAEs occurred in 8.9% of patients (N=17) with tumor progression (7.3% of the safety set) and general disorders and administration site conditions (3.1%) being the most common SAEs. 14 patients died due to tumor progression and 1 patient died of multi-organ-failure. No SAE or death was assessed as related to study medication. In 2.6% of patients an AE was considered at least possibly related to Epoetin alfa. Thrombovascular events occurred in 1.6% of the study population during the study (N=3). **Conclusions:** Results from this non-interventional study indicate that Epoetin alfa as used in the everyday clinical practice setting improves hemoglobin levels in cancer patients under chemotherapy and helps to maintain quality of life.

Disclosure: Gauler,T.: Honorare: Ortho Biotech Division of Janssen-Cilag GmbH Frohn,C.: Anstellungsverhältnis oder Führungsposition: Ortho Biotech Division of Janssen-Cilag GmbH

P524

Epidemiological register of the image ofantiemetic-strategies in the everyday routine

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With the approval of the first 5-HT3 antagonists 11/1990 and first neurokinin-1 receptor antagonists 11/2003 potent antiemetics are available. By an optimized antiemetic regimen even in cisplatinum-based chemotherapy (CTX) nausea/emesis can be avoided in up to 70-90% of patients. In this study, the everyday routine of antiemetic therapy in patients with a platinum- or anthracycline/ cyclophosphamide-based CTX is checked and correlated with the guidelines. 3/09 was the first review. 487 Patients and 1105 antiemetic treatments were evaluated. 70% of the patients were female, 30% male. The median age was 57.6 years. 48 % had breast cancer, 12% colorectal cancer, 7% NSCLC and 5% ovarian cancer. In correlation to the entities women were more frequently treated with anthracycline/cyclophosphamide-based CTX, while men more often received platinum-based CTX. In the evaluated collective 15.4% received cisplatin, 42.9% anthracycline/cyclophosphamide-based CTX and 28.5% carbo/oxaliplatinum-based CTX. 5-HT3 antagonists were used in almost all antiemetic treatments (93.5%). Aprepitant was used in 40% of the antiemetic regimens. In cisplatin-based CTX the percentage was 70.4%, in anthracycline/cyclophosphamide-based CTX 54.4% and in carbo- /oxaliplatinum based CTX 12.3%. In anthracycline/cyclophosphamide-based CTX the use of aprepitant increased steadily after the 1st CTX-administration from 48.4% in the 1st to 72.7% in the 4th CTX-administration. In cisplatinum-based CTX the use of aprepitant remained stable at around 70%, in carbo-/oxaliplatinum-based CTX the use of aprepitant increased from 12 to 17%. The increase of the use of aprepitant in anthracycline/cyclophosphamide-based CTX can indicate that the initial antiemetic regimen was insufficiently effective. Nevertheless the safety and the efficacy of antiemetic regimens with and without aprepitant are estimated equal by many physicians. It may be interesting in the final analysis to compare the impressions of the patients to those of the physicians regarding these aspects.

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P525

Evaluation of Complementary Treatments in Oncology, especially Traditional Chinese Medicine

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Introduction: Complementary treatments are used by more than 50% of cancer patients and more than 90% of breast cancer patients regard them as helpful. This means, that most of our patients have already made their decision and often use this treatments without knowledge of the attending physician At present we can only advise patients to avoid additional treatments during chemotherapy because of unknown interactions. The available data are often conflicting. Missing are data to assess value and safety in connection with standard treatments. This should be evaluated according to the established standards. Even if treatments are more individualized, clinical endpoints as quality of life, overall survival and time to progression can be used. Traditional Chinese Medicine (TCM) can be used as a model for complementary treatment. TCM is based on a conclusive concept and diagnostic procedures and treatments are standardized. There are hints, that there is a specific effect in cancer patients: 1. From TCM-decoctions cytotoxic drugs have been developed (Irinotecan, Topotecan, Indirubin). 2. Some extracts influence cell differentiation (ATRA, ATO). 3. They probably influence angiogenesis (Hydroxycamptotecin). 4. They probably interfere with tumor – host interaction 5. Preclinical date show effects on the immunsystem. **Methods:** Two aspects have to be evaluated: 1. Is the treatment effective? (What the patient wants to know) 2. Is any effect specific or unspecific? (What science and finance want to know) In a controlled study we will compare standard treatment for metastatic breast cancer with standard treatment combined with TCM- decoction.

Patients will be stratified according to their treatment of choice (arm A & B) or randomized, if they agree (arm C & D). This study concept comes close to the present treatment reality and avoids selection bias as far as possible (rejection to randomization, additional treatments without information). End points are quality of life, overall survival and time to progression. Safety is ensured by continuous control of blood levels of cytotoxic drugs during treatment. By comparison of the 4 treatment arms we can evaluate effectiveness and to some degree specific effects. **Results:** A pilot study with 79 patients in Jinan, China has shown that the combined treatment concept does improve nausea and vomiting as well as the leukocyte nadir during combination chemotherapy. **Conclusion:** To advise patients it is necessary to evaluate treatments, which are regularly used. This is possible by using standard protocols. Special requirements of patients have to be considered for randomization. Safety has to be ensured by controlling the effectiveness of the standard treatment.

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P526

Reduced transfusion (RETRA) rate with Darbepoetin alpha (DA) 500 µg given every 3 weeks for chemotherapy induced anaemia (CIA)

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Background: CIA requiring red blood cell transfusions (RBCT) is a common complication of myelosuppressive chemotherapy affecting up to 60 % of pts with solid tumours. RETRA, a non-interventional observational study, was initiated to observe if DA (Aranesp®) 500µg administered every 3 weeks according to label and current guidelines maintains a low RBCT rate. **Methods:** RETRA was initiated in 21 centres in Austria to prospectively collect data in pts with solid tumours suffering from CIA (Hb<11 g/dl as per EORTC guidelines). As primary endpoint, RBCT rate was assessed after 12 weeks of DA treatment and 4 weeks of follow-up. Appropriate descriptive and exploratory statistical analyses were done; the Kaplan-Meier method was used to analyze time to RBCT. SAS version 9.1 and R version 2.7.2 was used for analysis. **Results:** Between March 2006 and September 2008, 268 patients started DA treatment. Of these, 76% reached the primary endpoint. Mean baseline Hb was 10 g/dl (SD: 0.80), 14% of the patients had ECOG=2 at baseline. The proportion of patients who had RBCT was 16% (95% CI: 12-21%), with 9%, 11%, 23%, and 33% in breast, ovarian, NSCLC and colorectal cancer pts respectively. The median time to RBCT was 32 days (95% CI: 22-51 days). Baseline Hb was marginally lower in 41 DA+RBCT pts (9.6±0.9 g/dl) than in 214 DA pts (10.0±0.8 g/dl). DA increased Hb by a mean of 1.1 g/dl after 12 weeks, and 1.3 g/dl at the end of study, similarly to that observed for DA+RBCT pts (0.7 and 1.4 g/l, respectively). DA was initiated in 88% of pts at the Hb level recommended in the EORTC guidelines (i.e. 9.0-11.0 g/dl). A ceiling Hb = 12.0 g/dl was reached in 32% at end of study (32% for DA, 30% for DA+RBCT) but Hb>13.0 g/dl occurred in only in 9% (9% for DA and 12% for DA+RBCT). **Conclusions:** If administered according to label and current guidelines, DA effectively increases Hb to the recommended level without RBCT, and potentially harmful RBCTs – as described in a recent meta-analyses (Ludwig et al, JCO 2009) – were required only in few pts.

Disclosure: No conflict of interest disclosed.

P527

A multicenter observational study to investigate the efficacy and tolerability of Privigen, a novel polyvalent intravenous immunoglobulin (IVIG) preparation

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Introduction: Privigen is a 10% liquid preparation of polyvalent human IgG for intravenous administration, which has been granted market authorization in April 2008. The use of a novel stabiliser, L-proline, fully preserves IgG functional activity without refrigeration, making Privigen ready to use. Privigen is licenced as maintenance therapy in primary or secondary immunodeficiencies and as immunomodulatory therapy in autoimmune or inflammatory diseases. **Methods:** This is an interim analysis of an ongoing multicenter observational study to evaluate the efficacy and tolerability of Privigen in 14 German sites. Data for this interim analysis were collected from the start of the study in July 2008 to February 2009. **Results:** 30 patients (14 m, 16 w, mean age 57 years) received a total of 108 Privigen infusions with a mean dose of 15 g. The indications for the IVIG treatment were primary immunodeficiency (N=3), secondary immunodeficiency (N=13), immune thrombocytopenic purpura (N=5) and other autoimmune diseases (N=13). During the course of treatment, the mean duration of infusion could be reduced continuously from 109 min at the first application to 78 min at applications 4-6, indicating good tolerability. The efficacy of Privigen was judged by the physicians as very good in 74 %, as good in 7 %, as moderate in 15 % and as insufficient in 4 % of the cases. The tolerability of Privigen was judged by the physicians as very good in 85 %, as good in 4 %, as moderate in 7 % and as insufficient in 4 % of the cases. For only 2 patients (7 %), adverse events possibly or probably related to privigen (nausea in both cases) have been reported as of the cut-off date of this interim analysis. **Conclusion:** The present data demonstrate very good efficacy and tolerability of Privigen in the majority of patients requiring IVIG treatment for different indications.

Disclosure: Pfründer,D.: Anstellungsverhältnis oder Führungsposition: Angestellter von CSL Behring
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P528

Use of palifermin in a patient with severe psoriasis.

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Introduction: Palifermin is a recombinant keratinocyte growth factor (KGF) which has lately gained increasing interest in the treatment of hematological malignancies as well as solid tumors for prevention of chemo- or radiotherapy-induced oral mucositis. Alterations in the KGF signaling pathway have been proposed to account for epidermal hyperplasia associated with psoriasis. Psoriasis is characterized by an increased epidermal proliferation in the skin and an abnormal differentiation sequence of keratinocytes. A severe exacerbation of psoriatic skin lesions due to an increased KGF stimulation might be expected in psoriasis patients undergoing a treatment with palifermin. This is the first known case report about the use of palifermin in a patient with severe psoriasis. **Methods:** We treated a 45-year old male patient with aggressive non-hodgkins-lymphoma with high dose BEAM chemotherapy (carmustine, cytarabine, etoposide, melphalane on days -7 till -2) followed by an autologous stem cell transplantation (day 0). To prevent severe oral mucositis, the patient received KGF 60µg/kg body weight three consecutive days before (days -10 till -8) and after (days 1 till 4) administration of chemotherapy. At admission, the patient presented with disseminated, huge active psoriatic lesions and an additional psoriatic arthritis under a daily medication with 5mg oral prednisolone. Regarding his medical history, a severe psoriasis had been diagnosed 30 years ago and diverse treatments including common topic treatments, systemic corticosteroids in varying doses, methotrexate and the anti-TNF-alpha-antagonist infliximab had been used with few success. **Results:** Surprisingly, no exacerbation of psoriatic skin lesions after KGF application could be detected during the whole period of hospitalization. However, with the application of chemotherapy starting at day -7, a strong immunosuppressive agent was administered making an increased epidermal proliferation unlikely. Preclinical data have yet shown that the maximal increase of epithelial thickness in the buccal mucosa (as an indicative of pharmacodynamic response) occures already within 18 to 48 hours after the injection of palifermin. However, no augmented plaque production, redness or increase in growth of skin lesions could be observed or photodocumented in our patient on day -10 to -8, which represents the interval of KGF treatment even before start of chemotherapy application and the supposed time of maximal increase of epithelial thickness. **Conclusions:** We conclude that administration of palifermin is feasible in patients with severe psoriasis undergoing high-dose chemotherapy.

Disclosure: No conflict of interest disclosed.

P529

Is a platelet transfusion trigger below 10 G/l thrombocytes of relevant perilous risk in outpatient care? – Prophylactic platelet transfusions and platelet transfusion trigger in Aplastic Anemia (AA) in an outpatient setting.

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Introduction: Recent publications recommend a strong restriction of platelet concentrat (PC) transfusions with a transfusion trigger ≤ 10 G/l without major bleeding signs. We agree with the necessity of restriction due to potential adverse events, availability and costs of PC. However, many of the studies addressing adverse events of PCs do no longer reflect current practices, in particular they focus on inpatient treatment which allows regular clinical assessment of signs for thrombocytopenic bleeding. We report our data about chronic platelet transfusion therapy in adult AA outpatients to show that platelet transfusion recommendations have to be evaluated for different underlying diseases and situations. **Methods:** Analysis of patient (pt) characteristics and transfused PC units. Therapy efficacy and adverse events were evaluated by blood count, vital parameters, clinical symptoms, CMV-/HBV-/HCV-/HIV –status and antibody testing. **Results:** In total we examined 247 patients (pts) with a median duration of outpatient transfusion therapy of 256 d (1-4954 d). 12 pts (7 male/5 female) of this cohort were diagnosed with AA. Their patient characteristics were: median age: 63 (20-76) years; no stem cell transplantation; median duration of PC transfusion therapy: 13 (6-171) mo; median number of transfused PC units: 101 (16-328) with a median of 52 (2-328) pooled PC and 4 (1-175) apheresis PC. Median increment after the first PC was 51 (17-68) G/l. There was no hint for a decrease of clinical efficacy during transfusion therapy with PC. In none of the pts a seroconversion in CMV-/HBV-/HCV-/HIV –status was observed. No new HLA-antibodies occurred. 15 transfusion reactions after PCs in total were observed. None of these reactions was severe. 6 pts are transfusion independent now. 4 pts died due to infections caused by AA related neutropenia. One pt died due to a subarachnoidal hemorrhage after reduction of the transfusion trigger to <10 G/l after 11 mo without complications upon a transfusion trigger of < 20 G/l (328 P-PC). **Conclusions:** 1) Our data show that outpatient transfusion therapy is safe and efficient even in long term therapy. 2) We strongly recommend individualized transfusion guidelines based on the underlying disease and the patient situation. 3) Especially in outpatient care without fulltime observation and with more physical strain than in hospital a higher transfusions trigger may be necessary.

