Rehabilitation of breast cancer – medical evidence based concepts of rehabilitation

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In Germany every year approximately 74500 patients suffer from breast cancer. They represent the largest group within the oncological rehabilitation. Oncological rehabilitation of patients with breast cancer is a complex task with different requirements for patients, referer and service provider, the fulfilment of which can raise the question of medical evidence. The initial matter under consideration is to secure the success of the treatment in Acute Care, through the connection with the Acute Care Hospitals as part of cooperation with Breast Centres, the rehabilitation clinics commit themselves to guideline based advice and documentation of the treatment. In Rehabilitation clinics anti-hormonal therapies, therapies with Trastuzumab or Bisphosphonates are often being continued. Especially with the long-term adjuvant therapy concepts exists a great desire of the patient for information, which can only be adequately answered with the knowledge of each relevant Study. Patients come to us with heterogeneous somatic, psychological and social consequences caused by breast cancer or resulting from the therapies received. Based on the international classification of functional capability, disability and health, implemented especially in the Social Security Code IX and formulated by the Rehabilitations Service Providers (Pensions Insurance and Health Insurance), determine the tasks of rehabilitation by the impairment of physical function and structures and the resulting limitations in Activity and interaction, also considering the personal and environmental background. Breast cancer is the first oncological disease the German pension insurance has established a Rehabilitation standard for. The aim of this Rehabilitation – Therapy – Standard is to put the rehabilitative treatment of breast cancer on a scientific and medical evidence based basis and to improve the quality of rehabilitative care. Compliance to the Therapy Standard or the level to which the Rehabilitation-Therapy-Standard is fulfilled will be recorded in the discharge letter, evaluated and sent in the form of a report to the Rehabilitation-Quality-Control of the participating Hospitals/Clinics. Lectures and Training courses on healthy living are elements of the Rehabilitation, they need medical evidence, especially regarding the patients important questions on the subject of the influence of a possible recurrence. Disclosure: No conflict of interest disclosed.

Material and methods: Ex vivo modified grafts were tested in murine GvHD models using CD4+CD44+HLA-DR3+/4+CD8+T cells (triple transgenic mice, TTG) as donors, and Balb/c mice as recipients. A GvHD model by transplantation of 4x10^9 human PBMCs into NOD/SCID mice and a murine P815-Balb/c leukemia model to study the Graft-versus-Leukemia-Effect were generated. Survival, aGvHD development, leukocyte subset recovery (also regulatory T cells [Tregs]), and chimerism were analyzed for 60 days. Distribution of donor cells was confirmed by flow cytometry, (immuno-)histology, and real-time PCR.

Results: Stable engraftment of TTG-C57Bl/6 donor cells (H-2kd [C57Bl/6], human CD4, HLA-DR), a decrease of cd4, and development of a gut of aGvHD indicates a full Ttg donor hematopoiesis. The survival rate was significantly increased in Balb/c recipients transplanted with TTG donor cells+antibodies (0 to 90%, p<0.001, n=28). Without antibodies, aGvHD mice died within 35 days. In P815-Balb/c leukaemic mice+antibodies, survival was significantly higher compared to controls (60% vs. 0%, p<0.001, n=6). Donor-derived CD4+, CD25-, Fox-P3- Tregs could be measured (10% of lymphocytes; p=0.002, day 55) and the Fox-P3-RNA was up-regulated in liver and spleen due to modulation of the antibodies. Using grafts from Balb/c-Ttg chimeras after 40 days for transplantation without anti-human CD4 re-inubcation in Balb/c mice does not induce any aGvHD development even when higher T-cell numbers are used. In NOD/SCID mice, human CD45+, CD19+, CD4+, and CD8+ cells were detected after transplantation of 4x10^9 PBMCs. Without antibodies, NOD/SCID mice develop aGvHD and died within 10 days compared to the antibody controls (survival 0% vs. 100%, p<0.05; p<0.001).

Conclusion: We showed prevention of GvHD in a full MHC-mismatch and NOD/SCID mouse model by ex vivo modulation of Hsc grafts by ex vivo specific anti-human CD4 antibodies. To our knowledge, such a therapy for induction of tolerance to recipient’s tissues in Hsct is new.

Disclosure: No conflict of interest disclosed.

Clinical studies exploring the impact of Natural killer (NK) cells during hematopoietic stem cell transplantation (HSCT) have provided promising results. It is known that NK cells are a heterogeneous population and can be divided into functionally distinct NK cell subpopulations. Murine NK cells can be separated along their expression of CD27 and CD11b and CD117 (c-kit). However, the functional relevance of the distinct NK subsets in graft-versus-host-disease (GVHD) has not been investigated in detail so far. We have established different protocols for isolation and expansion of murine NK cell subpopulations. These NK subsets were further analyzed in vitro and in vivo in an allogeneic murine GVHD model. The four different NK cell subsets provide differences in their genomic, phenotypic and functional properties. Data clearly demonstrate that CD11b+ NK cells express multiple genes of cytotoxic pathways and develop the highest tumoricidal capacity. We observed up to 60% tumor lysis by CD27-CD11b+ NK cells compared to 40-45% by CD27+CD11b+, about 25% by CD27-CD11b- and 10% by c-kit+CD11b- NK cells at an effector-target ratio of 5:1. Interestingly, CD27+ NK cells provided the highest IFN-g production upon incubation with tumor cells and/ or IL-2. We further analyzed the migratory capacity and tissue homing of the different sorted NK cell subsets in bone marrow transplantation in vivo. Interestingly, CD11b+ NK cells migrate to the GVHD target organs, whereas CD27+ NK cells preferentially home to the bone marrow. Finally, we investigated the role of distinct NK cell subpopulations in the development of GVHD in a fully MHC mismatched HSCT mouse model. In summary, our comparative study outlines that only the CD11b+ NK cells that migrate to the peripheral GVHD target organs and provide the most efficient cytolytic capacity
provide GVHD protection. These new insights are highly relevant for the selection of the optimal NK cell preparation in the field of cellular therapy.

**Disclosure:** No conflict of interest disclosed.

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### V322

**Influence of microRNAs expression in graft-versus-host disease after allogeneic transplantation**

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**Background:** MicroRNAs (miRNAs) are a family of 19–24 nucleotide non-coding RNAs, which affect the regulation of gene expression in eukaryotic cells by binding to a 3'-untranslated region which target messenger RNAs. MiRNAs play important roles in many cellular processes such as development, stem cell division, apoptosis and cancer. We profiled miRNAs expressed on patients who underwent allogeneic hematopoietic stem cell transplantation (HSCT).

**Methods:** Here we analyzed in a retrospective study 50 patients (pts) for miRNAs expression (miRNA-21; -128; -146; -155; and -181) in whole blood that underwent allogeneic HSCT and analyzed their outcome. MiRNA expressions were performed by miRNA-specific real time RT-PCR.

**Results:** In this cohort 15 pts received grafts from HLA-identical siblings (30%), 25 pts from matched (50%) and 10 pts from mismatched (20%) unrelated donors. Transplantat consisted of unmanipulated peripheral blood stem cells (n=41, 82%) or bone marrow (n=9, 18%). Of all pts, 12 (24%) had relapsed and 3 (6%) died of February 2012. There was no significant correlation between miRNA expressions from siblings and unrelated donors. For miRNA-21, we found a moderate down-regulation in mismatched unrelated donors (p<0.08) versus matched donors. Analysis of each miRNAs for relapse or TRM showed no statistically differences. Among these pts, 45 (90%) developed acute GVHD (18 pts had an acute GVHD of grade ≥2). There was a trend correlation between low level of miRNA-181 (327 ± 117% vs. 537 ± 575%; p<0.07) and miRNA-128 (158 ± 92% vs. 297 ± 485%, p<0.06) of pts with acute GVHD ≥ 2. In pts with severe acute GVHD (grade >3) comparing all other pts, we found significant reductions of miRNA-146 (3355% vs. 1526%, p<0.001), miRNA-21 (904% vs. 5526%, p<0.001) and miRNA-155 (5.66% vs. 3.52, p<0.04). However, no statistically differences were found in the miRNA expressions in regards to chronic GVHD.

**Conclusions:** These results suggest that pts with low expression of miRNA-146, miRNA-21 and miRNA-155 confirms a relevant association of the development of acute GVHD and miRNA profilings could be an early indicator of severe acute GVHD.

**Disclosure:** No conflict of interest disclosed.

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### V323

**Monitoring of T-cell receptor beta repertoire during allogeneic stem cell transplantation by next generation sequencing**

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Allogeneic hematopoietic stem cell transplantation (aHSCT) of peripheral blood stem cells after G-CSF mobilisation includes transplantation of large numbers of donor-derived mature T cells. We applied a multiplex polymerase chain reaction assay in combination with next generation sequencing (NGS, Illumina HiSeq 2000) to determine the composition of the donor T-cell repertoire before and after aHSCT based on the rearranged TCR beta genes and their complementarity-determining region 3 (CDR3). For this purpose, we used DNA extracted from donors before and after G-CSF mobilisation as well as DNA from the peripheral blood of patients after aHSCT. Blood samples of the patients were collected at defined time points after aHSCT and the CD4- and CD8-positive T cells were analysed separately after sorting to high purity by flow cytometry. Following intensive bioinformatics analysis of the NGS data, we found a very similar V- and J-segment usage in CD4- and CD8-positive T cells but the overall diversity of the TCR beta repertoire was higher in CD4- than in CD8-positive T cells. Interestingly, stem cell mobilisation by G-CSF induced only minor differences in the V- and J-segment usage. Most strikingly, our sequencing approach allows the tracking of the development of T-cell repertoire in the stem cell recipients on a single cell level. Thus, our data provide an extremely deep insight in the mechanisms of reconstitution of the immune system in patients undergoing aHSCT and will help to identify the T-cell clones responsible for the development of graft versus host disease.

**Disclosure:** No conflict of interest disclosed.

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### V324

**Functionally impaired GPI-anchor negative EBV-specific T cells after Alemtuzumab mediated T-cell depletion**


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**Introduction:** Alemtuzumab, a monoclonal anti-CD52-antibody, is frequently used to deplete T cells (TC) in the context of allogeneic hematopoietic stem cell transplantation (HSCT). We recently showed long-term persistence of CD52-negative TC in patients after Alemtuzumab mediated T-cell depletion (TCD). The lack of CD52 resulted from missing glycosyl-phosphatidyl-inositol (GPI)-anchors on the cell surface. These persisting GPI-anchor negative TC exhibit a reduced CMV-specific T-cell function. Since EBV-reactivation and EBV-associated diseases (e.g. post transplantation lymphoproliferative disorder, PTLD) are life-threatening complications after HSCT, we also investigated on EBV-specific T-cell function of GPI-anchor negative TC.

**Methods:** Patients were screened for EBV-reactivation with PCR. PBMC were frozen at different time points after HSCT. We analyzed patients with EBV-viremia (n=6), EBV-associated PTLD (n=4) as well as patients without EBV-reactivation (n=5). EBV-specific TC were identified by peptide/HLA-A2 Tetramer staining. We analyzed peptide-specific cytokine production by intracellular FACS and IFNgamma ELISPOT assay. EBV-peptide loaded dendritic cells, peptide loaded K562-cells as well as autologous EBV-blasts (LCI) were used as stimulators in the functional assays.

**Results:** EBV-specific TC were predominantly found in the GPI-anchor positive TC independent of whether the patients had EBV-reactivation or not. In patients with EBV-PTLD, no EBV-specific TC were found prior to the occurrence of the disease. Beyond that, in patients with progressive or persistent PTLD, EBV-specific TC were first detected after clinical recovery. In general, GPI-anchor negative TC showed a reduced EBV-specific cytokine-production in the functional assays.

**Conclusion:** We confirmed that GPI-anchor negative TC reconstituting after Alemtuzumab mediated TC-depleted HSCT show an impaired antiviral function against EBV. Our data support the hypothesis that these functionally impaired GPI-anchor negative TC, persisting years after HSCT, may be responsible for infectious complications in patients undergoing Alemtuzumab based conditioning regimen. We demonstrated earlier that application of donor lymphocyte infusions (DLI) replenishes the GPI-anchor positive TC-compartment. Therefore, we hypothesize that DLI could also improve immunologic control of virus associated complications in this setting.

**Disclosure:** No conflict of interest disclosed.

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Abstracts
Expression of and CD8+ T cell responses to Wilms' tumor gene 1 (WT1) in patients with AML or MDS after chemotherapy and allogeneic stem cell transplantation

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Introduction: Leukemic blasts overexpress immunogenic leukemia-associated antigens like Wilms' tumor gene 1 (WT1). Several epitopes of WT1 can be recognized by autologous CD8+ T cells. Expression of WT1 has been extensively studied in patients with AML and MDS, but only few information is available on the expression and immunogenicity of WT1 in the context of allogeneic stem cell transplantation.

Methods: In the present study, we analyzed the correlation between the clinical course of 55 patients suffering from AML/MDS with the expression of WT1 before and after treatment, either with allogeneic stem cell transplantation (SCT) preceded by chemotherapy or with chemohemotherapy alone. Transcripts were measured by quantitative real time PCR (RQ-RT-PCR) from RNA of peripheral blood mononuclear cell (PBMC) and bone marrow mononuclear cell (BMMC) samples. Furthermore, we determined the presence or absence of WT1-specific CD8+ T cells by tetramer staining (flow cytometry) and enzyme-linked immunospot (ELISPOT) assays and correlated them with the outcome of patients.

Results: After therapy, WT1 transcripts were either still elevated or reduced to normal, as considered as those expressed by healthy donors. Reduction to normal levels correlated with a longer survival (P<0.01). Increment of WT1 transcripts eventually resulted in a clinical relapse and subsequent death of the patients. In patients with a longer survival and continuous complete remission (CR) on stem cell transplantation, higher and enduring frequencies of WT1-specific CD8+ T cells than in patients developing a relapse were detectable after three to nine months after transplantation. These cells were effector T cells secreting interferon gamma and granzyme B.

Conclusion: WT1 is a suitable marker for the detection of minimal residual disease (MRD) after allogeneic SCT or chemotherapy. WT1-specific CD8+ T cells might contribute to the maintenance of a CR. Furthermore, specific T cell responses against WT1 can be elicited and these specific CTLs may be raised from cross-reactivity. In addition, an inflammatory and T cell stimulatory cytokine milieu might contribute to the favorable outcome of patients. Relapses predicted by RQ-RT-PCR for WT1 might be treated by immunotherapy approaches such as antigen-specific donor specific lymphocytes (DLIs) and peptide vaccination.

Disclosure: No conflict of interest disclosed.

Fortbildung
Multiples Myelom

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Recent results obtained from massive parallel sequencing of primary myeloma cells challenge our traditional view of myeloma. When treating a patient with myeloma, we are very likely facing a mixture of genetically and biologically distinct malignant plasma cell populations instead of a typical clonal disease derived from one malignant clone. This implicates to base antimalyeloma therapy on common biological features of malignant plasma cells and to involve agents that interfere with this biology at multiple levels, rather than targeting an individual protein or mutation. Key biological features for myeloma cell survival include the crucial balance between protein biosynthesis and destruction, which involves the unfolded protein response and proteasomal degrada-

tion, as well as the multiple interactions between myeloma cells and the bone marrow microenvironment. Indeed, the protease inhibitor bortezomib and the immunomodulatory drugs (IMiD) thalidomide and lenalidomide, that together have resulted in a remarkable improvement in the clinical outcomes of myeloma patients, have their key molecular targets in the regulation of protein homeostasis (bortezomib via direct proteasome inhibition, IMiDs via interfering with protein ubiquitination that precedes proteasomal degradation), and also modulate the interactions between myeloma cells and the microenvironment. We shall here review the current status of the ongoing efforts to further improve this treatment paradigm, focussing on the preclinical and clinical development of next generation proteosome inhibitors and IMiDs, as well as on other drug types that interfere with the protein homeostasis and/or the tumor environment of myeloma, such as inhibitors of deubiquitinating enzymes upstream of the proteasome, HDAC-inhibitors that prevent myeloma cells from bypassing the proteasome, and inhibitors of DKK-1 and BTK, examples for agents that target cytoskeleton or accessory cells in the microenvi-
ronment. Finally, rationally based combination strategies in this context and their current status of development will be discussed.

Disclosure: No conflict of interest disclosed.

Freie Vorträge
Akute myeloische Leukämie II (experimentell und klinisch)

Exome sequencing identifies a unique association of GATA2 zinc finger 1 mutations with biallelic CEBPA mutations in acute myeloid leukemia (AML)

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Introduction: Cyogenetically normal acute myeloid leukemia (CN-AML) with biallelic CEBPA gene mutations (biCEBPA) represents a distinct disease entity with a favorable clinical outcome. So far, it is not known if other genetic alterations cooperate with biCEBPA mutations during leukemogenesis.

Methods: To systematically identify somatic mutations, we performed whole exome sequencing of five biCEBPA patients using the Illumina Genome Analyzer IIx Platform. By comparing the exome sequence of diagnostic AML samples with the exome sequence of the corresponding remission samples we were able to identify leukemia-specific sequence variants.

Results: We detected somatic GATA2 zinc finger 1 (ZFY1) mutations in 2 out of 5 cases. GATA2 and CEBPA are interacting transcription factors crucial for hematopoietic development. Inherited or acquired mutations in both genes have been associated with leukemogenesis. Further mutational screening detected novel GATA2 ZFY1 mutations in 13 of 33 biCEBPA positive CN-AML patients (13/33; 39.4%). No GATA2 mutations were found in 38 CN-AML patients with a monallelic CEBPA mutation and in 89 CN-AML patients with wild-type CEBPA status. In Kaplan Meier survival analysis, the presence of
GATA2 mutations (n = 10) did not negatively impact on the favourable clinical outcome of biCEBPA patients (n = 26). Co-immunoprecipitation experiments confirmed that all GATA2 ZF1 mutants tested were still able to interact with CE BPA. In reporter gene assays, all tested GATA2 ZF1 mutants showed reduced capacity to enhance CE BPA-mediated activation of transcription. Gene expression profiling revealed that CE BPA target genes are differentially expressed in biCE BPA patients with additional GATA2 mutations. These findings suggest that the GATA2 ZF1 mutations may collaborate with biCEBPA mutations to deregulate target genes during malignant transformation. Thus, we provide evidence for a unique genetic subgroup of AML.

Conclusion: In contrast to the results of most other high throughput sequencing studies in AML, which have painted a picture of increasing genetic complexity, our results suggest that there are indeed striking associations of defined genetic lesions in subgroups of AML. The specific association of mutations affecting two interacting regulators of hematopoiesis introduces a novel concept for leukemogenesis. The simultaneous mutational targeting of two transcription factors that function in the same differentiation pathway in AML.

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Susanne Schnittger: Employment or Leadership Position: MLL Münchner Leukämielabor GmbH

V340
Patterns and prognostic impact of cytogenetic subclones in acute myeloid leukemia (AML)
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Introduction: Subclonal heterogeneity within tumors has been recognized as a driving mechanism of cancer development and progression, since genetic variability may create subclones with a proliferative advantage. In acute lymphoblastic leukemia (ALL) an ancestor clone often gives rise to several subclones with some of them being dead ends while others lead to further subclonal diversification. In this study, we addressed the subclonal architecture in acute myeloid leukemia (AML).

Patients and methods: This analysis included all patients enrolled in the two consecutive AML96 (n = 1862) and AML2003 (n = 1179) trials of the German SAL study group. Patients had a confirmed diagnosis of non-promyelocytic AML and were previously untreated. Conventional karyotyping of metaphases was performed prior to treatment start. To avoid bias ISSH and molecular diagnostic analyses were not considered.

Results: Subclones were detected in 420/2639 (16%) of all evaluable patients. When only abnormal karyotypes were included, the frequency was 420/1266 (33%). As for distinct cytogenetic aberrations according to the WHO classification, the frequencies of subclonal formation in karyotypes with t(8;21), inv(16), t(16;16), t(9;11), inv(3) and t(3;3) ranged from 29% to 39%. In karyotypes with two distinct subclones, a mother-daughter pattern prevailed, with the subclone harbouring additional cytogenetic aberrations. In karyotypes with multiple subclones a branched pattern predominated over a linear pattern. Overall, subclone formation emerged as an adverse prognostic factor, predicting an inferior overall survival (OS) (p = 0.001 in AML96; p = 0.15 in AML2003) as well as an inferior event free survival (EFS) (p = 0.001 and p = 0.02, respectively) among patients with abnormal non-core-binding factor karyotypes. Exclusion of patients receiving allogeneic stem cell transplantation (ASCT) increased the predictive power (p = 0.001 (OS and EFS) in AML96; p = 0.001 (OS), p = 0.002 (EFS) in AML2003). When only patients receiving ASCT were considered, subclones lost their prognostic impact (p = 0.12 (OS), p = 0.21 (EFS) in AML96; p = 0.44 (OS), p = 0.69 (EFS) in AML2003).

Conclusion: Patterns of clonal architecture in AML are similar to those observed in ALL. This finding corroborates the proposed analogy between tumor development and species evolution with its random genetic variability and subsequent selection of the fittest. Importantly, subclone formation is associated with an extremely poor prognosis in AML.

Disclosure: No conflict of interest disclosed.
Secondary acute myeloid leukemia in children


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Secondary acute myeloid leukemia (sAML) following a primary malignancy, are rare in children. Between 1993 and 2011 107 patients (n=50; m=37) with a sAML have been enrolled to the AML-BFM 93 (n=38), 98 (n=37) and 2004 (n=32) trials. Treatment recommendation included double induction and alloSCT in 1st CR. The median age at diagnosis was 11.2 years (2.9–26.5 y). By morphology, 53% of patients showed AML FAB M4/M5, 21% FAB M1/2, 7% FAB M0, 7% M6/M7 and 4% have not been classified. Genetic data are available from 67% of the patients. Most frequently, aberrations involving 1q/2q were found in 32% of the patients, followed by normal karyotype (18%), monosomy 7 and complex karyotype (6%).

Results: sAML most frequently occurred following systemic malignancies (46%) such as acute lymphoblastic leukemia (32%), non-Hodgkin-lymphoma (9%) and Hodgkin disease (3%). Other primary malignancies were neuroblastoma (11%), osteosarcoma (9%), brain-tumors (8%), Ewing’s sarcoma (7%), soft-tissue sarcoma (7%), germ cell tumors (5%), nephroblastoma (4%) and carcinoma (4%). Compared to the general incidence, brain-tumors, especially osteosarcoma (4% vs. 9%), germ cell tumors (1% vs. 5%) and neuroblastoma (4 vs. 11%) seem to be overrepresented. The median interval between primary malignancy and sAML was 2.9 yrs (0.13 to 22.4 yrs). Compared to the total group the interval was significantly shorter in osteoand Ewing’s sarcoma (1.8 yrs, 0.8 to 6 years) and longer in neuro-/nephroblastoma (3.3 yrs, 1.4 to 22.3 yrs) or brain-tumors (3.8 yrs, 0.6 to 10.7 years).

Unfortunately, sAML showed a high rate of early death (n=17; 16%) and non-response (n=37; 42%) to induction. CR and partial response (PR), defined as “no evidence of leukemia blasts”, were achieved by n=42 (39%) and n=11 (10%), respectively. The 5-year overall survival (OS) improved from study AML-BFM 93, 98 to 2004 from 84%, 30% to 44% at 2004: p log rank<0.009. Allogeneic SCT have been performed in 43% of the patients either in 1st CR (29%) or following PB/RN (14%). Whereas children with SCT in 1st CR or PR had an OS of 55%/49% (AML-BFM 2004 69%/44%; AML-BFM 98 44%/13%) and 53% at 17 years, respectively, none of the patients transplanted with NR survived (median survival 6 months). In summary, sAML is still associated with a poor prognosis, mainly due to early death and non-response. If remission could be achieved, alloSCT allows long-term survival in 55% of the children.

Disclosure: No conflict of interest disclosed.

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Allogeneic transplantation in the elderly with AML/MDS: feasible and successful

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Introduction: AML & MDS are diseases of the elderly and because of the increasing age of our population more and more fit patients are presenting with standard chemotherapy incurable malignancies. Using toxicity reduced conditioning regimens it is possible to offer this patient group an allogeneic transplantation (alloTX). Efficacy and impact of influencing factors have to be evaluated.

Methods: Overall, 201 pts. (median age 66 y (range 60–76.6)) received their first alloTX after fludarabine-based conditioning regimens with a graft (PBSC in 96.5 %) from a sibling (29%) or UD (71%; mismatch 15%). Diagnosis were de novo AML (37%), sAML (37%), tAML (5%), MDS (16%) and MDS (5%). Remission at TX was mainly advanced disease stage >CR1/2 (88%), also reflected by the fact that 54% had persistent induction failure or relapse. Additionally 32% were untreated. Cyclosporin based GVHD prophylaxis (in 99%) of pts. was maintained by Campath (66%), MMF (7%) and ATG – F (24%).

Results: Leucocyte-engraftment was observed in median at day +13 (range 8–25) and platelets ≥ 20.000 at day +11 (7–113). Two primary graft failure occurred, both were salvaged by a second donation of the donor. In the 26 30 diagnostic procedures 94% CR (in 4% not applicable) were seen; acute GVHD II–IV occurred in 27% and III–IV in 13% as well or non (66%) and extensive (23%) cGVHD. Alive are 37 % at a median of 1250 days (105–3689); main cause of death was relapse/progression (25%). The NRM was 38%; main causes were: infection (25%), aGVHD w or w/o infection (3.5%) and cGVHD w or w/o infection (3%). In univariate analysis no significant differences for OS and PFS could be detected for Soror Index, marrow blasts at TX, graft, donor (SIB vs UD), diagnosis, different cytogenetics, graft analysis (concerning CD 34 and CD 3 count) and time depending cGVHD.

Conclusion: In this large cohort of consecutive elderly AML/MDS patients we show that alloTX is effective and feasible, independent of remission at TX and age per se.

Disclosure: No conflict of interest disclosed.

V343

Decitabine alone or in combination with ATRA: results of a phase II trial in 227 older medically non-fit AML patients, and a randomized, 4-arm follow-up study (DECIDER trial)

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Acute myeloid leukemia (AML) of older, medically non-fit patients still poses a highly unmet clinical need, and only few large prospective studies have been performed in this indication. With the established activity of hypomethylating agents such as Decitabine (DAC) in MDS and AML with 20–30% bone marrow blasts, we asked whether this drug is also active in patients with >30% blasts. To evaluate efficacy and toxicity of DAC in untreated AML patients >60 years ineligible for induction chemotherapy, 227 patients (median age 72 years), many with comorbidities, adverse cytogenetics and/or preceding MDS were

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Abstracts

V344 Screening for toxicity in elderly cancer patients – is there a clinical need?

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The demographic changes and the age dependent increase in the incidence rates of malignant tumours, will result in a substantial increase in the number of old cancer patients within the coming decades.

Increased toxicity is one of the main reasons to be uncertain about the adequate treatment of old cancer patients and not to treat old cancer patients with the same treatment as younger ones.

Recent studies addressed the questions, which factors are associated with increased toxicity in elderly cancer patients. Their results will be presented in detail. Some of these factors are belong to the Comprehensive Geriatric Assessment (CGA), which should be part of a structured diagnosis and treatment of elderly cancer patients.

However the question which threshold of toxicity will results in a change of treatment decision has not yet been addressed. Besides risk of toxicity, aim of treatment and attitude of patients are important part of clinical decision making.

In conclusion, CGA helps to identify elderly cancer patients at increased risk of toxicity. However alternative treatment strategies have to be validated in clinical trials.

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V345 Cachexia and nutrition in the elderly cancer patient

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Elderly are at risk for malnutrition and cachexia with increasing age and this will aggravate as cancer patients. Malnutrition is associated with more changes in the planned therapy and unfavorable outcome. In a geriatric assessment a nutrition score looking especially for malnutrition should be included. If we associate the classification of the elderly in Go-Go’s, Slow-Go’s and No-Go’s with the risk of malnutrition, the first group will mainly be well nourished, the second group is at risk and the last group will mainly be malnourished. What is the aim of nutrition in this patient groups? To avoid further weight decline and to support quality of life. This is very difficult if the patient is not receiving antitumor therapy as well to reduce the “inflammation”, the main promoter of cachexia. Patients should receive nutrition counselling, if they are malnourished, at risk and if they are not eating enough for > 5–7 days. Diet should consist of many small meals of tasty and balanced foods. If they cannot eat enough supplements, enteral or parenteral nutrition should be added. Important is to use a nutrition assessment, as the Mini Nutritional Assessment to identify patients at risk. The known BMI should not be used, because elderly are shrinking and therefore the BMI is increasing, despite weight loss. Further parameters influencing the oral intake are reduced appetite, taste, thirst and depression, infections, a bad dental record and not fitting dental prosthesis. Especially protein deficiency and the consecutive muscle loss are of concern in geriatric patients called starvation and sarcopenia. Expected daily kaloric intake is less than in younger cancer patients with 20 kcal/kg body weight. Further a higher protein intake is appreciated e.g. 1.2 gram m/kg b.w. and depending on the kidney function. Fluid intake should be 20 ml/kg b.w. and more as well depending on therapy side effects as diarrhoe, emesis, kidney function, fever and B-symptoms. Higher nutrition intake is less effective without increasing physical activity. Therefore all patients should be enforced to increase their daily activity with muscle training.

Conclusion: Malnutrition and cachexia are important problems in geriatric oncology and for patients’ outcome, but it should be recognized. Every elderly should get a nutrition assessment and if necessary nutrition counselling. Foods with high protein content and physical activity, additionally, should be combined for therapy.


V346 Update: Current clinical trails in elderly patients for multiple myeloma and myelodysplastic syndrome

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Multiple Myeloma (MM) and myelodysplastic syndrome (MDS) are bone marrow disorders affecting mainly elderly patients with a median age at diagnosis of 70 and 71 years, respectively. For both diseases, emerging treatment concepts have substantially improved prognosis, however this was first mainly translated into the younger patient group by introducing autologous (MM) and allogeneic (MDS) transplantation strategies. So far, the elderly patient group remained underrepresented in clinical trials. However, most recently, upcoming new drugs allowed substantial changes in treatment of both diseases also for the elderly patient group, resulting in improved progression free and overall survival. Treatment duration and maintenance treatment are important issues under investigation and discussion in both diseases. For MM addition of the so called new therapeutic drugs thalidomide, bortezomib and lenalidomide to conventional dose melphalan and prednisone showed improved response rates and prognosis in large clinical phase III trials. Future concepts will include a new generation of myeloma specific drugs such as antibodies (e.g. elotuzumab) and next generation proteasome inhibitors (e.g. carfilzomib) with suasive early results. For MDS, introduction of the epigenetic drug azacitidine lead to a substantial benefit for patients with intermediate-2 or high-risk disease independent of age and comorbidities as shown in several recent trials. Supportive care remains a key issue in both diseases and was also recently evaluated. In this session, most important results of recent studies regarding standard care and future concepts will be discussed.

Disclosure: Katja Weisel: Advisory Role: Janssen, Celgene; Financing of Scientific Research: Janssen, Celgene; Expert Testimony: Celgene

96 Onkologie 2012;35(suppl 6):1–259 Abstracts
Pharmacotherapy in elderly cancer patients – pitfalls and practical aspects

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Aging is a highly individualized process, leading at different pace to decline and sometimes ultimately exhaustion of physical and mental resources. Older age is associated with higher rates of comorbidity, often resulting in polypharmacy. In analyses and registries, the mean number of drugs of German patients > 70 years is 3–4, excluding “over the counter” drugs, potentially interfering with drugs used for the treatment of cancer. Furthermore, a number of physical changes occurring in aging individuals can have a profound effect on pharmacokinetics and pharmacodynamics. While body fat generally increases, body water diminishes, leading to altered distribution of lipophic drugs. Elderly patients are often at risk of hypoaalbuminemia, a fact that should be acknowledged when drugs showing high protein binding are used. The most significant change form the pharmacological point of view, however, is the gradual decrease in renal function, even in healthy elderly persons. This can, on the one hand, preclude the use of nephrotoxic drugs such as cisplatin (that in many cases can be substituted for carboplatin), and can, on the other hand, lead to accumulation of renally eliminated drugs. Already in 2007, the International Society of Geriatric Oncology, SIGOG, published recommendations for the adjustment of dosing in elderly cancer patients with renal insufficiency. The seemingly higher toxicity of the oral compound capetitabine in aged patients, for example, is in fact an indirect effect of decreased renal function, and not a direct consequence of age. In line with this, many analyses have failed to demonstrate an effect of age itself on pharmacokinetics. Undoubtful, however, are changes in pharmacodynamics, e.g. a more profound effect of many cytotoxic agents on bone marrow function in elderly patients. Physicians must be aware of this elevated risk of hematologic toxicity, and consequently either have a lower threshold for the use of G-CSF, especially in the curative setting, or should consider upfront dose reductions, in particular in medically non fit patients. Recently, for example, the British FOCUS2 trial could demonstrate that starting at 90% of the usual dose in elderly patients with metastatic colorectal cancer with the option to increase the dosage to the normal dose in the absence of severe toxicity is both a safe and effective approach in metastatic colorectal cancer.


Freie Vorträge
Chronische lymphatische Leukämie I

V348
Abundance of a NOTCH1 mutated clone affects clinical outcome of patients with chronic lymphocytic leukemia

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Introduction: The transmembrane receptor NOTCH1 operates as a ligand-activated transcription factor controlling developmental processes, proliferation and apoptosis. In the context of cancer, activating NOTCH1 mutations are the most frequent oncogenic events in T-cell acute lymphoblastic leukemia and have recently also been implicated in chronic lymphocytic leukemia (CLL). The prevailing CLL NOTCH1 mutation is a deletion of a CT dinucleotide (N1ΔCT) leading to a truncation of the NOTCH1 protein (p.P2515Rfs*4). Here, we studied this mutation in our cohort of CLL patients.

Methods: We assayed for the presence of the N1ΔCT mutation by using newly established restriction fragment length polymorphism analysis (RFLP) and amplification refractory mutation system PCR (ARMS) methodology. Presence of the N1ΔCT mutation detected by RFLP and ARMS was confirmed by conventional Sanger sequencing. Longitudinal abundance of the N1ΔCT mutated CLL clone was semiquantitatively assessed by RFLP and ARMS assays.

Results: Using RFLP analysis we were able to detect the N1ΔCT mutation in 4.5% (n=11/247) of CLL patients. These N1ΔCT RFLP positive patients showed significantly shorter treatment-free survival (TFS; p=0.01) and overall survival (OS; p=0.01). Interestingly, the more sensitive ARMS method revealed an additional n=15 patients with a N1ΔCT mutated CLL subclone resulting in a total mutation rate of 10.5% (n=26/247). TFS and OS of these solely N1ΔCT ARMS positive patients with a presumably lower mutated clonal abundance were not significantly altered. Longitudinal semiquantitative analysis of the clonal N1ΔCT abundance was performed on a subset of n=13 patients and revealed an increasing clonal size in all cases (Fig. 1). However, we did not observe a N1ΔCT ARMS positive patient turning positive by RFLP analysis during longitudinal follow up.

Conclusions: Our results show that high abundance of a N1ΔCT mutated CLL clone correlates with a more aggressive disease course. In addition, our data imply that in a subgroup of CLL patients the N1ΔCT mutation remains sub-clonal and does not contribute to disease progression.

Disclosure: No conflict of interest disclosed.