Disclosure: Höchsmann,B.: Anstellungsverhältnis oder Führungsposition: Angestellter IKT Ulm; Honorare: für Vorträge
Schrezenmeier,H.: Anstellungsverhältnis oder Führungsposition: Ärztlicher Leiter des IKT Ulm

Wissenschaftliches Symposium
Prädiktive Faktoren in der systemischen Therapie des Lungenkarzinoms

V530

Predictive Factors for Monoclonal Antibodies in the Therapy of Metastatic NSCLC

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In 2007 the official approval of bevacizumab in combination with chemotherapy initiated a new era of "antibody-based therapies" in metastatic non-small cell lung cancer (NSCLC). In earlier trials, fatal tumor bleeding occurred in a limited number of patients with centrally located, squamous cell cancers treated with bevacizumab. Today, histology is regarded as an important predictor of toxicity, and squamous cell histology (about one third of all metastatic NSCLC) is a contraindication for bevacizumab. In contrast, no reliable molecular marker is available today to predict the clinical benefit (tumor response or survival) with bevacizumab. Several candidate markers are under investigation, including plasma levels of VEGF, circulating endothelial cells (CECs), and endothelial progenitor cells (EPCs). Hopefully, these markers can be validated prospectively in the near future, which would allow to restrict bevacizumab treatment to patients who benefit from the drug. Cetuximab binds to the epidermal growth factor receptor (EGFR), and thereby inhibits tumor growth. Cetuximab is currently approved in chemotherapy-refractory metastatic colorectal cancer, and in head and neck cancer in combination with radiotherapy. Approval for cetuximab in patients with NSCLC is pending, and is expected based on the results of the phase III trial FLEX, including patientes with EGFR-positive tumors by immunohistochemistry. The results from the FLEX trial did not show any prodictive role for EGFR-FISH or KRAS mutations. Other markers, including polymorphisms of the Fc-gamma receptor which may be associated with antibody-dependent cellular cytotoxicity, are currently investigated. Of note, rash after the first cycle of cetuximab significantly correlated with clinical benefit in the FLEX trial. Further prospective evaluation is needed to determine whether rash is a valid surrogate marker for tumor response, and whether cetuximab can be stopped in patients not developing rash. Other antibodies are in clinical development. CP-751871, targeting the insulin-like growth factor 1 receptor (IGF-1R), showed promising clinical activity in combination with carboplatin and paclitaxel in a phase II trial. Interestingly, response rate was highest in patients with squamous cell cancer. Expression of IGF-1R may depend on tumor histology, and ongoing phase III trials are expected to show whether squamous histology and/or IGF-1R expression are predictive markers for CP-751871 and other drugs targeting IGF-1R. In conclusion, from an oncologists view, a crude diagnosis of "NSCLC" is no longer acceptable. Pneumologists are now frequently asked to perform biopsies rather than to collect cytology samples, whereas pathologists increasingly use immunohistochemistry to make a distinct diagnosis of the histological subtype.

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V532

Molecular Predictors of Chemotherapy in Non-Small-Cell Lung Cancer

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Tumor progression and resistance to therapeutic interventions are major obstacles to improving the outcome for patients with non-small-cell lung cancer (NSCLC). The identification of genes that may play a role in disease response may lead to molecularly-based decisions on the use chemotherapeutic agents. Among several candidate genes, two, the excision repair cross completing group 1 gene product (ERCC1) and the regulatory subunit of ribonucleotide reductase (RRM1), have been investigated in greater detail. ERCC1 functions as the 5' endonuclease of the nucleotide excision repair complex, and it is important for the repair of DNA damage caused by inter- and intra-strand cross-links induced by platinum-agents. High levels of ERCC1 are associated with reduced efficacy presumably through the increased efficiency of repair of platinum-induced DNA damage. RRM1 functions as the regulatory subunit of ribonucleotide reductase (RR) and controls substrate specificity and on/off function of RR, while the catalytic subunit (RRM2) converts nucleoside diphosphates to the corresponding deoxynucleotides. Results from multiple independent laboratories and clinical studies have unequivocally demonstrated that RRM1 is the dominant determinant of the chemotherapeutic agent gemcitabine, a nucleoside analogue. Gene expression-based therapeutic decision-making has already been explored in two clinical trials in patients with advanced NSCLC, and both have reported favorable disease response rates with this approach.

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Wissenschaftliches Symposium
Recent developments in understanding Myeloma pathophysiology

V535
Role of FISH in myeloma risk stratification

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For many years, factors related with demographics, features of the tumor clone itself, and laboratory abnormalities were analyzed to determine the outcome of patients with MM at presentation. The most powerful classification system was obtained by a combination of serum $ß_2$-M and serum albumin (International Staging System [ISS]). Comprehensive analyses of cytogenetic abnormalities in MM identified patients with a t(4;14), t(14;16) and/or 17p-deletion as the group of patients with the worst prognosis suggesting that novel approaches are required for the treatment of such high-risk patients. Using this prognostic information, the Mayo Group has defined two patient groups in newly diagnosed MM, which can be categorized as high-risk or standard-risk (mSMART classification). At this stage, this information can provide the framework for an individualized treatment approach, but it needs to be considered that current prognostic information is predominantly based upon patient populations treated with chemotherapy. Clinical evidence suggests that bortezomib may overcome the poor prognosis associated with unfavorable chromosomal abnormalities (t(4;14) and t(14;16) as well as deletions of 17p or 13q), both in the setting of relapsed/refractory MM and newly diagnosed disease. With respect to untreated patients, promising results have been reported from the VISTA trial: Patients treated with MP + bortezomib had a 32% CR rate independent of the cytogenetic risk category (high-risk [t(4;14), t(14;16), deletion 17p] versus standard-risk [all others]). Moreover, time-to-progression and overall survival were also similar in both risk groups. Bortezomib-based combination regimens have also shown promising results as induction regimens prior to autologous transplantation, but these results are primarily based upon response, and longer follow-up regarding progression-free survival is needed. There is emerging evidence that also lenalidomide is beneficial for MM patients with such high-risk chromosomal features: A retrospective analysis of relapsed/refractory patients treated with lenalidomide/dexamethasone showed no impact of parameters like t(4;14), del(13q), and high beta-2-microglobulin on survival. However, a recent report by the IFM Group indicated that in patients treated with lenalidomide plus dexamethasone, the presence of del(13) and t(4;14) resulted in a significant reduction in response rates and survival. Overall, further studies with larger patient numbers and longer follow-up are needed to confirm these results and to assess the effect on progression-free and overall survival. The consensus panel of the 2009 International Myeloma Workshop suggested that FISH should be performed in all myeloma patients at diagnosis, but with the limited data available, it is premature to draw definite treatment decisions according to specific cytogenetic abnormalities.

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Vorträge
Young Masters

V538
The immunomodulatory agent FTY720 induces apoptosis of Philadelphia chromosome-positive acute lymphoblastic leukemia cells and overcomes imatinib resistance in vitro

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Introduction: Imatinib has become standard therapy for de novo Philadelphia chromosome-positive ALL (Ph+ ALL) but acquired resistance to this tyrosine kinase inhibitor (TKI) occurs in 80% of patients with mutations in the tyrosine kinase domain (TKD). In contrast, primary resistance appears to be multifactorial but has been incompletely elucidated. FTY720 (fingolimod) is an immunomodulator, a synthetic compound produced by modification of a natural immunosuppressor. It interferes with T-cell trafficking and is used in organ transplantation. FTY720 induces apoptosis through activation of the phosphatase PP2A in a variety of B-cell malignancies independently of Bcl-2 expression. We investigated the potential effect of FTY720 in a non-mutated imatinib resistance Ph+ ALL cell line and in long-term cultured primary human Ph+ and Ph- ALL cells. **Methods:** We used the Sup-$B15_{RT}$ cell line as a model of imatinib resistance. It was derived from the $SupB15_W$ cell line (already characterized B-precursor Ph+ ALL) by gradually increasing the exposure to imatinib. $SupB15_{RT}$ cells are cross-resistant to the second generation TKI without usually involved mechanisms of imatinib resistance, e.g. Bcr-Abl gene amplification, point mutations in the TKD mutations, or Bcr-Abl overexpression. We also employed long-term cultured primary human ALL cells obtained from patients Ph+ (n=2) and Ph- cells (n=3). Cells were treated with FTY720 (1 to 10 µM) for 7 days. Cell viability was achieved by trypan cell counting. Apoptosis was determined using the Annexin V-FITC apoptosis kit. Abl phosphorylation was analyzed by Western Blot. **Results:** FTY720 induced dose-dependent apoptosis in all cases of Ph+ ALL cells (n=4) and in none of Ph- cells (n=3). The apoptotic effect was maximum after 48 hours. In $SupB15_W$, 75% of cells were apoptotic with late apoptosis (53 %). The proliferation was already inhibited by 2.5 µM. In $SupB15_{RT}$, the apoptotic effect is different with less apoptosis (39%) but mostly early apoptosis (27%). The antiproliferative effect was identical. In $SupB15_W$ and in $SupB15_{RT}$ cells, the level of Abl phosphorylation is not modified by FTY720. **Conclusion:** FTY720 is an antiapoptotic and antiproliferative agent in Ph+ ALL but not in Ph- ALL cells. It overcomes non-mutational imatinib resistance. It could be a therapeutic agent in Ph+ ALL. FTY720 induces cell death by a mechanism apparently different and unclear between imatinib-sensitive and -resistant Ph+ ALL cells.

Disclosure: No conflict of interest disclosed.

V539
Prognostic significance of whole body magnetic resonance imaging in 250 patients with asymptomatic monoclonal plasma cell disorders

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Introduction: The use of whole body magnetic resonance imaging (wb-MRI) enables the examination of almost the entire bone marrow compartment in patients with monoclonal plasma cell disease. Focal lesions detected by spinal MRI have been demonstrated to be of prognostic significance in symptomatic myeloma. The present study investigates the prognostic significance of focal lesions in wb-MRI in patients with asymptomatic monoclonal plasma cell disease. **METHODS:** Wb-MRI with T1 and T2 weighted sequences was performed in 250 patients with either monoclonal gammopathy of undetermined significance (MGUS, n = 84), solitary plasmacytoma (n = 17) or asymptomatic multiple myeloma (aMM, n = 149). The prognostic significance of the presence and absence, as well as the number of focal lesions for progression into a higher stage of disease or into symptomatic myeloma, respectively, were analyzed. Furthermore, multivariate analysis of additional prognostic factors for aMM was performed. **RESULTS:** Focal lesions were present in 9% of MGUS-patients and 28% of aMM-patients. In the plasmocytoma group, lesions additional to the initially diagnosed plasmocytoma were detected in 35% of patients. Among 185 patients without any focal lesions, 65 (35%) presented with a diffuse bone marrow infiltration in MRI. The presence of focal lesions per se and an increasing number of lesions were an adverse prognostic factor for both progression into a higher stage of disease and development of symptomatic myeloma ($p < 0.001$). Further adverse prognostic factors for progression free survival in patients with aMM were diffuse infiltration pattern in MRI, mono-

clonal protein of 40g/l or more and a degree of plasma cell infiltration in bone marrow of at least 20%. In accordance to previous reports, patients with progressive disease accounted for just about 1% per year (2 of 84) in the MGUS group in our cohort. In addition, the number of plasmocytoma patients was relatively low. Therefore, no multivariate analysis in these subgroups could be performed. **CONCLUSIONS:** Both presence and number of focal bone marrow lesions detected in wb-MRI are highly significant adverse prognostic factors for patients with monoclonal plasma cell disease who would not require systemic therapy according to current standards. We recommend performing wb-MRI in all patients with monoclonal plasma cell disease in order to assess individual risk profiles. However, the currently available evidence does not yet justify the initiation of treatment based on MRI findings alone.

Disclosure: No conflict of interest disclosed.

V540

Transcription factor Fra-2 and its possible role in invasion and metastasis of breast cancer

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Introduction: Fra-2 (Fos-related antigen 2) is a member of the Fos family of AP-1 transcription factors which are often up-regulated in human breast cancer. The results of previous studies with clinical samples and experimental studies of breast cancer cell-lines suggested that it might be involved in the regulation of tumor invasion and metastasis in breast cancer. **Methods:** In order to analyze the impact of Fra-2 on the aggressive behavior of breast cancer cells, we established stable transfectants of the weakly invasive MCF-7 cells and as well in the highly invasive MDA-MB231 breast cancer cells with Fra-2 overexpression. Additionally we generated MCF-7 stable transfectants with inducible Fra-2 transcription controlled by the tetracycline-inducible tetON-system. The consequences of Fra-2 upregulation on the biology of the breast cancer cells were analysed by MTT assays (proliferation) and Matrigel invasion assays (invasion and motility). In addition, possible target genes which were differentially regulated in stable transfectants with Fra-2 overexpression were identified by microarray analysis and, partly Western blots. **Results:** Cell proliferation was not influenced by Fra-2. In contrast, the invasive potential of the cells was increased by Fra-2 in MDA-MB231 and MCF7 cells. By using the GeneChip Human Genome U133A 2.0 array (Affymetrix), we identified several genes which are known to be involved in cell adhesion or invasion and which were up- or downregulated in stable transfectants, i.e. ICAM-1, L1CAM, ALCAM, CEACAM6 and CX43 etc. **Conclusions:** In clinical breast cancer samples, upregulation of Fra-2 protein expression has been observed and was shown to be associated with a more aggressive phenotype. Our data of the experimental studies with breast cancer cell-lines indicates that Fra-2 may play an important role in tumor progression by transcriptional regulation of genes which are involved in cell adhesion and invasion.

Disclosure: No conflict of interest disclosed.

V541

A distinct polymorphic region in the EFGR ligand binding site predicts the occurrence of skin rash in patients with advanced HNSCC

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Background: Cetuximab, a monoclonal antibody targeting epidermal growth factor receptor (EGFR) is the first molecular targeting approach for the treatment of head and neck squamous-cell cancer (HNSCC) that demonstrated clinical efficacy with prolonged progression-free and overall survival. The most common side effect of cetuximab is moderate to severe skin rash. In the current study we analyzed whether cetuximab-induced skin rash is correlated with distinct genetic variations within the EGFR gene and focused our analyses on gene polymorphisms known to modulate EGFR expression levels, its capacity of ligand binding or its mitogenic signaling activity. Furthermore the intensity of skin rash and gene polymorphisms were correlated with progression free survival (PFS) and overall survival (OS). **Methods:** 50 patients enrolled in a single-arm phase II multicenter study for second-line treatment of stage III/IV metastatic or recurrent SCCHN with cetuximab/docetaxel were genotyped for EGFR intron 1 CA-single sequence repeat (CA-SSR) polymorphism and the single nucleotide polymorphism R521K within EGFR exon 13. Association between genotypes and incidence/grade of skin rash classified by Common Toxicity Criteria (CTC) was assessed by Pearsons chi-square test. Survival analysis were performed by Kaplan Meier. **Results:** The relative genotype distribution within our patient cohort was comparable to that reported by the HAPMAP consortium for a European reference population. Overall, thirty-eight patients (76 %) developed skin rash within 6 weeks of treatment. For the CA repeat polymorphism (minor allelic sum 27-33 CA-SSR, major allelic sum 34-40 CA-SSR) we failed to observe an association with skin toxicity (p=.17), PFS (p=.18) and OS (p=.055) In contrast, the R521K variant (Lys allele) was significantly associated with reduced skin toxicity (p=.012). In fact, skin rash of grade >1 developed in only 7/27 (25%) of patients with homozygous Lys/Lys or heterozygous Lys/Arg genotypes but in 14/23 (60%) of patients with homozygous Arg/Arg genotype. PFS (p= .14) and OS (p= .10) were not associated with the SNP R521K. PFS (p .015) and OS (p .031) were, however, significantly associated with the occurrence of skin rash. **Conclusion:** Our study suggests that the EGFR R521K but not the CA repeat polymorphism is a useful predictive marker for skin toxicity in HNSCC. Furthermore the occurrence of skin rash is positively associated with PFS and OS. The evaluation of its correlation with EGFR expression, ligand binding and signaling activity is currently ongoing.

Disclosure: No conflict of interest disclosed.

V542

Pharmacogenetic profiling of peripheral neuropathy in elderly patients (>65years) with advanced gastric cancer receiving oxaliplatin based chemotherapy

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Introduction: Peripheral neuropathy (PNP) is a dose-limiting side effect of oxaliplatin based chemotherapy. High grade PNP may compromise quality of life especially in elderly patients (pts). A randomized multicenter phase II study was conducted to compare fluorouracil, leucovorin, oxaliplatin with or without docetaxel (FLO vs. FLOT, respectively) in elderly pts with advanced gastric cancer (AGC). Our purpose was to identify pharmacogenetic markers as predictors of high grade PNP within this study. **Methods:** 143 pts were enrolled in this study. Pts. were numerically >65 years or numerically >59 years but classified biologically >65 years as defined by an Instrumental Activities of Daily Living score of <8. PNP was classified according to an oxaliplatin specific scale. Genotyping was performed using PCR-based RFLP or TaqMan®-based allelic discrimination. 20 polymorphisms in 13 genes being part of the metabolism of the applied drugs or DNA repair were analyzed. Statistical analyses were based on stepwise multivariate cox regression models and included genotypes and clinical parameters. **Results:** Median age was 71 years (range 60-83). Pts received in median 6 cycles of treatment (range 1-12). 130 pts were evaluable for PN at time of analyses. Of these, 68 received FLO and 62 received FLOT. Cumulative grade 3 PNP occurred in 49% of pts without a significant difference between FLO and FLOT receiving pts (44% and 53%, respectively, p=0.4). Genotypes of TS and MTHFR could be identified as independent risk factors for grade 3 PNP by multivariate analyses. Pts carrying a TS promoter genotype known to be associated with low TS expression (2R/2R, 2R/3RC, 3RC/3RC) were

at higher risk for developing grade 3 PNP compared to pts without one of these genotypes (OR 3.0 [95%CI 1.27; 7.06], p=0.01). Pts carrying MTHFR1298AC or CC genotypes were also at higher risk for experiencing grade 3 PNP compared to pts with the wildtype MTHFR-1298AA genotype (OR 3.1 [95%CI 1.26; 7.60], p=0.01). In fact, 89% of pts that experienced grade 3 PNP were carriers of at least one of these risk genotypes. **Conclusion:** Polymorphisms of TS and MTHFR might be associated with grade 3 PNP in AGC pts receiving oxaliplatin based chemotherapy.

Disclosure: No conflict of interest disclosed.

V543

HOXB4 with deletion of its proline-rich N-terminal region causes acute myeloid leukemia in mice

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Background: HOXB4 is among the most important stem cell regulatory genes described so far and is able to amplify long-term repopulating stem cells (SC) without inducing malignant transformation. So far the underlying mechanisms of the SC amplificatory impact of HOXB4 are poorly understood. Aim. To understand the stem cell regulatory characteristics of HOXB4, we performed a mutational study by deleting its proline-rich region, which has been described to act as a transcriptional activation domain in many other proteins, like non-homeobox genes (e.g. p53, AP2) and other homeobox genes (e.g. HOXD4 and HOXA13). **Methods:** We performed in vitro and murine bone marrow transplantation experiments transducing murine 5-FU enriched HSCs retrovirally with the HOXB4 wild-type (wt) and several mutants, including a ?proline HOXB4 mutant (?P), where the proline-rich sequence between the amino acidic positions 71-120 in the exon 1 is deleted. **Results:** When HOXB4-?Pro (n=14) was overexpressed in normal murine progenitor cells, a significant decrease (75fold, p<0.05) of the 12 days ?-CFU-S frequency was observed compared to the HOXB4-wt (n=5). However, HOXB4-?Pro still generated significantly more ?-CFU-S in comparison to the GFP control (n=11) (35fold, p<0.0003). In addition, CRU assays were performed by transplanting mice with serial dilutions of 5-FU isolated bone marrow progenitor cells, in order to evaluate the effect of the HOXB4-?Pro on long-term repopulating stem cells. At 16th week post transplantation there was no significant difference in the CRU frequency between HOXB4wt (CRU 1/834, n=18) and HOXB4-?pro (CRU 1/413, n=18). However, in mice transplanted with HOXB4wt (n=12) 45.3 % of the circulating cells belonged to the transduced compartment compared to 19.2% in the HOXB4-?pro group (n=13) (p<0.006), whereas the lineage distribution within the transduced compartment did not differ between both experimental arms at this time point. Of note and in contrast to HOXB4wt, mice engrafted with HOXB4-?Pro BM cells (n=9) developed myeloproliferation with a significant increase of Mac-1 and Gr-1 positive cells over time in the PB (29% Gr1 and 43% Mac1 wk 4-16 compared to 71.2% Gr1 and 86.6% Mac1 week 36-56 wk, p<0.004). Ultimately, the HOXB4-?Pro mice developed acute myeloid leukemia without maturation, as confirmed by immunohistochemical analysis after a median latency time of 279 days (n=9), while the mice transplanted with HOXB4wt expressing BM cells did not develop any disease after an observation for more than 466 days (n=5, p<0.05). The AML in HOXB4-?Pro mice was readily transplantable (66.5 days for 2nd Tx, n=6; 43 days for 3rd Tx, n=4) (p<0.05 compared to 1st recipients). Conclusions. Our results demonstrate that the N-terminal proline-rich region of HOXB4 has an important function for the stem cell amplifying function of HOXB4 and loss of this domain converts HOXB4 into a leukemogenic gene.

Disclosure:

Freie Vorträge
Allogene Transplantation klinisch I

V544

Long-term follow-up results on survival, GvHD and late effects after bone marrow or peripheral bood stem cell transplantation for treament of leukemia – an EBMT late effects working party study.

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Introduction: The majority of allogeneic hematopoietic stem cell transplants nowadays use peripheral blood instead of bone marrow transplantation (BMT). Increased incidence of graft-vs.-host-disease (GvHD) has been described when comparing allogeneic peripheral blood to bone marrow transplantation (PBSCT). Long-term data on outcome and late effects of allogeneic PBSCT as compared to BMT are scarce. This study presents long-term outcome data from the largest randomized study comparing PBPCT and bone marrow transplantation (BMT). Patients and **Methods:** Between 1995 and 1999, 329 patients with leukemia were treated in a randomized phase III study of the European Group for Blood and Marrow Transplantation (EBMT) and transplanted with peripheral blood or bone marrow from HLA-identical sibling donors. Follow-up data were collected on 87.2% (176/202) patients surviving more than 3 years post-transplant at a median of 9.3 years after transplantation. **Results:** Ten-year overall survival (OS) was 49.1% for PBSCT and 56.5% for BMT (p = 0.27). 10-year LFS was 42.3% after PBPCT versus 45.5% after BMT (HR 0.88; CI 95% 0.65-1.19; p = 0.40). The cumulative incidence of relapse at 10 years was 23.5% and 19.5% after BMT and PBSCT, respectively (p = 0.456). A trend for improved LFS was seen for patients with AML [10-year LFS estimate 62.3% versus 47.1% for BM and PB recipients, respectively (p = 0.16)] and ALL [28.3% for BM recipients compared to 13.0 % for PB recipients (p = 0.13)] , while there was no difference in LFS for CML pateints. The cumulative incidence of secondary malignancies at 10 years was 4.8%; 3.2% after BMT and 6.8% after PBSCT, respectively (p = 0.17). More patients after PBSCT developed chronic GvHD (72.6% vs. 54.1%, p = 0.013) and extensive disease, respectively (56.3% vs. 30.4%; p=0.002). In addition, patients receiving PBSCT had a higher risk for GvHD involvement of skin, liver and oral mucosa and significantly more patients needed immunosuppressive treatment five years after transplantation [26.3% PB, 12.2% BM (p = 0.02)]. Nonetheless, there was no difference in the performance status, return to work, incidence of bronchiolitis obliterans, cataract and hematopoietic function. **Conclusion:** More than nine years after transplantation, OS and leukemia-free survival remain similar after BMT and PBSCT. Differences in the incidence of chronic GvHD and duration of immunosuppressive treatment did not affect survival, general health status and the occurrence of late events. In patients grafted for acute leukemias a trend for superior LFS after BMT became apparent while patients with CML had comparable results with BMT or PBSCT.

Disclosure: No conflict of interest disclosed.

V545

Shift to an alternative donor does not improve the outcome after second allogeneic stem cell transplantation (alloSCT) in acute leukaemia relapsing after a first alloSCT – a risk factor analysis by the German Stem Cell Registry (DRST)

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Introduction: Relapse of acute leukaemia is a major cause of treatment failure after alloSCT. During recent years, the introduction of reduced conditioning regimen and the broader availability of alternative donors have increased the possibilities to perform a second alloSCT as salvage treatment in this challenging situation, using different preparative regimen and/or different stem cell donors. **Methods:** To evaluate the present role of a second alloSCT (tx2) for the treatment of relapse after first alloSCT (tx1), we performed a nationwide retrospective analysis based on the German registry for stem cell transplantation (DRST). Data were completed by the respective centres according to a specifically designed questionnaire. **Results:** 214 patients (69% AML, 31% ALL, median age at tx1 37y) from 24 centers were identified. Donor at tx1 were HLA identical siblings (41%), matched unrelated (39%), mismatched family or unrelated (17%) or syngeneic donors (3%). Conditioning at tx1 was standard intensity (SIC, 61%), intermediate (IIC, 20%) or reduced intensity (RIC, 19%). Median remission after tx1 was 7 months, median time from relapse to tx2 was 73d. At tx2, patients were aplastic (4%), in CR (20%) or showed active disease (76%). In 61%, the same donor was used for tx1 and tx2, whereas a different donor was chosen in 39%. Conditioning at tx1/tx2 were SICI/SIC (8%), RIC/RIC (19%), less intensive at tx2 (mostly IIC or RIC after SIC, 71%), or more intensive at tx2 (SIC after RIC or IIC, 3%). Following tx2, CR was achieved in 58% of patients, out of which 81% relapsed again. This resulted in leukemia as the most frequent cause of death. With a median FU after tx2 of 23 months, median OS after tx2 is 117d. In a univariate analysis (log rank), OS after tx2 depended on stage at tx1 (CR vs. active disease, p=.001), stage at tx2 (CR vs. aplastic/active disease, p=.028) and duration of remission after tx1 (<=6m (1y OS 7%) vs. 6-12m (23%) vs. >12m (38%), p<.001). There was no significant difference regarding age (<> median), AML vs. ALL, change of the donor, family versus unrelated donor, or time point of alloSCT (<>2002). The strategy to reduce intensity of conditioning at tx2 compared to tx1 reached borderline significance (p=0.16). In a multivariate analysis (Cox Regression Model), time of remission after tx1 was the only significant factor for OS (p<.001, hazard ratio .61, 95%CI .49-.76). **Conclusion:** Survival of acute leukaemia after second allogeneic SCT is determined by the duration of remission after tx1. Switching to an alternative donor did not improve the results in our series. Further analysis is required to evaluate the role of RIC regimen for tx2.

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Outcome and Risk Factor Analysis in 173 Patients with AML and MDS with Poor Risk Cytogenetics Receiving a Uniform Conditioning Regimen for Allogeneic Stem Cell Transplantation (SCT)

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Introduction: Patients with AML and MDS with unfavorable cytogenetic aberrations seemed to benefit particularly from a sequential preparative regimen (FLAMSA-RIC, Schmid et al, JCO 2005) for allogeneic SCT. However, the poor risk subgroup still represents a heterogenous population that might be further subdivided. **Methods:** We investigated the role of potential risk factors for outcome after allogeneic SCT in 173 patients with poor risk cytogenetics (NCCN criteria), who uniformly had received the FLAMSA-RIC conditioning in 11 centres between 1999 and 2008. In addition to other risk factors, distinct cytogenetic aberrations observed at a sufficiently high frequency (-5, 5q-, -7, 7q-, and complex karyotype) were evaluated for their influence on OS. **Results:** Patients suffered from progressive MDS (13%), de novo AML (49%), sAML/MDS (25%), sAML/MPS (3%) and tAML (10%). Stage at transplant was untreated disease (19%), primary induction failure (34%), cCR1 (19%) and relapsed disease (28%). Median age was 53 (18-71) years. 57% patients had a complex aberrant karyotype, 32% and 41% had abnormalities of chromosome 5 and 7. Median time from diagnosis to transplantation was 4,6 months, median follow up was 3.5 years. Overall and leukemia free survival (OS/LFS) was 41.1%/35.5% at two, and 31.5%/27.6% at four years. With respect to cytogenetics, 3 prognostic groups could be identified: Patients with an unfavorable karyotype (including complex) without alterations of chromosome 5 and 7 showed a 2y OS of 60.4%. Similarly, patients with an isolated monosomy 7 had a 2y OS of 61.6%. In contrast, outcome of patients with a complex karyotype that included aberrations on chromosome 5 and/or 7 was significantly inferior (2y OS: 21%; p=.001). Among patients with MDS, transition into sAML was deleterious for outcome, as 2y OS decreased from 61% to 26.4% (p=.005). A cox regression model for OS identified disease subtype (MDS > de novo AML > sAML, p=.036, HR 1.43, 95% CI 1.02-2.00), stage at transplant (CR > untreated > refractory/relapsed disease, p=.009, HR .64, 95% CI .45-.89), and the prognostic cytogenetic subgroups as described above (p=.010, HR 1.49, 95% CI 1.01-2.02) as independent factors for OS. **Conclusions:** Allogeneic SCT following the FLAMSA-RIC regimen is an effective treatment for MDS and AML with unfavorable karyotype. Against the background of a uniform conditioning regimen, clinically and cytogenetically defined subgroups with different prognosis can be identified.