V349
Allogeneic stem cell transplantation (alloSCT) may improve the natural course of poor-risk chronic lymphocytic leukemia (CLL) as defined by the EBMT consensus criteria:
Update of a retrospective donor vs no donor comparison

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Objective: of this retrospective study was to study if alloSCT can improve the dismal natural course of poor-risk CLL by assessing its impact in an intent-to-treat donor-vs-no-donor comparison.

Methods: In a single centre analysis, course and outcome of all patients with CLL referred for evaluation of alloSCT indication between June 2005 and July 2011 were recorded. Indication was either one of the three EBMT consensus criteria or Richter’s transformation. Primary endpoint was overall survival (OS) of those patients for whom a 9/10 or 10/10 matched donor could be found within 3 months compared with that of patients without donor, measured from the 3-month landmark after donor search initiation.

Results: Altogether 119 patients were referred in the six year time period. Excluding 2 patients who turned out to have a mantle cell lymphoma, an indication for donor search was seen in 98 patients (84%). Of the 98 patients with indication, for 7 patients the donor search was not initiated. A donor could be identified within 3 months for 69 of the remaining 91 patients (76%). Of these, 17 patients (25%) did not proceed to transplant. With a median observation time of 24 (3–77) months, OS at 2 years from start of search was 70% for the patients who had a search indication (96 without lost to follow-up) opposed to 92% for the patients without indication (12 without lost to follow-up) (p=0.12). There was no impact of type of EBMT criterion and age on the outcome of patients with search indication. Although patients with and without a donor were well balanced for CLL-specific prognostic factors, OS at 2 years from the 3-month landmark after start of search was significantly better in patients with a donor than in those without (82% vs 56%; p=0.0088).

Conclusions: Survival of patients with poor-risk CLL and alloSCT indication for whom a donor cannot be found is significantly inferior compared to patients with a donor. This observation provides first comparative evidence that alloSCT indeed may have the potential to improve the natural course of poor-risk CLL.

Disclosure: No conflict of interest disclosed.
V350
Patients with chronic lymphocytic leukemia (CLL) and an early relapse do not benefit from treatment with (R-)CHOP or other chemo(immuno)therapies that contain three or more cytotoxic agents and/or an anthracycline – a metaanalysis of the German CLL Study Group (GCLLSG)

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Introduction: Despite an improvement of treatment outcomes in patients with CLL, almost all patients (pts) will eventually relapse. According to ESMO-guidelines, repetition of 1st-line therapy is a reasonable approach in case of a relapse ≥ 24 months after initial treatment [Eichhorst et al., Ann Oncol., 2011], but little is known which therapy to choose in case of an earlier relapse.

Methods: Among 1558 pts enrolled in 5 prospective trials of the GCLLSG, 315 pts received 2nd-line treatment within 24 months. Ten pts receiving relapse therapies that contain ≥ 3 cytotoxic agents and/or an anthracyclines (e.g. CHOPR, CHOP or FCM) have an impaired OS in comparison to standard-chemo-immunotherapies or single-agent alemtuzumab. However, the poor outcome of early relapsing pts underscores the need for alternative treatment approaches with either allogeneic stem cell transplantation or novel drugs.

Results: 1st- and 2nd-line therapies of 315 pts with an early relapse are depicted in figure 1. 2nd-line therapies were heterogeneous. Most common therapies were combination of cyclophosphamide, doxorubicine, vincristine, and prednisolone either with rituximab (CHOPR, n = 32) or without (CHOP, n = 24) and alemtuzumab (n = 27). Treatment regimens were summarized to 3 different groups: therapies containing an antibody with/without <3 cytotoxic agents, single-agent chemotherapies, and therapies containing anthracyclines and/or ≥ 3 cytotoxic agents. Treatment free survival for all three groups was 24.5, 18.7 and 16.4 months (p = 0.009) and overall survival was 78.3, 58.2, and 42.0 months (p = 0.012, see figure 2).

Aside from a higher median age in the single-agent chemotherapy group no differences in other baseline-characteristics were found.

Conclusion: Pts with a relapse <24 months who receive therapies that contain ≥ 3 cytotoxic agents and/or anthracyclines (e.g. CHOPR, CHOP or FCM) have an impaired OS in comparison to standard-chemo-immunotherapies or single-agent alemtuzumab. However, the poor outcome of early relapsing pts underscores the need for alternative treatment approaches with either allogeneic stem cell transplantation or novel drugs.

Disclosure: Paula Cramer: Other Financial Relationships: Travel Grants by Roche and Mundipharma

Barbara Eichhorst: Advisory Role: Gilead and Pharmacies; Financing of Scientific Research: Roche and Mundipharma; Expert Testimony: Roche and Mundipharma; Other Financial Relationships: Travel Grants by Roche and Mundipharma.

Fig. 1. TFS and OS for 3 different treatment groups (for abstract V350).
### V351

#### Chemoimmunotherapies prolong overall survival in patients with chronic lymphocytic leukemia irrespective of time point of administration – results of a metaanalysis of the German CLL Study Group (GCLLSG)

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**Introduction:** Repetition of first-line therapy is a reasonable approach in patients with chronic lymphocytic leukemia (CLL) if relapse occurs ≥ 24 months. This recommendation of ESMO guidelines has not yet been confirmed by clinical trials. Furthermore the most beneficial composition and sequence of regimens regarding treatment free (TFS) and overall survival (OS) remain unclear.

**Methods:** From 1659 consecutive patients included in five different study protocols of the GCLLSG designed for first-line and relapse therapy we selected 1558 patients who received at least one therapeutic regimen. 101 patients had to be excluded never having received treatment in one of the trials. 704 patients received at least one relapse regimen. For statistical analysis Kaplan-Meier estimators and curves were used including log-rank tests.

**Results:** Patients were stratified according to their first-line treatment. Comparing the different regimens from the first study generation (CLL4 trial) fludarabine (F) vs. fludarabine + cyclophosphamide (FC); CLL5 trial: F vs. chlorambucil (Cib) to regimens from the second generation (CLL6 trial: FC vs. FC + rituximab; CLL2M: bendamustine + rituximab; CLL2L: FC + alemtuzumab) TFS and OS steadily increase along with the advances in CLL research. Focusing on the impact of different treatment regimens irrespective of the time point of their administration, patients who received an antibody-based regimen at least once (n=909) had a significantly longer OS than those who had never been treated with an antibody (OS after 60 mos.: 75.7% vs. 64.1%, p<0.006). This was independent from the time point of administration (first-line or relapse). 202 patients received combination therapy with cyclophosphamide, doxorubicin, vincristine and prednisone (CHOP) with or without CD20-antibody. However, OS was significantly shorter in patients treated with any CHOP-containing regimen (p<0.0001) compared to those never having been treated with such a regimen. However, this observation might reflect a bias due to the selection of high risk patients. No differences in survival were observed in patients receiving a mitoxantrone-containing regimen in comparison to those never having received anthracyclines.

**Conclusions:** Advances in the development of strategies for first-line therapies result in prolongation of both treatment free and overall survival in patients with CLL. Chemoimmunotherapies prolong survival irrespective of the time point of administration.

**Disclosure:** Susanne Isfort: No conflict of interest disclosed.

**Michael Hallek:** Advisory Role: Gilead and Pharmacyclics; Financing of Scientific Research: Roche und Mundipharma; Expert Testimony: Roche und Mundipharma; Other Financial Relationships: Reisekosten: Roche und Mundipharma.

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**V352**

**Evaluation of free light-chain abnormalities as prognostic marker in Binet A stage chronic lymphocytic leukemia**

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**Introduction:** In recent studies, abnormalities of serum free light chains (FLC) were suggested as novel marker with independent prognostic value in B-cell chronic lymphocytic leukemia (CLL). We utilized the large, multicenter CLL1 study, to determine the prognostic value of FLC abnormalities in early stage CLL.

**Methods:** The randomized phase III CLL1 trial was conducted to assess if use of fludarabine prolongs progression free survival (PFS) in early stage CLL pts with a high risk (HR) profile. HR was defined based on thymidine kinase (TK), beta-2-microglobulin (B2MG), lymphocyte doubling time, and bone marrow infiltration pattern. FLC and λ were measured in serum samples of a representative subset (n=169, 27%) of the CLL1 study cohort comprising pts in Binet A stage randomized to the watch&wait strategy (both low and high risk profile as defined by the CLL1 study protocol). Published reference ranges were used to classify pts in 4 distinct subgroups: normal FLC, abnormal FLC scλ ratio (FLCR) with and without absolute FLC elevation (monoclonal and ratio-only, respectively), and normal FLCR with absolute FLC elevation (polyclonal). PFS and overall survival (OS) were measured as time from study entry to occurrence of progression or death from any cause, respectively.

**Results:** Median age of the 169 pts at study entry was 61 years (range 35–75), whereof 97 (57%) were male, and 24 (14%) were HR according to the CLL1 study protocol. Median FLC κ and λ were 12.2 mg/L (range 2.1–59.3) and 12.1 mg/L (range 2.0–142.0), respectively. Normal FLC were observed in 123 pts (73%), while in 26 (15%) an absolute FLC elevation (κ or λ) was present. FLCR was abnormal in 32 (19%) pts. Subgroups of FLC abnormalities included: 14 (8%) monoclonal, 18 (11%) ratio-only, 14 (8%) polyclonal. FLC abnormalities were significantly associated with IgVH status and CLL1 high risk profile and FLC levels were significantly correlated with B2MG (summed FLC κ and λ, Spearman r=0.38, p<0.0001). Progression was observed in 84 pts (50%), 21 (12%) died. In univariate analysis, FLC ratio-only and monoclonal changes were significantly associated with PFS (hazard ratio 2.40, 95%-CI 1.33–4.34 and 2.22, 95%-CI 1.13–4.37, respectively). No significant association was found in multivariable analysis.

**Conclusions:** In line with previous reports, FLC abnormalities were associated with established prognostic markers. However, we did not observe an independent prognostic value with regard to PFS or OS.

**Disclosure:** No conflict of interest disclosed.
Progranulin plasma concentration is a novel independent prognostic marker in chronic lymphocytic leukemia

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Introduction: Progranulin (Pgrn) is a 88 kDa secreted protein with pleiotropic functions including regulation of cell cycle progression, cell motility, wound repair and tumorigenesis. Using microarray based gene expression profiling we have recently demonstrated that GRN is significantly higher expressed in CD38+ZAP-70+ as compared to CD38-ZAP-70- CLL cells.

Methods: We measured Pgrn plasma concentrations by enzyme-linked immunosorbent assay (ELISA) in the Essen CLL cohort of 131 patients (pts) and examined Pgrn for association with established prognostic markers, time from diagnosis to first treatment (TTFT) and overall survival (OS). Employing the nosorbent assay (ELISA) in the Essen CLL cohort of 131 patients (pts) and of DNMT3A are frequently found in AML and are associated with poor prognosis of malignant hematological diseases like AML. DNA methyltransferases (DNMTs) catalyze the transfer of a methyl group from S-adenosylmethionine to cytosine residues in DNA to form 5-methylcytosine. DNA hypermethylation is involved in the development of many tumor or normal stem cells, giving rise to embryonic stem (ES) cells or differentiated cells. To determine the repopulation capacity of the leukemic cells we isolated the leukemic cells and transplanted GFP-positive primary leukemia cells into sublethally irradiated secondary wildtype recipients. Leukemia of both, wildtype and DNMT3B-overexpressing donors was transplantable and lethal. However, DNMT3B0 leukemia cells were impaired in leukemia development in secondary recipients. Secondary recipients of leukemic DNMT3B0 cells died significantly later (p=0.002). Taken together, these findings demonstrate that DNMT3B expression impairs leukemia maintenance. Loss of DNMT activity might contribute to the pool size of leukemia initiating cells.

Disclosure: No conflict of interest disclosed.

Freie Vorträge Stammzellen II (experimentell)

V354 DNMT3B overexpression is associated with prolonged survival in myeloid leukemic mouse model

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DNA hypermethylation is involved in the development of many tumor or malignant hematological diseases like AML. DNA methyltransferases (DNMT) play an important role in regulation of DNA methylation. Mutations of DNMT3A are frequently found in AML and are associated with poor prognosis. Here, we report the effects of DNMT3B overexpression on leukemogenesis using a Tetraclivin-inducible DNMT3B mouse model. In this mouse model, treatment with doxycycline suppressed DNMT3B expression whereas absence of doxycycline led to overexpression of DNMT3B on the mRNA and protein level. DNMT3B overexpression was not toxic since hematopoietic progenitor colony formation in vitro did not differ between DNMT3B expressing and physiologically expressing cells. To analyse the impact of DNMT3B overexpression on leukemogenesis, we retrovirally co-transduced lineage-negative bone marrow cells of wildtype and DNMT3B mice with a MSCV cMyc-bcl2 and a MSCV-IATA-GFP containing vector. 5 x 105 sorted GFP-positive cells were transplanted into sublethally irradiated wildtype recipients. Both recipients of transduced wildtype cells and recipients of transduced DNMT3B0 cells developed leukemia with a tendency of delayed leukemogenesis in DNMT3B overexpressing mice. GFP positive leukemia cells were sorted and doxycycline regulated DNMT3B expression was verified by Western blot analysis in vitro.

Reduced representation bisulphate sequencing (RRBS) confirmed widespread DNA hypomethylation on leukemias arising in the presence of the DNMT3B transgene. To determine the repopulation capacity of the leukemic cells we isolated the leukemic cells and transplanted GFP-positive primary leukemia cells into sublethally irradiated secondary wildtype recipients. Leukemia of both, wildtype and DNMT3B-overexpressing donors was transplantable and lethal. However, DNMT3B0 leukemia cells were impaired in leukemia development in secondary recipients. Secondary recipients of leukemic DNMT3B0 cells died significantly later (p=0.002). Taken together, these findings demonstrate that DNMT3B expression impairs leukemia maintenance. Loss of DNMT activity might contribute to the pool size of leukemia initiating cells.

Disclosure: No conflict of interest disclosed.
Abstracts

V356 Role of protein kinase C (PKC) in homologous and heterologous desensitization of G protein-coupled receptors in hematopoietic stem and progenitor cells

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Introduction: Hematopoietic stem and progenitor cells (HPC) express a variety of G protein-coupled receptors (GPCR), which all could play important roles in HPC trafficking. Regulation of differential expression and function of GPCR is largely unknown. We analyzed crosstalk of CXCR4 and CysLT1, the two GPCRs with the strongest cellular responses in HPC, and characterized the underlying signal transduction pathways.

Methods: Human G-CSF-mobilized CD34+ HPC were isolated from the peripheral blood. Calcium fluxes and actin polymerization in response to receptor activation were measured using a fluorescent calcium indicator and phalloidin-FITC. Phosphorylation of kinesins involved in signal transduction was assessed by Western blot.

Results: Activation of CXCR4 as well as CysLT1 resulted in homologous desensitization as measured by absence of calcium fluxes and actin polymerization after repeated stimulation. Heterologous cross-desensitization of CXCR4 by antecedent activation of CysLT1 and vice versa of CysLT1 by activation of CXCR4 was not observed. In contrast, heterologous desensitization of CysLT1-mediated actin polymerization was induced by antecedent activation of CXCR4, but not vice versa. This observation correlated with the inhibitory effect of protein kinase C (PKC) blocking with BisI and PKCeta pseudosubstrate, which was only observed by analyzing actin polymerization in response to CXCR4, but not to CysLT1 activation. Calcium fluxes mediated by either CXCR4 or CysLT1 were even increased by PKC blocking, indicating that the process of receptor internalization itself was also PKC-dependent. Interestingly, Pyk2 phosphorylation was abrogated by pertussis toxin (Gi protein inhibitor) only after activation of CXCR4, but not CysLT1, revealing a differential involvement of G proteins as well.

Conclusions: In CD34+ HPC, homologous desensitization of CXCR4 and CysLT1, which is required for rapid termination of receptor activation, is due to PKC-dependent receptor internalisation. Heterologous desensitization of CysLT1 after antecedent activation of CXCR4 is restricted to actin polymerization and also mediated by PKC signaling. As signalling of CXCR4 is not influenced by antecedent CysLT1 activation, and actin polymerization represents a prerequisite for cell migration, a hierarchy exists in the control of HPC migration by different GPCR, with a dominant role for CXCR4, which confirms the particular role of CXCR4 in stem cell trafficking.

Disclosure: No conflict of interest disclosed.

V357 Cellular aging during culture expansion of Mesenchymal Stromal Cells – A tightly regulated epigenetic process

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Introduction: Cells in culture undergo continuous changes which have to be taken into account for cellular therapy. We have recently demonstrated that long-term culture of mesenchymal stromal cells (MSC) is associated with specific senescence-associated DNA methylation (SA-DNAm) changes. These epigenetic modifications are highly consistent and can even be used to track cellular aging during culture expansion (Koch et al., Aging Cell 2012;11:366–9). On the other hand, pluripotent stem cells evade replicative senescence and the molecular mechanisms for this phenomenon are still unknown.

Methods: Here, we investigate SA-DNAm changes in MSC upon long-term culture, irradiation-induced senescence, immortalization and reprogramming into induced pluripotent stem cells (iPSC). DNAm profiles were analyzed with a novel high density DNA-methylation array covering more than 450,000 CpG sites.

Results: SA-DNAm changes are highly reproducible and occur particularly in intergenic and non-promoter regions of developmental genes. We demonstrate that ionizing irradiation, although associated with a very similar senescence phenotype, does not affect SA-DNAm. Furthermore, overexpression of the catalytic subunit of the human telomerase (hTERT) results in telomere extension but does not influence SA-DNAm. In contrast, we demonstrate that reprogramming into iPSC reverses almost the entire set of SA-DNAm changes.

Conclusions: Our results indicate that replicative senescence is associated with an epigenetically controlled process which stalls cells in a particular functional state, whereas irradiation-induced senescence and immortalization are not causally related to this process. The specific erasure of SA-DNAm upon reprogramming into iPSC may play a central role for rejuvenation and escape from replicative senescence.

Disclosure: No conflict of interest disclosed.
amount was sufficient for microarray analysis. Gene CHP arrays are ongoing and will be presented.

Disclosure: No conflict of interest disclosed.

V359 Immune injury by allogeneic CD4+ T cells leads to host hematopoietic stem cell dormancy and prevents engraftment of donor cells

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Introduction: The susceptibility of bone marrow (BM) to injury by T cells is long recognized, e.g., in aplastic anemia, and the inhibitory effect of IFNg on the proliferative activity of hematopoietic stem cells (HSC) is established. How donor T cells given within allogeneic hematopoietic cell transplantation (HCT) affect the recipient BM microenvironment and influence HSC activity has not been elucidated.

Methods: We examined the effect of graft T-cell subsets on blood reconstitution and engraftment of donor HSC in MHC-matched, minor-antigen-mismatched mouse models of non-myeloablative HCT. BALB.K (H2k) and BALB.B (H2b) mice were prepared with sublethal radiation at a dose that allows donor cells (AKR/J [H2k] and C57BL6 [H2k]) to engraft but also permits survival of host HSC and immune populations.

Results: Unexpectedly, donor T cells did not generally improve engraftment. In fact, donor CD4+ cells, even at low doses, initiated a chain of immunogenic events causing severe BM aplasia. Subsequent recovery of blood formation, particularly B lymphopoiesis, was markedly delayed despite no clinical signs of graft-vs-host disease. Specifically, CD4+CD25 cells interacted with immune-competent host cells, stimulating dendritic cells to produce excessive amounts of IL-12. IL-12 in turn, activated donor CD4+ cells to produce IFNg. This Th1-response was confined to the BM but absent in the spleen. In this cytokine-rich BM microenvironment host hematopoiesis was arrested at the stage of short-term HSC, while there was a lack of more mature multipotent progenitors, indicating that differentiation to replenish the blood pool was prevented. Cell cycle analysis revealed that HSC were at a resting stage. Quiescence of host HSC resulted in detainment in their niches and consequent failure of donor HSC to engraft. Our findings explain why recipients of HSC alone engrafted, while those given HSC+CD4+ cells failed to establish donor chimerism.

Conclusion: We postulate that HSC respond to certain inflammatory conditions by enhanced dormancy, which results in preservation of HSC in their niche. Our model corroborates the importance of IFNg in marrow aplasia and further shows that IFNg can be a regulator of hematopoiesis post-HCT. In the setting of reduced-intensity HCT, inflammation caused by interactions between donor T cells and residual host cells can result in persistent occupation of host HSC in their marrow-niche and consecutive failure of donor HSC to engraft.

Disclosure: No conflict of interest disclosed.

Fortbildung

Globulomaste / Tumour des Nervensystems

V381 Globulomaste: Standards of care and novel therapies

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Globulomastas are the most common and highly malignant primary brain tumors of unknown cellular origin. Median survival in population-based studies is still less than one year. Cytoreductive surgery, focal radiotherapy, and concomitant and adjuvant alkylating agent chemotherapy using temozolomide are standard of care for favourable prognosis patients. In general, gliblastomas can be classified by morphology, imaging parameters, clinical character-istics, single biomarkers or complex molecular signatures. Age and Karnofsky performance score provide important prognostic information. In contrast, neither histological subtyping of gliblastoma nor specific imaging features have assumed prognostic relevance. Among multiple candidate biomarkers, only promoter methylation of the O6-methylguanine methyltransferase (MGMT) gene, helps for clinical decision making. Patients with gliblastomas with MGMT promoter methylation derive more benefit from alkylating agent chemotherapy. In the growing population of elderly patients with gliblastoma, combination radiochemotherapy has not been shown to be superior to monotherapy and may be less well tolerated than either radiotherapy or chemotherapy alone. Here, MGMT promoter methylation may assume its major role as a predictive biomarker. Registration trials for two anti-angiogenic compounds, bevacizumab and cilengitide, have completed enrolment. Biomarkers for benefit from anti-vascular endothelial growth factor therapies have not been introduced into the clinic. Positron emission tomography for the detection of a,b integrins might be used to select patients for treatment with anti-integrins such as cilengitide. Screening for a specific type of epidermal growth factor receptor mutation, EGFRVIII, is explored as a biomarker for selecting patients for vaccination in two randomized clinical trials. Despite extensive efforts at defining biological markers as a basis for selecting therapies, most treatment decisions for gliblastoma patients are still based on age and performance status today. However, several completed or ongoing clinical trials may enrich the repertoire of criteria for clinical decision making and selection of therapies in the near future.


Fortbildung

Sarkome

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Osteosarcoma and Ewing sarcoma most commonly arises during adolescence, but can affect all ages and around one third of patients are adults. Treatment consists of both local therapy (surgery for osteosarcoma, surgery and/or radiotherapy for Ewing sarcoma) and chemotherapy, which is usually administered both before and after local treatment. Treatment of children and adolescents is routinely performed within the framework of prospective clinical trials. While young adults are commonly treated using the very same pediatric, investigator-initiated study protocols obtained one way or another, enrolment into the clinical trials themselves is, unfortunately, all too often neglected. While it is often believed that toxicity is higher for young adults than for children or adolescents, recent reports point into the opposite direction. Tumor size and size, the presence or absence of primary metastases, and the extent of histologic response to preoperative chemotherapy have been proven to correlate with prognosis in both diseases, while it is still being investigated if this also holds true for molecular biologic parameters and, if yes, which might be the ones most relevant. For more than three decades, high-dose methotrexate, doxorubicin, and cisplatin have formed the backbone of most osteosarcoma protocols, while chemotherapy for Ewing sarcoma is based on doxorubicin, alkylators (ifosfamide or cyclophosphamide), vincristine, actinomycin D, and lately topotecane. The potential benefits of adding other agents or, in case of Ewing sarcoma, high-dose chemotherapy are still under investigation, and claims of improved outcomes by newly licensed drugs require substantiation. It is also still open if postoperative treatment modifications based on the extent of histologic tumor response to preoperative chemotherapy can impact prognosis favorably. For osteosarcoma, this question is being addressed in the European-American Osteosarcoma Study, EURAMOS-1, which managed to recruit 2,260 patients from 326 centers in 17 countries over the past six years, including 432 from 85 German institutions, 38 from 8 Swiss centers, and 28 from 5 Austrian sites. The EURO-B.O.S.S. study is open for adults aged 41–65 years and offers an age-adjusted approach for older patients with osteosarcoma and other spindle cell sarcoma of bone. The EWING 2008 study offers a risk-based approach for
patients with Ewing sarcoma and includes an investigation of whether high-dose therapy improves outcomes.

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V365

**Soft tissue sarcoma in children and adolescents**

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Soft tissue sarcomas (STS) are a heterogeneous group of mainly mesenchymal tumors with more than 90 different entities. The incidence varies depending on age: whereas rhabdomyosarcoma (RMS) dominate in children <15 yrs of age, in adolescents and young adults (AYA 15–30yrs) RMS, synovial sarcoma, soft tissue Ewing tumors (STET), malignant peripheral nerve sheath tumors, fibrosarcoma and “others” are equally distributed. Children with STS are treated almost exclusively in clinical trials, like Cooperative Soft Tissue sarcoma Study Group (CWS) in Germany, Austria, Switzerland, Poland and Sweden (cws. ologohospital-stuttgart.de). By means of those a cure rate for RMS, SySa and STET from 60–80% has been achieved. Unfortunately, prognosis in AYA with STS is much poorer and did not improve in the last two decades probably due to their low participation in clinical trials and heterogeneous treatment both in pediatric and medical departments. In the CWS data base patients treated according to a study protocol show a much better prognosis in comparison to patients who were registered as so called "observation patients" with major violations of treatment recommendations. Accurate pathologic diagnosis of sarcomas represents the cornerstone of subsequent clinical decision making and should take place in reference pathologic center. Despite progress in molecular genetics, which allow for more precision in diagnosis and knowledge about biological differences between single STS types, in most clinical trials STS are being still lumped together. The CWS trials have treated patients with RMS, SySa, STET in similar way and called them RMS-like STS. All others STS were lumped as "non-RMS-like STS," similar to medical oncologist who lump even all STS since the therapeutic recommendation have been guided according to grade and not classification. Hopefully, the dramatic progress in identifying the critical biologic pathways active in specific sarcoma, will allow to develop better risk adapted and targeted therapies. Hereby the adequate tumor tissue collection and storage as well as the ability to correlate outcome with biological markers and treatment is critical for further progress. The CWS Study Group has launched 1990 a Sarcoma Tissue Bank which allows for search of prognostic relevant biologic markers and supporting translational research in the field.

**Disclosure:** No conflict of interest disclosed.

V372

**Vitamin D triggers Cathelicidin induced killing of B-cell derived malignant cells by human macrophages**

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**Introduction:** Distinct macrophage subsets have been linked with either protective or pathogenic roles in cancer. Tumor-associated-macrophages (TAM) isolated from solid tumours frequently have an immuno-suppressive and tumor promoting M2-like phenotype. It is unclear which tumoricidal effector mechanisms are compromised in thses immune cells. In haematopoietic lymphatic malignancies such as Burkitt’s lymphoma (BL) infiltration by macrophages is a characteristic morphological hallmark, while the phenotype and the relevance of TAM as part of the stroma are poorly understood. In this study we investigated the phenotype of the lymphoma associated macrophages (LAM) and the mechanisms by which macrophages are able to influence the growth of tumor cells.

**Methods:** We included 19 patients diagnosed with BL and, as a control group, 20 patients with benign reactive lymphadenopathy. Phenotypic characterizations of LAM were evaluated by immunohistochecmistry and by qPCR in paraffin embedded tissues. To investigate the role of distinct macrophage subsets in tumorigenesis in BL, we generated macrophages from PBMC either by GM-CSF to create the M1 phenotype, or by M-CSF to obtain the M2 phenotype, concomitantly them with several BL cell lines and analyzed tumoricidal effects by FACS, qPCR and immunofluorescence.

**Results:** (i) we demonstrate that that infiltrating macrophages in BL display an anti-inflammatory M2-phenotype characterized by the expression of surface marker CD68 and CD163. (ii) we identified impaired vitamin D metabolism in LAM and M2 macrophages (low expression of Vitamin D receptor and Cyp27B1, increased expression of Cyp24A1). (iii) these macrophages display a reduced cytotoxic potential towards lymphoma cells which was dependent on the expression of the vitamin D dependent peptide cathelicidin. Fourthly, we could recently demonstrated that excess supplementation of calcitriol increased the cytotoxic effect of M1- and M2 macrophages, dependent on cathelicidin secretion, against BL cells.

**Conclusions:** These results suggest a mechanism in which vitamin D is required for innate immunity to overcome the ability of lymphoma cells to evade macrophage-mediated antitumoral responses. The present findings underscore the importance of adequate amounts of vitamin D for sustaining innate immunity and imply that the therapeutic activation of the vitamin D pathway may even result in triggering tumoricidal effector mechanisms of LAM.

**Disclosure:** No conflict of interest disclosed.

V373

**Identification and characterization of Fbxo25 as a tumorsuppressor in B cell lymphoma**

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H1- associated protein X-1 (HAX-1) has recently been identified as a potent pro-survival protein implicated in different aspects of hematopoiesis. Specifically, Hax-1 functions in B-cell development and survival, while inactivating Hax-1 mutations have been identified as causative aberrations in severe congenital neutropenia (SCN), an autosomal recessive trait, indicating a role in myeloid progenitor cell survival. In line with an anti-apoptotic function, high levels of Hax-1 expression have been detected in different malignancies, particularly in lymphomas of the B-cell line. The mechanism of how Hax-1 abundance is regulated during the apoptotic response and whether Hax-1 can promote lymphomagenesis has remained unknown. Here we show that Hax-1 is targeted for ubiquitylation and proteasomal degradation in response to apoptotic stimuli by the orphan SFCprb-5325 E3 ubiquitin ligase (Skp1-cullin-1-F-box complex defined by the F-box protein Fbxo25). Upon apoptotic stimuli, Fbxo25 translocates to mitochondria to bind Hax-1, thereby mediating its SCFprb-5325 dependent ubiquitylation and degradation. Using mass spectrometry based phosphorylation analyses, we identify phosphorylation of Hax-1 by glycosyn synthesis drive 3 (GSK3) as a priming event of the Fbxo25-Hax-1 interaction and the subsequent degradation process. Functional experiments reveal that SFCRNA-mediated depletion of Fbxo25 in Em-myc B cells significantly increased lymphoma development and tumor activity in vivo, while Fbxo25 overexpression resulted in a proliferative disadvantage of targeted cells and delayed tumor formation in a syngeneic mouse model.
model. Moreover, analyses of CGH array data sets of different primary B-NHL-samples revealed a significant enrichment of Fbxo25 deletions, particularly in mantle cell lymphomas (MCL). In line with this observation, immunohistochemistry staining of 46 human MCL-samples identified Fbxo25 deficiency in 36 percent of investigated samples. Our work thus identifies Fbxo25 as a novel tumor suppressor in B-NHL, and establishes the GSK3- Fbxo25-Hax-1 axis in B-cell lymphomagenesis with potential therapeutic implications in patients with Fbxo25 deficient B-NHL.

Disclosure: No conflict of interest disclosed.

V374
An oncogene-based RNAi screen identifies a tumour suppressor network in B-cell lymphoma relying on the polyamine-hypusine axis

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Tumour suppressor genes encode a broad class of molecules whose mutational attenuation contributes to malignant progression. In the canonical situation, the tumour suppressor is completely inactivated through a two-hit process involving a point mutation in one allele and chromosomal deletion of the other. Here, to identify tumour suppressor genes in lymphoma, we screen a short hairpin RNA library targeting genes deleted in human lymphomas. We functionally identify those genes whose suppression promotes tumorigenesis in a mouse lymphoma model. Among the new tumour suppressors are adenosylmethionine decarboxylase 1 (ADMA1) and eukaryotic translation initiation factor 5A (eIF5A), two genes associated with hypusine, a unique amino acid produced as a product of polyamine metabolism through a highly conserved pathway. Through a secondary screen surveying the impact of all polyamine enzymes on tumorigenesis, we establish the polyamine-hypusine axis as a new tumour suppressor network regulating apoptosis. Unexpectedly, heterozygous deletions encompassing ADMA1 and eIF5A often occur together in human lymphomas and co-suppression of both genes promotes lymphomagenesis in mice. Thus, some tumour suppressor functions can be disabled through a two-step process targeting different genes acting in the same pathway.

Disclosure: No conflict of interest disclosed.

V376
Reduction of NR4A1 is associated with poor survival aggressive lymphomas

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Introduction: NR4A1 and NR4A3 belong to the orphan nuclear receptors. Their function as critical tumour suppressor genes was demonstrated by the rapid development of acute myeloid leukemia (AML) in NR4A1 and NR4A3 double knock-out mice and by their reduced expression in leukemic blasts from human AML patients. The aim of our study is to comprehensively study and functionally characterize NR4A1 and NR4A3 expression in Non-Hodgkin’s Lymphomas (NHL).

Methods: NR4A1 and NR4A3 expression were determined on mRNA- and protein levels in most lymphoid malignancies of B cell type. Additionally direct sequencing, methylation specific PCR and microRNA analyses of potential interacting NR4A partners were performed. For functional characterization NR4A1 was over expressed in a Sc-1 lymphoma cell line by using an inducible lentiviral construct, and additionally lymphoma cell lines (Sc-1 and Ly8) and immortalized B cells (UH3) were treated with Cytosporone-B (CnB), a non physiological NR4A1 ligand, followed by apoptotic assays (cleaved caspase 3, Sub-G1 peak determination, and the Annexin V staining).

Results: We found a more than 50% reduction of both, NR4A1 and NR4A3, in B-CLL (71%) and Follicular Lymphoma (70%), and more pronounced in diffuse large B cell lymphoma (DLBCL) (74%) compared to normal controls. Mutational analyses in selected cases revealed four of 16 aggressive lymphoma samples mutated (one missense- and three silent mutations (=25%)). In a patient cohort of 83 DLBCL, NR4A1 and NR4A3 were more than 50% down-regulated in 88% and 74% of patients, respectively. A low expression of NR4A1 was significantly associated with non-germinal center B-cell like subtype (p<0.001) and with poor overall survival (p=0.042, HR=2.2, CI=1.01–4.9). Functional in vitro characterisation demonstrated that NR4A1 induction in the transduced Sc-1 lymphoma cell line led to a significant reduction of cell growth (14%) and of apoptosis (18%).

Discussion: Our data indicates that NR4A1 has pro-apoptotic functions and that CnB induces NR4A1 mediated apoptosis in lymphoma cells. Hence, regulation of NR4A1 by CnB is a promising target for the development of new therapeutic drugs.

Disclosure: No conflict of interest disclosed.
V377

MCL1 is deregulated and mediates therapy resistance in subgroups of diffuse large B-cell lymphoma

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Introduction: To elucidate, if deregulation of the apoptotic machinery contributes to the molecular pathogenesis of subtypes of diffuse large B-cell lymphoma (DLBCL), we evaluated the role of the anti-apoptotic BCL2 member MCL1 that is deregulated in various forms of cancer.

Methods: We evaluated MCL1 expression in the activated B-cell-like (ABC) and germinal center B-cell-like (GCB) subtypes of DLBCL by gene expression profiling and immunohistochemistry. Array comparative genomic hybridization (aCGH) data was analyzed for MCL1 chromosomal gains/amplifications. To determine the functional role of MCL1, we used small hairpin RNAs (shRNAs) as well as specific inhibitors and chemotherapeutic agents.