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Reduced-toxicity conditioning with treosulfan and fludarabine in allogeneic stem cell transplantation for myelodysplastic syndromes: final results of an international prospective phase II trial

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Based on previous preclinical and clinical data which revealed treosulfan a promising alternative conditioning agent prior to allogeneic haematopoietic stem cell transplantation, a prospective, multicentre phase II protocol was conducted to assess the safety and efficacy of treosulfan-based conditioning in MDS patients. Eleven centres from 4 countries participated. In total, 45 patients with a median age of 50 years (range: 22 - 63 years) were included. Of the donors 33% were matched related and 67 % unrelated (MUD). Thirteen per cent of the patients had received AML-like induction therapy and 7 % low-dose chemotherapy. The IPSS risk groups were: 7 % "low", 44 % "Int-1", 31 % "Int-2" and 18 % "high". At study entry 44 % of the patients had 5 % or more blasts in the bone marrow. Treosulfan (14 g/m², 2 hour infusion) was administered on days -6 to -4 and fludarabine (30 mg/m², 0.5 hour infusion) on days -6 to -2. GvHD prophylaxis consisted of ciclosporine-A and a short course of MTX as well as ATG-Fresenius® (10 mg/m² i.v., days -4 to -2) in case of MUD. The graft was PBPC in 89 % and BM in 11 %. This final analysis is based on a median follow-up of 25.7 (range: 12.2 – 41.4) months. The median times to neutrophil (> 0.5 x 10^9/l) and platelet (> 20 x 10^9/l) engraftment were 18 and 17 days, respectively. The cumulative incidence (CI) of complete donor chimerism was 73 % on day +28 and 93 % on day +100. The frequencies of adverse events CTC grade III/IV (exceeding 5 %) occurring during 28 days post-transplantation were 80 % for CTC category "infection"; 22 % "gastrointestinal"; 9 % "pulmonary/upper respiratory" and 7 % "neurology". Frequencies for grade III/IV hyperbilirubinaemia, mucositis/stomatitis and seizures were 13 %, 0 % and 0 %, respectively. There were two cases of mild VOD which resolved before day +20 after the transplantation. The CI of grade II - IV aGvHD was 24 % and that of grade III – IV 16 %. The CI of cGvHD at 2 years was 59 % and that of extensive cGvHD 28 %. The CI of non-relapse mortality was 9 % at 100 days and 17 % at 2 years, and that of relapse/progression 16 % at 2 years. Accordingly, the Kaplan-Meier estimates of OS and DFS at 2 years were 71 % and 67 %. These final phase II data confirm favourable safety and efficacy of the treosulfan-based conditioning therapy in MDS patients. Overall, promising survival results in this MDS patient population give a reasonable base for comparative allogeneic transplantation trials.

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Physical exercise as adjuvant therapy for patients before, during and after allogeneic hematopoietic stem cell transplantation

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Introduction: Before, during and after allogeneic hematopoietic stem cell transplantation (allo-HSCT) patients experience considerable physical, psychological and psychosocial distress. Besides GvHD and infections, particularly reduced physical performance and functioning as well as high levels of fatigue affect patient's quality of life negatively. Referring to this, physical exercise constitutes a potentially promising intervention to moderate such side effects. **Methods:** In a multicenter RCT, founded by the German José Carreras Leukaemia Foundation, 112 patients were equally randomised to an exercise (training: 5 days/week) and a treatment-as-usual group (TAU). The enrolment took place in the framework of medical checks before allo-HSCT. Patients started to train in home-based setting before admission to and finished training 6-8 weeks after discharge from hospital (self-directed with an intervention manual and DVD). During the outpatient study phase both groups were contacted weekly via telephone, whereas in the inpatient setting supervised training was performed twice a week (2 x/week social contacts in the TAU). Physical performance was assessed via 6-minute walk test and handheld dynamometer, fatigue via Multidimensional Fatigue Inventory (MFI) and QoL via EORTC-QLQ-C30. Overall distress was ascertained by the NCCN distress thermometer. **Results:** The exercise group (in comparison with TAU) showed significantly better results for endurance performance at the end of study (p= .024) and over study time (p= .006). A better course of muscle strength could be reported during inpatient setting (p= 0.42). Concerning fatigue, patients of the intervention group showed significantly superior values for all measurements after HSCT and for course over time of enrolment (p= .004 to .026). Also physical functioning was significantly better in the experimental group at the end of treatment (p= .033). At least the reduction of global distress take better courses over study time for the training group (p= .013). Referring to perceived distress, the intervention seems to be effective for physical and emotional problems. Physical fitness correlates highly significantly (r= .361 to .618) with all reported symptoms/variables **Conclusions:** Physical exercise is beneficial for patients under allo-HSCT, even if the intervention is only partly supervised. For the first time it could be shown that physical exercise can alter fatigue in the context of allo-HSCT.

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Allogeneic Stem Cell Transplantation after Reduced-Intensity Conditioning in Patients with Myelofibrosis

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Background: Allogeneic stem cell transplantation (SCT) after standard myeloablative conditioning regimen can cure patients with myelofibrosis, but due to the high treatment related mortality elderly patients are excluded from this approach. Patients and **Methods:** Between 2002 to 2006, 103 patients with primary myelofibrosis or post ET/PV myelofibrosis and a median age of 55 years (range, 32-68) were included in a prospective multicenter trial to determine the toxicity and efficacy of a busulfan/fludarabine-based reduced-intensity conditioning regimen (RIC) followed by allogeneic stem cell transplantation from related (n=31) or unrelated donor (n=69). **Results:**All but two (2%) patient showed leukocyte and platelet engraftment after a median of 18 and 21 days, respectively. Acute graft-versus host disease (GvHD) grade II to IV occurred in 26%, and chronic GvHD in 42% of the patients. Cumulative incidence of non-relapse mortality at one year was 16% (95% CI: 9-23%) and

significantly lower for patients younger than 50 years of age (4% vs. 24%) (p=0.01) and with a completely matched donor (12% vs. 38%) (p=0.003). The cumulative incidence of relapse at three years was 22% (95% CI: 13-31%), and influenced by Lille-risk profile (low: 14%, intermediate: 22%, and high: 34%) (p=0.02), and by splenectomy (51% vs. 20%) (p=0.005). The estimated five-year event-free- and overall survival was 51% and 67%, respectively. In a multivariate analysis age (HR: 1.075) (p=0.001) and HLA-mismatch donor (HR; 2.672) (p=0.001) remained significant factors for survival. **Conclusions:** A RIC-regimen allows allogeneic stem cell transplantation even in elderly patients with myelofibrosis and can induce long-term freedom from disease.

Disclosure: No conflict of interest disclosed.

Freie Vorträge
CLL experimentell

V550

Deregulation of miRNA is mediated by epigenetic changes and disrupts suppression of the oncogene PLAG1 in chronic lymphocytic leukemia

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Introduction: MicroRNAs play a key role in cellular regulation and development of neoplastic disorders including chronic lymphocytic leukemia (CLL). However, underlying mechanisms of miRNA deregulation and functional miRNA-target interactions remain to be elucidated. **Methods:** Primary cells of 50 treatment-naïve CLL patients and peripheral B-cells of 14 healthy donors were applied for miRNA-expression profiling applying bead chip technology. DNA methylation was assessed by MassARRAY technique. Luciferase reporter assays, Western blot and real-time PCR analysis were carried out for PLAG1 in CLL. **Results:** Comparing purified CLL cells versus healthy donor peripheral B cells a general decrease of miRNA expression in CLL cells could be observed. Particularly in CLL cells a set of 7 up- and 19 down-regulated miRNA was identified. Intriguingly, promoters of 7 down-regulated miRNAs (miR-125, -126, -130a, -139, -181a2/b2, -326, -582) showed gain of methylation in CLL cells, compared to normal B cells. Among upregulated miRNAs in CLL, loss of methylation was seen in miR-141, -451 and –598. Re-transfection of depleted miRNAs indicated reduced survival of primary CLL cells. To elucidate functional implications of miRNA deregulation a target prediction algorithm for multiple miRNAs was developed and revealed DNA-binding proteins and transcription factors as major targets of miRNAs deregulated in CLL. A highly significant binding prediction at the 3´UTR of the pleomorphic adenoma gene 1 (PLAG1) indicated an oncogene not yet described in the context of lymphatic neoplasias. Luciferase reporter assays including site directed mutagenesis of binding sites in the PLAG1 3´UTR revealed a significant regulation of PLAG1 by miR-181a, miR-181b, miR-107 and miR-424. While expression of PLAG1 mRNA was not affected, PLAG1 protein expression was shown to be significantly elevated in CLL cells compared to healthy donor B cells. **Conclusion:** Deregulation of miRNA is a key feature of CLL and is predominantly characterized by loss of miRNA expression. Hypermethylation of promotors for down-regulated miRNAs and vice versa hypomethylation in promotors of up-regulated miRNAs indicate epigenetic changes as an underlying mechanism of miRNA deregulation. As a consequence reduced miRNA expression implies direct effects on CLL cell survival. Regarding subsequent specific miRNA-target interactions we could demonstrate a disruption of miRNA-mediated translational control as cause for overexpression of the oncogenic transcription factor PLAG1. PLAG1 overexpression represents a putative novel mechanism of CLL pathogenesis and will be further elucidated in transgenic *in vivo* models.

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17p Deletion in CLL: Detailed Analysis of TP53 Mutations, Alternative Mechanisms of p53 Inactivation, Clone Size and Clonal Evolution

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While it is generally accepted that inactivation of p53 (by mutation) underlies refractoriness of CLL with 17p deletion, few studies have analyzed a large cohort of CLL patients with 17p deletion with respect to TP53 mutations and investigated mechanisms of p53 inactivation. In order to assess the incidence of TP53 mutations in CLL with 17p deletion we identified a large cohort of CLL cases with 17p deletion (n=217) and studied TP53 mutations in 124 of these patients. We used DHPLC to screen for TP53 mutations (Exons 2–11). A sub-group of cases were also studied by direct sequencing and with an array based TP53 mutation platform (AmpliChip Roche molecular systems). Detailed genetic studies (VH-mutation status, ZAP70, FISH) were available for the patients. The median size of the 17p- clone was 73% (10–97%). In addition to the 17p-, 13q- was observed in 99 cases (46%), but 11q-, +12, 6q-, and +8q were rarely seen (8.8%, 11.5%, 5.1%, and 3.7%, resp.). Most cases with 17p deletion had an unmutated VH status (75%). We found mutations in the protein coding region of TP53 in 100 of 124 CLL patients (81%) with 17p deletion. The majority of mutations were located in the DNA binding domain of p53 and were missense mutations (72%). In the vast majority of the cases the estimated clone size of the TP53 mutation correlated very closely with the percentage of cells with a single signal (FISH results). The size of the 17p- clone was significantly higher in cases where a mutation was detected (65.5%) compared to cases where a mutation was not found (42.7%; p=0.0003). When we separated the cohort into quartiles based on the 17p- clone size we found increasing proportions of TP53 mutation, suggesting that sensitivity may cause the lower rate of TP53 mutation detection. As further evidence that mutations may also be observed in cases with 17p deletion in a sub-clone, we also observed mutations in cases with less than 20% of cells (5/11) carrying the 17p deletion. The analysis of follow-up samples in a number of these cases showed definite evidence for selection. In spite of this clear evidence for a classical TP53-related tumor suppressor mechanism underlying the resistance to chemotherapy in cases with 17p deletion, there remain cases where no mutation in the exons of TP53 can be detected, suggesting that in these cases alternative mechanisms lead to inactivation of p53. When we assayed the p21/p53 levels after irradiation, we found evidence of abnormal p21/p53 induction in 4/5 cases with 17p deletion but no TP53 mutation. The current study supports the role of p53 inactivation by TP53 mutation underlying the chemo-resistance of CLL with 17p deletion. The extent of mutations of the remaining allele and the demonstration of co-existing mutations even in cases with deletions in only the minority of cells suggest that p53 is the main biological target of 17p deletion and its clinical consequence.

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The Microenvironment Differentially Impairs Passive and Active Immunotherapy in B-CLL – Potential Therapeutic Synergism of CXCR4 Antagonists

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B cell chronic lymphocytic leukemia (CLL) represents one of the most common lymphoid malignancies in adults and is characterized by the accumulation of mature, $CD5^+$ B cells. Direct contact with stroma cells protects CLL cells from chemotherapy-induced apoptosis *in vitro*. Blockade of CXCR4 signaling antagonises stroma-mediated interactions and

restores CLL chemosensitivity. *In vivo*, the combination of CXCR4 antagonists with conventional chemotherapy may also effect efficient mobilization of hematopoetic progenitor cells with severe subsequent hematotoxicity of the administered cytotoxic agents. Therefore, combinations of CXCR4 blockade with cytoreductive treatment with selective activity on CLL cells may avoid potential hematotoxicity. Hence, we tested CXCR4 antagonists in the context of passive and active immunotherapeutic approaches. We evaluated how efficiently rituximab, alemtuzumab, and cytotoxic T cells killed CLL cells cocultured with stromal cells in the presence and absence of a CXCR4 antagonist. Stromal cell contact attenuated rituximab- and alemtuzumab-induced complement-dependent cytotoxicity (CDC) of CLL cells. Addition of CXCR4 antagonist abrogated the protective effect of stroma (mean relative viability±SEM after alemtuzumab treatment: w/o stroma 21%±4, w/stroma 37%±6, w/stroma and CXCR4 antagonist 27±6; $p<0.05$). To gain insight into the mechanisms underlying the protective effects of the stroma, we analyzed the protein expression of anti-apoptotic molecules in stroma-CLL cocultures. In line with previous reports we found a stroma-mediated upregulation of antiapoptotic Mcl-1 after 24 hours. The CXCR4 antagonist TN14003 reversed Mcl-1 upregulation, suggesting that stromal cell-induced apoptosis resistance is mediated by Mcl-1 upregulation. In contrast to fludarabine- and antibody-dependent CDC, the presence of stromal cells did not limit antibody-dependent cell-mediated cytotoxicity (ADCC; mean relative viability after alemtuzumab treatment: w/o stroma 86%±2, w/stroma 89%±2; n.s.) or T cell-mediated cytotoxicity (mean relative viability after incubation with allogenic T cells: w/o stroma 74%±6, w/stroma 69%±7; n.s.). Consequently, the addition of CXCR4 inhibitors did not enhance ADCC or T cell-mediated cytotoxicity. Since ADCC appears not to be the predominant effector mechanism of antibody therapy of CLL, our data identify the combination of CXCR4 antagonists with passive - but not active - immunotherapy as a promising potential treatment concept in CLL.