Results: We detected that MCL1 is differentially expressed in subtypes of DLBCL. ABC DLBCLs express MCL1 at significantly higher mRNA levels compared to GCB DLBCL patient samples assessed in 350 DLBCL patients (p=2.7 x 10^-9). Immunohistochemistry confirmed high MCL1 protein expression in ABC DLBCL in an independent patient cohort (n=249; p=0.001). To elucidate, if copy number alterations might contribute to aberrant MCL1 expression, we analyzed aCGH data from 203 DLBCL patient samples. Strikingly, we detected recurrent gains/amplifications of the MCL1 locus in 25.7% of ABC DLBCLs compared to only 12.5% of GCB DLBCLs (p=0.034). Additional analyses revealed that constitutive STAT3 signaling contributes to high MCL1 expression in ABC DLBCL. To investigate the functional role of MCL1 in DLBCL, we knocked down its expression using shRNAs that mediate RNA interference. Knockdown of MCL1 by two independent shRNAs induced apoptotic cell death in DLBCL cell lines with MCL1 expression but not in controls. In line with these data, only MCL1 expressing cell lines were sensitive to treatment with the BH3-mimetic Obatoclax. Further analyses revealed that MCL1 expression decreases sensitivity to chemotherapeutic agents that are commonly used in the treatment of DLBCL patients, clearly implying a role of MCL1 in mediating therapy resistance. In line, MCL1 expressing patients are characterized by adverse survival compared to cases that are MCL1 negative assessed in 233 DLBCL patients treated with R-CHOP (p=6.8 x 10^-4).

Conclusions: MCL1 is deregulated in ABC DLBCLs and contributes to therapy resistance. Our data suggest that specific inhibition of MCL1 might be utilized therapeutically in a subset of DLBCLs.

Disclosure: No conflict of interest disclosed.

V380

Case report 3: Therapy of a patient with choriocarcinoma at advanced stage

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We report the case of a 17 year old patient admitted to our hospital with an externally diagnosed mass in the left testicle, retroperitoneal, pulmonary and cerebral metastases, a highly elevated beta-HCG of >2 million IU/L, and a rapidly deteriorating state of health. He presented with tachycardia with signs of a cardiac failure and symptoms of a diabetes insipidus. The patient was transferred to ICU, where laboratory analyses revealed a beta-HCG induced hypothyroidism and affirmed the diabetes insipidus. On ICU, in addition to ventilation due to intermittent respiratory failure, the patient received treatment of diabetes insipidus and hypothyroidism. A three day cycle of cisplatin and etoposide was initiated as a pre-phase to more intensive chemotherapy. Furthermore, the patient was monitored for signs of pulmonary failure, intracranial hemorrhage, and tumor lysis syndrome. After successful stabilization, the patient received a full course of VIP, followed by stem cell mobilization and stem cell harvest in our oncology unit. In the further course of treatment,
he received 3 cycles of high-dose VIP and autologous stem cell transplantation. With this treatment, the patient achieved a marker negative partial remission, with residual lesions in the retroperitoneum (2.9 cm) and the lungs (biggest lesion 2.0 cm). He underwent inguinal orchietomy of the left sided testicle, revealing small areas of teratoma, and retroperitoneal lymphadenectomy, revealing only necrotic tissue. Resection of residual pulmonary lesions was omitted. Our multidisciplinary tumor board recommended additional cranial irradiation, a recommendation that was followed by the patient. Currently, the patient is disease-free and without signs of long-term toxicity for >8 months with the exception of recurrence of mild diabetes insipidus that is treated medically with oral desmopressin. In the mean time, he has passed his final exams at school. The difficulties at the start of the treatment start in this case, as well as controversies of the individualized treatment approach, especially the necessity of the upfront treatment intensification with high-dose chemotherapy and the decision for additional cranial irradiation will be discussed with data from the literature. In the absence of data from randomized trials for GCT patients with CNS metastases and “very poor prognosis”, the evidence mainly comes from case series and retrospective subgroup analyses that will be presented.

Disclosure: No conflict of interest disclosed.

Freie Vorträge
Chronische lymphatische Leukämie II

V381 Toll-like receptor 9 agonists induce polarization and migration of chronic lymphocytic leukemia cells

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Human leucocytes have the ability to migrate in order to perform their physiological functions. Prior to migration, they have to undergo polarization by forming a leading edge and an uropod via cytoskeletal reorganization. Here, we investigated the ability of chronic lymphocytic leukaemia (CLL) cells to polarize and the impact of Toll-like receptor 9 (TLR9) agonists on the polarization process. Immunomagnetically purified CLL cells were cultured (1.5x10^5/ml) for 48h in serum-free medium in the presence of the TLR9 agonists (0.25 µM) CpG A (ODN 2216), CpG B (ODN 2006), CpG C (ODN 2395) or PBS (control), respectively. Time-lapse video-microscopy was used to monitor cell motility and polarization in vitro. Cell motility was analysed by manual tracking, while the frequency of polarized cells was counted manually. Upon cultivation in vitro, CLL cells spontaneously adopted a polarized cell shape, where the percentage of polarized cells varied among CLL patients with a median of 28.4% (range: 10.1–3.8 9%, n=5). Upon stimulation with TLR9 agonists the median percentage of polarized cells was increased by CpG A to 34.9% (p=0.04), by CpG B to 52.3% (p=0.006) and by CpG C to 55.6% (p=0.008). On time-lapse microscopy, polarized cells migrated significantly faster and longer distances compared to their unpolarized counterparts. Therefore, a higher percentage of polarized cells at a given point of time could be correlated with increased migration of the whole cell population. In a second set of experiments we aimed to explore the impact of TLR9 agonists in our recently described CLL NOD/SCID/γC−/− (NSG) xenograft model. 5x10^6 freshly isolated PBMCs from CLL patients were stimulated for 2 hours with CpG A, B, C (5 µM) or PBS and subsequently intravenously injected into 8–12 weeks old NSG mice. Four hours post transplantation, the bone marrow and spleens of the recipient animals were analysed by flow cytometry for the presence of human CLL cells. We found that CpGs B and C significantly increased the homing capacity of CLL cells to the BM of NSG mice (CpG A p=0.036, CpG C p<0.001, n=4).

Conclusions: In summary, upon in vitro cultivation, CLL cells spontaneously adopted a polarized cell shape which correlated with their migratory behaviour. TLR9 agonists induced a pro-migratory phenotype in CLL cells in vitro and increased their capability to home to the BM of NSG mice in vivo.

Disclosure: No conflict of interest disclosed.

V382 TOSO-deficient B cells show impaired development in vivo

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In the recent years, TOSO – alias FAS apoptosis-inhibitory molecule 3 – has been controversially discussed whether it exerts anti-apoptotic effect or is the long-sought-after bona fide Fas-receptor. Since our group previously identified TOSO to be overexpressed in CLL and to be correlated with progressive disease, it is indispensable to clarify the biological significance of TOSO, particularly in the CLL relevant B cells. Therefore, we generated a B cell-specific knockout mouse model and cross-bred FAIM3-flxed C57BL/6 mice with CD19-specific Cre recombine expressing mice. B cells from the TOSO^ΔΔ/ΔΔ (KO) mice were isolated and gene expression analysis was performed using mRNA microarray platform. Convincing results were verified by flow cytometry and blood count was carried out in addition.

Peripheral blood of the TOSO deficient mice displayed decreased level of lymphocytes, in which B cells were determined as reduced entity (p=0.0333). Other cell types, like NK and T cells, remained thereby unaffected.

Downstream effects of TOSO were validated via microarray-based gene expression analysis. Results displayed a clear clustering of deregulated genes compared to control mice. Nearly 500 genes showed expression alterations. Genes involved in the NF-kB pathway and migration processes were down-regulated in TOSO^ΔΔ/ΔΔ mice, suggesting that TOSO represents an important factor in these processes. These results were confirmed by flow cytometry analysis. Thus, our results might reveal a new function of TOSO in migration, pro-survival signaling and B cell homeostasis, supporting the anti- apoptotic feature of TOSO in B cells.

Disclosure: No conflict of interest disclosed.

V383 Ca2+/NFAT signaling regulates the expression of CD38 and ZAP70 in murine B cells and controls B1a cell homeostasis

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Upon dephosphorylation by calcineurin, NFAT proteins translocate to the nucleus where they orchestrate diverse transcriptional programs. Although identified originally in T cells, it is now clear that NFAT transcription factors also possess important roles in other cells of the hematopoietic system. Here, we have analyzed the role of NFAT2 in B cell development. To achieve NFAT2 deletion limited to the B cell lineage, we bred NFAT2^flx/flx mice to CD19-Cre mice. B cells from these mice were isolated using CD19-labeled magnetic beads and subjected to analysis by flow cytometry. While CD19^+ splenocytes from conditional NFAT2 knock-out mice occurred in normal numbers, these cells showed significantly reduced expression of CD38 and ZAP70 upon stimulation. The reduction of these proteins could also be detected in B cells isolated from peripheral blood and from bone marrow and was confirmed by western blotting and quantitative RT-PCR. CD38 and ZAP70 are well characterized prognostic factors in CLL and their expression has been shown to correlate with poor survival. Our data indicate that the expression of these markers is at least in part regulated by NFAT signaling and that deregulation of this pathway can contribute to their overexpression.

Convincing results were verified by flow cytometry and blood count was carried out in addition.

Peripheral blood of the TOSO deficient mice displayed decreased level of lymphocytes, in which B cells were determined as reduced entity (p=0.0333). Other cell types, like NK and T cells, remained thereby unaffected.

Downstream effects of TOSO were validated via microarray-based gene expression analysis. Results displayed a clear clustering of deregulated genes compared to control mice. Nearly 500 genes showed expression alterations. Genes involved in the NF-kB pathway and migration processes were down-regulated in TOSO^ΔΔ/ΔΔ mice, suggesting that TOSO represents an important factor in these processes. These results were confirmed by flow cytometry analysis. Thus, our results might reveal a new function of TOSO in migration, pro-survival signaling and B cell homeostasis, supporting the anti-apoptotic feature of TOSO in B cells.

Disclosure: No conflict of interest disclosed.
in the abundance of B cell subpopulations in bone marrow, we detected an almost complete absence of B1a cells in the peritoneal cavity, clearly demonstrating the requirement of NFAT2 in the development of this subclass. B1a cells are a phenotypically and functionally distinct population of B cells which are long-lived and typically express CD5, CD43 and high levels of surface IgM together with low surface IgD and B220. A human B cell equivalent of the murine B1a cell has been suggested as the leukemic precursor cell of CLL. To delineate the role of NFAT2 in the development of B1a cells we determined the abundance of B1 progenitor cells (B1P) in bone marrow and spleen by FACS analysis. In NFAT2 knockout mice we observed a significant reduction of the frequency of B1P cells in bone marrow (0.8% vs. 4.7%) and spleen (0.16% vs. 0.82%). In summary, our data provide strong evidence that NFAT2 is critical for the expression of CD38 and ZAP70 in B cells and substantially controls B1a cell homeostasis implicating Ca2+/NFAT signaling as a potential target for the treatment of CLL.

Disclosure: No conflict of interest disclosed.

V384
Identification of novel tumor associated antigens for chronic lymphocytic leukemia by HLA ligandome analysis

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Introduction: Results of bone marrow transplantation as well as remission phenomena after viral infections suggest that chronic lymphocytic leukemia (CLL) might be targeted effectively by T cell based immunotherapy. For this goal the identification of tumor associated HLA-presented peptides, which are able to induce a tumor-specific T cell response, is indispensable. However, only few tumor associated antigens for CLL are described. Thus the aim of this study was to identify novel tumor associated antigens employing, for the first time, the approach of direct elution and analysis of HLA class I ligands from the surface of primary CLL cells.

Methods: HLA class I ligands were isolated from MACS-purified PBMCs of CLL patients using immunoprecipitation. Liquid chromatography/mass spectrometry (LC/MS) based peptide sequencing was used to identify HLA presented peptides. The obtained data were mined for leukemia associated peptides by comparison of HLA ligandomes of CLL cells with that of PMBCs and B cells from healthy donors, investigation of gene expression databases and literature research. In addition, HLA quantification experiments on the cell surface of CLL cells and autologous healthy B cells were performed using a flow cytometric indirect immunofluorescence assay.

Results: Quantitative results showed similar amounts of HLA class I (p = 0.23) and II (p = 0.33) molecules on CLL cells compared to normal B lymphocytes, even with a trend to higher HLA amounts on CLL cells. We were able to identify a total of more than 2500 peptides from 8 CLL patients. Several new ligands derived from established leukemia-associated antigens (e.g. Fibromodulin), from proteins showing an overexpression on mRNA level in CLL (e.g. SET proto-oncogene) and from new, potentially CLL associated peptides (LEUMAPs) identified by cross checking for occurrence on healthy tissues, especially B lymphocytes and PBMCs, were obtained. The sequences of identified LEUMAPs were verified by LC/MS-based peptide sequencing of their synthetic counterparts. Testing for immunogenicity by immunological assays is presently ongoing.

Conclusions: This study paves the way for the development of future peptide based immunotherapy of CLL by confirming that there is no loss or down-regulation of HLA on CLL cells and by identifying new leukemia associated antigens, which for the first time were directly obtained from the HLA ligandomes of CLL patients.

Disclosure: No conflict of interest disclosed.

V385
The B-cell receptor-controlled ceramide: glucosylceramide equilibrium decides on drug sensitivity of primary CLL cells

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Introduction: Survival of chronic lymphocytic leukemia (CLL) cells is triggered by several stimuli like engagement of the B-cell receptor (BCR), CD40 ligand (CD40L)-CD40 interaction or stimulation by interleukin (IL)-4. However, little is known about downstream metabolites involved in downstream signaling. Ceramide, as the central molecule in sphingolipid metabolism, promotes apoptosis. In contrast, glucosylceramide, a derivative of ceramide, stimulates cell proliferation.

Methods and results: In order to investigate a different regulation of sphingolipid components in CLL under the influence of these mentioned stimuli, we applied liquid chromatography electrospray ionization tandem mass spectrometry to 8 CLL samples. Analysis revealed a significant decrease of pro-apoptotic ceramide in BCR/IL-4/CD40L-stimulated primary CLL cells compared to native controls (p = 0.0258 for IgM, p = 0.0478 for IL-4, p = 0.0114 for CD40L). Anti-apoptotic glucosylceramide levels were significantly increased after BCR cross-linking (p = 0.0435). We identified via qRT-PCR that UDP-glucose ceramide glucosyltransferase (UGGC) catalyzes the synthesis of glucosylceramide out of ceramide and glucose. Besides specific UGGC inhibitors, in particular the imino sugars N-(Butyl) deoxygalactonojirimycin (OG1-β) or N-(n-Nonyl)deoxygalactonojirimycin (OG2-β), we demonstrated by flow cytometry analysis that BCR-mediated UGGC expression was further inhibited by the novel PI3Kdelta and BTK inhibitors CAL-101 and PCI-32765. Specific inhibition of UGGC as well as of PI3Kdelta and BTK reverted BCR-induced chemoresistance of CLL cells (82.9% to 72.3% for CAL-101 and 82.9% to 74.1% for PCI-32765). ABT-737 was recently shown to mediate apoptosis via ceramides. Our data reveal ABT-737 as interesting match for PI3Kdelta and BTK inhibition resulting in synergistic apoptosis, even under protection by pro-survival influences of the BCR (p < 0.0001 for CAL-101 and p = 0.0004 for PCI-32765).

Conclusion: We identified the UGGC-related ceramide:glucosylceramide equilibrium as a downstream molecular switch of BCR signaling and key regulator of CLL cell survival and apoptotic resistance. Controlling the balance of both metabolites is crucial for the mode of action of the novel kinase inhibitors CAL-101 and PCI-32765. This study provides potential new targeted therapeutic approaches for treatment of CLL beyond current chemotherapy-based concepts.

CMW and LFF contributed equally.

Disclosure: No conflict of interest disclosed.
**Abstracts**

**V386**

**Chronic lymphocytic leukemia cells receive Insulin-like growth factor receptor-I dependent survival signals that are sensitive to inhibition by sorafenib**

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**Purpose:** Chronic lymphocytic leukemia (CLL) is characterized by the accumulation of monoclonal B lymphocytes. Despite major advances in the field, there is no curative treatment for CLL to date, and new strategies are needed. The multikinase inhibitor sorafenib, targeting RAF, platelet-derived growth factor receptor (PDGFR), KIT, FMS-like tyrosine kinase 3 (FLT3), and vascular endothelial growth factor receptor (VEGFR), has been approved for the treatment of renal cell carcinoma and hepatocellular carcinoma. Recent studies have shown that CLL cells might be also susceptible to this compound. In this study we identified the Insulin-like growth factor receptor-I (IGF-IR) pathway as potential additional target of sorafenib inducing cell death in CLL cells.

**Methods:** Peripheral blood CLL samples were incubated with 10 µM of sorafenib or 50 µM AG1024 (IGF-IR inhibitor) for 24 hours. Apoptosis induction by sorafenib or AG1024 was determined by annexinV/PI staining. IGF-IR and Insulin receptor (IR) expression was monitored by flow cytometry.

**Results:** Treatment of CLL cells with sorafenib significantly increased apoptosis by 72%±7% (n=10, p<0.001 compared to vehicle control). This apoptotic effect resulted in inhibition of phosphorylated RAF-1, BRAF, MEK, and ERK, established downstream signals of the RAF-kinase pathway. Moreover, sorafenib treatment decreased phosphorylation of SRC and AKT, molecules implicated with IGF-IR and IR signaling. The latter were strongly expressed in primary CLL cells (IR 81%±5% positive cells, p<0.0006; IGF-IR 75%±4% positive cells, p<0.0068, n=7) compared with healthy B cells (IR 15%±0.6, IGF-IR 13%±1.5% positive cells). Similar to sorafenib, 24h treatment of CLL cells with AG1024 significantly increased apoptosis by 78%±4% (n=10, p<0.001 compared to vehicle control) and resulted in decreased phosphorylation of RAF-1, MEK, ERK, SRC, and AKT. Both compounds, sorafenib and AG1024 also downregulated the expression of IGF-IR by 49%±9% (p=0.07, n=6) and by 7% (p=0.2, n=6), respectively, suggesting that Sorafenib acts via inhibition of the IGF-IR pathway. Ongoing experiments verify these results in primary CLL cells with a newly designed inducible vector system.

**Conclusions:** Our data identify sorafenib as a potent inducer of CLL cell death and demonstrate that CLL cells receive IGF-IR- and RAF-dependent survival signals sensitive to inhibition by sorafenib.

**Disclosure:** No conflict of interest disclosed.

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**V387**

**Insulin like growth factor receptor I (IGF-IR) and vascular endothelial growth factor receptor 2 (VEGFR-2) are expressed on the circulating epithelial tumor cells of breast and prostate cancer patients**

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**Background:** Circulating epithelial tumor cell analysis is a promising diagnostic field for estimating the risk for metastatic relapse and progression in patients with malignant disease. CETCs characterization can be used as a liquid biopsy for prognostic and predictive purposes in breast, prostate and other cancers. IGF-IR and VEGFR-2 play an important role in the growth of tumor and progression of cancer disease. Therefore the purpose of the current study was to investigate their expression on the CETCs.

**Methods:** CETCs were determined from blood of 60 patients suffering from breast and prostate cancer. The number of vital CETCs and the expression of IGF-IR and VEGFR-2 were investigated using the Mantrac® method.

**Results:** IGF-IR expression on the surface of CETCs was detected in 76% of patients whereas expression of VEGFR-2 was observed in 83% of patients with breast cancer. 100% of patients with prostate cancer showed a IGF-IR and VEGFR-2 expression on the CETCs. The number of living CETCs was two-fold higher in prostate cancer patients, in comparison to breast cancer patients. A statistically high correlation was found between IGF-IR and VEGFR-2 on the CETCs. Moreover, sorafenib treatment decreased phosphorylation of SRC and AKT, which indicated an oncogene in myeloid leukaemia where high expression was found to predict poor outcome. However, in ABC the evidence is increasing, that expression in the basal disease subtype and associated with poor outcome in estrogen receptor-negative breast cancer patients (Patel et al., Oncogene, 2011).

**Conclusion:** Our results demonstrated for the first time the expression of IGF-IR and VEGFR-2 on the CETCs in patients with breast and prostate cancer and thus constitute the basic for using anti-IGF-IR and anti-angiogenic therapy for their elimination.

**Disclosure:** No conflict of interest disclosed.

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**V388**

**Expression and role of the transcription factor EVI-1 in human breast cancer**

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**Introduction:** EVI-1, a member of the SET/PR domain family of transcription factors located on the human chromosome 3q26, has been mainly studied as an oncogene in myeloid leukaemia where high expression was found to predict poor outcome. However, EVI-1 expression has been reported in a variety of other tumour types, including breast cancer. Recently elevated expression of EVI-1 was observed in the basal disease subtype and associated with poor outcome in estrogen receptor-negative breast cancer patients (Patel et al., Oncogene, 2011). Here we explore the expression and functional role of EVI-1 in breast cancer.

**Methods:** EVI-1 gene and protein expression was analyzed by real-time PCR and western blot in breast cancer cell lines (MCF7, MDA-MB468, MDA-MB231, SKBR3, BT549, T47D, BT474, HS578T) and patient samples. EVI-1 expression was modulated in MDA-MB231 and T47D breast carcinoma cells by transduction with inhibitory siRNA or lentivirus mediated shRNAs against EVI-1. Cells treated with control siRNAs and non-coding shRNA control lentiviruses were used as controls. Cell cycle, Bcl2 and apoptosis (% sub-G1 positive cells after PI treatment, Annexin-V/7-AAD staining. Caspase 3/7 and PARP activity after exposure to Staurosporine and TRAIL) assays were performed.

**Results:** EVI-1 expression was detected in most of the analyzed primary breast cancer samples (10/12) and cell lines (5/8). Interestingly, a trend towards higher expression in primary samples as compared to cell lines was observed.
Knockdown of EVI-1 expression in MDA-MB231 cells induced a G1-arrest and retarded cell proliferation. Moreover, EVI-1 knockdown cells displayed enhanced apoptosis sensitivity in response to both intrinsic (e.g. staurosporine) and extrinsic (e.g. TRAIL) stimuli. Notably EVI-1 did not significantly modulate the expression of tumor stem cell antigens (CD24, CD44), tumor sphere formation in vitro and expression of stem cell related genes (e.g. SOX2, OCT3/4, ALDH1).

**Conclusion:** In this study, we show that expression of the EVI-1 oncogene is a common event in breast cancer and modulates proliferation and apoptosis of the malignant cells. Currently, we are analyzing the molecular mechanisms by which EVI-1 mediates its effects in breast cancer cells and further analyze whether it plays selective roles in breast cancer tumor stem cells.

**Disclosure:** No conflict of interest disclosed.

**V389**

**CYP2D6*4-genotype influences tamoxifen efficacy in advanced breast cancer**

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**Introduction:** The influence of CYP2D6-genotype on the efficacy of tamoxifen (Tam) has been extensively analyzed in the adjuvant setting of breast cancer with conflicting results. However, there is only scarce data regarding this potential influence in the setting of advanced breast cancer (ABC). Thus, our objective was to analyze efficacy of palliative treatment with Tam in regard of the CYP2D6-genotype. We hypothesized that Tam is more effective in EM patients than in patients with impaired CYP2D6-activity. Here we present preliminary data of our analysis concentrating on CYP2D6*4.

**Methods:** ABC patients with prior or ongoing palliative Tam treatment (20mg/d) were eligible for this analysis. Genomic DNA was extracted from whole blood (n=51) and from formalin-fixed, paraffin-embedded tissue (n=42). CYP2D6*4 was determined by PCR-RFLP in blood samples. In tissue samples, genotyping was done using a pre-developed TaqMan® SNP Genotyping assay. For internal quality control, selected samples were determined by both methods. The primary efficacy endpoint was progression free survival (PFS); secondary endpoint objective response rate. The clinical charts were retrospectively analyzed according to PFS and treatment effects. Genotyping was performed blinded and clinical data were analyzed separately.

**Results:** 93 patients were identified. Median age at diagnosis was 49y (26-86y). In 6 patients genotyping did not show conclusive results, these patients were excluded from further analysis. Genotyping results were as follows: 59% wt/wt, 31% wt/*4, 10% *4/*4. PFS was significantly shorter in patients with *4/*4 compared to wt/wt (HR=2.68, 95% CI (1.21–5.03), p=0.015), and showed a decreasing trend in patients with wt/*4 (HR=1.65, 95% CI (0.96–2.83), p=0.071). In carriers of at least one *4-allele (wt/*4 + *4/*4) PFS was also significantly shorter than in wt/wt patients (HR=1.82, 95% CI (1.11–3.01), p=0.018). Compared to wt/wt, the rate of progressive disease more than doubled in carriers of at least one *4-allele (13% vs 31.4%, Pearson’s chi-squared p=0.044).

**Conclusions:** Our preliminary results show a significant influence of CYP2D6*4 on PFS and objective response rate in ABC patients treated with 20mg Tam daily in the palliative setting. Influence of CYP2D6-genotype has been studied extensively in the adjuvant setting with contradictory results. However, in ABC the evidence is increasing, that CYP2D6 has a significant influence on efficacy of Tam.

**Disclosure:** No conflict of interest disclosed.

**V390**

**Bismuth-213 radiolabeled anti-HER2-antibodies break chemo- and radioresistance in anti-HER2-resistant breast cancer cells**

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**Introduction:** Trastuzumab (anti-HER2-antibody, anti-HER2) is widely used for treatment of HER2-positiv breast cancer. Unfortunately, resistance to anti-HER2 followed by therapeutic failure is common. Targeted alpha-therapy seems to be a promising treatment modality to break chemo- and radioresistance in cancer. Therefore, we tested the cytotoxic potential of the alpha-emitter [Bi-213]anti-HER2 (Bi-213-antiHER2) to overcome resistance in anti-HER2-resistant breast cancer cell lines. Furthermore, we clarified the molecular mechanism of cell death induction by [Bi-213]anti-HER2.

**Methods:** JIMT-1 (anti-HER2-resistant) or SKBR-3 (anti-HER2-sensitive) breast cancer cell lines were treated with different activity concentrations of [Bi-213]anti-HER2 in a range of activities (2000–4000 kBq/mL). Induction of cell death was measured by flowcytometry. Involvement of apoptotic pathways was assessed by Western blot analyses at different time points.

**Results:** Treatment of the breast cancer cells JIMT-1 (anti-HER2-resistant) and SKBR-3 (anti-HER2-sensitive) with [Bi-213]anti-HER2 led to an increased cell death induction in both cell lines compared to unlabelled anti-HER2. [Bi-213]anti-HER2 stimulated apoptotic cell death in anti-HER2-sensitive as well as anti-HER2-resistant breast cancer cells. By this way [Bi-213]anti-HER2 restored deficient caspases activation in anti-HER2-resistant breast cancer cells. Displayed by molecular analyses the intrinsic mitochondrial pathway of apoptosis was activated. After treating breast cancer cells with [Bi-213]anti-HER2, caspase-9 was activated. In the apoptotic executioner cascade, caspase-3 was activated and its substrate PARP was cleaved into its inactive fragments. Additionally, downregulation of the death inhibitory protein Bcl-x, and the strong caspase inhibitor XIAP, both contributing to tumours’ resistance, was occurred by [Bi-213]anti-HER2 in the anti-HER2-resistant and anti-HER2-sensitive cells.

**Conclusion:** These findings indicate that [Bi-213]anti-HER2 induces apoptosis in anti-HER2-sensitive as well as in anti-HER2-resistant breast cancer cell lines via the intrinsic apoptotic pathway. [Bi-213]anti-HER2 overcomes anti-HER2-resistance. We conclude that [Bi-213]anti-HER2 is a novel approach in the treatment of untreatable anti-HER2-resistant breast cancer.

**Disclosure:** No conflict of interest disclosed.

**V391**

**Targeting oncoprotein stability overcomes lapatinib-resistance due to ERBB2 kinase domain mutations**

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ERBB2 kinase domain mutations were recently reported in some solid cancers. Moreover, certain ERBB2 mutations were shown to cause lapatinib resistance in vitro thus predicting their emergence in treated patients. We have recently shown that ERBB2-L755S, ERBB2-L755P and ERBB2-T798M mutants cause lapatinib resistance by activating the kinase conformation (DFG-in), which is incompatible with lapatinib binding (DFG-out). Using an in vitro cell-based drug resistance screen we also showed that these lapatinib resistant mutations might cause secondary resistance in patients treated with lapatinib. Thus, it is important to search for alternate treatment strategies to overcome lapatinib resistance.

ERBB2 kinase is a client for HSP90 and is degraded by HSP90 inhibitor treatment. We thus tested if targeting ERBB2 mutation stability by inhibiting HSP90 overcomes lapatinib resistance. Since the kinase domain is important for the ERBB2 interaction with HSP90, we tested whether ERBB2 kinase domain mutants retained their interaction with the chaperone. Co-immunoprecipitation analysis showed that the interaction of ERBB2 mutants with HSP90 is intact.
Importantly, HSP90 inhibitor treatment resulted in the degradation of lapatinib-resistant ERBB2 mutants as observed with the wild type ERBB2 kinase. Thus, HSP90 inhibitors may offer an alternative treatment option to overcome primary or secondary lapatinib resistance in patients harbouring ERBB2 mutations. Moreover, combined targeting of different physiological aspects (enzyme activity and protein stability) may prevent the emergence of secondary drug resistance due to kinase domain mutations.

**Disclosure:** No conflict of interest disclosed.

**V392**

**CMD-coated magnetic nanoparticles – A tool for quantitative magnetic assisted depletion of circulating epithelial tumor cells from peripheral blood**

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**Background:** Cancer is one of the main causes of death and it is well known, that most of the cancer patients die from distant metastases. Once the primary epithelial tumor is established, cells may begin to dissociate from the tumor and spread to other parts of the body. These circulating epithelial tumor cells (CETCs) are able to form metastases. However, the separation of CETCs from healthy leukocytes and their characterization is a major challenge. In hematol- and oncology magnetic nanoparticles are regularly used for labeling and detection of cells. We could already show that the selective enrichment of CETCs, using magnetic nanoparticles coated with carbosymethyl-dextran (CMD) is an effective enrichment method.

**Methods:** We analyzed whole peripheral blood samples from 280 breast cancer patients. Peripheral blood leukocytes were prepared by erythrocyte lysis. The isolated peripheral blood leukocytes containing the putative CETCs were incubated for 4 min with CMD-coated magnetic nanoparticles in PBS/EDTA. The labeled cells were separated by using a SuperMACS Separator (Miltenyi-Biotec, Bergisch-Gladbach, Germany) and were quantified as positive (retained fraction) and negative fraction (effluent fraction). The cells of both fractions were analysed by flow cytometry (FACSCalibur, Becton-Dickinson, USA) and were quantified as positive (retained fraction) and negative fraction (effluent fraction). The cells of both fractions were analysed by flow cytometry (FACSCalibur, Becton-Dickinson, USA) and were quantified as positive (retained fraction) and negative fraction (effluent fraction).

**Results:** Using magnetic nanoparticles we can enrich CETCs selectively. Approximately 114% of the total cell count was found in the positive fraction. All in all we analysed 280 breast cancer samples and were able to collect 75±28% of the entire CETCs in the positive fraction on average. We observed a difference in the enrichment efficiency of CETCs with regard to the individual therapy status of the patient. Pre-operative patients (n=10) or patients in follow-up care (n=50) show a better selective enrichment of CETCs in the positive fraction (pre-operative 93±33%; follow-up care 92±16%) than patients undergoing chemotherapy (n=23, approx. 33±16%).

**Conclusion:** We are able to enrich CETCs from peripheral blood blood quantitatively using CMD-coated magnetic nanoparticles from the leukocyte fraction with a single incubation and separation step. This procedure shows the highest efficiency for patients before surgery or in follow-up care. In the future, this noninvasive method may be a tool to effectively eliminate CETCs from the periphery.

**Disclosure:** No conflict of interest disclosed.

**V395**

**Stem cell therapy – indications to allogeneic transplantation in myelodysplastic syndrome and acute myeloid leukemia**

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Myelodysplastic syndrome (MDS) and acute myeloid leukemia (AML) represent the most prevalent indications for allogeneic stem cell transplantation (alloSCT) worldwide. In Germany, around 50% of all indications for alloSCT comprise patients (pts) with MDS (~15%), AML (~35%). This primarily reflects the broadly accepted curative potential of alloSCT in these disease entities, in which cure by conventional therapy is either not attainable or only infrequently reached. The indication to alloSCT in MDS relies predominantly on well-established adverse prognostic disease features indicating a high risk of leukemic transformation or of life-threatening complications due to MDS-related cytopenia. Although not derived from controlled clinical trials, the indication to alloSCT is generally accepted in suitable pts with an International Prognostic Scoring System (IPSS-MDS) score >1, in which marrow blast content and, in particular, adverse karyotype are the most decisive prognostic features. Owing to the fact that MDS affects predominantly elderly and comorbid pts, the performance of alloSCT could be extended considerably by the development of reduced-intensity preparative regimens, which together with more effective immune pharmacologic prophylaxis of graft-versus-host disease has clearly improved transplant results in this patient population, particularly after alloSCT from matched unrelated donors (MUD). Prospective trials comparing conventional therapy and alloSCT in elderly pts with unfavorable MDS are currently under way to define its therapeutic significance more precisely. In primary postremission therapy of AML, alloSCT has an established therapeutic benefit for younger (≤60 years) pts with unfavorable prognostic disease features at diagnosis, which is primarily based on adverse cytogenetic anomalies (in particular complex aberrant and/or monosomal karyotype). In younger pts with normal karyotype AML, molecular anomalies, like FLT3 internal tandem duplications, MLL gene rearrangements, or RUNX1 muta-
tions are generally indicating alloSCT due to the dismal prognosis with con-
ventional postremission therapy. In elderly AML pts, who in principal have a
worse outcome with conventional therapy, the role of alloSCT is currently
evaluated in prospective randomized trials. For nearly all primary refractory or
advanced stage AML pts, alloSCT from matched related or unrelated donors
still is the sole curative therapeutic approach.

Disclosure: No conflict of interest disclosed.

V396
Indications of allogeneic stem cell transplantation in
myeloproliferative neoplasia

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Philadelphia-negative myeloproliferative neoplasms (MPNs) are rare diseases
characterized by a clonal expansion of one or more bone marrow hematopoi-
etic cell lines. These diseases are usually diagnosed in elderly patients and are
generally not curable with conventional therapy. Polycythemia vera (PV) and
essential Thrombocythemia (ET) are associated with a proliferation of the
erthro- or megakaryopoietic cells respectively. Primary myelofibrosis (PMF)
however is characterized by a proliferation or depletion of one or more cell
lines, a proliferation of collagen fibers in the bone marrow and an extramedul-
 lary hematopoiesis. In PV and ET, the life expectancy is mostly not signifi-
cantly limited. On the contrary, in many patients with PMF the survival can be
dramatically reduced. Allogeneic stem cell transplantation (ASCT) is the only
treatment for these diseases with curative potential. However this treatment
modality is associated with a considerable treatment-related mortality. Therefore,
ASCT is indicated only in those cases where the risk of transplantation
is well justified through limited life expectancy. In this session, the indica-
tions of ASCT in MPNs will be summarized and discussed.

Disclosure: No conflict of interest disclosed.

V397
Adjuvant therapy of endometrial cancer: present state and
controversials

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Endometrial cancer is a major cause of morbidity and mortality for women
worldwide, with nearly 200,000 cases diagnosed every year. 75% of newly
diagnosed women can be cured with surgery alone. However it is now recog-
nized that endometrial adenocarcinomas represent a heterogeneous group of
diseases that can be distinguished both by clinical and biologic characteristics.
Type I tumors are believed to develop in an estrogen-related manner, tend to
be well differentiated, are associated with mutations in PTEN, K-ras, and beta
catenin, and harbor microsatellite instability. Type II tumors typically arise in
atrophic endometrium through a mechanism unrelated to estrogen exposure.
These are usually endocrine receptor negative, poorly differentiated and asso-
ciated with p53, p16 inactivation, HIR2/neu overexpression and reduced expres-
sion of E-cadherin.

The current treatment for endometrial cancer involves the use of surgery,
radiation therapy, hormone therapy and chemotherapy. The role of full surgical
staging remains controversial. Two recent studies did not show improvement in
disease-free or overall survival after lymphadenectomy in early-stage dis-
ease.

The use of radiotherapy in early-stage disease has been evaluated in three
major trials, all of which demonstrated that adjuvant radiotherapy improved
local disease control and recurrence-free survival but did not improve overall
survival at 5 years. Multiple randomized studies have evaluated chemotherapy versus, or
combined with, radiotherapy, GOG-122 compared whole abdominal radiation with
doxorubicin/cisplatin chemotherapy in patients with optimally debulked stage

Disclosure: No conflict of interest disclosed.
to indicate that Carboplatin and Paclitaxel or Cisplatin and Ifofamide may be active in this subgroup and may be explored for future adjuvant trials. Ifofamide is generally perceived to be an unpleasant drug to administer in the adjuvant setting and therefore Carboplatin/Paclitaxel will probably emerge as the preferred option. There is some dispute as to whether the addition of an anthracycline may offer benefit but the TEC regime may be worth while. Endometrial stromal sarcomas are the least common of the tumour groups and often express high levels of oestrogen and progesteron receptors. Therefore treatment with anti-oestrogens or progrestagens may be considered. Again numbers are too small to permit any randomised clinical trials. It is therefore quite difficult to advise whether adjuvant hormonal therapy should be given but in patents with high risk tumours who express ER PR positivity it seems reasonable to consider adjuvant Tamoxifen or Megestrol Acetate or a combination. Other drugs that have been explored include the aromatase inhibitors and the GHRH analogues.

For recurrent disease careful review of pathology and imaging is required and these cases should ideally be discussed at the Tumour Boards. If there is a long treatment free interval and the tumour expressed oestrogen or progesteron receptors then hormonal therapy as described above would be worth while. Some of these tumours may actually relapse at very prolonged and delayed intervals and these patients can be controlled by hormonal therapies.

When there is earlier and more aggressive diffuse relapse systemic chemother-apy is normally the treatment of choice if the patient is fit enough. Whilst anthracyclines, platinum and Ifofamide and taxanes are the most active, recent data would suggest that Carboplatin and Paclitaxel with or without an anthracycline should be considered as the treatment of first choice. Response rates of around 60–70% are reported. Palliative radiotherapy for symptomatic metastases is also to be considered and for patients with isolated pelvic recurrence radiotherapy with concomitant chemotherapy should also be considered. The role of the new targeted anticancer agents remains unresolved in this setting but these are good areas for investigation in clinical trials. A number of agents including the M-Tor pathway inhibitors, Pazopanib have been investigated in recent trials. The author is of the view that these should not be used outwith the clinical trials setting at the present time.

Disclosure: No conflict of interest disclosed.

V389
New substances in the systemic therapy of endometrial carcinoma
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The last decades have brought us significant advancements in our understanding of endometrial cancer (EC) which is the most common gynaecological malignancy. Targeted therapies have yet to be implemented in EC. Two major types of EC have been recognized with specific features and different changes in the genetic setting. The most frequent genetic alteration of endometrioid endometrial cancer (type I) is PTEN. PI3CA and K-ras mutations are less common but are often associated with PTEN. Other genetic endometrioid endometrial cancer (type I) is PTEN. PI3CA and K-ras muta-ent changes in the genetic setting. The most frequent genetic alteration of

Disclosure: No conflict of interest disclosed.

P401
D-L-Methadone inhibits tumor growth significantly in acute lymphatic leukemia xenograft mouse model as well as in glioblastoma xenograft mouse model and improves therapeutic success of doxorubicin in vivo
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Introduction: Resistances to current treatment regimes are a major concern in oncology. Novel strategies are needed to improve therapeutic success. Cancer cells like leukemia and glioblastoma overexpress opioid receptors on their cell surface. Opioid receptors are the target of the opioid D-L-methadone. D-L-methadone induces apoptotic cell death in leukemia and glioblastoma cell lines as well as in patient-derived cells in vitro. By this way, D-L-methadone seems to be a promising approach for killing tumor cells. To prove the clinical relevance, we analyzed the anti-tumor efficacy of D-L-methadone in treatment of leukemia and glioblastoma in mouse models in vivo. Methods: Patient-derived ALL-xenografts were transplanted into NSG-(NOID/SCID/IL2r null)-mice and glioblastoma cells U87MG were transplanted into nude-mice. Opioid receptor-expression was analyzed using flowcytometry. At different time points after D-L-methadone and/or doxorubi-cin treatment tumor volume of xenografted tumors were measured. Serum level of methadone was analyzed by mass spectrometry. Results: In vitro, we found that D-L-methadone induces apoptosis and sensiti-tizes leukemia and glioblastoma cells for doxorubicin-induced cell death. Similar effects were found in patient-derived leukemia and glioblastoma cells ex vivo as well as in the highly resistant glioblastoma-initiating-stem cells. D-L-Methadone reversed deficient caspases activation by doxorubicin in leukemia and glioblastoma cells. Cotreatment of D-L-methadone and doxorubicin provoked downregulation of the anti-apoptotic proteins XIAP and Bcl- 2, both contributing to tumors’ resistance. In vivo, D-L-methadone or combined treatment using D-L-methadone and doxorubicin inhibited the growth of leukemia and glioblastomas significantly. Up to 4 h after D-L-methadone application, we found plasma concentrations of methadone in the range of 50–200 ng/mL in leukemia-xenografted NSG-mice and in glioblastoma-xenografted NUDE-
mice in a range of 30–1600ng/ml. These findings correlated with the concentrations showing cytotoxicity in vitro. 

Conclusions: Our studies demonstrate that D.L-methadone and co-treatment using doxorubicin and D.L-methadone significantly inhibited tumor growth in vivo. These findings suggest that D.L-methadone provides the foundation for new strategies using D.L-methadone as an additional antinecancer drug in cancer therapy to improve therapeutic success especially when conventional therapies are less effective.

Disclosure: No conflict of interest disclosed.

P402
Inhibition of Calcineurin/NFAT signaling breaks resistance of Ph+ ALL to imatinib

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Introduction: Approximately 20 percent of adult ALL cases are positive for the Philadelphia chromosome t(9;22) and express a Bcr-abl fusion protein with constitutive tyrosine kinase activity. While the majority of these leukemias initially respond well to imatinib, most of them develop TKI-resistance over time. NFAT is a family of highly phosphorylated transcription factors residing in the cytoplasm of resting cells. Upon dephosphorylation they translocate to the nucleus where they orchestrate diverse transcriptional programs. Several recent studies have demonstrated that NFAT signaling is involved in the pathogenesis of a wide array of hematological malignancies.

Methods: In this study, we investigated the role of NFAT signaling in the development of TKI-resistance in Ph+ ALL. We analyzed several Ph+ ALL cell lines, which had been shown previously to be resistant to imatinib, as well as primary leukemia samples from patients with TKI-resistant disease. NFAT expression and aberrant nuclear translocation was assessed by Western Blotting and the NFAT2 mRNA level was confirmed by quantitative RT-PCR. The cells were subsequently grown under optimized cell culture conditions in the presence or absence of TKI and cell viability was assessed using propidium iodide staining and flow cytometry. Proliferation assays were performed after 48–72 h.

Results: All cell lines and primary leukemia samples showed marked expression and evidence of aberrant nuclear translocation of NFAT2 when analyzed by Western Blotting. Even maximum concentrations of imatinib had only minor effects on cellular proliferation documenting resistance to TKI inhibition. To inhibit the calcineurin/NFAT signaling cascade as a potential therapeutical target, we treated the cells with the calcineurin inhibitor CSA in addition to imatinib. The combined treatment showed a dramatic effect on ALL cell proliferation, resulting in almost complete cell death after 48 h. This effect could be reproduced using tacrolimus indicating that it was specifically mediated through calcineurin inhibition and not by potential off-target activities of CSA.

Conclusions: Our data provide strong evidence that Calcineurin/NFAT signaling contributes to the proliferation of TKI-resistant Ph+ ALL cells and that its inhibition can break treatment resistance to TKI. Targeting NFAT signaling in combination with Bcr-abl inhibition might therefore be a novel option in the treatment of Ph+ ALL.

Disclosure: No conflict of interest disclosed.

P403
EVI-1 modulates apoptosis sensitivity in acute lymphoblastic leukemia cells via direct regulation of BCL-x

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Introduction: EVI-1, a member of the SET/PR domain family of transcription factors located on the human chromosome 3q26, is one of the most potent oncoproteins associated with myeloid malignancies and a predictor of poor clinical outcome in acute myeloid leukemia. Data on the expression and role of EVI-1 in lymphoid disorders are largely missing.

Methods: EVI-1 gene expression was analyzed by real-time PCR in lymphoid cell lines and primary cells from patients with pediatric acute lymphoblastic leukemia (ALL), chronic lymphocytic leukemia, acute myeloid leukemia and control healthy bone marrow. Protein expression was assessed by immunoblot. EVI-1 gene expression was modulated in NALM-16 and REH pre-B-ALL cells by transduction with inhibitory shRNA and lentivirus-mediated EVI-1 overexpression. Cell cycle, BrDU, apoptosis (% sub-G1 positive cells after PI treatment, Annexin-V/7-AAD staining, Caspase 3/7 and PARP activity after exposure to Staurosporine, Etoposide, Dacarbazine, Cytarabin and TRAIL), gene expression and chromatin immunoprecipitation assays were performed.

Leukemicigenic activity was assayed upon transplantation in NOD/SCID/IL2R-α-/- (NSG) mice using CD45, CD10, CD19 and TdT to detect human leukemic cells by FACS and histopathological staining.

Results: High EVI-1 expression was detected in a subset of pediatric ALL patients (5/33) and was associated with high risk disease (p=0.02). Knockdown of EVI-1 expression lead to increased spontaneous apoptosis and enhanced apoptosis sensitivity in response to staurosporine, TRAIL and chemotherapeutically, while overexpression exhibited protective effects. In vivo, inhibition of EVI-1 severely impaired leukemogenesis and conferred a survival benefit. Analysis of organ sections of mice transplanted with EVI-1 knockdown cells showed inhibition of organ infiltration by leukemic cells and enhanced levels on activated Caspase-3. Moreover, EVI-1 modulated expression of apoptosis-related genes like BCL2, BCL-x, XIAP, NOXA, PUMA, TRAIL-R1 and was shown to directly associate with the promoter of the anti-apoptotic protein BCL-x in chromatin immunoprecipitation assays.

Conclusion: Our data demonstrate that the EVI-1 oncogene can be expressed in ALL cells and promotes their leukemogenic properties by modulating apoptosis sensitivity via direct regulation of BCL-x. Future studies in larger patient cohorts are needed to explore the prognostic impact of EVI-1 expression in lymphoid neoplasia.

Disclosure: No conflict of interest disclosed.

P404
Induction of NK cell ADCC against Her2/neu expressing ALL blasts by Trastuzumab in vitro and long term clinical follow-up of Her2/neu positive ALL patients

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Introduction: Her2/neu (p185g,c2) is overexpressed and involved in disease pathophysiology in various epithelial malignancies and can successfully be
targeted with the monoclonal antibody Trastuzumab. Her2/neu is also expressed on acute lymphoblastic leukemia (ALL) cells in about one third of patients, and previous reports observed a tendency to a bad prognosis of patients with Her2/neu ALL, but as of now no study has performed survival analyses. Moreover, the binding of Trastuzumab to ALL blasts and its potential to induce antibody-dependent cellular cytotoxicity (ADCC) of NK cells has not been studied.

Methods: Patients records and flow cytometry data on Her2/neu expression at time of initial diagnosis of 57 patients (37 men, 20 women, median age 40 years, range 37–83 years) were analyzed. Statistical analyses were performed using unpaired Student’s T tests and Kaplan Meyer regression analysis. Activation, ADCC and cytokine release of allogeneic NK cells in cultures with patient leukemia cells upon exposure to Trastuzumab and Rituximab were determined by flow cytometry, cytoktotoxity assays and ELISA, respectively.

Results: Her2/neu was detected on ALL blasts in 15/57 patients (26%). The clinical course was followed for a median duration of 44 months (range 0.5–356 months). At the end of follow-up, 9 patients in the Her2/neu group (60%) and 25 patients in the Her2/neu group had died (60%). Median survival time was 41 months in the Her2/neu group (range 3.5–138 months) and 43 months in the Her2/neu group (range 0.5–356 months, p=0.81). Median time to relapse was 15.5 months (range 0.5–138 months) and 22.5 months (range 0.5–97 months, p=0.77) in the Her2/neu and Her2/neu group, respectively. Treatment of primary ALL cells with Rituximab and Trastuzumab increased in vitro NK cell reactivity with comparable effects being mediated by both antibodies and the combination of both resulting in further increased ADCC and cytokine production.

Conclusion: Her2 surface positivity was not correlated with disease free and overall survival in our patient cohort. However, as Trastuzumab potently induces NK reactivity against ALL cells as shown in our experiments, targeting Her2 may, alone or in combination with Rituximab, be a promising strategy for treatment of patients with Her2 positive ALL.

Disclosure: No conflict of interest disclosed.

P406
Knockdown of NOXA in patient-derived ALL cells reveals that distinct signaling pathways are activated by drug combinations compared to single drugs

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During polychemotherapy, cytoxic drugs are given in combinations to enhance their anti-tumor effectiveness. For most drug combinations, underlying signaling mechanisms responsible for positive drug-drug interactions remain elusive.

Here, we prove a decisive role for the Bcl-2 family member NOXA to mediate cell death by certain drug combinations, even if drugs were combined which acted independently from NOXA, when given alone. In proof-of-principle studies, Betulinic acid, doxorubicin and vincristine induced cell death in a p53- and NOXA-independent pathway involving mitochondrial pore formation, release of Cytochrome C and Caspase activation. In contrast, when Betulinic acid was combined with either doxorubicin or vincristine, cell death signaling changed considerably; the drug combinations clearly depended on both p53 and NOXA. Similarly and of high clinical relevance, in patient-derived childhood acute leukemia samples the drug combinations, but not the single drugs depended on p53 and NOXA, as shown by RNA interference studies in patient-derived cells.

Our data emphasize that NOXA represents an important target molecule for combinations of drugs which alone do not target NOXA. NOXA might play a special role in regulating apoptosis sensitivity in the complex interplay of polychemotherapy. Deciphering the differences in signaling of single drugs and drug combinations might enable designing highly effective novel polychemotherapy regimens.

Disclosure: No conflict of interest disclosed.

Posterdiskussion
Akute myeloische Leukämie

P407
Molecular cytogenetic screening for TET2 deletions serving as example for targeted diagnostics in patients with AML

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Introduction: In the last decade a whole bunch of novel somatic mutations recurrent in patients with acute myeloid leukemia were detected. Some of these mutations, like FLT3, NPM1, CEBPA or IDH1 and IDH2, have shown prognostic significance. Nevertheless, prospective screening for the numerous mutations is currently still time-consuming and cost-intensive. Therefore, targeted individualized diagnostic screening is of utmost interest. In this light we analyzed TET2 deletions in patients with AML and chromosome 4 aberrations deriving from the following aspects: 1. Acquired deletions and mutations in the TET2 gene, located on q424, are frequent in individuals with MDS and MDS progressing to secondary AML (sAML). 2. Recent reports have shown concomitant submicroscopic deletions in association with chromosomal translocations/insertions in several leukemia subtypes.

Methods: The databases of the three SAL trials AML96, AML2003 and AML60+ were searched for patients with structural 4q-aberrations (abnormal 4q- and trisomy 4, respectively. In case of available bone-marrow samples collected at diagnosis these specimens were screened for TET2-deletion by interphase FISH (XL TET2, MetaSystems, Germany).
Results: The Frequency of patients with abnormal 4q (n = 39) and +4 (n = 52), respectively, referring to those with aberrant karyotypes (in total n = 1560 patients) were 3% for each category. In 2891 cases a search for TET2 deletion could be performed with five patients (18%) showing a heterozygous TET2 loss. One of these patients had the diagnosis of MDS RAEB-2 and four an AML with myelodysplasia-related changes. Four out of these 5 deleted cases had complex aberrant karyotypes. Four patients died (n=2 patients during induction chemotherapy, n=1 patient in relapse six months after diagnosis, n=1 patient 5 years after diagnosis). One patient received an allogeneic HSCT in CR1 two months after diagnosis of sAML and is still alive at last follow-up.

Conclusions: Our investigations on AML patients with structural aberrations on chromosome 4q or +4 demonstrate a TET2-deletion in a reasonable frequency preferably in AML patients with myelodysplasia-related changes. The here described individualized, evidence adapted diagnostic screening using selected molecular markers might be a time and cost effective approach to rare AML entities in the future. In addition, in AML patients with abnormal 4q the detection of a TET2 deletion might help to clarify the history of the disease.

Disclosure: No conflict of interest disclosed.

P408 EZH2 mutations in childhood acute myeloid leukemia

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Introduction: Recently, we identified novel inactivating mutations of the histone methyltransferase gene EZH2 in myeloid disorders, most commonly in myelodysplastic syndromes, myeloproliferative neoplasms and the overlap myelodysplastic/myeloproliferative neoplasms (Ernst et al. Nat Genet 2010; 42:722–726). Here, we sought to determine the frequency and prognostic impact of EZH2 mutations in a well defined cohort of 75 randomly selected childhood acute myeloid leukemia (AML) patients (median age 10.3 years, range 0.0–20.1, 52% female).

Methods: PCR-reactions were performed using standard conditions with primers covering all 20 exons of the EZH2 gene, including its intron-exon boundaries to detect possible splice mutations. Sanger sequencing was carried out bidirectionally using an ABI 3600 Genetic Analyzer (Applied Biosystems).

Results: We identified one EZH2 mutation in a single childhood AML case. The patient was a 16-year-old female patient with FAB M2 AML without Auer rods, no involvement of the central nervous system but complicated by confirmation of leukemic blasts in bilateral pleural effusions. Cyto遗传ic analysis showed the karyotype 45,X;18(21)q12;q22. The mutation was a homozygous in-frame insertion of 6 bp within EZH2 exon 20 that was confirmed in a second independent analysis. On protein level, the mutation inserts the two amino acids lysine and threonine at positions 743 and 744 (SET domain), respectively, resulting in an abnormal EZH2 protein with a length of 753 amino acids. Analysis of a subsequent remission sample showed an absence of the mutation indicating that the EZH2 mutation is somatically acquired.

Conclusions: To our knowledge, this is the first report to describe a somatic EZH2 mutation in pediatric AML. Although EZH2 mutations seem to be rare in childhood AML our findings indicate that EZH2 mutations might contribute to the disease in specific cases. The incidence of EZH2 mutations in childhood AML needs to be assessed in a larger cohort of patients particularly in children with core binding factor leukemia.

Disclosure: No conflict of interest disclosed.

P409 Delta9-THC modulates methylation of oncogenes and tumorsuppressors in acute leukemia

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Introduction: Some cannabinol derivatives were shown to mediate antitumor efficacy. We have reported earlier that the CB1/2 cannabinoid receptor agonist Delta9-Tetrahydrocannabinol (THC), the major psychoactive component of marijuana, induces apoptosis in various leukemia cell lines (Kampa et al., DGHO annual meeting 2011). We now show that THC induces apoptosis in primary leukemic blasts, preferably in unidifferentiated leukemia. Further we here investigated the molecular mechanisms underlying treatment response.

Methods: CB1/2 protein expression was analyzed by flow cytometry. Ex vivo blasts derived from patients with acute leukemia were treated with THC in serial dilutions. Antiproliferative effects and induction of apoptosis were assessed using XTT-based and annexinV-based techniques. The CB1 antagonists LY320135 and JTE-907, a selective CB2 inverse ligand agonist, were used to confirm involvement of CB1 (mainly expressed in brain tissue) and CB2 (expression in hematologic/lymphoid cells). Global DNA methylation gene arrays using cell lines as well as patient samples were performed to unravel changes in methylation status upon THC treatment.

Results: CB1 as well as CB2 are highly expressed on leukemia cells in a subpopulation of investigated patients. Potent antiproliferative and proapoptotic effects of THC were observed with highly CB1/2-expressing cells in a dose dependent manner. Pretreatment with LY320135, but not with JTE-907, resulted in a dramatic abrogation of the antileukemic effect of THC monotherapy, arguing that THC-induced apoptosis is mediated via the cannabinoid receptor CB1. As more responders had lymphoid- or mixed phenotypes, we speculated that epigenetic modifications might be associated with MLL (mixed lineage leukemia) methyltransferase function. Global DNA methylation gene arrays verified that THC modulates global methylation of genes – including demethylation of histone deacetylases and tumorsuppressors.

Conclusion: Our results demonstrate that THC mediates strong ex vivo anti-leukemic activity in a subset of patients identified to express CB1 and provide new insights into the underlying functional mechanisms. Clinical evaluation of cannabinol receptor agonists as low-toxic agents could thus be considered in selected cases of patients with acute leukemia.

Disclosure: No conflict of interest disclosed.

P410 MicroRNA-143 interferes with ERK signaling in granulopoiesis of CD34+ hematopoietic progenitor cells and is downregulated in AML

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Mitogen-activated protein kinase (MAPK) pathways are a family of related and sometimes interconnected pathways and one of the most studied. Over the last years, extensive work has established that these proteins play a critical role in G-CSF mediated maturation of neutrophil granulocytes. Understanding the mechanisms by which the MAPK pathways are regulated represents an important area of investigation. MicroRNAs, a class of small non-coding RNAs, have been found to play an important role in the regulation of diverse cellular processes by binding to target mRNAs leading to their translational repression. Derepression of certain microRNAs thereby may lead to disrupted signal pathways, such as MAPK-signaling, and to tumorigenesis. However, the role of microRNAs in hematopoietic differentiation and blood cancer development remains largely unknown. In this study we performed a global screen to iden-
tify microRNAs involved in G-CSF-regulated MAPK-pathways in CD34+ hematopoietic progenitor cells. Here we found microRNA-143 (miR-143) to be frequently upregulated in G-CSF stimulated CD34+ with a strong correlation to CD15 expression. We could also show the granulopoietic association of miR-143 in several hematopoietic cell line models and AML patient samples. Especially, AML patient samples FAB M4 and M5, which show monocytic phenotypes, had a significant lower expression level of miR-143 compared to the other AML FAB types. In addition we show that miR-143 is upregulated in APL patients after ATRA treatment.

By an *in silico* prediction we found MAPK protein family members (eg. MAPK1, MAPK3 and MAPK7) as predicted targets of miR-143. Western blot analysis of AML patient samples and G-CSF stimulated CD34+ cells clearly show an inverse correlation of miR-143 and MAPK7 (ERK5) protein expression. By transient overexpression of miR-143 we could show a strong downregulation of ERK protein expression in NB4 cells. These studies suggest that miR-143 upregulation by G-CSF may be an important regulatory step for permitting neutrophil differentiation. This information may prove useful for the understanding of conditions in which neutrophil proliferative/differentiative balancing is dysregulated, such as in myeloid leukemia and myelodysplastic disorders.

**Disclosure:** No conflict of interest disclosed.

**P411**

**Transcription factor C/EBPα-induced microRNA miR-30c inactivates Notch1 during granulopoiesis and is downregulated in acute myeloid leukemia**

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**Introduction:** The transcription factor CCAAT Enhancer Binding Protein alpha (C/EBPα) is crucial for normal granulopoiesis and frequently disrupted in acute myeloid leukemia (AML). Mutations in the *CEBPα* gene are reported for about 10% of all AML. Loss of expression or function of C/EBPα leads to a block of myeloid differentiation. MicroRNAs inhibiting translation of mRNA into protein were identified as critical players in stem cell development and granulocytic differentiation. We and others have already shown that C/EBPα exerts its effects during granulopoiesis by regulating microRNAs such as miR-223 and miR-34a.

**Results:** In a global microRNA-array screen we found miR-30c as a novel target of C/EBPα during granulocytic differentiation. Wild-type C/EBPα-p42 upregulates miR-30c expression, whereas the C/EBPα-p30 mutant, found in AML does not. Furthermore, G-CSF upregulates miR-30c expression during granulocytic differentiation of primary human CD34-positive progenitor cells. C/EBPα induces miR-30c and downregulates Notch1, a putative target of miR-30c, on protein, but not mRNA level. A block of miR-30c by LNA pre-vents C/EBPα-induced downregulation of Notch1 protein expression. miR-30c is significantly downregulated in various subtypes of AML. Especially in AML patient samples with CEBPA mutations, miR-30c is significantly downregulated in comparison to AML patients without any CEBPA mutation. In mice, miR-30c shows a high expression in LSK (including hematopoietic stem cells), GMP (granulocytic monocyteic precursors) and granulocytes. An induced knock-out of C/EBPα in mice leads to a significant downregulation of miR-30c expression in bone marrow cells.

**Conclusions:** Our data indicates that C/EBPα-induced miR-30c inactivates Notch1 during granulopoiesis and is downregulated in AML. These data reveal the importance of deregulated microRNA expression in leukemia and may provide novel biomarkers and therapeutic targets in AML.

**Disclosure:** No conflict of interest disclosed.

**P412**

**Phosphorylated Heatshockprotein-90 is overexpressed in core binding factor leukemias (CBFL) and can effectively be targeted by the HSP90 inhibitor IPI-504**

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**Introduction:** Activating mutations of the KIT class III receptor tyrosine kinase (TK) are associated with the pathophysiologaly of acute leukemia, especially CBFL, and systemic mastocytosis (SM). Despite considerable antiproliferative and proapoptotic activity of several KIT TK inhibitors in vitro, clinical efficacy in AML and SM is in general moderate. We hypothesized that resistance to therapy is promoted by activation of alternative signaling pathways. Previously we reported that KIT TK inhibition results in significant upregulation of phosphorylation of heat shock protein (HSP) family members and that KIT is a client protein of phosphorylated (p) HSP90 putatively stabilizing KIT protein function in the presence of KIT TK-inhibitors (Kampa-Schittenhelm et al., ASH annual meeting 2010). This prompted us to further test the HSP inhibitor IPI-504 in KIT dependent CBFL, including the leukemic stem cell fraction.

**Methods:** Protein expression levels of (p)HSPs in leukemic blasts of CBFL patients were studied by flow cytometry focusing on the CD34+/CD38– leukemia stem/progenitor cell fraction. Cellular proliferation and induction of apoptosis in leukemia cells treated with the HSP90 inhibitor IPI-504 was determined by XTT- and annexin V-based assays.

**Results:** (p)HSP90/60 levels are preferentially upregulated in CBFL associating with KIT dysregulation. Consequently, HSP90 inhibition with IPI-504 potently degrades KIT expression causing a direct antiproliferative and anti-apoptotic effect in CBFL in vitro and ex vivo models. Efficacy of IPI-504 was potentiated when combined with TK inhibitors. Importantly, high expression of (p)HSP90 and HSP60 was in particular observed in the CD34+/CD38– leukemia stem cell fraction arguing for a function as protection mechanism to uphold (leukemic) stem cell function in the presence of cell stress such as antileukemic treatment.

**Conclusion:** HSPs are upregulated in CBFL, and IPI-504 induces antiproliferative and proapoptotic effects in primary leukemia samples. Importantly, the malignant stem cell pool in KIT-associated acute leukemia in particular expresses high levels of (p)HSP90, rendering HSP90 inhibition an attractive novel strategy to overcome the therapy-refractory behavior of malignant stem cells. These results provide a rationale for the evaluation of HSP90 inhibitors such as IPI-504 in CBFL.

**Disclosure:** No conflict of interest disclosed.

**P413**

**Role of the Apoptosis-stimulating protein of p53 2 (ASPP2 or TP53BP2) as a prognostic marker in acute myeloid leukemia**

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**Introduction:** Inactivation of the p53 tumor suppressor pathway by loss-of-function mutations is a frequent event in many cancers and promotes tumorigenesis and resistance to chemotherapy by impaired induction of apoptosis. But also cancer subtypes harboring a p53 wildtype isoform lack proper induction of programmed cell death. We and others have previously shown in a series of neoplasms that dysfunctional expression of apoptosis stimulating proteins of p53 (ASPP) may be involved. Acute leukemias typically lack p53 mutations and we have previously demonstrated that ASPP2 silencing in vitro contributes to attenuated therapy response (Kampa et al., DGHO annual meeting 2011). Here we assessed ASPP2 mRNA and protein expression in a larger cohort of patients with acute leukemia and studied association of aberrant ASPP2 expression with prognostic risk groups.
Methods: Quantitative RT-PCR was performed to analyze ASPP2 mRNA levels in 58 leukemia patients and blood donors. Protein expression levels were evaluated in treatment naive as well as anthracycline-treated patient samples using flow cytometry and immunoblotting. Small-interference (si) RNA experiments were employed to reveal involvement of ASPP2 in therapy response and tumorigenesis in functional viability assays using primary patient samples.

Results: mRNA and protein expression of ASPP2, a proapoptotic member of the ASPP family and haploinsufficient tumor suppressor, varies widely in leukemia specimens derived from newly diagnosed patients. Low ASPP2 expression associates with more aggressive course of disease. Moreover, failure to upregulate ASPP2 translation upon chemotherapy leads to higher risk of therapy resistance in *in vitro* and *ex vivo* leukemia models, which is in line with siRNA experiments abrogating ASPP2 transcription that confirmed a higher prevalence of resistance towards chemotherapy. Interestingly, siRNA-pretreated cells revealed accumulation of polyplid cells after anthracycline therapy, indicating mitotic failure due to lack of proper induction of apoptosis upon DNA damage, arguing for a role of ASPP2 dysfunction in early tumorigenesis.

Conclusions: Our results demonstrate aberrant ASPP2 expression in a subset of patients with acute leukemia. Furthermore we show a correlation of ASPP2 expression levels with therapy response. Prospective clinical studies are warranted to evaluate the role of ASPP2 as a prognostic marker in leukemia.

Disclosure: No conflict of interest disclosed.

P414

**Germline mutations in cancer predisposing genes** **BRCA1, BRCA2, BARD1 and TP53** **in patients with therapy-related myeloid neoplasms**


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**Introduction:** Therapy-related myeloid neoplasms (t-MNs) account for about 10% of all MDS/AML cases. They are regarded complex diseases originating from an interplay between exogenous toxicities and a susceptible organism. We hypothesized that in a subset of cases t-MNs develop in the context of hereditary cancer predisposition syndromes.

**Methods:** We systematically evaluated pedigrees of adult and pediatric patients with t-MNs for cancer incidences and the possibility of a hereditary cancer predisposition syndrome. In addition, we performed comprehensive mutational analyses by sequence and MLPA analyses and assessed deleterious heterogeneous germline mutations for loss of heterozygosity (LOH) in sorted CD34+ leukemic cells by SNP array.

**Results:** A nuclear pedigree was obtained in 51/53 patients with t-MNs resulting in a total of 828 individuals analyzed. With a standardized incidence ratio of 1.03 (95% CI, 0.74–1.39), the tumor incidence of first-degree relatives was not increased when compared to the population-based tumor registry of the Austrian province Tyrol. However, six pedigrees were suggestive of a hereditary breast and ovarian cancer syndrome, three of a Li-Fraumeni like syndrome and three index patients showed multiple primary neoplasms. Overall, we tested thirteen patients for **BRCA1**/2, 49 patients for **TP53** as well as **CHEK2** c.1100delC and eleven patients for the **BARD1** C577S variant. Mutational analyses revealed two pathogenic nonsense **BRCA1** (c.3112G>T, E1038X, c.521C>T, R1751X), one novel non-synonymous **BRCA2** (c.4027A>G, K1343E), two **BARD1** (c.1670G>C, C557S) and four deleterious **TP53** germline mutations (g.18508_18761delinsGCC; c.847C>T, R283C; c.845_848dupGCCG, R283fs*22; c.1146delA; K382fs*40) in 9/53 (17%) index patients with t-MNs. The majority of mutations were found in patients with breast cancer as primary malignancy (6/14, 43%). LOH in leukemic cells was demonstrated for the **BRCA1** c.3112G>T and **TP53** c.845_848dupGCCG mutations, respectively.

**Conclusions:** A higher than anticipated percentage of patients with t-MNs carries cancer susceptibility mutations which are likely to contribute to therapy-related leukemogenesis. Given an estimated prevalence of 1:20.000 in the general population, **TP53** germline mutations are highly enriched (4/53, 8%) in our cohort of t-MN patients. These results may also have far reaching consequences for surveillance of both, patients and families.

Disclosure: No conflict of interest disclosed.

P415

**Resistance to AC220 in FLT3-ITD** **AML is mediated by a secondary FLT3-ITD F691L mutation**


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**Introduction:** As **FLT3** mutations can be found in appr. 30% of all AML patients and FLT3-ITD is associated with an inferior clinical prognosis, FLT3 is considered as an attractive therapeutic target. Several TKIs targeting FLT3 have been developed and tested in AML patients.

**Case:** A 48-year-old female patient presented with a FAB M1 AML. BM cytogenetics revealed a normal karyotype whereas BM molecular cytogenetic analysis revealed a mutation of NPM1 and an FLT3-ITD mutation. After induction therapy according to the RAITFY study protocol no complete remission was achieved and salvage chemotherapy was started. After two months, a refractory disease was diagnosed and an allogenic HSCT was performed. On day 88 post transplant a relapse was diagnosed. After 4 weeks of Sorafenib treatment and a second allogenic HSCT a complete molecular remission was achieved lasting for three months. Upon relapse, treatment with AC220 was initiated within a clinical trial. After initial response to AC220 a relapse was diagnosed with 60% BM and 67% PB blasts 4 months later. During the course of AC220 treatment FLT3-ITD was sequenced in blast cells from the patient’s PB. Before AC220 therapy no secondary mutation in FLT3-ITD was detectable, whereas at the time of relapse during AC220 treatment a novel FLT3-ITD F691L was identified. FLT3-ITD F691L was cloned, and drug response to AC220 was examined in Ba/F3 cells. Whereas FLT3 activity as well as the proliferation of cells expressing unmutated FLT3-ITD was greatly reduced upon AC220 treatment at nanomolar concentrations, the highly resistant F691L mutation shifted AC220 response 100-fold. This FLT3-ITD F691L mutation induced strong AC220 and Sorafenib resistance *in vitro*, but retained sensitivity to PKC412 and Sunitinib. Based on these findings, treatment with Sunitinib was started. Initially the patient responded. However, after a period of 2.5 months another relapse was diagnosed and the patient subsequently died.

**Conclusion:** Our analyses underline the potency of the inhibitor AC220 to inhibit unmutated FLT3-ITD at low nanomolar concentrations. We show that FLT3-ITD secondary mutations F691L and F691I induce strong resistance to this compound. Importantly, these secondary FLT3-ITD mutations are sensitive to Sunitinib and PKC412. Thus, secondary mutations warrant careful consideration of the specific TKI to optimize the treatment of AML.

Disclosure: No conflict of interest disclosed.