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V553

Polyreactivity of B-cell receptors in chronic lymphocytic leukemia correlates with aggressive clinical course of the disease

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Introduction: Emerging evidence suggests that the development and course of chronic lymphocytic leukemia (CLL) may be driven by antigenic stimulation through the B-cell receptor (BCR). Here we set up a model system of epitope recognition in CLL to explore how diverse epitope recognition in CLL is and whether the epitope recognition pattern has clinical relevance. **METHODS:** BCRs from six randomly chosen CLL patients were cloned and recombinantly expressed as IgG1 Fab fragments. Combinatorial phage peptide libraries with five different insert designs were constructed and selected on the Fab fragments. We tested the binding of phage displayed epitope-mimics to the respective Fab fragment by ELISA as well as to the native BCR on the cells of CLL patients. Cell-bound phage displayed epitope-mimics were separated from unbound phage by differential centrifugation and bound phage were quantified by bacterial infection. **RESULTS:** We selected epitope-mimicking peptides from phage display libraries on six CLL BCRs. The selected peptides bound to the recombinant BCRs as well as to the native BCRs on the respective CLL cells. To model epitope recognition in a larger cohort of CLL patients we chose six "index" epitope-mimics and evaluated their binding in a set of 100 unrelated CLL cell samples. All CLL samples recognized one or several index epitopes. Some of the CLL samples showed marked polyreactivity whereas other samples were mono- or oligoreactive. We determined whether the degree of BCR polyreactivity correlates with the clinical course of the disease using *time to first treatment* (TTFT) as surrogate marker of disease progression. We found that CLL patients expressing BCRs reactive with each of the epitope-mimics had a significantly worse clinical course than less reactive control patients (median TTFT 27 months versus 87 months). Moreover, CLL patients whose cells express BCRs reactive with five or more epitope-mimics were also characterized by an aggressive clinical course as compared to patients reacting with less than five epitopes (median TTFT 24 months versus 97 months). These outcomes were unrelated to known prognostic markers such as BCR mutational status. **CONCLUSIONS:** We introduce a system for modelling and monitoring of BCR epitope reactivity in CLL. Our findings indicate that a polyreactive epitope recognition pattern may be a determinant of an aggressive clinical course in this disease.

Disclosure: No conflict of interest disclosed.

V554

SYK tyrosine kinase plays a role in CXCR-4 chemokine and $_{4}$ $_{1}$-integrin signaling in B chronic lymphocytic leukemia

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B cell chronic lymphocytic leukemia (B-CLL), one of the most common lymphoid malignancies in adults, is characterized by the expansion of mature, $CD5^+$ B lymphocytes. Recently, we identified SYK (spleen tyrosine kinase), a key mediator of B cell receptor (BCR) signaling, as candidate for targeted therapy in CLL. Moreover, the first clinical trial of the SYK inhibitor Fostamatinib Disodium (FosD) demonstrated high clinical activity. After initiation of the therapy though, an abnormal lymphocyte migration-behaviour with increased circulating leukocytes during lymph node regression was observed (Friedberg et al, ASH meeting 2008). Therefore we analyzed the role of SYK beyond BCR signaling in chemokine- and integrin signaling, both axes essential for CLL cell migration and adhesion towards their protective microenvironment. The homing of CLL cells towards the protective niches is mediated by the chemokine CXCL12 and its receptor CXCR4 as well as the cell-cell contact dependent crosstalk, e.g. via the integrin very late antigen-4 (VLA-4), which interacts with VCAM-1 on stromal cells. Immunoblotting and flow cytometry revealed significant SYK phosphorylation, reflecting its activation, when CLL cells were cultured in VCAM coated wells ($p<0.05$) or stimulated by CXCL12 ($p<0.01$). Furthermore, the phosphorylation of Akt, a downstream target of chemokine and integrin signaling, was induced by CXCL12 or VCAM stimulation. Concomitant SYK inhibition by R406 (Rigel Pharmaceuticals) abrogated this phosphorylation. Binding affinity of integrins is determined by their conformation, which is regulated by extracellular signals. Conformation-specific antibodies recognize activity dependent epitopes and detect the high affinity state of VLA-4. We found a small (5%) but significant reduction ($p<0.05$, $n=10$) in the expression of this epitope on CLL cells pre-treated with R406. In line with this, SYK inhibition resulted in a significant reduction of adhesion capacity towards VCAM measured in flow chambers. In addition, the migration capacity of CLL cells towards CXCL12 in transwell chambers was markedly reduced ($p<0.01$). In conclusion, our data show that SYK, beyond BCR signaling, is involved in integrin inside-out and outside-in signaling as well as in chemokine signaling in CLL cells. Since all of these axes may play an important role for CLL-cell adhesion and migration to the lymph nodes and bone marrow, this might explain the observed leukocyte flare during lymph node regression after SYK inhibition observed in the first clinical trial.

Disclosure: No conflict of interest disclosed.

V555

The tumor suppressor mechanism in 13q14.3 involves monoallelic expression, non-coding RNA genes and epimutations affecting the majority of chronic lymphocytic leukemia (CLL) patients

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Introduction: Deletions in chromosomal band 13q14.3 occur in more than 50% of chronic lymphocytic leukaemia (CLL) patients, indicating a tumor suppressor mechanism (TSM) in this region. Intriguingly, candidate genes including miR15a and miR16-1 lack point mutations in the majority of patients, yet the candidate genes are significantly down-

regulated in CLL patients. We have recently shown that already in healthy tissue one gene copy is randomly chosen for silencing. Aims: To identify an epimutation in the critical region in 13q14.3 **Methods:** DNA- and Histone-methylation of all CpG islands in the region was analysed using aPRIMES and ChIP-qPCR as screening tools, BioCOBRA as a quantitative high-throughput method and bisulfite sequencing for validation. **Results:** Two candidate regulatory elements with abnormal chromatin in CLL patients were identified (n=80, median 57% DNA-methylation, range 0-100%) as compared to healthy probands (n=20, median 88% DNA-methylation, range 74-100%, p<0.003). Interestingly, this epimutation can be found in all cytogenetic subgroups of CLL patients and is independent of IgV(H) mutation status, making it a prime candidate for an underlying epigenetic defect in CLL. **Conclusions:** We propose a model for the TSM in 13q14.3 where i) in healthy B-cells, only one gene copy is active while the second is epigenetically silenced, ii) 13q14.3 harbors an epimutation that is present in all cytogenetic subgroups of CLL patients and that iii) deregulates expression of long ncRNA genes directly and candidate tumor suppressor genes indirectly.

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Wissenschaftliches Symposium
Marrow niche for hematopietic and leukemia stem cells

V556
Real time imaging of hematopoietic stem cells

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We have developed *ex vivo* real-time imaging technology to study the homing of purified GFP-expressing (GFP^+) HSCs. We found that transplanted HSCs tended to home to the endosteum (an inner bone surface) in irradiated mice, but were randomly distributed and unstable in non-irradiated mice. Moreover, GFP^+HSCs were more frequently detected in the trabecular-bone area (TBA) compared to compact-bone area (CBA), and this was validated by live imaging bioluminescence driven by the Stem-Cell-Leukemia (*Scl*) promoter-enhancer[7]. HSCs home to bone marrow (BM) through the vascular system. We found that the endosteum is well-vascularized and that vasculature is frequently localized near $N\text{-cadherin}^+$ pre-osteoblastic cells, a known niche component. Thus bone marrow can be separated into central marrow zone and the endosteum zone, and the latter normally maintains HSCs but promotes their expansion in response to BM damage.

Disclosure: No conflict of interest disclosed.

V557
Dynamic Interactions Between the Nervous and Immune Systems with the Microenvironment, Regulate Normal and Leukemic Stem Cells

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Functional, preclinical models for normal and leukemic human stem cells using immune deficient NOD/SCID mice as recipients, revealed that their repopulation is dependent on SDF-1/CXCR4 interactions. Homing, retention, release and stem cell mobilization are tightly regulated processes, which involve bone turnover and an interplay between cytokines, chemokines, adhesion molecules and proteolytic enzymes. The roles of CD44, MT1-MMP and RECK in stem cell migration will be discussed. Most blood forming stem cells are retained in the bone marrow (BM), anchored to specialized niches via adhesion interactions, which prevent their motility and proliferation. However, low levels of motile progenitors migrate in the circulation as part of homeostasis. These low levels are dramatically amplified during alarm situations in response to stress signals due to injury, bleeding and infections, as part of host defense and repair mechanisms. These stress signals are mimicked by repeated G-CSF stimulation in order to mobilize stem and progenitor cells to the circulation in order to harvest them for clinical transplantation protocols. Stem cell adhesion interactions with the stromal niche supporting cells need to be dynamic in order to allow the undifferentiated cells to proliferate, differentiate and migrate. Osteoclasts have a dual role in host defense: bone remodeling and regulation of stem cells by freeing them form their inhibitory anchorage to stromal cells. Thus, osteoclast/osteoblast interactions also regulate BM leukocyte production on demand. Both osteoclasts, osteoblasts and stem and progenitor cells functionally express receptors for neurotransmitters. Immature human CD34+ cells dynamically express dopamine and epinephrine receptors and inflammatory, myeloid cytokines such as G-CSF and GM-CSF increase catecholaminergic receptor expression in order to facilitate leukocyte production and trafficking. This up regulation activates the progenitor cells in response to stimulation by the neurotransmitters and induces their motility and proliferation via Wnt signaling. Thus**,** regulation of leukocyte production and trafficking by stem cells in the BM reservoir is dynamic and involves mutual, reciprocal interactions between the nervous and immune systems with the stromal microenvironment throughout the body. Both normal and leukemic human stem and progenitor cells functionally express neurotransmitter receptors, which are involved in regulation of their motility and proliferation. In conclusion, normal and leukemic human stem cells are directly and indirectly regulated by dynamic interactions of the nervous and immune systems with the microenvironment.

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Wissenschaftliches Symposium
Anaplastische Gliome: Perspektiven der Behandlung – Lehren aus den aktuellen Phase III-Studien

V559
Novel molecular signatures beyond 1p/19 deletions

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1p/19 deletions are the predominant target of molecular analyses in oligodendroglial and diffuse astrocytic tumors of WHO grades II and III. This is due to all, diagnostic, prognostic and predictive impact of 1p/19q deletions in these tumors. In brief: combined 1p/19 deletions are very frequent in oligodendroglioma and oligoastrocytoma but rare in astrocytoma; patients with 1p/19q deletions appear to have a better prognosis than patients with similar tumors but without these molecular lesions; 1p/19q deletions are associated with a more favourable response to chemo- and radiotherapy. Recent studies revealed sporadic mutations in the gene for isocitrate dehydrogenase 1 (*IDH1*) in gliomas. Mutations exclusively occur in codon 132 contributing to the isocitrate binding site and render the enzyme dysfunctional in respect to isocitrate decarboxylation. The pathogenic role of *IDH1* mutations in gliomas is not yet established and different hypotheses focus on increased sensitivity to reactive oxygen species, HIF up-regulation and deficiency of peroxysomal alpha oxidation. *IDH1* mutations have been detected in the majority of astrocytomas, oligodendrogliomas and oligoastrocytomas but only rarely in glioblastomas and other neuroectodermal tumors. This molecular trait groups closely together set of tumors which have been expected to differ based on the previous 1p/19q data sets. As such it is not of diagnostic value in the separation of astrocytic from oligodendroglial tumors. However, analysis of a phase 3 trial suggested, that *IDH1* mutations represent a novel independent positive prognostic factor in anaplastic gliomas more powerful than that of combined 1p/19q deletion. Similar to *IDH1*, somatic mutations have been also observed for *IDH2* in glioma patients, albeit in a much lower frequency. Current data indicate that *IDH2* mutations are associated with an oligodendroglial tumor phenotype and with WHO grade III in these tumors.

Disclosure: No conflict of interest disclosed.

Freie Vorträge
Aggressive B-Zell-Lymphome

V562

The Addition of Rituximab to First-Line Chemotherapy (R-CHOP) Results in Superior Response Rates, Time to Treatment Failure and Response Duration in Patients with Advanced Stage Mantle Cell Lymphoma – Long-Term Results of a GLSG Trial

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Introduction: The addition of rituximab to chemotherapy (R-CHOP) has been shown to improve response rates in mantle cell lymphoma (MCL), but prolongation of response duration or overall survival was not observed (Lenz et al., JCO 2005). In a similar randomized comparison of 90 patients, again the addition of rituximab to MCP showed a tendency towards higher CR rates, but no improvement of overall response rate, progression free, or overall survival (Herold et al., ICML-10, 2008). **Methods:** We present an update of a previously published trial randomly comparing efficacy and safety of R-CHOP to CHOP induction in previously untreated patients with advanced stage MCL. **Results:** Of the 123 evaluable patients, 63 patients were randomized to R-CHOP. Median age was 62 years, and risk profiles of the two treatment arms were comparable. Overall response rates were 92% vs. 75% for R-CHOP vs. CHOP (p = 0.0139) and complete remission rates 33% vs. 8% (p = 0.0008). After a median follow-up of 66 months, median time to treatment failure was prolonged from 14 months for CHOP to 28 months for R-CHOP (p = 0.0004). Similarly, median response duration was prolonged from 18 (CHOP) to 29 months (R-CHOP, p = 0.0078). So far, no significant improvement of overall survival has been observed with median 77 vs. 59 months (p = 0.27) after R-CHOP and CHOP, respectively. Toxicity was not significantly higher for R-CHOP treated patients. **Conclusions:** After longer follow-up, superior remission rates, time to treatment failure, and response duration of combined immuno-chemotherapy were confirmed. However, in contrast to other lymphoma entities, improvement of overall survival has not yet been proven in MCL patients. Therefore new therapeutic options are urgently warranted to further improve the long term outcome in this otherwise dismal disease.

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V563

Molecular remission is an independent predictor of clinical outcome in patienst with MCL: Results of the randomized intergroup trials of the *European MCL Network*

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Detection of minimal residual disease (MRD) by RQ PCR is an important tool for therapy monitoring in B-cell lymphoma and may predict the clinical course. Within the two randomized intergroup trials of the *European MCL Network* investigating the impact of different combined immunochemotherapy protocols for patients with stage II-IV mantle cell lymphoma (MCL) followed by autologous stem cell transplantation for patients <65 yrs and a rituximab or interferon maintenance treatment for patients > 65 yrs prospective analysis of MRD was performed by quantitative RQ-PCR The prognostic impact of MRD using clone-specific *IGH* or *BCL1-JH* quantitative Real-Time PCR was analysed in 259 patients with mantle cell lymphoma (MCL) treated within the two randomized trials of the European MCL Network *MCL Younger* (18-65 years, R-CHOP+/-R-DHAP followed by autologous stem cell transplantation (ASCT) n=160) or *Elderly* (>65 years, n=99 R-CHOP vs. R-FC followed by maintenance). After rituximab based induction treatment 106/190 (56%) responding patients achieved a molecular remission (MR). Notably, *MCL Elderly* patients achieved a MR (41/65, 63%) more frequently compared to *MCL Younger* patients (MR 46/91, 51%) despite a higher number of patients with an adverse MIPI score (MIPI high risk 49% vs. 12%). MR was associated with a significantly improved remission duration (RD) in both trials (RD at 2-years 87% vs. 61%, p=0.0043) and emerged to be an independent prognostic factor for RD in multivariate analysis (HR 0.4, 95%CI 0.1-0.9, p=0.027). Notably, MR in the BM was highly predictive for prolonged RD independent of the clinical response (CR, Cru or PR) (RD at 2-years 100% in MRD negative CR and 88% in MRD negative PR, compared to 78% in MRD positive CR and 53% in MRD positive PR, p=0.0015). MRD was also predictive for outcome when assessed in the post induction period (2-year RD 89% for patients with MR during first year follow-up compared to 55% in MRD positive patient, p=0.0001) in both trials. ASCT in *MCL Younger* patients increased the proportion of patients in MR from 55% prior to high dose therapy to 72% thereafter. We conclude that sequential MRD monitoring is a powerful predictor for treatment outcome in MCL.

Disclosure: No conflict of interest disclosed.

V564

Sex-specific effects of rituximab on treatment outcome of elderly patients with diffuse large B-cell lymphoma: A retrospective analysis of the RICOVER-60 trial of the German High-Grade Non-Hodgkin Lymphoma Study Group (DSHNHL)

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Introduction: In the RICOVER-60 trial, where 1222 elderly (61-80 year-old) patients with untreated CD20-positive aggressive NHL were randomized to receive 6 or 8 cycles of CHOP-14 with or without 8 applications of rituximab, best results were obtained with 6xR-CHOP-14 in all subgroups including patients who did or did not receive additional radiotherapy to bulky disease. To study the impact of sex on treatment outcome, patient characteristics and results were analyzed according to the patients´gender. **Methods:** A retrospective analysis has been performed to compare the outcome from female and male patients treated in the RICOVER-60 trial. **Results:** Female patients within the rituximab arms presented a significantly higher LDH and lower performance status compared to the male counterparts (Elevated LDH: 57.2% vs 43.1%, p=0.001; ECOG >1: 18.6% vs. 11.1%, p=0.009). This significance was transferred into the whole population even though the two patient groups did not differ within the non rituximab arms. Female patients experienced more grade 3&4 leukocytopenias than men (64.5% of cycles vs. 45.9%, p<0.001) and the time with median of leukocytes < 2000/mm^3 was longer (2 vs. 0 days). This was associated with more grade 3&4 infections (7.4% of cycles vs. 6.0%, p=0.020), but there was no difference with respect to therapy-associated deaths rates (7.2% vs 7.8%, p=0.654). Female patients had a higher 3-year PFS (67.5% vs. 61.0%; p=0.062) and OS (74.2% vs. 68.4% p=0.086). These differences were largely due to a greater improvement by the addition of rituximab on the outcome of females: while the difference in 3-year

PFS between female and male patients was 5.2% (p=0.448) in patients receiving CHOP-14 only, this increased to 7.6%(p=0.053) when rituximab was added. Although a less favourable outcome would have been expected for female patients according to the described differences in LDH and performance status. In a multivariate analysis adjusting for the IPI-relevant risk factors LDH, ECOG performance status, advanced stage and >1 extranodal involvement, the relative risk for progression in male compared to female patients was not significantly elevated after CHOP-14 only (1.127; p=0.348), but was significantly higher when rituximab was added (1.592: p=0.004). **Conclusion:** Rituximab improves outcome both in elderly female and male patients treated with CHOP-14; however, the positive effect of rituximab is more pronounced in female patients and renders male sex a significant risk factor. This observation together with the low toxicity of rituximab justifies a trial with higher doses of rituximab for male patients. *Supported by Deutsche Krebshilfe.*

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V565

Immuno-Chemotherapy with Rituximab, Methotrexate, Lomustine, and Procarbazine in Patients older than 65 years with Primary CNS Lymphoma

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Introduction: Primary CNS lymphoma (PCNSL) has a poor prognosis dispite good initial response to steroids and whole brain irradiation (WBRT). Addition of high-dose methotrexate (MTX) to WBRT has improved the prognosis of patients with PCNSL, resulting in median survival rates of up to 60 months (mo). However, most patients eventually relapse. Surviving patients, particularly the elderly undergoing combined radio-chemotherapy, are at substantial risk of developing leukoencephalopathy. We developed a protocol with high-dose MTX combined with the lipophilic alkylating agents procarbazine and lomustine and the anti-CD20 monoclonal antibody rituximab (R-MPL protocol), adapted especially for older patients with PCNSL. Here we report the results of 26 patients treated with combined immuno-chemotherapy with R-MPL protocol in a monocentric pilot study. **Methods:** Patients >65 yrs with PCNSL received up to 3 cycles of R-MPL protocol: rituximab (375mg/m² d-6, 1, 15, 29), MTX (3g/m² d2, 16, 30), procarbazine (60 mg/m² p.o., d1-10) and lomustine (110 mg/m² p.o., d1). Cycles were repeated on d42. Inclusion criteria were age >65 yrs and biopsy-proven PCNSL. There was no minimum Karnofsky Performance Score. **Results:** Until now 26 patients (median age 75 yrs., range 65-83 yrs.) completed the R-MPL protocol. 24 patients were eligible for response evaluation, 2 died, one of pulmonary embolism 2 weeks after initiation of treatment, the other suffered a sigmoid perforation. Objective response was seen in 22 of 24 evaluable patients (91,7%) with 16 CR and 6 PR. 5 patients did not tolerate MTX after 1 (n=4) and 2 (n=1) applications due to cholestatic hepatitis (n=1) and renal impairment (n=4). neither was evaluable for response. Two patients with refractory disease were successfully salvaged with AraC/thiotepa (n=1) and HDT and ASCT (n=1), respectively. 5 patients experienced relapse and could not be salvaged. After a median follow-up of 25 mo (range 2-33), 12 patients (46,2 %) are alive and disease-free. The median survival is 19 mo. Preliminary evaluation shows a 12-mo overall survival of 69% and a 24-mo survival of 47%. Until now we have observed no severe leukoencephalopathy. Our most recent follow-up data will be presented in detail. **Conclusion:** This immuno-chemotherapy protocol is safe and highly efficacious in treating elderly patients with PCNSL. A prospective phase-II trial will be initiated.

Disclosure: No conflict of interest disclosed.

V566

Treatment of HIV-associated lymphoma in the era of highly active antiretroviral therapy – preliminary results of the German HIV Lymphoma Cohort

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Background: HIV-associated lymphomas are a significant factor of mortality and morbidity in HIV-infected patients. Optimal treatment remains controversial, especially with regard to the use of intensive regimens and the use of rituximab. **Methods:** This ongoing observational study includes all HIV-infected patients with Non-Hodgkin's Lymphoma (NHL) and Hodgkin's Lymphoma (HL) diagnosed since 2005 in 24 participating centres. Characteristics of patients are analyzed, including histology, risk factors such as International Prognostic Index (IPI) score, use of specific polychemotherapy (PCT), and antiretroviral therapy (ART). Patients are followed prospectively every six months. **Results:** As of December 2008, 208 patients (175 NHL, 33 HL) have been enrolled. At the time of lymphoma diagnosis, only 35 % of NHL patients (more patients with HL: 73 %, p<0.001) had prior ART exposure, and only 22 % of the NHL patients (HL 53 %, p<0.01) had a viral load below 50 HIV RNA copies/ml. After a median follow-up of 15.4 months, 64 (31 %) patients had died. Overall survival (OS) was worse in NHL patients than in HL patients (p=0.03). NHL cases were either treated with a CHOP-based PCT (74 %) or an intensified protocol adapted from the German study group for adult acute lymphoblastic leukaemia (GMALL protocol, 26 %), leading to a complete remission (CR) rate of 72 % (HL 93 %, p=.01). Among NHL cases, CD20+ cases had higher CR rates (74 % vs. 47 %, p=0.04) and a significantly better survival than CD20- cases (p<0.001). In CD20+ cases, the use of rituximab was associated with higher CR rates (74 % vs. 52 %, p=0.02) and with better OS (p=0.02). In total, there were 10 PCT associated deaths which were not related to specific PCT regimens or to the use of rituximab, to CD20 expression, or to other factors. **Conclusions:** Whereas the majority of NHL cases is diagnosed in ART naïve patients, HL mainly occur in patients receiving a virologically effective ART. The high early mortality rate in NHL cases underlines the need for intensive efforts to improve the outcome of this population, especially in patients with CD20- NHL. In CD20+ NHL, outcome was significantly better and was further improved by the use of rituximab. Our data indicate that treatment with rituximab is not associated with increased mortality from infection in patients with NHL.

Disclosure: Hoffmann,Chr.: Beratungstätigkeit:Abbott, Bristol-Myers Squibb, Essex, Gilead, Hoffmann La-Roche, MSD, Pfizer, Tibotec; Honorare: Abbott, Boehringer, Bristol-Myers Squibb, Essex, Gilead, Hoffmann La-Roche, MSD, Pfizer, Tibotec; Finanzierung wissenschaftlicher Untersuchung: Abbott, Boehringer, Bristol-Myers Squibb, Essex, Gilead, Hoffmann La-Roche, MSD, Pfizer, Tibotec
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V567

Rituximab-containing therapy for Non-Hodgkin`s Lymphoma: opportunistic infections and unexpected toxicity

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Introduction: Rituximab (R) is standard treatment for a variety of B-cell Non-Hodgkin lymphoma (NHL). Adverse events are usually mild to moderate. In rare cases, however, R may be associated with opportunistic infections (OI) or severe unexpected toxicity (UT). **Methods:** The records of consecutive pts treated at 2 institutions from 01/06 to 12/08 with R-containing chemotherapy or R-maintenance therapy (R-M) for NHL were analyzed for OI and severe UT. UT was considered as related to R if it could not be explained otherwise. **Results:** 99 pts were included in the cohort study. Pts received a median of 6 cycles (range 1 - 18) of R. A total of 517 cycles of R were evaluable for OI or UT. 7 of 99 pts (7%) (2 fe-

males, 5 males) with a median age of 69.5 yrs (range 41-76) experienced OI (n=3) or UT (n=4). OI consisted of pneumocystis jirovecii pneumonia (PCP) after 5x R-CHOP-14 for diffuse large B-cell lymphoma (DLBCL), Epstein-Barr-virus (EBV)-associated hepatitis after 5x R-CHOP-21 for relapsed follicular lymphoma (FL) and generalized herpes zoster following 6x R-bendamustine (RB) plus 1x R-M for recurrent BALT-lymphoma. PCP and Herpes zoster resolved under antimicrobial therapy while EBV-hepatitis improved spontaneously. R was terminated in the pt with PCP but could be restarted in the latter 2 pts. UT consisted of interstitial pneumonitis (IP) in 2 pts after 8 and 6 cycles of R-CHOP for DLBCL, a case of congestive heart failure (NYHA III°) after 6x R-CHOP + 2x R-M for FL and a case of grade 4 pancytopenia lasting for 22 days following 2x R-FC for chronic lymphocytic leukemia. IP completely resolved after initiation of prednisone (n=1) or under empiric antimicrobial therapy (n=1). Congestive heart failure improved under appropriate therapy. This pt received 2 more cycles of R-M. Pancytopenia slowly recovered under therapy with G-CSF, R was terminated. Furthermore, 2 additional pts were transferred to our department for therapy of enterovirus-induced encephalitis after 6x R-CHOP-21 + 2x R-M for FL (n=1) and cerebral toxoplasmosis in a pt heavily pretreated with R-containing therapy for relapsed mantle cell lymphoma (n=1). **Conclusions:** OI and severe UT are rare but potentially fatal complications. Awareness of OI/UT, rapid diagnostic proceedings and, whenever possible, initiation of therapy are essential. In selected cases careful reexposure of R may be feasible.

Disclosure: No conflict of interest disclosed.

Freie Vorträge
AML experimentell II

V568
SRC is a critical signalling mediator in FLT3-ITD positive AML

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Activating mutations of FLT3 are frequent in patients with AML. Two types of mutations are most common: Internal tandem duplications (ITD) of the juxtamembrane domain in approximately 30% of patients and point mutations within the second tyrosine kinase domain (TKD) in about 7% of AML patients. Patients carrying the FLT3-ITD mutation have a significantly worse prognosis whereas FLT3-TKD mutations do not appear to influence the clinical outcome. Studies have shown that mice receiving a transplant of bone marrow expressing FLT3 ITD develop a myeloproliferative disease. In contrast, mice which were transplanted with FLT3 TKD infected bone marrow, suffer from a lymphoid disease. Thus, both FLT3 mutations seem to exert different biological functions. Interestingly, FLT3-ITD but not FLT3-TKD or FLT3-WT leads to a strong activation of the STAT5 signalling pathway. Therefore, STAT5 activation may be responsible for the observed differences in biology. Here we investigated the signalling pathways leading to STAT5 activation downstream of FLT3-ITD. FLT3-ITD does not bind STAT5 directly nor does it activate the classical JAK2 pathway. Instead FLT3-ITD utilizes c-Src to activate STAT5: Co-immunoprecipitations and GST pull downs revealed a strong and exclusive interaction between Src and FLT3 ITD, which is mediated by the Src-SH2 domain. This interaction is absent in FLT3-TKD or FLT3-WT after ligand stimulation. The sequence duplication in FLT3-ITD leads to additional potential Src-SH2 binding sites. We identified tyrosines 589 and 591 of FLT3-ITD to be essential for Src binding and subsequent STAT5 activation. Specific Src inhibitors or Src-siRNA blocked STAT5 activation and growth induced by FLT3-ITD but not FLT3-TKD. FLT3-ITD positive cells with a stable Src knockdown injected into syngenic mice led to a leukemic disease with a significant delayed onset and prolonged survival in comparison to the control group. Finally, a combination of FLT3 and Src inhibitors was tested. This combination was highly efficient in FLT3-ITD positive cells but not in FLT-TKD positive cells. Together these findings show that Src plays an important role in the signalling of FLT3-ITD but not FLT3-TKD. Thus, Src might be an interesting therapeutic target for FLT3-ITD positive AML.

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V569
Expression, function and regulation of Autotaxin, a relevant motility and survival factor, in FLT3-ITD positive Acute Myeloid Leukemia and primary hematopoietic stem cells

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With an incidence of about 25 %, the internal tandem duplication (ITD) mutation of the FLT3 receptor gene represents one of the most common abnormalities in adult patients with acute myeloid leukemia (AML). Using microarray analysis, we recently showed that leukemic samples of patients with FLT3-ITD mutations have significantly upregulated expression levels of *Autotaxin* (*ATX*). The ATX protein acts as a secreted lysophospholipase D (lysoPLD) generating bioactive lysophosphatidic acid (LPA) from its precursor lysophosphatidylcholine (LPC). High ATX expression is associated with aberrant motility and increased cell growth in a variety of cancer cells. However data on ATX in myeloid leukemias, especially in AML, are missing. To study the role of ATX in detail, we overexpressed two alternatively spliced transcripts in several human leukemic cell lines where ATX was endogenously absent. The expression of ATX transcripts was confirmed at both mRNA and protein levels by RT-PCR and Western blotting, respectively. Transwell migration assays in the presence of LPC or LPA were performed to study ATX's role on cell motility. Proliferation and clonogenic potential were investigated using MTT and colony forming assays. High ATX expression was primarily found in malignant cells, whereas ATX was also detectable in CD34+ cells. FLT3-ITD transgene expression induced ATX expression in OCI-AML3 cells. *Vice versa*, inhibition of FLT3-ITD by sublethal doses of PKC412 in MV4-11 cells resulted in a significant reduction of ATX with subsequent loss of LPC induced specific chemotaxis. Moreover, we could show that the Jun N-terminal kinase (JNK) represents an important link between FLT3 and ATX. The ATX transgene expression increased colony-forming capacity in the presence of LPC or LPA by 40% and 75%, respectively. LPA increased chemotaxis in human leukemic cell lines and human CD34+ progenitors in a dose dependent manner and induced significantly higher migratory rates by at least 50%. LPC induced chemotaxis by 80-200% only in cells where ATX was present. However, the LPC/LPA induced chemotaxis could be blocked by pertussis toxin (PTX) and Ki16425 demonstrating the involvement of PTX sensitive LPA1 receptors in this process. Our data suggest that the production of bioactive LPA through ATX is involved in controlling proliferation and migration of haematopoietic stem cells and its deregulation may contribute to the pathogenesis of AML, specifically in FLT3-ITD positive disease.