P416

**Physiologic hypoxia of 6% O2 regulates FLT3-ITD expression and function in AML cell lines harbouring a FLT3-ITD mutation**

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**Introduction:** FLT3-ITD are found in appr. 40% of normal karyotype AML and confer to a bad prognosis. While initial chemosensitivity is not impaired in these patients, the risk of relapse is substantially higher. The reason for the higher rate of relapse in FLT3-ITD patients is currently unknown, but might...
result from hitherto unknown interactions with the microenvironment (i.e. hypoxia) that provide a survival advantage in FLT3-ITD positive AML blasts.

**Material and methods:** FLT3-ITD positive AML cell lines MV4-11 (ITD +/+ ) and Molm13 (ITD −/+ ) were compared to FLT3-WT cell lines OCI-AML3 and KG1a. Experiments were carried out in parallel under standard laboratory conditions and reduced oxygen environment of 6% O2. Applied methods comprised cell culture (cell proliferation determined by Tripans blue exclusion), FACs (Cell cycle analysis, apoptosis) and western blot.

**Results:** FLT3-ITD cell lines MV4-11 and Molm13 showed a decrease in proliferation after 48-72 hours of 6% O2 while in FLT3-WT cell lines OCI-AML3 and KG1a the proliferation was not impaired. This decrease in proliferation was not due to increased apoptosis, as there was no increase in annexin-V expression. Instead, FLT3-ITD cell lines showed an arrest at 6% O2 in G1 phase. This effect seemed to be lost after long term culture. To exclude the possibility that FLT3-ITD signalling conferred to the cell cycle arrest, FLT3-ITD was inhibited by sorafenib at nanomolar concentrations, however with no effect on proliferation. Instead, total FLT3 protein expression was down regulated in FLT3-ITD cells, cultured under hypoxic conditions. The down regulation was most pronounced in MV4-11, carrying the mutation in both alleles. In the FLT3-WT cells, no downregulation of FLT3 was seen, suggesting that this loss of FLT3 only affects FLT3-ITD. Consequently, FLT3 downstream targets JAK and STAT were less activated in the FLT3-ITD cell lines at 6% O2 as compared to 21% O2, possibly explaining the loss of proliferation.

**Conclusion:** Physiological hypoxia of 6% O2 regulates FLT3-ITD expression and subsequently JAK-STAT phosphorylation in AML cell lines harbouring an FLT3-ITD. This results in decreased proliferation and cell cycle arrest. Further investigation on the mechanisms of down regulation of FLT3-ITD and molecular consequences of this loss are ongoing.

**Disclosure:** No conflict of interest disclosed.

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**P417**

Quantitative expression of Toll-like Receptor -1, -2, -3, -5, -7, and -9 in blasts of patients with newly diagnosed or relapsed acute myeloid leukemia

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**Objectives:** Toll-like receptors (TLRs) are well known to play an essential role in the immune response. As previously described TLR-2, -4, and -9 are expressed on different levels in dendritic cells derived from blasts of patients with acute myeloid leukemia (AML). It is known that dendritic cells express TLRs which identify so called pathogen-associated molecular patterns (PAMPs) and by this way trigger the maturation of dendritic cells and production of cytokines. By this way the innate and adaptive immunity are activated. The aim of our study was to evaluate the expression of TLR-1, -2, -3, -5, -7, and -9 in blasts of patients with AML and compared it the expression of mononuclear cells of healthy volunteers.

**Methods:** We analysed the expression of the above mentioned TLRs with a quantitative real-time polymerase-chain-reaction in blasts generated of whole blood of 6 patients with newly diagnosed or relapsed AML, the AML cell line Kasumi 1, and monoclonal cells of healthy volunteers.ABL was used as endogenous reference. The median patients age was 57 years (range 38–60 years). Three patients had relapsed AML after allogeneic stem cell transplantation (aSCT). 2 patients a relapse before aSCT, and 1 patient had a newly diagnosed AML. AML classification was as follows: M4 n=2, M5 n=2, unspecified n=2.

**Results:** In AML blasts and in mononuclear cells of healthy volunteers all analysed TLRs were highly expressed. TLR-1 and -2 were seen to be expressed at a lower level than compared to TLR-3, -5, -8 and -9, respectively. The highest level of TLR expression was seen for TLR-7. We could see a slightly different expression of TLR-2 and -3 in healthy volunteers compared to patients with AML. For TLR-2 we had a higher expression in healthy volunteers with a median range of 9936% compared to 1888%. For TLR-3 the level for healthy volunteers was lower in contrast to the level of patients with AML (median 155862% and 487949%, respectively). But due to the small numbers of analyses this difference was not significant.

**Conclusion:** We could demonstrate in our study an expression of all tested TLRs in leukemia blasts. There was no significant difference compared to the expression of TLRs in healthy volunteers. We could detect a slight difference for TLR-2 and -3, but due to the small number of patients, further analyses are needed to confirm these results.

**Disclosure:** No conflict of interest disclosed.
Smac mimetic BV6 sensitizes human AML cell lines to Cytarabine induced apoptosis and affects proliferation and differentiation of human hematopoietic stem cells

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Introduction: Since most anti-cancer therapies primarily act by inducing apoptosis in cancer cells, defects in the apoptosis programs can lead to treatment resistance. "Inhibitor of Apoptosis Proteins" (IAPs) are a family of proteins, which block apoptosis by inhibition of effector caspases and are involved in regulation of NF-κB. In many tumors, including AML, IAPs are highly expressed which is associated with a poor prognosis.

Methods: AML cell lines were treated with BV6, a bivalent IAP Inhibitor, and Cytarabine. Apoptosis was determined by FACS. Western Blot and immunoprecipitation was performed to analyze target molecules of BV6 and effector proteins of the apoptosis machinery. CD34+ cells of healthy donors were seeded in CFU assays upon BV6 treatment. Colonies were analyzed by microscopy and FACS. Proliferation assays were performed upon BV6 treatment of multipotent progenitors isolated of cord blood.

Results: We found a concentration- and time-dependent induction of apoptosis in AML cell lines by Cytarabine or BV6. Importantly, BV6 sensitized AML cells for Cytarabine-induced apoptosis in a highly synergistic manner as demonstrated by the calculation of combination index (CI<0.1). BV6 and combination treatment led to Casp1, Casp2 and XIAP degradation, caspase 3, 8 and 9 activation and caspase-8/FADD/RIPI complex formation, indicating simultaneously induction of intrinsic and RIPI-dependent apoptosis. Interestingly, we discovered that BV6-induced cell death was even increased in the presence of the caspase inhibitor zVAD.fmk in some cell lines. This increased cell death was inhibited by Necrostatin-1, thereby pointing to a switch of cell death from apoptosis to necroptosis. In MPP we could detect a dose-dependent inhibition of proliferation upon BV6 treatment. This effect lasts at least 2 weeks and developed more pronounced over the time. BV6 had no effect on proliferation and differentiation in CFU assay after first plating. We detected decreased colony numbers and differentiation at low concentrations of BV6 after the first replating, indicating no direct apoptosis-inducing effect on hematopoietic progenitors but modulation of differentiation and self renewal capacity.

Conclusions: These findings show highly synergistic effect on apoptosis induction by BV6 and Cytarabine in AML cell lines. CFU and proliferation assays indicate a long term effect of BV6 in low concentrations on proliferation and differentiation of hematopoietic progenitors.

Disclosure: No conflict of interest disclosed.

Enhanced senescence of mesenchymal stromal cells from AML patients

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Introduction: The microenvironment in the bone marrow has tremendous effects on maintenance, regulation, and protection of hematopoietic stem cells (HSC). These features are not restricted to the healthy system, but might also have strong impact on progress and the development of resistance of leukemia. In this project, we compare mesenchymal stromal cells (MSC) from patients suffering from acute myeloid leukemia (AML) and healthy donors as surrogate niche models to mimic and analyze the environment of a leukemic stem cell niche. Through characterization of MSC from leukemic patients vs. healthy donors we want to identify how the stem cell niche is affected by AML.

Methods: MSC were isolated from human bone marrow samples of healthy donors and AML patients after ethical approval by red blood cell lysis. This allows preparation of MSC even with low volume samples. The cells were expanded and used for initial characterization, analysis of CFU-F frequency and long-term culture. RNA of early passages and senescent cells after long term culture was isolated to examine gene expression (GeneChip Human Gene 1.0 ST Array, Affymetrix) profiles.

Results: After isolation, the majority of AML-MSC already displayed a senescent phenotype and cell shape, decreased CFU-F frequency, and a significantly decreased proliferation capacity showing a decreased number of cumulative population doublings upon long-term culture. Comparison of gene expression profiles of healthy vs. AML-MSC, including early and senescent cell passages, showed clustering of AML samples and senescent passages of healthy donor derived MSC, confirming our previous observations on molecular level. 124 genes showed to be significantly differentially expressed between early passages of AML-MSC vs. MSC from healthy donors, with 60 of them overlapping with the profiles of healthy senescent samples. Among these genes were that is associated with growth size phenotype, aging, cancer, and cell-cell signaling and adhesion.

Conclusions: These data show that bone marrow MSC of AML patients already possess a senescent status or are more sensitive to cellular aging, possibly due to enhanced stress for a leukemic microenvironment. These characteristics and data will be used to search for new starting points of leukemic therapy, to inhibit the development of resistance and relapse, and to identify specific signals and interactions within a leukemic environment in the human bone marrow.

Disclosure: No conflict of interest disclosed.
APL. Our data reveal the importance of deregulated miRNA biogenesis in cancer and may provide novel biomarkers and therapeutic targets in myeloid leukemia.

Disclosure: No conflict of interest disclosed.

P422

AML cells differ in functional and molecular response towards hypoxia according to their differentiation

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Introduction: Mounting evidence implies a physiologically hypoxic environment in the bone marrow. Own and data of other groups suggest this hypoxia 6 % O₂; however this concentration is more likely to be the median of several different O₂ levels residing in the bone marrow. Especially the hematopoietic stem cell niche is assumed to bear oxygen levels of 1 % O₂ or even lower. Here, hypoxia is attributed to differentiation and proliferation. The effects of hypoxia, especially very low oxygen concentrations, on AML blasts or leukemic stem cells are however unknown.

Material and methods: Cell culture experiments were performed under standard laboratory conditions and reduced oxygen environment utilizing a human leukemia (AML) CD96 could be identified as LCS marker (PNAS 104: 11088, 2007). AML cell lines NB-4 and KG1a were used for experiments. Applied methods comprised cell culture, FACS (cell cycle analysis, apoptosis) and western blots for protein expression and phosphorylation.

Results: Under physiological hypoxia of 6 % O₂, 24–72 hours the AML cell lines OCI-AML3, NB-4 and KG1a showed unimpaired survival and proliferation compared to normoxia. However, exposure to 1 % O₂ all cell lines displayed a marked decrease in cell numbers compared with normoxic or physiological hypoxic conditions. While the CD34+ cell line KG1a featured a reduction of proliferation with good viability, NB-4 cells, a CD34- promyelocytic leukemia, showed decreased survival with strong induction of apoptosis. As potential reason of the reduced proliferation in CD34+ AML we observed a GI arrest, probably due to induction of cell cycle regulatory proteins p27 with consecutive decrease of cyclin E in at 1 % O₂. Additionally, these cells showed both increased activation of pro-survival pathways Akt and ERK at 1 % O₂ and induction of antiapoptotic proteins (particular XIAP) already at 6 % O₂. None of these activations and/or inductions were observed in CD34- NB-4 cells.

Conclusion: These data imply that depending on their maturation state AML cells react differently to hypoxia: while immature AML cells go into cell cycle arrest at very low levels of oxygen, higher differentiated AML cells seem unable to survive severe hypoxia due to differential activation/induction of pathways involved in apoptosis, cell cycle and survival. Further investigations on the molecular mechanisms and functional consequences are ongoing as well as verification of these results in primary cell AML cells.

Disclosure: No conflict of interest disclosed.

P423

Depletion of CD96 positive leukemic stem cells (LSC) provides a rationale for autologous SCT in AML

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Introduction: Enthusiasm for autologous stem cell transplantation (ASCT) is limited due to residual leukemic stem cells (LSC). For acute myelogenous leukemia (AML), CD96 could be identified as LSC marker (PNAS 104: 11088, 2007). Here, strategies for engineering autologous stem cell grafts deplete of CD96 positive AML-LSC using magnetic cell sorting (MACS) as well as for in vivo targeting of AML-LSC by antibody dependent cellular cytotoxicity (ADCC) are described.

Methods: The CD96 antibody TH111 was developed in our laboratory (Exp. Hematol. 26: 1209, 1998) and used to deplete by MACS early AML cell line KG-1a as surrogate for LSC. Efficiency of the procedure and its potential influence on the viability of normal hematopoietic progenitor cells (HPC) and differentiation were analyzed by flow cytometry and colony forming assays, respectively. ADCC-optimized and in vitro affinity matured CD96 antibodies were generated by recombinant DNA technologies. Antibodies were characterized by SDS page and flow cytometry for purity and specific binding. Antibody-mediated effector functions were analyzed in 51Cr-release assays.

Results: In order to test puring efficacy towards LSC of AML, a stem cell containing preparation was spiked with the CD96 positive AML cell line. Using CD96 antibody TH111, up to a 100-fold depletion of CD96 positive cells was achieved by MACS. Normal HPC were not affected as indicated by cell count, viability, and the potential to proliferate and differentiate. Chimeric antibodies containing wild type or affinity matured variable regions in combination with an ADCC optimized human IgG1 Fc were generated. Not only antigen binding affinity of the matured antibody was enhanced (EC50: 0.6 μg/ml vs. 2 μg/ml), but also NK cell mediated lytic properties against CD96 positive target cells were elevated (EC50: 0.02 μg/ml vs. 0.15 μg/ml; E:T ratio 2:5:1).

Conclusions: CD96 antibody TH-111 can be used efficiently to deplete LSC of AML from autologous stem cell containing preparations, without affecting normal HPC viability and proliferation or differentiation properties. A chimeric affinity matured antibody targeting CD96 was able to recruit donor NK cells and displayed enhanced lytic activity against CD96 positive targets. Particularly in combination with the application of CD96 antibody in vivo in a MRD-situation, this ability to clear CD96 positive LSC from autologous grafts may revitalize ASCT in AML patients.

Disclosure: No conflict of interest disclosed.
Conclusion: Thus, there is good evidence that the two novel AML stroma cell lines are suitable candidate cell lines to model the networks between AML stroma and cells of the immune system.

Disclosure: Tobias May: InSCREENEx GmbH, Inhoffenstraße 7, 38124 Braunschweig, Germany: employment, shareholder, filed patent for immortalization technology.

No conflict of interest disclosed.

P425 Sorafenib for primary refractory or relapsed FLT3-ITD+ acute myeloid leukemia: The Tübingen experience

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Introduction: Patients with acute myeloid leukemia (AML) harboring FLT3 receptor mutations have a poor prognosis. Promising response rates with single agent therapy using the multikinase inhibitor sorafenib have been reported in FLT3-ITD+ AML. We report our single center experience with sorafenib in patients with primary refractory or relapsed FLT3-ITD+ AML.

Methods: Patients with FLT3-ITD+ AML treated with sorafenib were identified from our institutional data base. Data were confirmed by retrospective chart review.

Results: 6 patients (4 female, 2 male, median age 47 years, range 38–68) diagnosed with FLT3-ITD+ AML between 2008 and 2011 were treated with sorafenib for primary refractory (n=3) or relapsed (n=3, 1 molecular relapse after hematopoietic cell transplantation, HCT) disease. 4 patients also showed mutations in the NPM1 gene and had a normal karyotype. At treatment initiation, 4 patients had 5–62% blasts in the peripheral blood and all but one patient (NPM1+ MRD) had 55–77% blasts in the bone marrow. Sorafenib was started at a dose of 200 mg (n=2) or 400 mg (n=4) bid. Main side effects were diarrhea, nausea, rash, palmar-plantar erythrodysesthesia and cytopenias. After a median of 24 days (6–26), clearance in the peripheral blood and after a median of 29 days (14–57) cytological complete remission (CR) in bone marrow was observed in all patients. Three patients (2 primary refractory AML, 1 relapse post HCT) could be treated in CR with further salvage therapy (2 allogeneic HCT, 1 experimental immunotherapy). After a follow-up of 72 and 90 days, two were alive and in CR; the other patient died from multi-organ failure 39 days after HCT. One patient with NPM1+ MRD post HCT achieved molecular CR, received no further therapy and is alive and well. One patient treated with sorafenib for relapse after HCT achieved initial CR, but relapsed again after 94 days. She was finally treated with AraC and idarubicin (7+3) resulting in CR. Salvage HCT is planned. One patient with primary refractory AML refused further treatment with sorafenib after achieving CR, relapsed and died after 377 days. In total, 4/6 primary refractory or relapsed AML patients are alive after initial salvage treatment with sorafenib after a follow-up of 72–458 days.

Conclusions: Treatment with sorafenib in advanced stage FLT3-ITD+ AML shows a high CR rate and enables further consolidation therapy such as allogeneic HCT.

Disclosure: No conflict of interest disclosed.

P426 Cladribine, cytarabine and idarubicin (CAI) for remission induction in patients with relapsed AML – a phase II study

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Introduction: Currently, there exists no standard therapy for patients with relapsed AML. High-dose cytarabine (AraC) in combination with an anthracycline or anthrachinone is chosen mostly. CR rates are lower than in newly diagnosed patients and range between 15% and 50%. Purine analogues like cladribine (2CdA) and fludarabine enhance intracellular concentrations of active AraC metabolites and thus can overcome resistance mechanisms. In clinical trials, 2CdA has shown single drug activity in AML with low toxicity. Therefore, a combination therapy with 2CdA, AraC and idarubicin (Ida) seems reasonable. Here we present data from a phase II trial evaluating CAI in relapsed AML patients.

Methods: CAI consisted of two courses 2CdA 5 mg/m²/12 h, d 1–3, AraC 1000 mg/m²/12 h, d1–3 and Ida 8 mg/m²/d, d1–3. After 8 patients (pts.), the prolonged duration of neutropenia especially in course 2 prompted us to change the protocol with 1) application of growth factors from day 15 onwards, and 2) omit Ida in course 2. Results: Eighteen pts. are included so far (56% male). Median age was 61 years (range 43–77 years). First relapse was diagnosed in 94% of pts., a 2nd relapse in one patient. Concomitant pulmonary disease was seen in 6 (33%) pts. and cardiac disease in 6 pts. (33%). To control hyperleukocytosis, AraC civi with a maximum dose of 170mg was given in two pts. After the first course, CR was achieved in 39%, CRi in 22%, and PR in 11% of pts. Four pts. were refractory and one patient died early. Median duration of neutropenia was 23 days (range 18–41d). Six pts. received a second course of CAI/CA.

At the end of treatment, response rate was 44% CR, and 61% CR/CRi/PR. In one patient with CRi after course 1, response was unknown at the end of CAI. Another patient with CRi after course 1 relapsed after course 2. Eight pts. received an allogeneic stem cell transplantation after CAI. Main non-haematologic toxicity grade 3 or 4 was infection in 96% of courses. Hepatotoxicity occurred in 13%, nausea in 33%, and diarrhea in 13% of courses. Cardiac toxicity or nephrotoxicity grade 3/4 were not observed.

Conclusions: Combination therapy with CAI appears to be feasible and successful in patients with relapsed AML. However, infections are a serious complication warranting intensive supportive care. The trial will be continued and duration of remission and overall survival will be evaluated later. Supported by Lipomed AG and Leukämie-Initiative Bonn e.V.


P427 Long-term outcome for patients with acute myeloid leukaemia and myelodysplastic syndrome after reduced-intensity conditioning for allogeneic hematopoietic cell transplantation

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Allogeneic hematopoietic cell transplantation (HCT) is an effective treatment for patients with acute myeloid leukaemia (AML) and myelodysplastic syndrome (MDS). Reduced-intensity conditioning (RIC) regimes have been developed with the aim to perform safer allogeneic HCT in patient populations previously discarded because of higher HCT-related mortality. Between April 1999 and December 2009, a total of 95 consecutive patients (44 male, 51 female) with de novo AML (n = 62), secondary AML (n = 23) and MDS (n = 10) underwent reduced-intensity conditioning (RIC) followed by allogeneic HCT. RIC consisted of fludarabine/body total irradiation of 2 Gy according to the Seattle protocol in 21 patients (22%), the FLAMSA protocol in 70 (74%) and other chemotherapy regimens in 4 patients (4%). All patients were ineligible for myeloablative HCT because of age or comorbidities. Donors were siblings in 31 (33%) patients and unrelated (URD) in 64 (67%) patients. The majority of patients received granulocyte-colony stimulating factor (G-CSF) mobilized peripheral blood stem cells (n = 91, 96%). Graft-versus-host disease prophylaxis consisted of cyclosporine A and mycophenolate mofetil in the majority of patients. With a median follow-up of 37 (range, 6–137) months, 39 patients (41%) are alive. Overall survival (OS) and disease free survival (DFS) rates at 5 years for the whole cohort were 40% and 57%, respectively. OS and DFS rates at 5 years for patients with AML was 43% and 56%, for patients with MDS 20% and 71%, respectively. The cumulative incidence of non-relapse mortality at 5 years was 30% and was significantly higher for patients with MDS (70%) than for patients with AML (24%; p<0.01). No difference in OS and DFS projected at...
Background: Prior trials have demonstrated efficacy and effectiveness of posaconazole in the prophylaxis of invasive fungal diseases (IFDs) in high-risk patients. Controversy exists about the cost effectiveness of posaconazole prophylaxis in neutropenic patients with a high risk of invasive fungal diseases. We performed an analysis comparing the direct costs of posaconazole prophylaxis against topical polyene (thrush) prophylaxis in patients with acute myelogenous leukemia (AML) and myelodysplastic syndrome (MDS).

Methods: Data of AML/MDS patients receiving remission-induction chemotherapy were analysed to compare hospital costs of patients before (2003–2005) and after (2006–2008) introduction of posaconazole prophylaxis. All cases were part of an earlier analysis demonstrating effectiveness of posaconazole over topical prophylaxis (Vehreschild et al., J Antimicrob Chemother. 2010 Jul;65(7):1466–71). Duration on general ward, intensive care unit, mechanical ventilation, diagnostic procedures and all anti-infective drugs were included into the cost analysis.

Results: Patient groups were well matched according to age, gender, underlying disease, and duration of neutropenia. The average cost per patient in the posaconazole group (n=76) and the topical polyene group (n=81) were 21,040 € (95% CI: 18,204–23,876 € ) and 23,169 € (95% CI: 19,402–26,937 € ) per patient, respectively. Antifungal treatment costs were nominally higher in the posaconazole group (4,380 € [95% CI: 3,678–5,482 € ] vs. 4,019 € [95% CI: 2,825–5,214 € ]). The costs for antibacterials (1,316 € [95% CI: 1,039–1,593 € ] vs. 1,333 € [95% CI: 1,238–1,827 € ] ) and antivirals (159 € [95% CI: 57–261 € ] vs. 165 € [95% CI: 75–254 € ] ) were numerically decreased in the posaconazole group. Average duration of ICU stays were 1.79 (95% CI: 0.68–2.90) days per patient for the posaconazole group compared to 3.83 (95% CI: 1.53–6.13) days per patient in the topical polyene group. Costs for diagnostic procedures were 611 € (95% CI: 478–744 € ) and 653 € (95% CI: 552–754 € ) per patient, respectively.

Conclusions: In our hospital, there was a trend towards cost-saving by posaconazole prophylaxis in patients receiving remission-induction chemotherapy. These cost savings were primarily caused by a shorter overall length of stay and the less frequent ICU treatment of patients receiving posaconazole. Posaconazole improved patient outcomes without adding the treatment costs of AML patients.


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Global heart failure after induction therapy for acute myeloid leukemia

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Introduction: The use of anthracyclines (AC) is limited by a well-known dose-dependent cardiotoxicity. However, acute heart-failure (HF) due to AC is a rare but severe event. Here we report on a patient with acute myeloid leukemia and HF after first exposure to idarubicin.

Case report: A 21 year old male patient received induction chemotherapy analogue to the OSHO #61 protocol because of acute myeloid leukemia, FAB M1 with trisomy 8 and 10. We administered Idarubicine 78 mg abs. and cytarabine 17.6 g abs. 9 days after chemotherapy he developed neutropenic fever and antibiotic therapy was changed to vancomycin and ceftazidime. 5 days later he complained dyspnea, symptoms of hypoxia as well as tachycardia. The computer tomography of the chest displayed no pathological findings, especially no pneumonic infiltrates. Due to rapid cardiopulmonal impairment the patient was displaced to the intensive medical care unit. Echocardiography revealed chamber dilation of the left ventricle, generalized hypokinetik wall motion and a reduced pumpfunction of the right ventricle. Left and right atrium showed a normal size and kinetic, and a left ventricular ejection fraction (EF) of 30%. The maximum of NT-proBNP was 14628 pg/ml (standard value <84 pg/ml). Prior to the development of HF the patient had no history of coronary artery disease or illegal substance abuse and a EF of 66%. Due to persisting sepsis, antimicrobial therapy was changed to tigecycline, cipro...
Azacitidine in an AML patient under hemodialysis

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Treatment of Acute Myeloid Leukemia undergoing hemodialysis is challenging since there are no specific management guidelines for the administration of cytotoxic agents. Standard chemotherapy protocols, such as a combination of idarubicin and cytarabine, could be inappropriate due to altered drug pharmacokinetics and an increased risk of complications e.g. infections because of the co-morbidities in these patients. Recently, hypomethylating agents such as azacitidine have been shown to be active in myelodysplastic syndromes and acute myeloid leukemias. Despite the fact that these agents are generally well tolerated, to our knowledge the use of Azacitidine has never been reported for patients with AML undergoing Hemodialysis. We report about the use of Azacitidine in a 63 year old man suffering from Diabetes, Hypertension and coronary heart disease. He suffered from chronic renal failure and hemodialysis was instituted in 2009. He was admitted to our hospital because of Fatigue and a Syncope in May 2011. A secondary acute myeloid leukemia, FAB M1 was diagnosed. The karyotype of the leukemic blasts was normal and no NPM1 or FLT3 mutation could be detected by molecular analyses. The initial leukocyte count was 20,000/ul with 40% myeloblast, hemoglobin level was 9 g/dl and thrombocyte count was 529,000/ul. After informed consent was given he was treated with Azacitidine at a daily dose of 50 mg/m² (100mg absolutely) intravenously daily for 5 consecutive days every 4 weeks. Azacitidine was administered after hemodialysis. A total of 8 cycles was applied. The treatment was favorably tolerated. During the treatment the leukocyte count went into the normal range with 40% granulocytes. The hemoglobin level remained stable at about 9 g/dl and the thrombocyte count remained in the upper normal range. No growth factor supply was necessary and the patient remained free of severe infectious complications over the whole treatment period.

In February 2012, 10 months after initial diagnosis, the leukemia progressed. It was refractory to an alternate chemotherapy with hydroxyurea and the patient succumbed to a fungal infection. Taken together this 5 day regimen of Azacitidine in a reduced daily dosage (50 mg/m²) is feasible in AML patients who are undergoing hemodialysis and shows antileukemic activity. Further analysis are necessary to define the optimal dose of Azacitidine in this high risk patients.

Disclosure: No conflict of interest disclosed.

LaSRT (Large-scale real-time titration): a recombinant virus titration method simple, safe and efficient for pre-clinic gene transfer research

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Background: Titer of recombinant virus (RV) is the most accurate and important index, however, classic titration method need to be improved.

Aims: To establish and evaluate a simple, safe and efficient titration method (LaSRT) for pre-clinic gene transfer research.

Methods: 1. Virus: Lentivirus with GFP maker was produced by PWPXL4, pMD2.G, pCMV/RS.V4.3 vector-system (Addgene Co.) and 293T/17 cell (ATCC). Retrovirus with puromycin resistant gene was generated by pMSCVpuro vector (Clontech Co.) and Plat-E cell (Cellbiolabs Co.), 293 cells (ATCC)
are used as titration target cells. Virus supernatants from 48h to 72h were used.

2. Classic titration: For lentivirus with GFP, FCM titration was used as control. 2 x 10e5 293 cells were transfected with serial dilutions of supernatant as 1ml, 100 μl and 10 μl (n=3). Titer (TU/ml)=(2 x 10e5 target cells) x (positive cells)/volume of supernatant (ml). For pMSCVpuro retrovirus, 2-week-clone-forming method were used as control.

3. LaSRT titration (see Fig. 1): plate 10 000 293 cells/well in a 96 wells-plate, each well with 180ul IMDM, 10% FCS and 4 μg/ml Protamate sulfate (Elkins-Sinn, Inc.); 24h, add 20μl virus supernatant to the first well then to limited dilute at 1:10 in the following wells (n=3). 3±2d, observe GFP+ cells under inverted fluorescence microscope, the positive cell numbers (M) would only be counted in the “counting well” (the last well where the M≥10, and no positive cells in the next well), the serial number (N) of the counting well is used for titer calculation.

4. Titer Transfusions (TU/ml)=M x10^N±1

5. For pMSCVpuro retrovirus, the only difference is to count survival cells in the counting well on +4d.

Results: No significant difference was seen between the results of pWPXlαD GFP-lentivirus got by FCM vs LaSRT. (5.3±1.5)x10^5 vs (5.1±1.3)x10^5, P>0.5. For pMSCVpuro retrovirus, the only difference is to count survival cells in the counting well on +4d.

Disclosure: No conflict of interest disclosed.

![Detection process of LaSRT for GFP recombinant virus](image)

Fig. 1. for abstract P432

P434

HLA-peptide multimer selection of adeno-virus specific T-cells for the purposes of adoptive T-cell therapy of adeno-virus infections post haematopoietic stem cell transplantation

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Introduction: Adeno-virus infection following haematopoietic stem cell transplantation affects 5–28% patients, with a mortality rate of 50%. There are currently no pharmaceutical agents licensed for treatment in this setting. T-cells are critical for the control of adeno-virus infection and thus adoptive T-cell therapy is an attractive therapeutic option. The role of CD8 T-cells in clearing adeno-virus infection is not fully understood as their low frequency hinders studies in healthy donors.

Methods: We generated HLA-α peptide multimers (tetramer) for 8 HLA Class I restricted epitopes from the adeno-virus hexon protein, which are highly conserved across adeno-virus species. Epitope specific T-cells from healthy donors were characterised in terms of frequency, phenotype and functionality using flow cytometry.

Results: Ad-specific tetramer staining T-cells have a minimally differentiated central memory phenotype; CD45RA+CD45RO-,CCR7+, CD62L+. 20d, add 20ul virus supernatant to the first well then to limited dilute at 1:10 in the following wells (n=3). 3±2d, observe GFP+ cells under inverted fluorescence microscope, the positive cell numbers (M) would only be counted in the “counting well” (the last well where the M≥10, and no positive cells in the next well), the serial number (N) of the counting well is used for titer calculation.

Summary/Conclusions: LaSRT is a better titration method for pre-clinic gene transfer research for the following reasons:

1. Simple and accurate, regardless big difference of original titer of RVs;
2. Safe, closed system till detection;
3. Efficient and economic, large scale samples can be tested with only a drop of virus;
4. Valid for different RV with a suitable maker, such as AV, AAV and LV with GFP. In near future, it can be easily developed to be automatic or semi-automatic detection as RQ-PCR and FISH.

Disclosure: No conflict of interest disclosed.

P435

Everolimus inhibits CMV specific CD8+ T cells in a dose-dependent manner

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Everolimus is a novel immunosuppressive drug which is approved for solid organ transplantation and recently used off-label for patients after hematopoietic stem cell transplantation (HSCT). Everolimus is also useful in reducing cyclosporine-related nephrotoxicity. As reactivation of cytomegalovirus (CMV) plays a pivotal role for the outcome of patients after HSCT, we investigated in vitro to which extent everolimus affects CD8+ T-cell responses specific to CMV.

Peripheral blood mononuclear cells (PBMCs) were taken from the blood of HLA-A2/CMV seropositive healthy volunteers by Ficoll–Hypaque density gradient centrifugation and isolated using MACS® by anti-CD8 antibody labeled magnetic beads to get a purified CD8+ population. The CD8+ cells were then stimulated with the HLA-A*0201-restricted peptide from CMVpp65 (NLVPMATV) in a mixed lymphocyte-peptide culture (MLPC). Carboxyfluorescein succinimidyl ester (CFSE) was used to analyze the proliferation of CMV specific CD8+ T cells. On day 6, CD8+ T cells were restimulated overnight by CMVpp65 peptide-pulsed K562-HLA-A2 transfectants and analyzed by enzyme-linked immunosorbent (ELISPOT) assay and multi-color fluorescence flow cytometry. ELISPOT assay was used to determine the frequency of CMV-specific IFNγamma-producing cells. Cells were stained with anti-CD137 antibodies and CMVpp65 tetramer to detect activated CMV specific CD8+ T cells by flow cytometry.
Everolimus concentrations from 0.1 to 50 nM corresponding to serum levels of the drug from 0.1 to 50 ng/ml inhibited the proliferation and IFNγamma secretion of HLA-A2 restricted CMVpp65 specific CD8+ T cells after one round of MLPC. The inhibition was dose-dependent and complete inhibition of the proliferation of CMV specific CD8+ T cells was observed within the therapeutical (3–8 nM) range, comparable to prednisolone, a well studied immunosuppressive agent. The frequencies of CD8+CMVtetramer+ T cells were equal to those of CD137+ CMV tetramer+ T cells, also comparable in numbers to CMV-specific IFNγamma-producing cells in ELISPOT assays. Thus, everolimus is a potent immunosuppressive drug for the treatment of rejection or graft-versus-host disease (GVHD) after HSCT. As a note of caution, attention must be paid to early detection and pre-emptive treatment of CMV reactivation in patients treated with everolimus as we demonstrate here that everolimus strongly hampers CMVpp65 specific CD8+ T cells.

Disclosure: No conflict of interest disclosed.

P434

Immune responses against several leukemia-associated-antigens (LAAs) in the course of allogeneic hematopoietic stem cell transplantation (allo-HSCT) and donor lymphocyte infusion (DLI) in patients with different haematological diseases

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Delayed donor lymphocyte infusion (DLI) already plays a key role in supporting the graft-versus-tumor effect after allogeneic hematopoietic stem cell transplantation (HSCT). The potential efficiency of DLI differs for each disease entity. The graft-versus-leukemia (GvL)-effect observed after DLI is based on the CTL-mediated immunity which is reactive against minor histocompatibility antigens (mHAg). To date, the role of leukemia-associated antigens (LAAs) in GvL has to be elucidated.

In this study, we analysed peripheral blood samples of a small cohort of four patients with AML, T-NHL, CML and Multiple Myeloma before and after DLI or allogeneic stem cell transplantation for specific T-cell responses against several LAAs. Immune reactions of CD8+ T-cells were measured in ELISPOT assays for INF-gamma and granzyme B. In addition tetramer assays were performed. Epitopes derived from several LAAs such as PRAME, mutated NPM1, Survivin, RHAMM, Proteinase 3, WT-1, hTERT and further antigens were tested. CD8+ T-cell responses against NPM1-P91, P93, P300, Proteinase 3, Survivin and WT-1 were detected in blood samples after preemptive DLI but not in samples before DLI in the patient with AML. In parallel, former detectable NPM1mRNA transcripts were no longer traceable – the patient achieved molecular complete remission – and former mixed chimerism became complete after DLI. For the patient with CML we could find an increase in cytotoxic T-cell responses against h-TERT and RHAMM-R3 in the course of allo-HSCT and after DLI. The samples of the patient with Multiple Myeloma and the one with T-NHL are currently under investigation.