Disclosure: No conflict of interest disclosed.

V570
Cbl mutants lead to myeloproliferative disease or to myeloid leukemia in a mouse transplantation model

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Purpose: Somatic mutations of Kit have been found in leukemias and gastrointestinal stromal tumors. The proto-oncogene c-Cbl negatively regulates Kit and Flt3 by its E3 ligase activity and acts as a scaffold for several signaling adaptor molecules. We recently identified the first c-Cbl mutation in human disease in an AML patient, called Cbl-R420Q. **Results:** We transduced primary murine bone marrow retrovirally with c-Cbl mutants and transplanted it into lethally irradiated mice. Almost all recipients of bone marrow cells transduced with Cbl mutants developed a lethal hematologic disorder with a mean latency of 341 days in the Cbl-R420Q group and 395 days in the Cbl-70Z group. Eleven out of 13 mice and 8 out of 11 mice died in the

Cbl-R420Q group and Cbl-70Z group, respectively. Two animals succumbed to a myeloid leukemia, the other mice developed a myeloproliferative disease. The leukemic mice showed a leukocytosis of up to 140.000/µL. They developed a splenomegaly with massive expansion of myeloid cells in liver and spleen. Histology sections of spleen, liver and bone marrow and FACS analyses of spleen, bone marrow and peripheral blood showed extensive infiltration of myeloid cells. **Conclusion:** Thus, transplantation of bone marrow cells expressing Cbl mutants leads to a myeloid leukemia or to a myeloproliferative disease with long latency and high penetrance.

Disclosure: Sargin,B.: Anstellungsverhältnis oder Führungsposition: Angestellt; Aktienbesitz: Daimler; Finanzierung wissenschaftlicher Untersuchung: Innovative Medizinische Forschung, IMF, Münster
Brandts,Chr.: Anstellungsverhältnis oder Führungsposition:angestellt; Beratungstätigkeit: Lilly; Aktienbesitz: Bayer; Finanzierung wissenschaftlicher Untersuchung: DFG, Carreras, Deutsche Krebshilfe; Gutachtertätigkeit: Blood, Leukemia

V571

Oncogenic RAS enables DNA damage- and p53-dependent differentiation of acute myeloid leukemia cells in response to chemotherapy

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Introduction: Acute myeloid leukemia (AML) is a clonal disease originating from myeloid progenitor cells with a heterogeneous genetic background. High-dose cytarabine is used as the standard consolidation chemotherapy. Oncogenic RAS mutations are frequently observed in AML, and patients with RAS mutations benefit most from high-dose cytarabine as postremission therapy (Neubauer et al., J Clin Oncol 2008). The molecular reason for this phenomenon is not well understood. **Methods:** We used bone marrow cells expressing a conditional MLL-ENL-ER oncogene to investigate the interaction of oncogenic RAS and chemotherapeutic agents. In addition, we used primary human inversion 16 positive AML samples with or without oncogenic RAS mutations to corroborate our findings. **Results:** Oncogenic RAS synergizes with cytotoxic agents in the activation of DNA damage checkpoints. This results in an Atm/r as well as p53-dependent genetic program that reduces clonogenicity and increases myeloid differentiation. Inhibition of Mdm2-mediated degradation of p53 further enhances this myeloid differentiation. In primary AML cases, oncogenic RAS also was associated with a more differentiated phenotype with regard to expression of differentiation markers. **Conclusions:** The data can explain the beneficial effects observed in AML patients with oncogenic RAS mutations treated with high dosages of cytarabine and suggest that induction of p53-dependent differentiation, e.g. by interfering with Mdm2-mediated degradation, may be a rational approach to increase cure rate in response to chemotherapy. The data also support the notion that the therapeutic success of cytotoxic drugs may depend on their ability to promote the differentiation of tumor-initiating cells.

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Neubauer,A.: Anstellungsverhältnis oder Führungsposition:C4 Beamter
Honorare:Verschiedene Firmen, mit dem hier vorliegenden Abstract kein Interessenkonflikt; Finanzierung wissenschaftlicher Untersuchung:DFG, BMBF, Carreras, Krebshilfe, Sander, Lokale Stiftungen, Roche (unrestricted grant); Gutachtertätigkeit:Gerichte, Landesärztekammer

V572

HOXA9 AND ITS NON-HOMEODOMAIN ISOFORM HOXA9T COOPERATE IN NORMAL AND MALIGNANT HEMATOPOIESIS

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Introduction: Hoxa9 is a homeobox transcription factor with a central role in both hematopoiesis and leukemia. High expression levels of HOXA9 in human haematopoietic cells are a characteristic feature of acute myeloid leukemia (AML). Previously a highly conserved al-ternative splice variant of Hoxa9 - truncated Hoxa9 (Hoxa9T) - has been identified that lacks the homeodomain (Fujimoto *et al*., 1998). So far the biological relevance of Hoxa9T in normal and malignant hematopoiesis is not known. **METHODS:** Expression analysis of HOXA9 and HOXA9T in cell lines and patient samples was performed by TaqMan quantitative RT-PCR. To investigate the *in vivo* function of Hoxa9T, we used the syngenic murine bone marow transplantation model, colony forming cell assay (CFC) and colony forming unit spleen assay (CFU-S). To understand the function of the naturally occurring splice variant of HOXA9, we used retroviral constructs of several murine Hoxa9 isoforms, namely the wildtype (Hoxa9 WT), the truncated (Hoxa9T) and a mutated full-length form (Hoxa9 FL) in which the pseudointron recognition sites are silenced to eliminate the splicing event. **RESULTS:** HOXA9 and HOXA9T are co-expressed in human leukemic cell lines as well as in 115 AML patient samples with normal karyotype. Interestingly, only a minor fraction of 12% of the analyzed AML patient samples showed a similar ratio between HOXA9- and HOXA9T-expression compared to highly purified CD34+ bone marrow cells of healthy donors (ratio=3.2). The vast majority of the patients (86%) showed an increase in this ratio due to an elevated HOXA9- to HOXA9T-expression, whereas 17% showed a decrease in this ratio. In clear contrast to Hoxa9 WT, Hoxa9T did not show any increased activity compared to the empty retroviral control in the CFC-assay or CFU-S assay. Expression of these Hoxa9-variants either alone or in combination with each other in mice revealed that Hoxa9 has to co-operate with its own spliced isoform to exert its full leukemogenic potential: Hoxa9T collaborated with ectopically expressed Hoxa9 WT or Hoxa9 FL in the propagation of AML in transplanted mice (n=10, Mdn latency 136 days and 144 days, respectively). In contrast, expression of Hoxa9 FL (n=22, Mdn latency 208 days) or Hoxa9T alone showed a reduced leukemogenic potential (n=17, Mdn latency not reached) compared to Hoxa9 WT (n=21, Mdn latency 135 days). **CONCLUSION:** These results indicate that the leukemogenic potential of Hoxa9 depends on its collaboration with its own spliced isoform.

Disclosure: No conflict of interest disclosed.

V573

Mesenchymal stroma cells from patients with myelodysplastic syndrome and acute myeloid leukemia show distinct cytogenetic and DNA-mutation data as compared with bone marrow leukemic blasts

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Introduction: Bone marrow mesenchymal stroma cells (BMSC) are key components of the hematopoietic microenvironment. BMSC from patients with acute myeloid leukemia (AML) and myelodisplasic syndrome (MDS) display functional and quantitative alterations. **Methods:** To gain insight into these questions, we carried out cytogenetic analyses, FISH, FLT3 and NPM1 mutation examinations of both hematopoietic (HC) and BMSC derived from 53 AML and 54 MDS patients and 35 healthy donors after *in vitro* culture expansion. **Results:** Clonal chromosomal aberrations were detectable in BMSC of 12 % of patients. Using FISH we have assume that cytogenetic markers in BMSC were always distinct as the aberrations in HC from the same individual. 17 % and 12% of AML patients showed FLT3 and NPM1 mutations in HC, respectively. In BMSC, we could not detect mutations of NPM1 and FLT3, independent from the mutation status of HC. For control analysis, BMSC cultures from 35 healthy donors were prepared under the same conditions. BMSC from healthy donors showed normal diploid karyotypes and absence of DNA-mutations of NPM1 and FLT3 genes. **Conclusions**: Our data indicate that BMSC from MDS and AML patients are characterized by genetic instability. Lack of aberrations as detected in HC and appearance of novel clonal rearrangements in BMSC may suggest enhanced genetic susceptibility and potential involvement of BMSC in the pathogenesis of MDS and AML.

Disclosure: Blau,O.: Anstellungsverhältnis oder Führungsposition: wiss. Mitarbeiter Universitätsmedizin Berlin; Beratungstätigkeit:---; Aktienbesitz:---; Honorare:---; Finanzierung wissenschaftlicher Untersuchung:---;Gutachtertätigkeit:---; Andere finanziellen Interessen:---;

Blau,I. W.: Anstellungsverhältnis oder Führungsposition: Oberarzt, Universitätsmedizin Berlin; Beratungstätigkeit: MSD, Ortho Biotech; Aktienbesitz:---; Honorare: MSD, Celgene, Orhto Biotech, Mundipharma; Finanzierung wissenschaftlicher Untersuchung:---; Gutachtertätigkeit:---; Andere finanziellen Interessen:---;

Fortbildung
Aufgaben der onkologischen Rehabilitation bei Patientinnen mit Mammakarzinom

V576
Arm morbidity after surgery for breast cancer. Therapeutic approach and residual functional impairment with respect to work and occupation.

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Improvements in the life expectancy after therapy of breast cancer shifts the focus of attention to quality of life issues. Quality of life is influenced mainly by pain, lymph edema, reduced arm mobility, reduced grip strength of arm and hand muscles and psychological factors. Breast cancer surgery compromises the mobility of the shoulder joint resulting in shoulder-arm morbidity in approximately 20% of all patients undergoing breast surgery. The main risk factor for the development of arm morbidity is the number of lymph nodes removed during the operative procedure. Therefore, sentinel lymph node biopsy techniques benefit the patient with reduced frequency of arm morbidity compared to classical axillary dissection. Factors unrelated to arm morbidity are age, stage of the disease, kind of breast surgery and radiotherapy. Arm morbidity related to breast cancer surgery must be differentiated from preexisting shoulder morbidity, for example periarhtropathy humeroscapularis, impingement disease, lesions of rotator cuff muscles or the biceps tendon, inflammation of bursas or shoulder joint arthrosis. In patients with preexisting shoulder morbidity, breast cancer surgery may worsen shoulder-arm functions. The probability of preexisting shoulder-arm comorbidity rises with the patients age. Shoulder-arm morbidity may develop to a chronic impairment preventing the patient from reintgration into daily life. Physiotherapy (arm and shoulder exercises) should be started accordingly as soon as possible after surgery in special rehabilitation institutions to provide for a fast recovery and to secure the patients self-help competence. Reintegration into work life and associated functional restrictions due to impaired physical capabilities are discussed with respect to the different occupational fields.

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V578
Sport and exercise after breast cancer – what can we achieve?

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Sport and exercise are important forms of therapy in rehabilitation measures for female patients with and after breast cancer. In literature we find primarily investigations into forms of aerobic exercises through which positive effects can be achieved in the areas of physical efficiency, bodily function, body image , self confidence, psychological stress, quality of life and fatigue. Positive effects for the mobility of the arm and shoulder area can be achieved with physiotherapy. Consideration for the significance of this was shown in the consensus proceedings for the guidelines for the rehabilitation of patients with breast cancer, by demanding 2 hours per week of both sport therapy and physiotherapy for at least 75% of the patients. Besides that sport and exercise have significance in relation to primary and tertiary prevention of breast cancer: in both areas relevant reductions of risk factors through physical activity are possible. The available studies show thereby different levels of effect relating to age and hormone receptor status. During the oncological rehabilitation measures most patients are physically active and achieve the above outlined positive effects short term. The aim during rehabilitation has to be to push and encourage the patient to adopt regular physical activity besides other lifestyle measures into their daily routine and thus to benefit long term from the above outlined effects.

Disclosure: No conflict of interest disclosed.

Vorträge
Best Abstracts

V580
A prospective, randomized trial of simultaneous pancreatic cancer treatment with enoxaparin and chemotherapy. First Results of the CONKO 004 trial.

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Objective: The treatment of patients (pts) with advanced pancreatic cancer (APC) is often complicated by venous thromboembolic events (VTE). Anticoagulation therapy with low molecular weight heparin (LMWH) may prevent VTE and is under discussion to improve overall survival (OS) in cancer. Based on our previous pilot study indicating the safety and feasibility of the LMWH enoxaparin added to chemotherapy in pts with APC we started the open, prospective, randomized, multicenter study to investigate the role of enoxaparin in these pts. **Methods:** Calculation was based on an expected reduction of the primary endpoint [symptomatic VTE (sVTE) within the first 12 weeks of treatment] from 10% to 3%. Toxicity, time to progression (TTP) and OS were among the secondary endpoints of the study approved by the ethics committees of the participating centers. Chemotherapy naive pts with histologically or cytologically confirmed APC were randomized to receive or not to receive additional LMWH (enoxaparin 1mg/kg once daily) starting simultaneous to palliative systemic chemotherapy. **Results:** In January 2009 after recruitment of 312 pts the study was closed. There were 22 sVTE in 152 pts of the observation group (O) and 8 in 160 pts of the enoxaparin group (E). ITT and PP analyses after a median follow-up of 30.4 weeks demonstrated significant risk reductions from 14.5 to 5.0 % (65 % RRR) and 14.5 to 3.8 % (74 % RRR) for E, respectively. Major bleeding events were 9.9% for O and 6.3% for E (ITT; n.s.). In each group there was one tumor-related fatal hemorrhage. Preliminary data show no difference in TTP (O:19 vs. E:22 w) and OS (O:29 vs. E:31 w). **Conclusions:** Enoxaparin is effective and safe in the primary prevention of sVTE applied simultaneously with cytotoxic chemotherapy in pts with APC. Final results on TTP and OS are pending.