Here, we could demonstrate for the first time polyclonal cytotoxic CD8+ T-cell responses against several known LAAs in a patient with AML with NPM1mRNA after preemptive DLI in molecular remission associated with MRD negativity. Whether specific cytotoxic T-cell responses against epitopes derived from the NPM1mRNA peptide or the polyspecificity of the cytotoxic T-cells against several LAAs are decisive in the elimination of the myeloid blasts with NPM1mRNA remains to be determined in a larger cohort of patients. In addition, boosting of T-cells against specific LAAs could possibly be an approach to enhance GvL-effect with reducing GVHD-effect at the same time, but the most suitable LAAs or combination of LAAs for each disease entity still has to be investigated.

Disclosure: No conflict of interest disclosed.

P437

Influence of CEACAMs expression in graft-versus-host disease after allogeneic transplantation

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Background: The carcinoembryonic antigen (CEA) family is involved in intercellular binding interactions that affect various normal and pathogenic processes associated with cellular growth and differentiation. In human, the CEA family are subdivided into the CEA-related-cell-adhesion molecules (CEACAMs) and the pregnancy-specific glycoproteins (PSGs). Never before the influence of CEACAMs on patients who underwent allogeneic hematopoietic stem cell transplantation (HSCT) was evaluated.

Methods: Here we analyzed in a retrospective study 33 patients (pts) for CEACAMs expression that underwent allogeneic HSCT for various diseases and analyzed their outcome. CEACAMs expressions were performed by flow cytometry and ELISA in whole blood, serum and urine samples.

Results: We report the analysis of 19 pts with acute leukemia (58%), 5 pts with chronic leukemia (15%), 4 pts with MDS (12%) and 5 pts with advanced NHL (15%). The median age at transplant was 50.5 (range, 18–69) years. In this cohort 12 pts received grafts from HLA-identical siblings (36%), 16 pts from matched (49%) and 9 pts from mismatched (15%) unrelated donors. Transplantant consisted of unmanipulated peripheral blood stem cells (n=26, 79%) or bone marrow (n=7, 21%). Of all pts, 7 (21%) had relapsed after transplant. Among these pts, 31 (94%) developed acute GVHD (21 pts had an acute GVHD of grade 2). There was no significant correlation between CEACAMs expression after transplant between the variant leukemic disorders in the whole blood and the urine samples. Analysis of each CEACAMs for relapse showed no statistically differences. For CEACAM-6, we found a moderate up-regulation in pts with acute GVHD ≥2 versus acute GVHD <2 (p<0.1). In pts with severe acute GVHD (grade ≥3) comparing all other pts, we found significant induction of CEACAM-1 (118.5 ng/ml vs. 198.3 ng/ml, p<0.05) in urine samples and CEACAM-1 (109.2 ng/ml vs. 157.5 ng/ml, p<0.04) in serum samples. However, no statistic differences were found in the CEACAM-1 in regards to chronic GVHD.

Conclusions: These results suggest that pts with high levels of CEACAM-1 confirms a relevant association of the development of acute GVHD and CEACAMs profilings could be an early indicator of severe acute GVHD.

Disclosure: No conflict of interest disclosed.

P438

A role for IL-10 in modulating murine Graft-versus-Host Disease

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Allogeneic hematopoetic stem cell transplantation (HSCT) is the treatment of choice for a variety of hematologic malignancies. Graft-versus-Host disease (GVHD) is a key contributor to treatment related morbidity and mortality and consequently limits the efficacy of allogeneic HSCT. Interleukin 10 (IL-10) is a well-known cytokine with immunoregulatory and anti-inflammatory properties, also important in context of GVHD.

To address the role of IL-10 in murine GVHD we used an acute MHC mismatch model: C57BL/6 recipients were lethally irradiated and receiver bone marrow and CD82- T cells from BALB/c donors or vice versa. Transplantation experiments with IL-10 deficient (IL-10−/−) donors and recipients on C57BL/6 or BALB/c background clearly show an important role for IL-10 in suppressing GVHD. In further studies we addressed potential sources of IL-10 in the context of GVHD. Donor type CD4+ FoxP3+ regulatory T cells (Treg) are known suppressors of T cell activation and proliferation in GVHD, but use
IL-10 independent mechanism as shown by us in vitro and in vivo. Further in vitro studies concerning the role of IL-10 derived from host dendritic cell indicate that no relevant amounts are released by CD11c+DC populations. In contrast, the course of GvHD in B cell deficient JHT mice is strongly aggravated suggesting a distinct role for B cells as key players during GvHD priming. To precisely define IL-10 dependent or independent mechanism of B cell mediated GvHD modulation we employ recipient mice lacking the ability to produce IL-10 exclusively in B cells. Taken together, our results provide the basis for an improved understanding of highly relevant mechanisms in GvHD paving the way for new treatment options to overcome current limitations of allogenic HSCT.

Disclosure: No conflict of interest disclosed.

P439 Azacytidine impairs NK-cell activity in AML and MDS patients undergoing MRD-based pre-emptive treatment after allogeneic stem cell transplantation

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The hypomethylating agent azacitidine (AZA) represents the standard treatment for many high-risk MDS and AML patients. However, its precise mechanism of action has not been fully understood yet. Human NK-cells play an important role in the regulation of immune responses against malignant cells. Their function is tightly controlled by a complex interplay of activating and inhibitory receptors – some of them being regulated by promoter methylation of the respective genes. We, therefore, explored whether AZA modulates in vitro NK-cell function as well as in vivo during minimal-residual disease (MRD)-guided treatment of imminent relapse in MDS and AML patients treated within the prospective RELAZA trial (NCT00422890).

Methods: After purifying NK-cells of healthy donors by MACS (magnetic cell sorting), cells were exposed in vitro to different concentrations of AZA (100 nM, 1 μM, 3 μM) with or without IL-2. In parallel, the NK-cell phenotype of stem-cell transplanted patients (n=12) with AML or MDS, undergoing MRD-guided treatment with AZA was monitored by FACS from peripheral blood samples on day 1, 5 and 7 of the first and second AZA cycle. All patients were still in complete haematological remission at the time of therapy.

Results: In vitro, we observed a significant reduction (3.1% to 1.8% p=0.028) of the immature and cytokine-regulating CD56bright NK-cell subpopulation with increasing concentrations of AZA. There was a trend towards a reduced expression of the death-ligand TRAIL, the activating receptors NKGD2 and NKp46 and for an increased expression of the inhibitory KIR CD158b1/b2, whereas we could not detect any changes in the expression of FAS-L, Perforin, Granzyme B, NKp30, NKp44, CD69, CD57, DNAM-1, CD16, and NKGA2-CD94. Confirmatory, we noticed a significant decrease in the expression of TRAIL (p=0.003), NKGD2 (p=0.03) and NKp46 (p=0.006) during AZA treatment in vivo. Interestingly, these changes appeared to be reversible before the next cycle. The observed reduction of NK-cell activating receptors and TRAIL during AZA treatment correlated with a reduction or stable course of MRD in all analyzed patients.

Conclusion: These data suggest that the clinical effects of AZA are not mediated by enhancing NK-cell activity. In fact, the drug may have inhibitory effects on NK-cell function which should be considered when applying AZA in the post-transplant setting.

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P440 The impact of Toll like receptor 4 polymorphisms on complications after allogeneic HSCT

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Toll like receptor 4 (TLR4) is an important pathogen recognition receptor. Recent evidence suggests that the ability to respond properly to TLR ligands may be impaired by single nucleotide polymorphisms within TLR genes. HSCT is associated with several complications such as graft-versus-host disease (GVHD) and an increased risk of infections. Therefore polymorphisms on TLR4 may influence individual susceptibility to GVHD or infectious complications. The purpose of this study was to investigate the influence of TLR4 polymorphisms on complications after allogeneic HSCT. A total of 264 patients undergoing allogeneic HSCT and their respective donors were retrospectively genotyped using pyrosequencing for two polymorphisms in TLR4 (Asp299Gly and Thr399Ile). Allele frequency of TLR4 was similar in the patients and donors group. The wild type AA and CC genotype was present in 89% of the patients. No homozygous mutated genotype was found for both polymorphisms. An increased relative risk of acute GVHD was observed in the group of patients with the AG genotype (RR 2.09 95% CI 1.17–3.37) although the difference was not significant (p=0.08) in univariate analysis. In addition, the incidence of fungal infections in patients bearing the AG genotype was significantly higher compared with the AA genotype (p=0.044). TLR4 Thr399Ile polymorphism was not associated with clinical complications or transplantation outcome. Other post transplant characteristics such as cytomegalovirus reactivation, chronic GVHD, overall survival and disease free survival were unrelated to the presence of these polymorphisms. Typing for the Asp299Gly polymorphism in TLR4 may result in a reduced incidence of fungal infections in patients undergoing allogeneic HSCT. A larger prospective study is required to define genetic susceptibility to complications after allogeneic HSCT.

Disclosure: No conflict of interest disclosed.

P441 A comprehensive approach for fast and sensitive chimerism analysis and detection of minimal residual disease using short insertion/deletion polymorphisms

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Analysis of chimerism in the post-transplant period after allogeneic blood stem cell transplantation (HSCT) has become the most important tool to assess treatment responses in patients suffering from leukemia. A multiplex PCR-based approach using sets of short tandem repeats (STRs) currently serves as standard for chimerism analysis. Although, STRs show high power of discrimination to donor and recipient, this method provides limited sensitivity that does not reach below 1%. To enable quantification of minimal residual disease (MRD), more sensitive and efficient approaches are required for early detection of imminent relapse.

We established a combined approach that links the multiplex PCR for initial genotyping with allele-specific qPCR (AS-qPCR) for subsequent detailed analysis to advance chimerism monitoring and MRD analysis. Insertion/deletion polymorphisms (INDELs/DIPs) were chosen as superior markers for this application. Since the STRs currently serve as standard for chimerism analysis, the method provides limited sensitivity that does not reach below 1%. To enable quantification of minimal residual disease (MRD), more sensitive and efficient approaches are required for early detection of imminent relapse.

We identified a set of 110 DIPs that were screened for their potential to identify the most informative allelic constellations for donor and recipient distinction. We identified 44 loci with high power of discrimination, which were chosen to establish the initial multiplex-qPCR approach for capillary electrophoreses. A gender-related locus was included for sex identification. For all multiplexed DIPs, AS-qPCR assays were developed and optimized toward specificity and sensitivity. Our data showed enhanced sensitivity and only minimum background amplification, mandatory for reliable results.
The presented work demonstrate the benefits and suitability to use insertion/deletion loci for chimerism analysis and MRD detection. The combined approach of multiplex-PCR with subsequent allele-specific real-time PCR analysis introduces a fast and cost effective molecular diagnostic tool, which mediates an effective initial genotyping and a quantitative monitoring of individual MRD down below 1%.

Disclosure: No conflict of interest disclosed.

P442
A simple, one step assay for simultaneous detection of hematopoietic chimerism, NPM1 and FLT3-ITD mutations after allogeneic stem cell transplantation

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Hematopoietic chimerism analysis is important in the follow-up of patients undergoing allogeneic stem cell transplantation (HSCT) because it can predict disease recurrence, graft-versus-host disease and graft failure. However, mixed chimerism (MC) states in which normal recipient hematopoietic cells are found has been described. To detect the patients who are at risk of leukemia relapse is vital to detect residual leukemia in the form of minimal residual disease (MRD). The objective of this study is to simultaneously detect residual leukemic cells in the form of MRD and host hematopoiesis in the form of MC in a rapid one step assay. A multiplex PCR for the detection of short tandem repeats (STR), NPM1 and FLT3-ITD mutation was performed in 122 samples from 12 selected patients. All the included patients had both mutations at diagnosis and 1 to 3 informative STR, NPM1, FLT3-ITD and chimerism status were individually analyzed in parallel using a standard singleplex PCR. After transplantation 10 patients showed a state of MC. In seven patients with MC both mutations were found at the same time of MC, while one patient with MC only a NPM1 mutation was detected at relapse. Two patients with MC did not show NPM1 or FLT3-ITD mutation at any time and these patients were not in relapse. Two patients in remission showed complete donor chimerism and were negative for FLT3-ITD and NPM1. Correlation coefficient of chimerism percentage between singleplex and multiplex PCR was 0.98, while the correlation coefficient for FLT3-ITD mutated/unmutated ratio was 0.89 for both PCR systems. Our one step assay provides clinical applicability for rapid simultaneous detection of chimerism status, NPM1 and FLT3-ITD mutation in AML patients undergoing HSCT.

Disclosure: No conflict of interest disclosed.

P444
Internal validation and quality control in quantitative hematopoietic chimerism testing

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Quantitative hematopoietic chimerism testing mainly relies on PCR amplification of polymorphic short tandem DNA repeats (STR) and capillary electrophoresis analysis of PCR products. Few studies had addressed STR performance in hematopoietic chimerism testing. Quality control (QC) procedures are routinely used in clinical laboratories. Applications of such procedures to hematopoietic chimerism are scarcely reported. The aim of this study was to assess the impact of STR validation parameters on the quantification of hematopoietic chimerism and to analyze the analytical process through the application of QC procedures. STR used in this study were SE33, D1S80 and THO1. In addition, PCR for amelogenin and FISH for X and Y chromosomes were used. Analytical chimerism testing was assessed using male/female artificial cellular mixtures prepared in known proportions (0% 0.5% 1% 3% 5% 10% 30% 50% 100%). To evaluate the clinical hematopoietic chimerism performance 152 samples from 96 sex-mismatched transplanted patients were used. The 2% DNA mixture was used to generate Levey-Jennings QC charts. A precision profile for each STR (mixed chimerism range 0.5–30%) was performed. Detection limits for SE33, THO1 and D1S80 were 81, 85 and 61 relative fluorescence units respectively. Analytical sensitivity of artificial DNA mixtures for all STRs was 1% (range 0.5–1.6%). Analytical sensitivity for FISH and amelogenin was 0.5% (range 0.1–1.1%). SE33 and THO1 did not show allelic imbalance while severe allelic imbalance (allele peak ratio <0.60) for D1S80 was detected at 0.25 ng of DNA template mass. The detected allelic imbalance resulted in a 50% overestimation in the mixed chimerism calculation. Sensitivity in clinical samples was 1% (range 0.4–2%) for all STRs. Correlation between the STR and non-STR markers (mixed chimerism range: 0–90%) in clinical samples was high (regression coefficient >0.90). At low mixed chimerism percentage (1%) THO1, SE33 and D1S80 variation coefficient was 23, 19 and 16 % respectively.

After performing Levey-Jennings QC charts and applying the Westgard multi-rule procedure violation of the 1.00 rule was observed for THO1 and 2 consecutive times for SE33 and D1S80, not complying with the 2.00 rule. STR validation is a critical step to detect intrinsic errors that may impact the final mixed chimerism result. Implementing standard QC procedures can identify systematic and random errors so corrective actions can be performed.

Disclosure: No conflict of interest disclosed.

P444
Only gene variants of IL10, IL23R and LCT 13910 influence transplant outcome of patients with HLA-identical unrelated donors as well as patients with sibling donors

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Introduction and methods: here we aimed to evaluate in 314 patients and their HLA identical unrelated donors (URD) and 285 patients and their HLA identical sibling donors (SIB) after T cell repleted myeloablative transplantation and use of GVHD-prophylaxis with only MTX and CSA or CSA and MMF. 48 different gene variants from which were reported to have influence on the outcome of transplant. Patients were transplanted for acute leukemia, CML, MDS, lymphoma and MM between Jan. 2000 and June 2010 at our center. Results: In the cohort of URD the occurrence of acute GVHD grade 2–4 was influenced adversely by gene variants on recipient side of LTA (40% vs 28%, P = 0.013), MBL2 codon550 (47% vs 31%, P = 0.03), MCP1 (69% vs 42%, P = 0.036) and NFRBHL1 (51% vs 34%, P = 0.018). Further, the occurrence of severe aGVHD 3–4 was influenced adversely by gene variants of MBL codon 550 (10% vs 23%, P = 0.025), MBL2 codon 4 (10% vs 36% P = 0.04), LCT13910 (9% vs 26%, P = 0.04) and CYBB1 (8% vs 20%; P = 0.05). Favorable effect was induced by gene variant of IL6 on aGVHD 3–4 (4% vs 19%, P = 0.039) in the URD setting, whereas none of these gene variants had an influence on aGVHD in the SIB cohort. Further, we found that the rate of 5-year none-relapse mortality (NRM) was associated adversely with the detection of variants of IL16 (60% vs 34%, P = 0.01) and MCP1 (58% vs 27%, P = 0.025), which also influenced the 5-year estimate for overall survival (OS) of patients (MCP1 40% vs 53%, P = 0.014 and IL16 46% vs 28%, P = 0.03) in the URD setting. On the donor side the occurrence of aGVHD grade 2–4 was influenced by MBL2 codon4 (69% vs 32%, P = 0.007), TLR2 (66% vs 41%, P = 0.02), TLR5 (75% vs 42%, P = 0.041). Acute GVHD 3–4 was influenced by IL23R favorably (0% vs 20%, P = 0.01) and adversely by IL18 (10% vs 36%; P = 0.01) in the URD setting. The 5-year NRM was associated with the detection of gene variants at donor side of CCR5 (53% vs 27%, P = 0.01), CTLA4 (23% vs 44%, P = 0.018), CYP1B1 (14% vs 26%, P = 0.04), TRL2 (2% vs 34%, P = 0.025). Also, IL10 gene variants at donor side influenced the 5-year OS significantly (23% vs 54%, P = 0.04) as well as the gene variants TLR2 (28% vs 50%, P = 0.04), IL18 Rap (40% vs 72%, P = 0.04) and FAS (60% vs 36%, P = 0.04). In the SIB cohort NONZ and GSTP showed to effect outcome. Only gene IL10, IL23R and LCT 13910 remained to be important in URD and SIB cohort in this analysis.

In conclusion we report here that gene variants have only a moderate influence on the transplant setting.

Disclosure: No conflict of interest disclosed.
Abstracts

P445

Immuno-monitoring of patients after reduced intensity conditioning and double cord blood transplantation for treatment of high risk acute leukemia

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Introduction: Since 1999 transplantation of double cord blood units (dUCBT) is used increasingly for the treatment of acute leukemias. Aside from engraftment, graft versus host disease and relapse, infections considerably influence outcomes. In this study we therefore monitored immune reconstitution of patients (n=6) after double cord blood transplantation and compared our results patients (n=40) receiving HLA identical (10/10) G-CSF mobilized peripheral blood monoclonal stem cells (PBSC). Patients: All dUCBT patients (aged 19 to 70) suffered from high risk leukemia and non-myeloablative and maximum of 2 HLA-mismatches was tolerated between the cord bloods and between each cord blood and the recipient. 4/5 patients tested positive for CMV IgG before transplantation. Patients (aged 22 to 73) treated with PBSC transplantation suffered from different malignant hematopoietic disorders and received myeloablative or non-myeloablative conditioning regimens.

Methods: Lymphocyte subsets of peripheral blood were analyzed by 7 color flow cytometry on days 30, 60, 90, 120 and 180 after transplantation. Using a standardized protocol we determined absolute cell counts of monocytes, T-cells, B- and NK-cells. HLA A2 positive samples were analyzed with multimers to detect CD8+ cells with antigen specific T-cell receptors for CMV (VLE,NLV) and EBV (GLC,CLG,YVL).

Results: The comparison revealed principal differences in immune reconstitution, reaction of CMV and EBV, and the achievement of full donor chimerism. All dUCBT patients reached full chimerism on d30 whereas 3/39 PBSCT did not engraft and 6/39 reached full chimerism later than d60. CMV was reactivated in 21/40 PBSCT and in 3/6 dUCBT patients. There was no evidence of EBV reactivation in dUCBT group, whereas 27/40 PBSCT patients reactivated. While CD8+ cells recovered to normal counts on d60 in PBSCT patients (mean=632±933), levels of dUCBT patients remained low in the four groups is shown in Table 1:

<table>
<thead>
<tr>
<th></th>
<th>Flu/Mel cells/µl median (range)</th>
<th>Flu/TBI cells/µl median (range)</th>
<th>Flu/Mel/AI-RIT cells/µl median (range)</th>
<th>Flu/TBI-RIT cells/µl median (range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD3+4+ T-cells</td>
<td>d=100 d=500 57 (22–81) 90 (27–151)</td>
<td>73 (35–99) 184 (72–366)</td>
<td>189 (85–1105) 102 (42–951)</td>
<td>251 (27–337) 186 (3–257)</td>
</tr>
<tr>
<td>CD3+8+ T-cells</td>
<td>d=100 d=500 13 (4–126) 240 (13–271)</td>
<td>139 (33–218) 732 (72–1469)</td>
<td>213 (74–6181) 100 (35–2917)</td>
<td>251 (8137–539) 215 (2–2062)</td>
</tr>
<tr>
<td>CD3+19+ B-cells</td>
<td>d=100 d=500 0 (0–1) 56 (3–891)</td>
<td>9 (0–190) 174 (1–520)</td>
<td>2 (0–46) 140 (8–440)</td>
<td>45 (4–155) 83 (5–851)</td>
</tr>
</tbody>
</table>

Disclosure: No conflict of interest disclosed.

Table 1. for abstract P446

P446

Impact of radioimmunotherapy with Yttrium-90-lbritumomab Tiuxetan as part of reduced intensity conditioning for allogeneic hematopoietic cell transplantation on immune reconstitution in patients with Non-Hodgkin-Lymphoma

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Introduction: The influence of combined use of radioimmunotherapy (RIT) and reduced intensity conditioning (RIC) on immune reconstitution after allogeneic hematopoietic cell transplantation (allo HCT) has not been evaluated.

Methods: We compared data on immune reconstitution in 14 patients receiving allo HCT at our institution from 2006-08 combining RIT using yttrium-90-lbritumomab tiuxetan (Y90-CD20, Zevalin®) and RIC with either fludarabine (Flu) (30 mg/m² day −8 to −4) or melphalan (Mel) (140 mg/m² day −3)/alumtuzumab (20–30 mg day −3 to −2) (n=7, Flu/Mel/Al-RIT-group) or with fludarabine (30 mg/m² day −8 to 4) (2 Gy total body irradiation (TBI) (n=7, Flu/TBI-RIT-group). Postgrafting immunosuppression consisted of cyclophosphamide alone (Flu/Mel/Al-RIT-group) or cyclophosphamide with mycophenolate mofetil (Flu/TBI-RIT group). The results were compared to 14 patients in a concurrent control group with similar conditioning regimens without RIT (n=7, Flu/Mel (without alemtuzumab), n=7, Flu/TBI). Differences in engraftment and immune reconstitution were evaluated.

Results: Diagnoses in the RIT-groups were high-grade NHL=n=6, low-grade NHL=n=2 and CLL=n=2. In the control group there were high-grade NHL=n=4, acute leukemia=n=6, MDS=n=1, multiple myeloma=n=1, Hodgkin lymphoma=n=1 and CLL=n=1. Engraftment to >500 granulocytes/µL was observed after a median of 20 (range, 13–26) in Flu/Mel/Al-RIT-group and 13 (range, 0–17) days in Flu/TBI-RIT-group and to >20,000 platelets/µL after a median of 11 (range 8–42) and 8 days (range, 0–16). In the control groups engraftment of granulocytes was observed after a median of 15 (range, 11–21) in Flu/Mel-group and 11 (range, 0–27) days in Flu/TBI-group and of platelets after a median of 21 (range, 5–29) and 10 days (range, 0–35). Immune reconstitution in the four groups is shown in Table 1:

<table>
<thead>
<tr>
<th></th>
<th>Flu/Mel cells/µl median (range)</th>
<th>Flu/Mel/AI-RIT cells/µl median (range)</th>
<th>Flu/TBI-RIT cells/µl median (range)</th>
<th>Flu/TBI cells/µl median (range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A = 8%</td>
<td>B: AML (n = 12), OMF (n = 4), NHL = 6 and CLL = 2</td>
<td>C: AML (n = 5), NHL = 6, CLL = 1</td>
<td>D: AML (n = 4), NHL = 6, CLL = 2</td>
<td>A: AML (n = 4), NHL = 6, CLL = 2</td>
</tr>
</tbody>
</table>

Conclusion: The combined use of RIT and alemtuzumab leads to a significant slower immune reconstitution after allo HCT. The immune reconstitution has to be correlated to outcome and the incidence of infections.

Disclosure: No conflict of interest disclosed.
P448
Reduced intensity conditioning with fludarabine and thiotapec for second allogeneic transplantation of relapsed patients with acute myeloid leukaemia

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Introduction: AML patients relapsing after allo-SCT have a poor prognosis. The potentially curative approach of a 2nd allograft is generally hampered by excessive treatment-related mortality and a high risk of relapse. We tried to address both problems with a novel reduced intensity conditioning regimen aiming for efficacy with low organ toxicity.

Methods: A 2nd allograft was offered to 58 AML patients with a median age (range) of 53 (23–69) years and a median time to relapse after the 1st allo-SCT of 326 (47–2189) days. Only 4/58 patients were transplanted in complete remission. Thiopeta 5 mg/kg on days –7 to –5 (15 mg/kg) and fludarabine 30 mg/m² on days –8 to –4 (150 mg/m², n=18) were given initially, while a lower fludarabine dose of 90 mg/m² (n=40) was given after June 2005 to reduce infectious complications. G-CSF-mobilised unmanipulated peripheral blood stem cells (bone marrow for 1 patient) from HLA-matched unrelated donors (sibling donors for 2 patients), different from these employed for the 1st allo-SCT (in 56/58 cases) were mostly used. GvHD prophylaxis consisted mainly of CsA and alentumizumab (10–40 mg). Median follow-up was 6.7 years. Competing risks were considered in data analysis.

Results: Response rates at 1 month were complete remission in 50 and persist-ent disease in 3/53 evaluable patients. No graft failures occurred. Despite heavy pretreatment and active disease in most cases, the regimen had an acceptable treatment-related mortality (95% confidence interval) of 31(21–46%) at 3 years, mainly attributable to infections. Most deaths were caused by relapse, which had an incidence of 56 (45–71%), resulting in an overall sur-vival rate of 18 (9–29%) and an event-free survival rate of 13 (5–23%) at 3 years. In multivariate analysis, overall survival improved with younger patient age (p=0.006), longer relapse-free interval after the 1st allo-SCT (p=0.0025) and the development of chronic GvHD after the 2nd allo-SCT (p=0.04), which affected 49 (36–66%) of patients at 3 years. Patients ≤65 years old who relapsed >12 months after the 1st allograft (n=20) had a 3-year overall survival rate of 41 (19–62%). Conventional cytogenetics and FLT3 mutation status did not affect outcome.

Conclusions: Our regimen is feasible and provides at least for a subgroup of relapsed AML patients a reasonable therapeutic option in an otherwise fatal situation. A better understanding of the underlying biology is needed to further lower the risk of relapse.

Disclosure: No conflict of interest disclosed.

P449
Impact of age on outcome after allogeneic hematopoietic stem cell transplantation using reduced-intensity conditioning with Fludarabine/Busulfan

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Introduction: The use of reduced intensity conditioning (RIC) regimens in allogeneic hematopoietic cell transplantation (HCT) allows transplantation of elderly or comorbid patients. RIC using fludarabine and busulfan (Flu/Bus) is frequently used in patients up to the age of 65. There is little published evi-dence in patients above the age of 65 and the influence of patient age on out-come remains to be evaluated.

Methods: Retrospective analysis of 74 consecutive adult patients (T=30, n=44) after allogeneic HCT using fludarabine (30 mg/m², day –5 to –4)/ busulfan (0.8 mg/kg day 6–7) for RIC at our institution between 2005–2011. As GvHD prophylaxis calcineurin inhibitor combined with mycophenolate mofetil (n=18) or methotrexate (n=55) and anti-thymocyte globulin was used.

Results: Median age of patients was 59 years (range, 30–74). Patients were grouped in 3 age categories: patients ≤60 years (group A, n=36), patients 60–64 years (group B, n=19) and ≥65 years (group C, n=19). Diagnoses in the different groups were: A: acute myeloid leukaemia (AML, n=10), osteo-myelofibrosis (OMF, n=17), myelodysplastic syndrome (MDS, n=8) and chronic myeloid leukaemia (CML, n=1); B: AML (n=12), OMF (n=4), MDS (n=3); C: AML (n=9), OMF (n=4), MDS (n=6). At time of HCT, 25% of the patients in A, 53% in B and 53% in C were in complete remis-sion. Grafts either from matched related (MRD, A=10, B=2, C=1), matched unrelated (MUD, A=12, B=7, C=7) or mismatched unrelated (MMUD, A=14, B=10, C=11) donors were used. The elderly subgroups showed a similar outcome compared to younger patients (3-year OS in A 53% vs. 48% in B vs. 63% in C, p=0.71). Cumulative incidence of non-relapse mortality (NRM) and relapse adjusted for competing risk was A, 29%; B, 17%; C, 11% and A, 17%; B, 35%; C, 30% at 3 years, respectively. The use of MUD or MMUD had no negative influence on OS (3-year OS in A: MUD 27%, MMUD 71% vs. MRD=57%, p=0.41; in B: MUD 34%, MMUD 56% vs. MRD=50%, p=0.92; in group C only one MRD was used. Incidence of acute graft versus host disease (GvHD) ≥H was in A=8%, in B=5% and in C=16%. Incidence of chronic GvHD was 47% in A (lim-it=12, extensive=5), 37% in B (limited=3, extensive=4), and 58% in C (limited=8, extensive=3).

Conclusion: RIC with fludarabine and busulfan is a treatment option for allo-geneic HCT in elderly or heavily pretreated patients. Age and donor source do not have a negative impact on survival.

Disclosure: No conflict of interest disclosed.

P450
Incidence and severity of oral mucositis after allogeneic stem cell transplantation with busulfan-containing conditioning is depending on application route and dose

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Introduction: Busulfan is often used in the conditioning for allogeneic transplantation. To improve bioavailability an intravenous formulation (Bivulsul) was introduced. Within the BMT programme at the University of Cologne oral Busulfan was used until 2004 and switched to Bivulsul thereafter. We assessed the effects of dose and formulation of Busulfan on mucosal toxicity.

Methods: 75 patients have received a conditioning containing Busulfan. Among these 16 received oral Busulfan (group A) at a fixed dose of 16 mg/kg with cyclophosphamide, 30 patients were treated with 12.8 mg/kg BW Bivulsul and cyclophosphamide (group B) and 29 patients received 6.4 or 8.6 mg/kg BW Bivulsul and Fludarabine (group C).

Results: In group A severe mucositis (grade III-IV) occurred in 69% of patients compared to 30% in group B and 21% in group C (p=0.001). On the other hand 50% in group B and 52% in group C developed no mucosi-tis in contrast to only 6% in group A. Median time to develop mucositis grade III/IV was 12 days for oral BU versus 16 days in both intravenous BU groups, p=0.048. The median duration of mucositis showed no sig-nificant difference between the groups. Total parenteral nutrition (TPN) was required in all 16 patients in group A, in 80% in group B and 62% in group C (p=0.013). The median duration of TPN was 27.5 days (range 7–47) for group A, 22 days (range 9–46) for group B and 11 days (range 4–76) for group C (p=0.015). The median time to the start of TPN was 6 days for group A, 8 days for group B and 9 days for group C (p=0.001). Intravenous opiates were necessary in 94% of patients in group A, 80% of group B and 76% of group C with no statistically significant difference. The median duration of opiate treatment was 15 days (range 5–26) in group A, 9 days (range 0–82) in group B and 6 days (0–18) in group C (p=0.001).
Conclusions: For patients receiving myeloablative conditioning with Busulfan, the use of the intravenous formulation had significant impact on reducing mucositis, parenteral nutrition or opiate analgesics use. Further reduction of toxicity was seen with lower doses of Busilvex. The improved toxicity profile of standard dose Busilvex compared to oral Busulfan allows the application of myeloablative conditioning with less toxicity, making Busyl reduced-toxicity, full intensity conditioning.

Michael von Bergwelt-Baildon: Financing of Scientific Research: Astellas

Posterdiskussion
Der spezielle Fall

P451 Sustained therapy response in a patient with metastatic urachal carcinoma using a cetuximab-based polychemotherapy

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Introduction: Urachal carcinoma is an uncommon tumor accounting for less than 1% of bladder tumors. According to the WHO classification urachal carcinomas are mostly adenocarcinomas arising from remnants of the urachus occurring at a median age of 50.6 years. Large prospective randomized trials evaluating systemic chemotherapy or targeted approaches are currently not available.

Methods: In May 2004 a 46 year old man was diagnosed with localized urachal carcinoma followed by complete resection of the primary tumor. Local recurrence, however, appeared three years later. After salvage surgery, adjuvant platln based chemotherapy and R0 resection of a solitary lung metastasis was performed. Symptomatic iliacal and mediastinal lymph node metastasis where diagnosed for the first time in May 2010. A tissue sample from the resected lung metastasis was then analyzed for EGFR expression as determined by IHC as well as EGFR and KRAS mutations. In June 2010 we started a palliative chemotherapy using FOLFOXIRI (Oxaliplatin, Irinotecan, 5-FU, leucovorin) plus cetuximab. After 6 cycles the therapy was deescalated to FOLFIRI plus cetuximab for further 6 cycles. The chemotherapy backbone was stopped in January 2011 and maintenance cetuximab bi-weekly was applied.

Results: Shortly after starting FOLFOXIRI chemotherapy, the patients symptoms improved rapidly. Besides PNP III no further grade 3/4 toxicities were observed. The patient developed a skin rash I° which persisted throughout the therapy. Best response in CT scans was a mild reduction in size of the major lymphatic lesions (<30% of the largest diameter of the left iliacal reference lesions), formally corresponding to a stable disease according to RECIST criteria. During follow up (and cetuximab maintenance) the patient remained free of symptoms and showed no signs of progression. Accordingly, a CT scan after 15 months of cetuximab maintenance (21 months after therapy start) showed no signs of progression or new metastasis compared to best response.

Conclusions: To our best knowledge, this is the first report demonstrating long term remission of metastatic EGFR-sensitive urachal carcinoma using a cetuximab based polychemotherapy. We encourage further evaluation of the applied regimen within clinical investigations. Along with this case report, a review of the literature for systemic therapy options in this rare entity will be presented.

Disclosure: No conflict of interest disclosed.

Fig. 1.
Disclosure: No conflict of interest disclosed.

**P453**

An uncommon cause of agranulocytosis: Autoimmune Neutropenia (AIN) in an adult – a case report

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**Background:** Autoimmune neutropenias (AIN) are a very rare cause of agranulocytosis in adults. Clinical presentation is heterogeneous varying from being asymptomatic to serious infectious complications with relevant morbidity and mortality. AIN are characterized by autoantibodies directed against neutrophils further resulting in their destruction.

**Case report:** A 40-year-old man was admitted to the hospital due to a febrile infection. His blood count showed a severe leukocytopenia (0.33 x 10^9/L) with an absolute neutrophil count of 0.05 x 10^9/L, a lymphocyte count of 0.31 x 10^9/L and a mild microcytic anemia (Hb 10.6 g/dl, MCV 73.3 fl). The LDH-level was normal (137 U/L) and there was no evidence of a PNH clone by flow-cytometry. There was no history of myelosuppressive medication. Physical examination demonstrated cervical lymphadenopathy, splenomegaly (20x8 cm) and an infected skin lesion of the ankle. There was no evidence for a viral infection. Bone marrow diagnostics demonstrated a complete depletion of the granulopoiesis and polyclonal lymphocytic infiltrates and a normal karyotype. A biopsy of a cervical lymph node showed major texture destruction with no evidence of lymphoma or lymphocytic hyperplasia. Autoantibodies against granulocytes were detected and finally confirmed the diagnosis of primary autoimmune neutropenia, as there was no evidence for a secondary systemic autoimmune diseases (e.g. rheumatoid arthritis, lymphoproliferative disorders). Thus immunosuppressive treatment with prednisone (1 mg/kg/day) in addition to G-CSF s.c. was initiated. Neutrophil count recovered completely after 5 weeks/days along with the anemia of chronic disease. Prednisone dose was tapered down and the patient remains now stable on azathioprine.

**Summary/Conclusions:** Autoimmune neutropenia must be considered as a differential diagnosis in the situation of agranulocytosis. Diagnosis of AIN is based on the detection of antineutrophil antibodies. Underlying disease must be ruled out. The primary treatment consists of G-CSF and immunosuppressive therapy (e.g. prednisone, methotrexate, cyclosporine A), especially in the cases of secondary AIN.

Disclosure: No conflict of interest disclosed.

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**P454**

Case report: A 48-year-old man with aggressive NK-cell leukaemia / lymphoma of the mesentery with angioinvasion

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A 48-year-old man was admitted to our hospital because of fever (39°C), night sweats and abdominal pain. He had been well until 2 weeks before presentation. On clinical examination we saw a critically ill patient with diffuse abdominal pain on palpation. Ultrasanography of abdomen was unremarkable. Abdominal CT-scans showed multiple, slightly enlarged mesenteric lymph nodes (<1.5 cm) and markedly thickened, dense mesenteric tissue.

**Laboratory findings:** White cell count (WBC) was 4.400 per mm³ (differential count: neutrophils 75%, lymphocytes 17%, monocytes 6%, eosinophils 1%, basophils 1%, no blasts), hemoglobin 12.2 g/dl, hematocrit 36% and platelet count 174.000 per mm³. ESR was 28 mm/h, CRP 18 mg/l. Serum levels for LDH (1025 U/l) and β2-Mikroglobulin (3.91 mg/l) were elevated. Testing for acute viral infections (HIV, HBV, EBV, CMV, HBV, HCV) was negative.

**Histology:** A diagnostic laparoscopy with excision of mesenteric tissue and mesenteric lymph nodes was performed. Histology, immunohistochemistry and molecular analyses detected a mesenteric neoplastic infiltrate of blasts with angioinvasion. The neoplastic cells expressed CD45, CD2, CD56 and cytoplasmic CD3 but no surface CD3. The cells were positive for the cytotoxic molecules granzyme B, TIA 1 and perforin. The lymphoma cells were negative for CD5, CD4 and TdT. Proliferation index was more than 90%. EBV in situ hybridisation showed bright nuclear signs. The mesenteric lymph nodes showed no manifestation of the lymphoma. Routine staging procedures for lymphoma excluded any other manifestation. Particularly involvement of peripheral blood, bone marrow, liver and spleen were ruled out.

**Diagnosis:** Aggressive NK-cell leukaemia / lymphoma of mesentery with angioinvasion, stage IVEB, IPI 3.

**Therapy:** After 4 cycles of chemotherapy with CHOP a complete remission was achieved (no constitutional symptoms, normal LDH, normal CT scans). A consolidation therapy with high-dose chemotherapy with autologous stem cell transplantation was planned. But 3 months after diagnosis the patient suffer a fulminant relapse with multiorgan failure and died.

**Summary:** We describe the rare case of a 48-year-old patient with an aggressive NK-cell leukaemia / lymphoma of mesentery with angioinvasion and fatal clinical course.

Disclosure: No conflict of interest disclosed.
P455
Supply of rare platelet concentrates for a patient with a high titre HPA-5a antibody between treatment and MUD transplantation over a period of 9 months: A case report

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Introduction: Patients with specific antibodies against human platelet antigens (HPA) need selected platelets in case of therapeutic treatment to prevent or reduce haemorrhagic symptoms. Patients with a rare anti-HPA-5a need donor with HPA-5b typing. Antigen frequency HPA-5b is 20% and for HPA-5a 99%; in Germany six potential donors were available at that time.

Case history: A 68-year-old woman was treated for secondary acute myeloid leukaemia (AML) following a myelodysplastic syndrome in 10/2010. Platelet count during AML therapy was refractory to transfusions with regular apheresis platelets. At this stage we detected a high titre anti-HPA-5a antibody (dilution 1:2048) in the patient's serum. HPA-5b (5a-negative) platelet concentrates had to be produced to gain satisfactory platelet count increments during the next months. In February 2011 it was decided to use allogeneic bone marrow transplantation (aBMT) for further therapy. 28 days prior to allogeneic blood stem cell transplantation the patient was treated once with Rituximab. Preceding the transplantation an antibody immunoadsorption was planned. However, 4 weeks prior to transplantation anti-HPA-5a had fallen to such a low titre that immunoadsorption was not affordable. In June 2011 the patient received a matched unrelated donor transplantation (10/10; peripheral blood stem cells) from a person being HPA-5a antigen positive. On day +28 the patient had trilineage engraftment and in the following weeks no persistent thrombocytopenia. Between July and November 2011 the anti-HPA-5a antibody was not detectable.

Methods & Results: Screening for anti-HLA was negative and for anti-HPA positive. An inhouse MAIPA assay showed specific reactions for anti-HPA-5a in combination with HPA-5a-positive reference cells. Allele-typing: positive for HPA-5a; negative for HPA-5b. Total transfusions (2-4.5x10^11 PLT per single donor unit): unsellected firstline therapy = 10; selected during AML therapy = 14; during mud-transplantation = 11.

Conclusions: The strong HPA-5a antibody titre decreased spontaneously over a period of 7 months using compatible PLT. For Rituximab, it is unlikely to cause a rapid clearance of HPA-antibodies, however Rituximab may have prevented the reapparance of the antibody after transplantation when HPA-5a-positive donor derived platelets were produced in the patient. Also HPA-5b donors are extremely rare in Germany, a sufficient PLT supply could be provided by Roche international. Before PE therapy R level was 30.8 µg/ml, started PE therapy. Because measurement of R level is routinely not available provided by Roche international. Before PE therapy R level was 30.8 µg/ml, started PE therapy.

Disclosure: No conflict of interest disclosed.

P456
Hepatitis B reactivation after Bortezomib therapy: a call for screening and prophylaxis

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Introduction: Reactivation of chronic inapparent Hepatitis B virus (HBV) infection may occur during or after immunosuppressive therapy, typically causing acute hepatitis with severe liver cell damage within the first 6 months after the end of therapy or at immune reconstitution. Bortezomib is associated with an increase of herpes zoster infections; the association of HBV reactivation with bortezomib has not been studied.

Methods: Description of a clinical case and discussion of available literature.

Clinical case: A 75 year old man was diagnosed with symptomatic DS stage IIIA multiple myeloma; inpatient treatment was begun with Bortezomib, Melphalan and Prednisolone (VMP) and radiation therapy to his lumbar spine in a regional cancer center. There was no hepatitis history, and no screening for previous hepatitis had been done. Therapy was continued on an outpatient basis and stopped after 6 cycles for serologic VGPR. 3 weeks after his last therapy, the patient started having rising liver enzymes but no jaundice. HBV reactivation was diagnosed 8 weeks after the end of chemotherapy with a high viral load, and entecavir 0.5 mg daily was immediately started. Transaminases normalized over the course of 5 weeks; HBV DNA became negative after 10 months of entecavir. No new chemotherapy was necessary so far.

Conclusions: HBV reactivation is a well known risk of immunosuppressive therapies, particularly when there is a relevant reduction of lymphocytes as in rituximab. Bortezomib may cause severe lymphopenia and is known for its risk of herpes virus reactivation, possibly due to a decrease of specific T lymphocytes; herpesvirus prophylaxis is therefore recommended. Screening for inapparent HBV infection has become standard before rituximab containing lymphoma therapy, but in many institutions is not routinely done outside clinical trials for other therapies. Although there are no published trials on HBV reactivation on or after bortezomib therapy, HBV screening should be routinely done before any bortezomib containing therapy.

Disclosure: No conflict of interest disclosed.
**P458**

**Treatment of Rituximab (R) induced Progressive Multifocal Leuкоencephalopathy (PML) with Plasma Exchange (PE) therapy (R Apheresis) – a promising tool in treatment of a live threatening side effect**


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**Introduction:** PML is a rare opportunistic disease which results from reactivation of latent JC polyoma virus (JCV). Carson et al. reported 57 cases after Rituximab (R) therapy in HIV negative patients (pts). No curative therapy for PML exists. In Natalizumab induced PML removal of the drug via PE is an important part of the therapy. Here we report a case of R removal with PE accompanied by our CDC-based B cell cross match assay for monitoring R removal from plasma.

**Case report:** A 70 y. o. m. with a history of marginal zone lymphoma was admitted with diagnosis of R induced PML to our department. Until Sept. 2010 he received 6 cycles of an Immuno-chemotherapy (R – Bendamustine) and 4 cycles of R maintenance therapy. CT scan showed a vgPR before neurological symptoms in Nov. 2010 occurred. Six weeks after the last R administration, pat. showed paraparesis of the legs and paresis of the left arm. Corresponding demyeilnation signs were observed in MRI of the brain and backbone. PML diagnosis was supported by detection of JC virus with polymerase chain reaction (PCR) in cerebrospinal fluid. A normal WBC with severe lymphopenia (0.7 g/l) could be observed. IgG, IgM, IgA and B cells were not detectable, CD 3+/CD 4+ count was very low (99 /µl). We immediately started a supportive therapy with Mefloquine and Mirtazapine, IVIG were substituted. While showing a high R concentration in pat. plasma we started PE therapy. Because measurement of R level is routinely not available we used our new developed CDC based assay to monitor anti CD 20 antibody concentration in pts. serum and compared our results with R serum levels, provided by Roche international. Before PE therapy R level was 30.8 µg/ml at the end of therapy (14th apheresis) 1.83 µg/ml. Improvement of neurologic symptoms and of the MRI lesions could be observed after 2 weeks. Lymphopenia disappeared, IgG and CD 4 count increased. After an inpatient rehabilitation program for 4 weeks he was discharged to his home. Unfortunately he died 2 weeks later due to pneumonia.

**Conclusions:** Because of rising use of R there is an increasing risk for development of PML. The only effective therapy for PML at this time is immune reconstitution. To accelerate immunoresponse, rapid elimination of R with PE therapy appears as an useful tool and warrants further investigation. Our CDC dependent assay is a rapid and cheap method to measure R level because of the lack of a commercial available R serum level testing.

**Disclosure:** No conflict of interest disclosed.

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**P459**

**Sarcoidosis after treatment with bevacizumab and chemotherapy – a case report**


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A female patient, born in 1964, was admitted to the Oncological Centre Berlin-Spandau in 2008 presenting with ovarian cancer. In May 2008 the tumor was resected. Histology showed high grade serous adenocarcinoma and in the TNM system: pT1a, pG1, pV0, pN0 (0/30), M0 G3 R0. 6 cycles Carboplatin AUC5, 175 mg/m² Paclitaxel and in the ICON 7 trial 18 cycles of bevacizumab (dose 7.5 mg/kg body weight) were administered. The patient was in complete remission.

During follow-up the patient developed isolated nodules in the spleen. In January 2012 splenectomy was performed. Histologically multiple partially confluent granulomas of sarcoidosis type were found. We performed a bronchoscopy and found in the BAL cytology lymphocytes with a ratio of CD4 / CD8 of T 5 to 1. The diagnosis of sarcoidosis stage 2 was now clinically confirmed.

It is debatable whether the alteration of the blood vessels by Bevacizumab may be a cause for alteration in the immunological system leading to inflammation and finally to sarcoidosis. The other possibility is that the development of sarcoidosis during cancer treatment is a coincidence.

**Disclosure:** No conflict of interest disclosed.
Cytokine analysis in Kikuchi-Fujimoto disease supports the potential relationship with systemic lupus erythematosus

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Introduction: Kikuchi-Fujimoto disease (KFD) or necrotizing histiocytic lymphadenitis is a rare idiopathic disease commonly presenting with flu-like symptoms and painful adenopathy (typically posterior cervical). KFD shares similar laboratory and clinical manifestations with systemic lupus erythematosus (SLE) and a long-term follow up of patients with KFD for an early detection of transformation into SLE is suggested. Reports on cytokine analysis in patients with KFD are exceptional.

Methods: A cytokine profile of a 51-year-old woman with a relapsing form of KFD was obtained. The time of sampling included disease exacerbation (lymphadenopathy, fever, skin rash, myalgia), corticotherapy and disease remission. Initially, corticotherapy was applied intravenously (methylprednisolone 60–120 mg daily, 5 days in total), followed by 6 weeks of peroral administration in gradually decreasing doses (methylprednisolone 24–2 mg daily). In our study, levels of interleukin-1β (IL-1β), IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-12, IL-13, IL-17, granulocyte colony-stimulating factor (G-CSF), granulocyte macrophage colony-stimulating factor (GM-CSF), interferon-γ (IFN-γ), monocyte chemotactic protein-1 (MCP-1), macrophage inflammatory protein-1α (MIP-1α) and tumor necrosis factor-α (TNF-α) were assessed using the Multiplex Suspension Array System. Measurements were made on frozen serum samples.

Results: The levels of free circulating serum IL-1β, IL-4, IL-17, G-CSF, GM-CSF and IFN-γ were below detection limits. The levels of free circulating IL-2, IL-5, IL-12 and IL-13 were elevated in some measurements, but did not correlate with the clinical course of the disease. Elevations of three groups of serum factors, which correlated to the clinical course of the disease, were observed: lymphocyte growth factors (IL-7), chemotactic factors (IL-8, MCP-1, MIP-1β) and inflammatory cytokines (IL-6, TNF-α). While elevated in the acute phase, they decreased during corticotherapy along with relief of symptoms.

Conclusions: KFD should be included in differential diagnosis of lymphadenopathy and fever of unknown origin. The clinical and laboratory findings often resemble malignant lymphoma, tuberculosis and SLE. In accordance with the published literature, our results support a hypothesis of IL-6 playing a role in the pathogenesis of KFD. Moreover, the acquired cytokine profile shows several similarities to SLE, thus confirming possible connections between these two entities.

Disclosure: No conflict of interest disclosed.

Chemotherapy with cisplatin/paclitaxel for the treatment of metastatic, sarcomatoid carcinoma of the Bartholin gland

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Introduction: Carcinoma of the Bartholin gland is a rare tumor accounting for 2 to 7% of all vulvar neoplasms and 0.001% of all female genital malignancies. It was first reported by Klob in 1864; until today only a few hundred cases are reported in literature and the level of evidence in treatment with chemotherapy is very low.

Case report: In June 2008 a low-differentiated sarcomatoid carcinoma of the Bartholin gland has been diagnosed in a 40-year-old woman without the evidence of distant metastases. She underwent local surgery in curative intention (R0-resection) and remained in the following time in complete remission (CR). In October 2011 skin metastases on the patient’s scalp were diagnosed and histological examination revealed malignant cells related to the carcinoma of the Bartholin gland. In November 2011 the patient presented at the emergency unit of our hospital following a focal convulsive attack. Cranial computed tomography (CCT) showed multiple metastatic lesions of the brain. CT of the body revealed multiple metastatic lesions in the lungs, kidneys and the pancreas. Therapy started first with whole brain radiation (WBR) containing of 10×3 Gray (Gy). Following WBR, palliative chemotherapy with cisplatin/paclitaxel (Cis/Pac), being a therapeutic option in carcinoma of the vulva, was initiated. Because of the rarity of this cancer of the Bartholin gland, there is no standard systemic chemotherapy approved to treat this tumor entity. After 3 courses of Cis/Pac, the staging showed a stable disease (SD) and there were no serious adverse effects due to the chemotherapy. After the fourth cycle of Cis/Pac, an increased creatinine level was noticed for the first time, and therefore cisplatin was replaced by carboplatin (Car). In ultrasonography an urine accumulation could be detected. The disposition of urethral splints followed, where upon only a moderate decrease of creatinine could be observed. Under continuation therapy with Car/Pac, a distinct hematotoxicity occurred: anemia CTC°4, leukocytopenia CTC°3 and thrombocytopenia CTC°3. A further staging by CT using a contrast-medium was not possible, due to the elevated creatinine level. However, after the fifth cycle of the chemotherapy in ultrasonography progressive disease (PD) with massive infiltration of the kidneys could be shown. Now, a change to a second-line chemotherapy is urgently warranted, the patient still being in a very good general condition (Karnofsky score: 90%).

Disclosure: No conflict of interest disclosed.

Disseminated Aspergillosis combined with Pneumocystis pneumonia in an immunocompromised patient: a diagnostic challenge

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Background: In severely immunocompromised patients, simultaneous or sequential secondary infections in Pneumocystis jirovecii pneumonia (PcP) are mostly caused by bacterial pathogens, whereas fungal pathogens, especially Aspergillus fumigatus, are rarely diagnosed.

Methods: We report on the clinical course of a 59 year old female patient with a history of relapsed astrocytoma III, treated with high doses of corticosteroids after initial surgery and subsequent radiation therapy. The patient had not been reliably on Pneumocystis infection prophylaxis. Chest computed tomography (CT) findings after admission to the hospital for dyspnea and fever showed suggestive lung infiltrates, and treatment with sulfamethoxazole/trimethoprim i.v. had been started immediately. Pneumocystis was identified by immunofluorescence in bronchoalveolar lavage (BAL) sample. The CD4 lymphocyte count at this time was 30/μl. Because of respiratory failure mechanical ventilation was started. After a short period of clinical improvement the patient’s condition worsened again severely and thus additional BAL and blood based microbiological, serological and molecular diagnostics as well as chest CT were repeated, showing a radiomorphological shift to multiple nodular pulmonary infiltrates.

Results: Blood culture findings showed negative results, BAL culture yielded Aspergillus fumigatus. Surrogate parameter analysis (galactomannan and a nested Aspergillus specific PCR assay) showed repeatedly positive findings both in blood and BAL samples prior to culture results. A commercially available multi-pathogen PCR assay showed positive signals for Aspergillus genome in blood samples late in the clinical course. Viral pathogens were not detected. Despite broad antifungal and antibacterial treatment the clinical condition of the patient worsened rapidly, and she died from disseminated aspergillosis inducing multi-organ failure, including extensive brain manifestation.

Conclusions: We report on a patient with Pneumocystis pneumonia and disseminated aspergillosis due to low CD4 lymphocyte count after intensive treatment with corticosteroids for relapsed astrocytoma. As both infections represent life-threatening conditions in severely immunocompromised patients, a heightened clinical awareness and a stringent diagnostic work-up encompassing aspergillosis diagnostics is crucial for adequate and early antifungal treatment and therefore patients survival.

Disclosure: No conflict of interest disclosed.
Mucormycosis in a patient with acute lymphoblastic leukemia
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Introduction: We report on a 42-year-old female patient diagnosed with acute lymphoblastic leukemia who developed fatal mucormycosis during treatment in the GMALL 07/2003 protocol.

Case: The patient was diagnosed with acute pro-B lymphoblastic leukemia (high risk) and was treated in the GMALL protocol 07/2003. Except of diabetes mellitus and asthma bronchiale no relevant ancillary diagnoses were present. The patient received induction therapy and consolidation I without any infectious complications. Complete remission was achieved after induction I. Due to absence of a stem cell donor and the high risk situation we applied second consolidation with IDA-Flag. During neutropenia we performed antimicrobial and antifungal prophylaxis with fluconazole. Eight days after starting treatment with IDA-Flag she developed fever. We initiated antibiotic therapy with pipercillin and tazobactam. In the course of treatment the patient developed severe pain in the right upper abdominal quadrant, hypothermia and an abrupt rise of the CRP. Therefore we escalated antimicrobial therapy to meropenem and caspofungin. The patient was treated with linezolid due to positive blood cultures with detection of VRE. CT-scans showed a transdiaphragmal liquid structure in the liver and the right lung. Sonography-guided puncture of the liver could not obtain specimen. Due to prolonged neutropenia and the deteriorating condition of the patient autologous stem cells were applied. Despite escalation of antimicrobial therapy and treatment at the intensive care unit the patient died 16 days after initiation of chemotherapy. Autopsy showed extensive fungal invasion of the liver and the lung; especially haemorrhagic infarction, blood vessel occlusions and transmural invasions by mucor species.

Conclusions: Aspergillus species and Candida species account for most cases of invasive fungal disease but various fungi, including mucor species may be involved. Concomitant diabetes mellitus is a major risk factor. Clinical diagnosis of mucor infection is difficult and the organism mostly fails to grow on cultivation media. Early treatment with preferably liposomal amphotericine B in mucormycosis is mandatory. However, the course of this rare infection is mostly fatal.

Disclosure: No conflict of interest disclosed.

Crosstalk of cancer cells and cancer associated fibroblasts in the p53 response to Cisplatin in intact tissues from NSCLC specimen
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TP53 mutations or other alterations of the p53 pathway are common in non-small-cell lung cancer (NSCLC). Until now most of the research work focused on cell autonomous functions of p53 in the cancer cell compartment. However, in NSCLC a significant proportion of the total tumor mass is represented by tumor stroma, in particular by cancer associated fibroblasts (CAFs). Here we investigated whether the activity of p53 in cancer cells may affect the response of their adjacent CAFs to chemotherapy. We prepared tissue slices from 28 patient derived lung carcinomas and cultivated them for 96 hours. As quality control we compared morphology, Ki67 and p53 immunostaining in all cultivated tissue slices with the corresponding tumor tissue material fixed immediately after surgery (routine material). No significant change was observed in morphology, proliferation or p53 immunostatus. We next investigated effects of cisplatin treatment in these in vivo cultivated tissues. Based on the p53 induction characteristics in cancer cells, we divided the samples in 3 classes. Class I tumors showed only low basal levels of p53 in tumor cell nuclei with an accumulation upon cisplatin treatment. Class II showed constitutively high levels with no further induction upon treatment and samples of class III showed no p53 staining at all. As expected, in all cases from class II tumors TP53 mutations could be detected whereas affiliation of samples to classes I and III was found to be independent of the TP53 mutation status. Unexpectedly, p53 accumulation in CAFs in response to cisplatin could only be detected in samples of class I tumors. This was mirrored by the p53 target p21 which again was selectively induced in CAFs of class I tumors. In contrast, no p53 or p21 induction could be detected in CAFs of class II and III tumors. TUNEL was employed to analyze induction of cell death upon cisplatin treatment in cancer cells and CAFs. Interestingly, all cases with a response in CAFs showed also a response in cancer cells arguing that not only p53 induction but also induction of cell death was markedly correlated between cancer and stromal cells. In conclusion, our data indicate the existence of a crosstalk between tumor cells and their adjacent CAFs which coordinates their reaction to cisplatin treatment.

Disclosure: No conflict of interest disclosed.

The long non-coding RNA MALAT-1 stimulates cellular migration and wound healing
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Introduction: The functions of so-called long intergenic non-coding RNAs (lincRNA) have remained elusive in many cases. MALAT-1 (Metastasis-Associated-in-Lung-Adenocarcinoma-Transcript-1) is a lincRNA with strong expression levels in various cell lines and tumor types. Previously, we demonstrated MALAT-1 expression levels to be associated both with patient survival and with tumor promoting effects in non-small lung cancer (NSCLC). Here we investigate the molecular impact of MALAT-1 on cellular gene regulation.

Methods: Both biological effects (cellular migration and wound healing) and gene expression were studied in murine fibroblasts (NIH 3T3) either transduced with PNCO-MALAT-1 expression vector or with empty control vector using the Mouse Gene 1.0 ST array (Affymetrix, Santa Clara, CA). 250 genes, which showed a twofold up-regulation or down-regulation were evaluated using the Ingenuity Pathways Knowledge Base.

Results: Strong expression of MALAT-1 in mouse fibroblasts (NIH3T3 cells) significantly increased migration and wound healing potential in vitro. Depending on MALAT-1 levels, gene expression differed. The identified genes were categorized into three “Top Bio Functions” groups (250 genes out of 29,000 genes displayed a log ratio ≥1). The three most important groups are: “Cellular Growth and Proliferation” (n=90), and “Cellular movement” (n=75), and “inflammatory response” (n=65).

Conclusions: These data demonstrate that strong MALAT-1 expression levels stimulate cellular migration and wound healing in vitro. The observed biological effects are supported by gene expression analysis and contribute to the idea of multidimensional effects of MALAT-1 on important cellular functions.

Disclosure: No conflict of interest disclosed.
Abstracts

Impact of interleukin-22 in human lung cancer

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Introduction: Interleukin-22 (IL-22) is an interleukin-10-related cytokine with unique functions on interleukin-22-receptor-1 (IL-22-R1) expressing epithelial cells. Recently, it has been suggested that IL-22 may be an autocrine factor in lung cancer. However, the prevalence of this cytokine and its role in the promotion of human lung cancer are not known.

Methods: Expression of IL-22 in primary lung cancer tissue was analyzed by immunohistochemistry. IL-22 serum levels were measured by ELISA. Expression of the IL-22-R1 was addressed by Western blot and quantitative PCR. Analysis of downstream signaling was performed by Western blot. Cell viability and impact on apoptosis was assessed by cell titer blue and annexin V-propidium iodide staining, respectively.

Results: We first screened two cohorts of 205 and 2145 lung cancer samples (on a tissue microarray) for IL-22 expression. IL-22 was detected most frequently in small cell (n=50) and large cell lung cancer (n=303) with 58% and 46% respectively. IL-22 expression did not correlate with survival time in any of these subtypes. 123 sera of lung cancer patients were analyzed for IL-22 concentrations. Among the subtypes analyzed large cell lung cancer patients had the highest mean serum level (548 pg/ml, n=4). Next, we addressed why, despite the expression of IL-22 as a putative protumoral factor, the course of the disease seems unaltered. We analyzed the effects of IL-22 in five human lung cancer cell lines (A549, HCC827, H1339, H1157 and LUC1-H191). IL-22-R1 was expressed in all analyzed cell lines but the expression level differed between the cell lines. High levels of IL-22-R1 were associated with a high cellular response rate to IL-22 exposure. IL-22 induced proliferation of these lung cancer cell lines. We found no increased resistance to chemotherapy by IL-22 treatment. In contrast, when cells were continuously exposed to cisplatin until they grew drug-resistant, we found a striking upregulation of the IL-22-R1 both on protein and mRNA level. IL-22-stimulated cisplatin-resistant cells exhibited higher proliferation rates than the non-resistant controls.

Conclusions: Our data give no evidence for IL-22 expression in tumor tissue as a prognostic factor in resectable lung cancer at the time of diagnosis. In contrast, our results indicate that in chemotherapy-resistant tumor cells, upregulation of IL-22-R1 and of IL-22-responsiveness may contribute to more aggressive behavior of the disease.

Disclosure: No conflict of interest disclosed.

Increasing of mutation detection rates in NSCLC by 454 sequencing

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Introduction: Lung cancer is the leading cause of cancerrelated mortality worldwide, with an overall five-year survival rate of 15% (1). Non-small cell lung carcinoma (NSCLC) constitutes approximately 75–80% of all lung cancers (1). Significant advances in treatment were recently achieved with drugs designed specifically to target molecules that regulate critical growth and/or survival pathways of cancer cells (1). The success of a treatment with the epidermal growth factor receptor (EGFR) tyrosine kinase inhibitors (TKIs) erlotinib and gefitinib depends on the mutational status of tumor-relevant genes like EGFR and KRAS.

Methods: Sanger sequencing was applied for mutational screening in EGFR (Exon 18, 19, 21) in 36 NSCLC. Next-Generation-Sequencing (NGS) was applied for mutational screening in EGFR (Exon 18, 19, 20, 21) and KRAS (Exon 2, 3) in 40 NSCLC. DNA was isolated from FFPE-tumor tissue, amplicons (300bp) were prepared and multiplex identifiers (MID) were added manually. After purification, the pooled library was sequenced using the GS junior (454 Life Sciences, Branford, CT, USA) with an aimed coverage of 1000-fold.

Disclosure: No conflict of interest disclosed.

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Results: Sanger sequencing: 1/36 NSCLC (2.8%) showed a mutation in EGFR. NGS: 11/40 NSCLC (27.5%) showed a mutation in EGFR and 4/40 NSCLC (10%) showed a mutation in KRAS. Some of these mutations only could be detected due to the high coverage.

Conclusions: Our data show that NGS is a feasible method in routine molecular pathology to examine mutational status of tumor-relevant genes in a cost effective and comprehensive manner. In contrast, conventional capillary sequencing techniques often lack the sensitivity and cost effectiveness to detect tumor mutations occurring at less than 20% frequency. With 454-sequencing, mutations can be detected against a wildtype-background of 99% (Coverage 1000x), which is very important in solid tumors. We could improve our mutation detection rate which is an important step towards personalized medicine.


Disclosure: Tanja Hinrichsen: Employment or Leadership Position: Angestellt am Zentrum für Humangenetik und Laboratoriumsmedizin Dr. Klein und Dr. Rost
Hanns-Georg Klein: Employment or Leadership Position: Inhaber Zentrum für Humangenetik und Laboratoriumsmedizin Dr. Klein und Dr. Rost
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P469 Early clinical evaluation of FGFR1 inhibition in lung cancer: Preliminary results of a phase I study in FGFR1 amplified lung cancer patients treated with BGJ398, a pan FGFR inhibitor

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Background: Recently, within the Cologne Lung Cancer Genome Project, focal amplification of fibroblast growth factor receptor 1 (FGFR1) was identified in a large set of squamous cell carcinomas (SCC) of the lung using high resolution copy number analysis and confirmed by fluorescence in situ hybridization (FISH) with a frequency of about 20%. In addition, among small cell lung cancer (SCLC) patients, 7% were identified with FGFR1 amplification. Significant inhibition of FGFR1 amplified lung cancer cells by FGFR-TKIs was shown both in cell lines and xenotransplant models.

Methods: BGJ398 is a highly selective, orally available, ATP-competitive pan FGFR inhibitor produced by Novartis Pharma. A personalized, first in man (FIM), ongoing phase I trial evaluates BGI monotherapy in adult patients with advanced solid tumors harbouring FGFR alterations (amplifications, mutations). The primary goal of the study is to determine the maximum tolerated and/or recommended phase II dose. Secondary objectives include safety, tolerability, pharmacokinetics and anti-tumor activity in FGFR-dependent cancer. Here we present results in FGFR1 amplified lung SCC and SCLC patients.

Results: Up to the data cut-off in February 2012, 7 patients with lung cancer, among them 6 patients with FGFR1 amplified SCC were evaluable. These patients were treated in dose cohort 6 (100 mg daily) and 7 (150mg daily). Treatment was in general well tolerated with mild signs of kinase inhibitor associated toxicity (fatigue, diarrhea, decreased appetite) and with hyperphosphatemia as toxicity specific for FGFR-inhibition. Clinical efficacy was heterogenous including patients with progressive disease, stable disease and one confirmed partial response (PR). The lung SCC patient with achieved PR received BGJ398 as a 3rd line treatment and is currently in a stable partial remission for already 8 months.

Conclusions: The evaluation of FGFR1 amplified lung cancer patients in this early clinical development phase will rapidly help to answer the question whether targeting FGFR1 represents the first effective personalized treatment approach in SCC of the lung. Preliminary observations demonstrate clinical efficacy including one confirmed partial response.

Jürgen Wolf: Advisory Role: Advisory board; Financing of Scientific Research: Vortrag; Other Financial Relationships: Reisekostenerstattung

P470 Evaluation of the clinical characteristics and natural history of patients with FGFR1 amplified squamous cell lung carcinoma


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Introduction: In lung squamous cell cancer (SCC) FGFR1 amplification has been described as a new potential targetable genetic aberration. Here we describe the natural history and the clinical characteristics of lung SCC patients harboring FGFR1 amplification.

Methods: Within the Network Genomic Medicine Lung Cancer, a local screening network encompassing hospitals and office-based oncologists in the catchment area of the Center for Integrated Oncology (CIO) Köln-Bonn, we screened 553 predominantly SCC lung cancer patients from 01/2011 to 01/2012. FISH analysis was used to detect FGFR1 amplification. The definition for

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FGFR1 amplification was as follows: 50% of tumor cells showing more than 5 copies of FGFR1, or more than 15% of tumor cells displaying clusters of FGFR1, or the ratio of FGFR1 copies to centromeric copies being above 2. Clinical data were collected from medical records, the epidemiological cancer registry of Northrhine-Westphalia and treating physicians.

Results: FGFR1 FISH analysis could be conducted in 95% of the screened cases and showed amplification with a frequency of 16%. The distribution of males and females in the amplified cases was balanced. At the time of lung cancer diagnosis the median age of the patients was 67 yrs (46–82). Initial stage of the patients was: 16% stage I; 17,3% stage II; 26,7% stage IIIA; 40% stage IIIB/IV; 97,3% of the patients were ever smokers with a median of 40 pack years. The median progression free survival for stage IIIB/IV patients was 11 months (95% CI 8–14; n=14). The median overall survival was not yet reached after a median follow-up time of 14 months (95% CI 11–17; n=24).

We also screened for coexisting driver aberrations in EGFR, BRAF, KRAS, PIK3CA; ALK and ERBB2. Two patients showed a coexisting PIK3CA (E545K, H1047R) mutation and one a coexisting BRAF (V600E) mutation.

Conclusion: We show the feasibility of screening for FGFR1 amplification under routine clinical conditions. FGFR1 amplifications are common (16%) in lung SCC and are associated with smoking. The implementation of FGFR1 screening is essential for recruitment of patients in clinical trials evaluating new FGFR1 directed targeted drugs in lung SCC.

Disclosure: No conflict of interest disclosed.

P471 Implementation of real-time genetic diagnostics and personalized treatment of non-small cell lung cancer (NSCLC) in a regional screening network

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Background: Personalized treatment of genetically defined NSCLC subgroups has the potential to improve the outcome of these patients substantially. Now, the implementation of high-quality molecular diagnostics and personalized treatment strategies in routine clinical practice also outside of highly specialized academic centers is a major challenge.

Methods: In March 2010 we established the Network Genomic Medicine Lung Cancer in the catchment area of our comprehensive cancer center, i.e. the Center for Integrated Oncology (CIO) Köln Bonn after approval through the local ethics committee (10-242). We systematically screened all lung adenocarcinoma (AD) patients for the presence of ALK translocations, mutations in KRAS, EGFR, BRAF and PIK3CA and for amplification of ERBB2. Squamous cell carcinoma (SQ) patients were analyzed for FGFR1 amplifications.

Results: Between March 2010 and December 2011 1990 NSCLC patients were genotyped. In 81% of all cases material was suitable for molecular analysis. The majority of samples analyzed were AD (1020) followed by SQ (403). In AD frequencies of genetic lesions were: KRAS (32%), EGFR (13%), EMLA-ALK (3.1%), BRAF (2.6%), PIK3CA (2.5%), ERBB2 (2%). EGFR mutations were highly enriched in the lepidic and micropapillary subtype of AD, in contrast in the solid subtype the frequency of driver mutations was rather low. In samples with pure SQ component we identified FGFR1 amplifications with a frequency of 19.1%. Overall, in 40% of all NSCLC samples potentially tractable oncospecific lesions were identified. All patients with AD translocations received crizotinib when clinically indicated (11/22). 75% of the patients with activating EGFR mutations and stage IIIB/IV received erlotinib or gefitinib. The survival of the EGFR mutated patients as well as of those with the EMLA-ALK fusion was significantly better compared to the other subgroups.