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Oettle,H.: Finanzierung wissenschaftlicher Untersuchung:Teilfinanzierung der IIT-Studie durch Firmen: Sanofi-Aventis, Lilly, Amgen

V581
Overall Mortality and Fungal Infection-Related Mortality in Patients Undergoing Myelosuppressive Chemotherapy in a Tertiary Care Centre during 12 Years (1995-2006): Final Analysis

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Introduction: Invasive fungal infections (IFI) contribute significantly to mortality and morbidity in patients (pts) receiving myelosuppressive chemotherapy for hematological malignancies. The aim of the present

study was to evaluate the incidence of IFI in our department from 1995 until 2006 and to analyze overall survival, infection-related mortality, and treatment changes over time of IFI. We now present the results of our final analysis. **Methods**: Data of chemotherapy courses given on the leukaemia ward were retrospectively collected with a standard questionnaire. Modified EORTC/MSG criteria for IFI were applied: A positive PCR for Aspergillus spp. in BAL samples was also defined as probable IFI. Data were analysed using SPSS 17 and GraphPad InStat V2.05. **Results**: In total, 1693 courses in 592 pts were evaluated. Most were given to treat AML (63%) or ALL (including Burkitt lymphoma; 30%). Pts with AML received antifungal prophylaxis with itraconazole while pts with ALL received oral amphotericin B. At least one IFI was observed in 139/592 pts (23%), and in 149/1693 (8.8%, 95%CI 8-10%) courses. Forty-two of these were proven, 32 probable and 75 possible IFI. The incidence of IFI increased in the more recent years: During 1995-2001, an IFI occurred in 65/919 courses (7.1%, 95%CI 5-9%) and during 2002-2006 in 84/774 courses (10.9%, 95%CI 9-13%), p=0.007. In contrast, mortality due to IFI decreased in the past years: 57% in years 1995-2001 and 29% in years 2002-2006, p<0.001. We also observed an increase in median overall survival in patients with IFI in the later years: 54 days (95%CI 26–82 days) before 2002 versus 229 days (95% CI 35–423 days) in years 2002-2006, p=0.001. By multivariate analysis, factors predictive for better overall survival were controlled disease after chemotherapy (HR 0.226, p<0.001), possible IFI (in contrast to proven/probable IFI, HR 0.511, p=0.002), age < 60 years (HR 0.611, p=0.015), and use of novel antifungals (HR 0.493, p=0.002). **Conclusions**: Compared to 1995-2001, the incidence of IFI increased, but IFI-related mortality decreased and overall survival in patients with IFI increased significantly in the later years. In our patients, improved survival data were associated with status of underlying disease (controlled), classification of IFI (possible), age < 60 years, and the use of novel antifungal agents.
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V582

Identification of unknown epigenetically regulated genes in non-small cell lung cancer

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Introduction: Lung cancer is the number one cause of cancer deaths worldwide. Besides mutations aberrant methylation of 5' CpG-islands leading to loss of gene expression is an important event in the pathogenesis of lung cancer. Recently, it has been reported that on average 600 CpG-islands are targets for methylation in malignant cells. However, to date, about 50 genes are known to be frequently methylated in lung cancer. Thus, our project is aimed to identify new epigenetically silenced cancer-associated genes in non-small cell lung cancer (NSCLC). *Methods:* The genome-wide gene expression pattern in A549 cells was analyzed before and after treatment with 5-aza-2'-deoxycytidine (AzadC), a methyltransferase inhibitor and/or Trichostatin A (TSA), a histon deacetylase inhibitor, using Affymetrix U133 plus 2.0 arrays. The genome-wide DNA methylation of A549 cells was determined by a combined methylated DNA immuno-precipitation/microarray approache (MeDIP-chip). Microarray data of certain genes were confirmed by methylation-specific PCR (MSP) in both lung adenocarcinoma cell lines and primary tumor samples of NSCLC patients. *Results:* The expression of 3824 genes was induced in A549 after treatment with AzadC and/or TSA. By MeDIP-chip we identified 1982 methylated genes in the cell line A549. Comparing the results of the microarray approaches 325 genes were found to be both methylated and drug induced. Ontology analyses revealed that these 325 genes are involved in certain molecular pathways which are important for the development of a malignant phenotype (e.g. apoptosis, cell communication and cell cycle). So far, the methylation status of 11 genes was confirmed by MSP in lung adenocarcinoma cell lines and in tumor and matching non-malignant lung tissue samples of several NSCLC patients. Methylation was only found in tumor, but not in non-malignant lung tissue samples suggesting that methylation of these genes is tumor specific. *Conclusion:* By using different microarray approaches we were able to identify a large number of unknown epigenetically regulated tumor associated genes which may play a role in the pathogenesis of NSCLC. Our results may aid to a better understanding of NSCLC.

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V583

Disruption of TGF-ß signaling prevents the generation of tumor-sensitized T-reg cells and facilitates therapeutic antitumor immunity.

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Introduction: Since we could show that T cells with an impaired TGF-ß receptor (dnTGFßRII) could be primed in reconstituted lymphopenic mice (RLM) even when reconstituted with splenocytes from tumor-bearing mice (TBM), we investigated the underlying mechanism. We hypothesized, that dnTGFßRII T cells were insensitive to tumor-induced Treg immune suppression mediated by TGF-ß or could not get induced to become T-regs. **METHODS:** To test this, systemic tumor was established by i.v. administration of B16BL6-D5 (D5) in female FoxP3/GFP+/+ Tg mice (FoxP3+ Tregs in these mice express green fluorescent protein/GFP). FoxP3+/GFP+ cells from TBM were sorted and used for reconstitution together with either naïve WT or naïve dnTGFßRII splenocytes of irradiated (500R) mice, which were then vaccinated with D5-G6, a mGM-CSF secreting subclone of D5. Tumor vaccine draining lymph nodes from RLM were harvested, activated with anti-CD3/anti-CD28 and expanded in IL-2 to generate tumor-specific effector T cells. To test wheter dnTGFßRII could become FoxP3+, GFP- cells from male naïve WT-GFP+/0 or dnTGFßRII-GFP+/0 spleen cells were in vitro activated in the presence of TGF-ß in order to induce FoxP3+ adaptive Treg cells. Furthermore, we reconstituted lymphopenic mice with naïve spleen cells together with $CD25^+$ cells from either WT TBM or dnTGFßRII TBM mice. **RESULTS:** Both effector T cells generated from RLM reconstituted with WT or dnTGFßRII splenocytes in presence of GFP+/FoxP3+ cells showed a significant reduction of tumor-specific IFN? release. WT spleen cells were induced to become GFP+/FoxP3+ in a TGF-ß dose dependent manner. In striking contrast, dnTGFßRII spleen cells showed significantly fewer GFP+/FoxP3+ cells. Additionally, CD25+ from TBM dnTGFßRII did not suppress the priming of therapeutic tumor-specific effector T cells. **CONCLUSION:** These results show that a) T cells insensitive to TGF-ß can still be suppressed by tumor-induced T-reg cells b) TGF-ß plays a crucial role in the de novo induction of "adaptive" T-reg.

Disclosure: Petrausch,U.: Anstellungsverhältnis oder Führungsposition: UniversitätsSpital Zürich
Fox,B.: Anstellungsverhältnis oder Führungsposition: Chief, Laboratory of Molecular and Tumor Immunology, Robert W. Franz Cancer Research Center, Earle A. Chiles Research Institute; Beratungstätigkeit: für Cell Genesys in der Vergangenheit

V584

Hematopoietic Development from Human Induced Pluripotent Stem Cells

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Embryonic stem cells (ESC) represent an unprecedented resource for cell replacement therapies. However, ESC-derived progenitors face immune barriers when transplanted into genetically non-identical hosts.

Recently, combinatorial overexpression of a limited number of embryonic proteins was found to reprogram somatic cells back to pluripotency, enabling the derivation of isogenic (patient-specific) human induced pluripotent stem (iPS) cells. While current research is focusing on improving reprogramming protocols (e.g. circumventing the use of oncoproteins and gene transfer through retroviruses), limited data is available on the *in vitro* differentiation efficacy of human iPS cells into specific lineages. We have previously shown in mouse ESC that the embryonic morphogens BMP4 and Wnt3a direct blood formation via activation of *Cdx* and *Hox* genes (Lengerke *et al*, Cell Stem Cell 2008). Ectopic expression of *Cdx4* and *HoxB4* enables the generation of mouse ESC-derived hematopoietic stem cells (HSC) capable of multilineage reconstitution of lethally irradiated adult mice. Here, we ask whether these signaling pathways direct hematopoietic development from human iPS cells generated in our laboratory (Park *et al*, Nature 2008). Our data shows robust differentiation of iPS cells to mesoderm and blood lineages, comparable to reports on differentiation of human ESC in this system. We detect robust formation of $CD34^+$ (28.9±12%), $CD45^+$ (26.8±13.4%) and $CD34^+CD45^+$ (16.1±13.7%) cells, and a high incidence of CFU-initiating cells in functional colony assays (95 colonies per 12500 EB-derived cells). Similar to our findings in mouse ESC, mesodermal and hematopoietic genes are expressed in waves, and expression was augmented by supplementation of cultures with BMP4. Mesodermal markers (e.g. *BRACHYURY*) were induced at day 2 and declined after day 9, when hematopoietic markers (*SCL)* appeared indicating conversion of mesoderm to progenitors of the blood lineage. Expression of all three human *CDX* genes (*CDX1*, *CDX2* and *CDX4*) peaked at day 6, suggesting that the function of *CDX* genes to pattern preformed mesoderm to blood fate is conserved in human embryogenesis. Taken together, our results show robust hematopoietic differentiation of human iPS cells and suggest that *in vitro* differentiating iPS cells are a potential resource for isogenic blood cell replacement therapies and can be used to study human developmental hematopoiesis and *in vitro* modeling of hematologic diseases.

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Lengerke,C.: Anstellungsverhältnis oder Führungsposition: Angestellt als Assistenzärztin an der Medizinischen Klinik Tübingen; Finanzierung wissenschaftlicher Untersuchung: fortüne Programm der Universität Tübingen; Gutachtertätigkeit

V585

CEBPA gene mutations affecting the TAD and bZIP region show differerences in their association with clinical parameters and prognosis: an analysis in 1779 patients

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Mutations of the *CEBPA* gene coding for the key myeloid transcription factor CCAAT/enhancer binding protein alpha have been reported in up to 20% of patients with AML and normal karyotype and appear to be associated with an improved outcome after chemotherapy. However, most studies published so far investigated selected patient populations or relatively small cohorts. In order to study this abnormality in an unselected cohort, including older patients and all karyotype abnormalities, we analyzed 1779 newly diagnosed AML patients for the predominant *CEBPA* mutations, namely insertion/deletion mutations affecting the transactivation domains (TAD) or the basic-/ leucine zipper-region (bZIP). Outcome was analyzed for those 1435 patients treated in the AML96 protocol of the SAL. Screening for *CEBPA* mutations was done on DNA isolated at the time of diagnosis using high resolution fragment analysis and direct sequencing. **Results:** Overall, 152 individual *CEBPA* mutations were identified in 103 of the 1779 patients (5.8%), 49 patients had combined TAD1/bZIP mutations, 24 had only a TAD-mutation and 30 had only a mutation in bZIP. Mutations were significantly more frequent in normal karyotype (NK) patients (84/836; 10%) compared to patients with aberrant karyotype (AK) (15/814; 1.8%; P<.001). Median age of patients with *CEBPA*-mutations was 49 years vs. 60 years in patients with wt-*CEBPA* (P<.001). This age difference was largely due to the lower age of patients with bZIP mutations (med. age 46 yrs.) or combined bZip/TAD1 (47.5 yrs.) mutations, whereas patients with only a mutation in the TAD-domain were significantly older (64 yrs.). Patients with TAD-mutations showed significant differences in WBC counts, rate of sAML and the frequency of additional NPM1-comutations from the other two mutation types. Overall, patients with CEBPA mutations had a significantly higher rate of complete remissions (CR *CEBPA*-mut: 74.7% vs. -wt: 49.4%; P<.001), which translated into a significantly better overall survival (OS: CEBPA-mut: median 33.6 months vs –wt: 12.6 months; P=.001). Interestingly, in patients with aberrant karyotype, CEBPA also predicted a significantly better OS (med. mutant not reached vs. 10.5 months for wt; P=.002), but was not associated with improved outcome in older patients (>60 years). When analyzed separately, the OS differed significantly between patients with bZip-mutations and TAD-mutations (med.: OS: bZip: 50.2 mo vs. TAD 12.2 mo; P=.05). In a multivariate analysis, CEBPA mutations represented an independent predictor of CR-rate and overall survival. In conclusion, our data indicate that CEBPA mutations show a distinct association with clinical parameters and suggest a previously unrecognized association between the localization of the mutation and their effect on the leukemia, which might be due to the differential formation of the truncated p30 variant of the protein.

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Fortbildung
Diagnostik und Therapie des Mulitiplen Myeloms: State of the Art

V587

Treatment of Younger Multiple Myeloma Patients

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Multiple myeloma (MM) is a malignancy that affects about 3,500 new individuals each year in Germany. The incidence increases with age, approximately 50% of patients present under the age of 65 years. The term "younger myeloma patients" is not consistently defined. In regard to autologous stem cell transplantation (ASCT), the upper age limit of "younger myeloma patients" is between 65 and 75 years. For allogeneic transplantation approaches the "younger myeloma patients" should not be older than 55 to 65 years. ASCT is considered the gold standard in first line therapy of "younger myeloma patients". The standard high-dose regimen is Melphalan 200 mg/sqm. The achievement of CR or at least 90% tumour reduction (Very Good Partial Remission) by this intensive treatment approach is associated with a longer PFS and OS. Therefore, new drugs like thalidomide, bortezomib and lenalidomide are incorporated before, during and after ASCT. First results show improved PFS related to the new drugs in ASCT based treatments. In some countries, health authorities or insurances cover induction treatments including new drugs. The optimal drug combination (two or three drugs) has not been established, yet. There is no consensus regarding double transplantation. Maintenance treatment appears to be effective in patients with residual disease after ASCT. Duration and dosages of maintenance regimens are under evaluation. Some trials now challenge the role of ASCT in front-line treatment by comparing conventional therapeutics in combination with novel agents in front-line therapy followed by ASCT in relapse versus the traditional front-line ASCT approach, including new drugs. Allogeneic Transplantation is a potentially curative approach in MM. However, current data indicate, that there is a strong need for further trials to optimize this effective treatment. The prognosis of younger MM patients was improved in the 90s as a result of optimization of the ASCT. Now, new drugs confer further improvement of PFS and OS.

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