Conclusions: The implementation of high-quality real-time molecular diagnostics is feasible in daily clinical routine and allows personalized treatment of patients with NSCLC in a health-care provider network resulting in an improvement of the overall survival.

Disclosure: No conflict of interest disclosed.

P472 MIMEB: A phase II trial to evaluate FDG-PET/FLT-PET and DCE-MRI for early prediction of efficacy in patients with advanced non-small cell lung cancer treated with erlotinib and bevacizumab

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Background: Molecular imaging tools gain in importance for assessment of pharmacodynamics, pharmacokinetics, prognosis and prediction of therapeutic outcome in patients with advanced NSCLC treated with targeted therapy. We set up a prospective clinical trial in order to assess the predictive value of early changes in tumor metabolism (by FDG-PET), proliferation (by FLT-PET), and tumor vascularization (by DCE-MRI) noninvasively during therapy with erlotinib and bevacizumab in previously untreated patients with advanced non-squamous cell NSCLC and to identify a subgroup of patients without a known predictive tumor-based molecular marker with clinical benefit from the combination therapy.

Methods: Patients with non-squamous NSCLC stage IV without prior systemic therapy received at least six weeks of combined erlotinib and bevacizumab. FLT and FDG-PET scans as well as DCE-MRI scans were performed at baseline, after one week of therapy and after six weeks of therapy. Standard uptake values (SUVs) of the PET scans and vascularization parameters of the DCE-MRI scans were analyzed, coregistered and compared with each other. Tumor specimens were analyzed in accordance with guidelines of a network screening panel. The primary objective of this trial was to evaluate the accu-
racy of FDG–FLT–PET and DCE-MRI for early prediction of nonprogression and PFS and its association with molecular markers.

**Results:** Of the 40 patients eligible, all received sequential FDG-PET analyses, whereas sequential FLT-PET was performed in 37 patients and DCE-MRI in 36 patients. Changes in imaging parameters during therapy were coregistered and correlated with non-progression after six weeks, progression-free survival and overall survival. Until May 2012, tissue samples from 30 patients have been genetically analysed. Preliminary results regarding the predictive value of FDG-PET, FLT-PET and DCE-MRI were successfully implemented in the treatment plan of a prospective phase II trial. Results of the ongoing analysis for evaluation of imaging analysis and correlation with tissue-based biomarkers will be presented.

**Conclusions/Perspective:** Imaging-based predictive biomarkers to identify the subpopulation of patients with pronounced benefit of the combination of erlotinib and bevacizumab as well as the association with mutational status will be presented.

**Disclosure:** No conflict of interest disclosed.

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**P473 Enhanced physical activity intervention in lung cancer patients during palliative chemotherapy – a randomized controlled trial**

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**Introduction:** This RCT tested the effects of a specially designed strength and endurance training on the independence and quality of life in lung cancer patients in stage IIIA/IIIB/IV during palliative chemotherapy. The aim was to break the vicious circle created through the connection of physical inactivity and the worsening of symptoms and side effects.

**Methods:** Between August 2010 and December 2011 lung cancer patients in stage IIIA/IIIB/IV with a good performance status receiving palliative chemotherapy treatment at the Vivantes Hospital Neukölln/Berlin, were randomly assigned into a group receiving an additional strength and endurance training and a group receiving only conventional physiotherapy. The Barthel Index and the EORTC QLQ-C30/LC13 questionnaire (Physical functioning p = 0.025, Haemoptysis p = 0.019, Pain in Arms or Shoulder p = 0.048, Peripheral Neuropathy p = 0.050, Cognitive functioning p = 0.050). Significant differences were found between the groups concerning the 6MWT, staircase walking and strength capacity (IG+CG). Additionally the level of dyspnoea decreased significantly in the IG while performing submaximal walking activities.

**Conclusion:** The training program has a positive impact on the patient’s independence in carrying out activities of daily living. Single factors of the patient’s quality of life can be significantly improved. Moreover it has a significant positive effect on the patient’s endurance and strength capacity and the patient’s dyspnoea perception. This study demonstrated that lung cancer patients receiving palliative chemotherapy should have enhanced physical activity intervention during their hospitalization.

**Disclosure:** No conflict of interest disclosed.

**P474 EGFR mutation testing in lung cancer patients of German outpatients centres – data from the clinical TLK Registry**

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**Introduction:** Tyrosine-kinase inhibitors (TKIs) are novel therapeutic options available for the treatment of lung cancer. Since 2005, they have been approved for the treatment of patients with advanced non-small-cell lung cancer (NSCLC) in Germany. However, patients with a mutated form of the epidermal growth factor receptor (EGFR) benefit more often from the treatment. To use these substances, knowledge about the EGFR mutation status is required. Here, we present data on the frequency and results of EGFR mutation testing in German outpatient centres.

**Methods:** The clinical registry on lung cancer (TLK Registry) conducted by iOMEDICO in collaboration with the Arbeitskreis Klinische Studien (AKS) prospectively collects data on the treatment of lung cancer as administered by haematology/and clinicians in routine practice in Germany. Patients are followed for 3 years. A broad set of data regarding demography, tumour characteristics, biomarkers, comorbidities, all systemic treatments, outcome data such as response rates, progression free survival and overall survival, etc. are collected. Since January 2010, 80 sites have actively recruited 1145 patients.

**Results:** Of the 904 NSCLC patients, 514 (57%) had adenocarcinoma. EGFR mutation was tested in 32% (n = 292) of all NSCLC patients and in 44% (n = 226) of those patients with adenocarcinoma. Adenocarcinoma was the most common histological subtype tested for EGFR mutation. Female NSCLC patients were tested more often than male NSCLC patients (41% vs. 28%, respectively). In patients with adenocarcinoma, 50% of female patients were tested in contrast to 40% of male patients. Of all patients tested, 16% carried the EGFR mutation. In the tested patients with adenocarcinoma, EGFR mutation was found in 22% of females and 14% of males. From 2010 to 2011, the frequency of EGFR testing has remained constant.

**Conclusion:** In German outpatient centres, one third of patients diagnosed with NSCLC are tested for EGFR mutation, with female patients and especially female patients with adenocarcinomas being tested more frequently. This indicates that patients with a higher risk for mutation (according to published data) are preferentially selected for EGFR testing.

**Disclosure:** No conflict of interest disclosed.

**P475 Report of patients with bronchogenic cancer in a single geriatric institution**

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Between 01.01.2011–31.12.2011 50 patients with bronchogenic cancer were treated in our geriatric institution (female: 22; male: 28; age range: 50–95 years): Histology: 4x SCLC, 36x NSCLC. 10 patients suffered from lung metastases which were encoded as bronchogenic cancer. Patients-characteristic: ECOG-status 1-3, median 2, Deficit in at least 1 ADL: 38/40, deficit in at least 1 IADL:Special features: 4/40 suffered from bronchogenic cancer as 2nd. neoplasia (3x head and neck tumor, 1x NSCLC 23 years after successful treatment of SCLC – continued smoker), 1/40 with 3rd. carcinoma after H&N and CRC). Further special features: 4/40 <60 year (2x male and 2x female): Male 57 y; G.W., NSCLC + WERNICKE encephalopathia, Male y. U.W., SCLC +
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Distribution pattern of metastases and their associations with survival of malignant pleural mesothelioma patients undergoing different treatment modalities

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Introduction: Optimal treatment for Malignant Pleural Mesothelioma (MPM) is still being discussed. Since the first administration of pemetrexed (PMX), bi- or multimodality therapy including chemotherapy (CTX), radiotherapy (RTX) and/or surgery is more often performed. This strategy increases overall survival (OS), but also the chance to develop metastases later in the course of the disease. Recent data about the distribution pattern of lymph node and distant metastases in MPM pts, who were treated in Germany, are limited, and the prognostic value of the initial distribution pattern of metastases is unclear.

Methods: This retrospective study included 132 MPM patients (pts) treated at the West German Cancer Center from 08/2001 to 11/2010. All pts initially received PMX-based CTX. Additionally, several pts underwent surgery (pleural pneumonectomy or pleurectomy/decortications) and/or radiotherapy. 34 pts (41%) received only palliative CTX, 47 pts (36%) underwent adjuvant RTX after CTX. 21 pts (16%) were surgically treated in combination with neoadjuvant CTX (n = 6) or in multimodality protocols (n = 15).

Results: At diagnosis, 94 pts (72%) had neither proven lymph node nor distant metastases. 24 pts (18%) presented only lymph nodes, 11 pts showed (8%) only distant metastases. 24 pts (18%) presented only lymph nodes, 11 pts showed (8%) only distant metastases.

Conclusions: The distribution pattern of lymph node and distant metastases in MPM pts is significantly associated with a worse median OS of pts (N1-N3M0: 14.0 mos) compared to pts with no metastases (N0M0: 21.1 mos; HR: 2.255, 95%CI: 1.186–4.287, P = 0.0131). Median OS of pts with only lymph node metastases (N1-N3M0: 14.0 mos) was shorter than that of pts having only distant metastases at diagnosis (N0M1: 20.4 mos), but this association was only of borderline significance (P = 0.0573). Occurrence of distant metastases without lymph node metastases did not influence OS (N0M1: 20.4 mos vs. N0M0: 21.1 mos; P = 0.922). Pts presented initially in 21% (n = 24) lymph node metastases, and in 11% (n = 14) distant metastases, mainly in the lung (n = 13), bones (n = 2) and peritoneum (n = 1). Until death 32 pts (24%) developed lymph node and 55 pts (42%) distant metastases, mainly in the lung (n = 24, 18%), liver (n = 14, 11%), or peritoneum (n = 13, 10%). Needle-track metastases arose in only 8 pts (6%).

Disclosure: No conflict of interest disclosed.

P477

Dual inhibition of MEK/ MAPK and PI3K/Akt in multiple myeloma

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Introduction: Aberrant signaling through the so-called RAS-dependent pathways via MEK/ MAPK and PI3K/Akt has been implicated in multiple myeloma (MM) cell growth and survival. Inhibition of these pathways might be a useful treatment option, although pre-clinical efficacy in primary MM cells varies and may be improved by drug combinations. However, combined inhibition of both of these pathways has not systematically been studied in MM.

Methods: We performed dual pharmacological blockade of MEK and either PI3K/mTOR or Akt in a large series of primary MM samples (n = 55) and MM cell lines (n = 11), using the small molecule MEK-inhibitors PD184352, PD0325901, PI3K/mTOR-inhibitor PI103 and Akt-inhibitor Akt-1,2. The rate of apoptotic cells was measured with flow cytometry staining for AnnexinV-FITC/PI. Peripheral blood mononuclear cells from healthy donors served as normal cell controls. Additionally, we screened the tested MM cell lines for activating point mutations in K- or N-RAS at positions 12, 13 and 61, and corre- lated the RAS-status with cell survival data.

Results: Single-agent inhibition of Akt or PI3K/mTOR induced apoptosis in up to about half of primary MM cases with a large range of activity, whereas MEK blockade yielded only minor to moderate cytotoxicity. Still, combined treatment with MEK and either Akt or PI3K/mTOR inhibitors significantly enhanced cell death in 75 % of the samples compared to Akt or PI3K/mTOR inhibition alone. Correlation with RAS mutation status revealed that the quarter of samples that was most resistant to combination treatment, all were RAS wildtype. Samples that were sensitive to combined treatment comprised RAS mutated as well as RAS wildtype cases.

Conclusion: Our comprehensive analysis with primary MM samples showed that combined targeting of MEK/ MAPK and PI3K/Akt signaling could be an effective anti-MM strategy. Our results suggest that mutated RAS serves as predictor for sensitivity to this combination treatment. However, genetic lesions other than in RAS are also expected to confer high sensitivity as sug- gested by the presence of sensitive but RAS wildtype samples. Patients with RAS mutated MM could therefore constitute a subgroup for which combina- tion treatment with MEK and PI3K/Akt inhibitors might prove particularly beneficial.

Disclosure: No conflict of interest disclosed.

P478

From CLL to multiple myeloma – Spleen Tyrosine Kinase (Syk) influences multiple myeloma cell survival and migration

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Introduction: Spleen Tyrosine Kinase (Syk) has proven to be an important target in various B-cell malignancies. Recent results have shown a relation between the CD31/CD238 pathway and Syk-signaling. Since CD38 is well present on plasma- and multiple myeloma (MM)-cells, we sought to elucidate the importance of Syk in MM as compared to CLL as published (Buchner M et al. Blood 2010:115:4497–506).

Methods: Experiments were performed using MM cell lines (MMC: L363, MM.15, MM.1R, RPMI8226, U266, IM-9) and bone marrow (BM) specimen from MM patients (pts). Healthy donor BM samples and CLL pts served as controls. Syk expression was determined via immunoblotting. Flow cytometry was used to evaluate cell viability after Annexin/PI-staining. Chemotaxis was
performed in a two-chamber migration plate to several chemotrafactants and detected by flow cytometry.

**Results:** MMCLs displayed varying expressions of Syk. We determined a variation from 0.5 (U266) to 2 (RPME8226) fold, compared to peripheral mononuclear blood cells (PBMC). Treatment with the Syk-inhibitor R406 for 24h led to significantly reduced viability in L363 (-9.0%, p<0.001 compared to vehicle control) and MM.1R (9.4%, p=0.01) as compared to MM.1S (+4.9%), which proved less sensitive to R406 (n=10). In combination with Bortezomib an accumulative apoptotic potency in L363 and MM.1R was detected. Further evaluation of the downstream mechanisms revealed McI-1 downregulation in L363 and MM.1R which appeared to be partly responsible for the R406-induced effect. Currently, additional downstream proteins (AKT, ERK) are tested. High CD38 expression was observed on four of five cell lines (L363, MM.1S, MM.1R, RPME8226). Syk inhibition did not result in a significant change of CD38 expression as detected in R406-treated CLL pts samples after 24, 48, or 72h in L363 cells. The migratory capacity of L363 to BM supernatant from healthy donors was significantly reduced after targeting Syk. Compared to CLL pts, MM BM pt samples appeared to have lower Syk and pSyk expression.

**Conclusions:** Syk expression in MMCLs (L363, MM.1S, MM.1R) could be shown to resemble that in CLL pts controls. Inhibition of Syk via R406 led to potent cytotoxic and antimigratory effect on MMCLs in vitro. Despite these findings we observed low expression of pSyk in MMCL and low pSyk and Syk in MM pt samples. Our data confirm the presence of Syk, but contradict the hypothesis of an important role of Syk for MM pathogenesis.

**Disclosure:** No conflict of interest disclosed.

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**P479**

**PAT-SM6 – a novel antibody targeting multiple myeloma**

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**Introduction:** Chemoimmunotherapy involving B-cell directed monoclonal antibodies has improved prognosis of lymphoid malignancies like ALL and B-cell lymphomas but remains largely unsuccessful for the treatment of multiple myeloma.

**Methods and results:** PAT-SM6, a fully human IgM antibody, revealed a homogeneous binding independent from stage of disease to primary MM cells. In contrast no binding was detected on primary non-malignant hematopoietic tissue including plasma cells. Further characterization demonstrated that PAT-SM6 binds to the tumor specific variant of the heat shock protein GRP78 stably expressed on the surface of primary MM cells. Moreover, antibody treatment of both MM cell lines and primary MM cells caused significant cell death (range 74.3–33%) and further analysis revealed the induction of apoptosis as main mode of action. In addition, cell death was increased by adding complement to the cell cultures resulting in significant complement dependent cytotoxicity (CDC). In summary, PAT-SM6 specifically targets myeloma cells, induces cytotoxicity by induction of apoptosis and CDC and therefore provides a promising approach for immune therapy of multiple myeloma.

**Conclusion:** These results are the basis for a phase I dose escalating study in patients with relapsed multiple myeloma which will be initiated soon at our centre.

**Disclosure:** Leo Rasche: No conflict of interest disclosed. Stephanie Brändlein: Advisory Role: Beratervertrag mit der Fa Patrys Limited Australien

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**P481**

**One third of Afroamerican patients with MGUS/MM are carriers of hyperphosphorylated paratarg-7, the first autosomal-dominantly inherited risk factor for hematological neoplasms, to develop monoclonal gammopathy of undetermined significance (MGUS) and multiple myeloma (MM)**

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**Background:** Hyperphosphorylated paratarg-7 (p-p7) is a frequent target of paraproteins in German patients with monoclonal gammopathy of undetermined significance (MGUS)/multiple myeloma (MM). The frequency of MGUS/MM is lower in Japan than in Europe, while it is higher in Afroamerican people. As p-p7, the first molecularly defined autosomal-dominant risk factor for any hematological neoplasm, is inherited in a dominant fashion, we determined the incidence of the p-p7 carrier state in a Japanese and in an Afroamerican population, and compared the frequency of p-p7-specific paraproteins and the p-p7 carrier state in those patients with MGUS/MM.

**Methods:** Peripheral blood from 111 Japanese patients with MGUS/MM, 65 Afroamerican people with MM/MGUS and 278 healthy blood donors was analyzed for the p-p7 carrier state by isoelectric focusing and for p-p7-specific antibodies by ELISA.

**Results:** The Japanese and the Afroamerican groups were compared with 252 German MGUS/MM patients and 200 healthy controls. Five of 111 (4.5%) Japanese, 22 (8%) Afroamerican and 35 (13.9%) German IgA/IgG MGUS/MM patients had a p-p7-specific paraprotein. The prevalence of healthy p-p7 carriers in the Japanese study group was 1/278 (0.36%) and in the Afroamerican group was 2/66 (9%), whereas it was 4/200 in the German group. The relative risk for p-p7 carriers developing MGUS/MM had an odds ratio of 13.1 in the Japanese, 3 in the Afroamerican and 7.9 in the German group.

**Conclusion:** The proportion of p-p7 carriers with a p-p7-specific paraprotein is lower among Japanese than in German patients with MGUS/MM, while it is higher in Afroamerican patients. p-p7 carriers in all ethnic groups have a high risk of developing MGUS/MM.

**Disclosure:** No conflict of interest disclosed.

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**P482**

**In multiple myeloma oligoclonal abnormal protein bands recognize recurrent myeloma antigens after allogeneic stem cell transplantation**

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**Introduction:** Allogeneic stem cell transplantation can offer long term remissions in multiple myeloma especially for patients with high-risk disease. After transplantation up to 70% of patients develop oligoclonal abnormal protein bands (APB) on immunofixation, which do not correspond to the patient’s paraprotein, but most likely to an overshooting antibody production by different regenerating B cell subclones. APB have been found to confer a good prognosis suggesting that these oligoclonal immunoglobulins could be involved in a specific humoral anti-myeloma immune response. We therefore set out to investigate the antigen-specificity of APB to find out if these immunoglobulins react with common myeloma antigens.
Abstracts

Methods: Immunoglobulins were chromatographically purified from the sera of patients with multiple myeloma after allogeneic stem cell transplantation. Patients with and without APB on immunofixation were included in this study. Reactivity of immunoglobulins with myeloma cell extracts was assessed by immunoblot. Immunoglobulin fractions showing reactivity with the myeloma extracts were subsequently used to probe two-dimensionally separated myeloma extracts. Immunodetected protein spots were excised and identified by mass spectrometry. Subcellular localization of individual antigens was assessed by immunofluorescence confocal imaging.

Results: We found that immunoglobulin fractions with APB recognized multiple recurring protein targets. In contrast, immunoglobulin fractions of myeloma patients without APB and those of healthy donors showed no or markedly reduced reactivity with such antigens. Many of the protein targets were upregulated or differentially expressed in myeloma. Proteins from the heat-shock family were most frequently identified. Other significant targets were neutral alpha-glucosidase, alpha-enolase, proliferating cell nuclear antigen and MAGEA4. Characterization of HSP60 as one APB target revealed an aberrant, tumor-specific membrane display pattern of this antigen, potentially explaining how this antigen is made accessible to the immune system.

Conclusion: Our findings suggest that the better prognosis of myeloma patients developing APB could be due to a specific anti-myeloma immune response.

Disclosure: No conflict of interest disclosed.

P483 Analysis of two assays for serum free light chain kappa and lambda using polyclonal and monoclonal antibodies

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Introduction: Serum free light chains (FLC) kappa and lambda are used for diagnosis and monitoring of multiple myeloma and related plasma cell disorders.

Methods: In this method comparison we measured 123 samples of 101 patients (n=44, f=57) from the Department of Hematology. The mean age was 68±11 years and diagnoses were: multiple myeloma, n = 66; monoclonal gammopathy of undetermined significance (MGUS), n = 20; Waldenström’s macroglobulinemia, n = 8; smoldering myeloma, n = 4; primary amyloidosis, n = 2; POEMS syndrome, n = 1. Polyclonal antibody-based assays (Freelite®. The Binding Site, Birmingham, UK) and monoclonal antibody-based assays (N Lates FLC, Siemens, Marburg, Germany) for free light chain (FLC) kappa and lambda were used. All samples were run on a BNII nephelometer.

Results: Correlations between both assays were R = 0.96 and R = 0.70 for FLC kappa and FLC lambda, respectively. Moreover, the concordance between the two methods was 95% for the FLC kappa/lambda ratio. Discordant results were observed for the FLC kappa/lambda ratio in 9 samples out of 9 patients (5 multiple myeloma, 1 smoldering myeloma, 1 MGUS, 1 POEMS syndrome, 1 Waldenström’s macroglobulinemia). All 9 patients revealed a positive serum immunofixation electrophoresis.

Conclusions: The clinical value of monoclonal antibody-based assays for the measurement of FLC kappa and lambda should be further evaluated.

Disclosure: No conflict of interest disclosed.

P484 Hepatitis B virus infection is associated with deletion of chromosome 8p21 in Multiple Myeloma

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Introduction: Serologic analyses within epidemiological cohort and case-control studies indicate an association between HBV infection and risk of multiple myeloma (MM). To verify the relationship with an independent approach, we investigated the correlation between Hepatitis B (HBV) positivity and specific chromosomal aberrations which were routinely determined in MM patients.

Methods: The patients group was derived from the 680 MM patients of the years 2004–2010 which were recorded in the clinical cancer registry of the NCT. The dataset was supplemented with data from a specialised clinical database of the Section MM. HBV status was determined identifying serologically HBsAg and anti-HBc, and interphase FISH analysis was performed on CD138-purified plasma cells using a comprehensive MM specific probe set. Descriptive tests for differences among HBV positive (HBsAg positive and/or anti-HBc positive) and HBV negative (HBsAg negative and anti-HBc negative) MM patients, by demographic and clinical characteristics, were performed using the SAS procedure FREQ and the c² test-based p-value. Group comparisons on prevalence were done by computing the odds ratios (OR) with 95%-confidence limits (95%-CL) using the SAS procedure LOGISTIC. The ORs were adjusted for age, gender and tumour stage (SSD).

Results: Overall, 46 (6.8%) of the 680 patients under investigation were HBV positive. The characteristic properties age, gender and SSD were fairly well balanced. Only five out of the 46 HBV positive patients had an active infection (HBsAg positive), while 41 had a resolved infection (HBsAg negative and anti-HBc positive). The FISH analyses for 5 frequently observed gains [1q21, 9q34, 13q13, 15q22 and 19q13] and 5 frequently observed losses [6q21, 8p21, 9q34, 11q23, 15q22 and 19q13] were available. The odds ratio for loss of 8p21 was significantly elevated and for loss of 13q14 non-significantly increased in HBV positive patients.

Conclusion: Both of these losses (8p21, 13q14) were shown associated to HBV positivity also within hepatocellular carcinoma. Thus, the present result is consistent with other HBV-related findings and supports previous indications to a positive association of HBV with MM. In summary, these observations suggest for the first time a virus involvement in the aetiology of MM.

Disclosure: No conflict of interest disclosed.

P485 Serum immunoglobulin alterations in patients with chronic hepatitis C

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Background and aims: Adaptive humoral immune responses play a central role in the pathogenesis and clinical course of patients with chronic hepatitis C virus (CHC) infection. CHC infection is associated with the development of
B cell lymphoproliferative disorders including monoclonal gammopathy of undetermined significance (MGUS). We aimed to investigate both polyclonal and monoclonal changes of serum immunoglobulins (Ig) in our Essen CHC cohort and correlate the results with the clinical course of the disease.

**Methods:** To detect monoclonal proteins standard serum electrophoresis was combined with parallel screening immunofixation using pentavalent antiserum. Free light-chains (FLC) κ and λ were measured in all samples. We used a summated FLC (sFLC, FLC κ+λ) concentration of >50 mg/l and a normal I/k ratio of 0.26–1.65 to define patients with a polyclonal FLC elevation.

**Results:** FLC measurements were available in 325 patients (mean age 50.2±13.5 y, male/female ratio 1:1.5). Median FLC κ were 20.7 mg/l (range 4.0–328), median λ 22.9 mg/l (4.9–1510). Ten MGUS cases were identified among 324 screened samples (prevalence 3.1% 95%-CI 1.5–5.6). Notably, n=7 had an IgM or IgA isotype and n=4 were <50 years old. A total of 9 (3%) cases showed a pathologic FLC ratio, polyclonal elevation was present in 121 cases (38.5%). Interestingly, CHC patients convoluted with HIV (53/328, 10.2%) exhibited higher sFLC levels than HIV negative CHC patients (median 66.8; range 24.8–289.7 mg/l vs. median 42.2 range 9–1533.6 mg/l; p<0.0001).

Data on antiviral treatment was available for 97 of 220 treated CHC patients (44.1%). Of note, a preliminary analysis on the outcome of antiviral treatment in these patients revealed a significantly higher prevalence of elevated sFLC levels in responding compared to resistant patients (29/76 (38%) vs. 2/17 (12%), p=0.04).

**Conclusions:** HCV induces both polyclonal and monoclonal changes in serum Ig proteins. Elevated polyclonal sFLC baseline levels could be tested as a new response predictor in HCV treatment.

**Disclosure:** Patricia Johansson: No conflict of interest disclosed.

Jan Dürrig: Expert Testimony: The Binding Site

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**P486**

**Conditional survival analyses in patients with multiple myeloma: a different way to define prognosis**

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**Introduction:** Prior analyses have advocated that mortality from major cancer has declined reflecting continuing progress in cancer prevention, early detection and treatment. Survival estimates are typically presented as the probability of surviving a given length of time after the diagnosis. In contrast, conditional survival (CS) describes the probabilities of surviving y additional years given patients survived x years. CS provides additional information about how the risk of death may change over time, taking into account, how long someone has already survived. CS analyses in multiple myeloma (MM) are as yet lacking.

**Methods:** We evaluated 816 consecutive MM patients (pts) treated at our department between 1997–2011. Via electronic tumor documentation system, age, gender, disease stage (Durie/Salmon (D/S)), time of death and last follow-up were assessed. We determined 5-years-CS (5y-CS) as the probability of surviving at least 5 more years as a function of years a pt had already survived since initial diagnosis (ID), i.e. 5y-CS for those, who survived 0, 1, 2, 3, 4 and 5 years after ID. 5y-CS was stratified according to age, and stage.

**Results:** The OS probabilities at 5- and 10-years were 50% and 25%, respectively. The 5y-CS probabilities remained almost constant (~53%) over the years a pt had already survived after ID. According to baseline variables, CS-estimates showed no gender difference. However, D/S stage I vs. stage II-III showed different 5y-CS estimates over the years (75% vs. 42%, respectively) for those who survived 1 year after ID. Age subgroups ≤60, 60–70 and >70-years showed substantially different 5y-CS-estimates, but also remained constant over the course of time with ~63%, 51% and 27%, respectively. The multivariable Cox model, including gender, year of admission, age and D&S, showed increased HR for both latter groups (p=0.001). At the 80 years decreased, but increased for those ≥70-years over the study period, illustrating that not only young and fit, but also elderly, comorbid and advanced pts are increasingly treated within large referral/university centers. Analyses stratified by age and stage revealed substantially different OS- and CS-estimates.

**Conclusions:** CS seems an attractive tool to predict outcome over time, supplements existing measures and may guide cancer survivors in planning their future. Ongoing analyses aim to distinctively define long-term survivors and to identify MM-related risks.

**Disclosure:** No conflict of interest disclosed.

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**P487**

**Neuropil Gelatinase-Associated Lipocalin (NGAL) as a marker of renal injury in multiple myeloma patients**

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**Introduction:** Acute kidney injury (AKI) is a common complication in multiple myeloma (MM) disease. In diagnosis and treatment, serum-creatinine is mainly used determining renal function but poorly reflects acute renal functional changes. Estimated glomerular filtration rate (eGFR) is more exact but has greater value in chronic kidney disease. As restoration of renal function has prognostic implication for MM patients (pt), novel biomarkers of kidney injury are of importance. Neuropil gelatinase-associated Lipocalin (NGAL) is an early and specific marker of AKI. It was recently evaluated in critically ill and organ transplanted pt. Here, we assessed urinary NGAL in pt with MGUS, asymptomatic and symptomatic MM disease.

**Methods:** 124 pt were evaluated. 9 pt had MGUS (4M/5F; median age 65y), 11 pt had asymptomatic MM (5M/6F; median age 63y) and 104 pt (56M/48F; median age 66y) had symptomatic MM either at primary diagnosis, in remission or in relapsed disease. Urinary NGAL was measured with an ELISA technique according to manufacturer instructions.

**Results:** 32 of 104 pt (30.8%) with symptomatic MM and 1/9 (11.1%) pt with MGUS had eGFR <60 ml/min/1.73m², 34/98 (34.7%) evaluable pt with symptomatic MM had proteinuria >200 mg/24h with detected Bence-Jones Protein (BJP) at the time of evaluation, 24 (33.8%) of the remaining 74 patients with proteinuria <200 mg/24h had BJ proteinuria at primary diagnosis. Median NGAL was 5.5 ng/ml (interquartile range 3.9–9.1 ng/ml) in pt with MGUS and 10.8 ng/ml (4.1–22.7 ng/ml) in pt with symptomatic MM disease. In symptomatic MM pt, median NGAL was 15.4 ng/ml (6.0–83.1 ng/ml) in pt with GFR <60 ml/min/1.73m² in pt with eGFR <60 ml/min/1.73m². For pt with BJ proteinuria, median NGAL was 19.5 ng/ml (7.8–58.9 ng/ml) pt with proteinuria <200 mg/24h showed median NGAL of 9.5 ng/ml (3.6–16.4 ng/ml). Using a cut-off for urinary NGAL at 30 ng/ml, which was shown to be predictive for AKI in other cohorts, 12/18 (66.7%) pt with NGAL >30 ng/ml had BJ proteinuria >200 mg/h, whereas 21/80 (26.7%) of pt with NGAL ≥30 ng/ml had BJ proteinuria >200 mg/24h. There were no differences in NGAL values regarding myeloma disease state.

**Conclusion:** Urinary NGAL is a promising biomarker for evaluation of renal injury in multiple myeloma disease. Our data show that NGAL is higher in pt with BJ proteinuria irrespective of renal function. Further prospective evaluation is warranted.

**Disclosure:** No conflict of interest disclosed.
P488
Treatment of multiple myeloma in Germany – an update of a representative multicentre health care survey

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Aim: The current survey was undertaken to gain insights into the changes of disease management of multiple myeloma (MM) over time and the implementation of new guidelines in clinical routine in Germany.

Patients and methods: 817 centres involved in the treatment of MM including university hospitals (UH), community hospitals (CH), and oncologists in practice (OP) were contacted. 15% of identified centres provided information on 1378 pts. corresponding to 13% of the expected national prevalence. Detailed data on 478 uns selected patients with treatment decisions in the first and second quarters of 2011 (start, change or end of therapy) in 58 representative centres (10 UH, 27 CH, 21 OP) were included in this analysis. Data was verified by central monitoring. For all comparisons a p-value of less than 0.05 was considered statistically significant. The results were compared to similar published surveys in 2004, 2006 and 2009.

Results: At the time of first diagnosis most patients (57%) were already in stage III (Durie-Salmon). Cytogenetic analysis was performed on 53% of pts. and 75% of these with FISH-based cytogenetic information. 13q deletions were identified in 38% of patients whose cytogenetic status was analyzed with FISH. The risk assessment has become well established (75% in 2011 vs. 39% in 2009). Overall 33% of patients were considered as candidates for stem cell transplantation. SCT was performed on 19% of the pts. and a SCT was scheduled as the next treatment curse for 9% of the pts. There is a remarkable shift in the treatment: Bortezomib was administrated to 67% pts. in first line and Lenalidomide to 27% pts. in second line at that time. The real treatment for defined subgroups was compared with the suggested therapy algorithm in DGH0 guidelines. The majority (56%) of symptomatic pts. is 70 years or older and have an ECOG 0–2. 72% of this subgroup were treated with Bortezomib as recommended in the guidelines. 34% of symptomatic pts. are younger than 70 yrs, with an ECOG 0–2 and with ISS 2–3. 70% of these groups received a treatment corresponding with the guidelines (BoAD, BoD3, BoCD or CAD). 48% of them were candidates for a SCT.

Conclusion: Regarding diagnostic measures and treatment algorithm, an increasing correspondence of clinical routine with the recommended guidelines was evident. Nevertheless, stem cell transplantation was considered in not all eligible candidates. Novel substances, however, were rapidly integrated into the treatment of MM.

Disclosure: No conflict of interest disclosed.

P489
Bendamustine and prednisone in combination with Bortezomib in the treatment of patients with relapsed/refractory multiple myeloma

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Introduction: Bortezomib is a proteasome inhibitor that has shown important clinical efficacy either as a single agent or in combination with other cytostatic agents in multiple myeloma (MM). The combination of bortezomib with bendamustine and prednisone (BPV) was assessed to determine the efficacy and toxicity of this regimen in patients with advanced MM.

Methods: Between January 2005 and December 2011, 78 patients (median age 62; range 31–81 years) with relapsed or refractory MM were treated with bendamustine 60–80 mg/qm on day 1 and 2, bortezomib 1.3 mg/qm on day 1, 4, 6, 9, and 11, and prednisone 100 mg on day 1, 2, 4, 8 and 11. Cycles were repeated every 21 days until maximum response or progressive disease. Previous therapy lines ranged from 1 to 9 (median 2), and included 31 thalidomide, 10 x lenalidomide, 14 x bortezomib, 24 x autologous PBSC, and 19 x autologous/allogeneic PBSC. 39 patients were refractory to the last treatment. 33 patients had preexistent severe thrombocytopenia, leukocytopenia or anemia (WHO grade 3 or 4). Response was assessed using EBMT criteria modified to include near complete remission (nCR) and very good partial remission (VGPR).

Results: The median number of the BPV-treatment was 2 (1–7) cycles. 54 patients (69 %) responded after at least one cycle of chemotherapy with 3 CR, 14 CR/PR, and 37 partial remissions. Median overall response rate was 46 % (0 % CR, 46 % PR). 31 % of the individuals tolerated was 4,5 (range 2 to 8) with a dose between 60 to 90 mg/qm (day 1+2). The median number of the BPV-treatment was 2 (1–7) cycles. 54 patients (69 %) responded after at least one cycle of chemotherapy with 3 CR, 14 CR/PR, and 37 partial remissions. Median overall response rate was 46 % (0 % CR, 46 % PR). 31 % of the individuals


P490
Efficacy and toxicity of bortezomib / dexamethasone chemotherapy in patients with advanced cardiac light chain amyloidosis

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Purpose: Advanced cardiac involvement has a poor prognosis in patients with light chain (AL-) amyloidosis. Since bortezomib is known to rapidly lower the light chain (AL)- amyloidosis by defining Mayo stage III for cardiac biomarkers (Dispenzieri et al., 2004).

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**Table 1.**

<table>
<thead>
<tr>
<th></th>
<th>Untreated / Treated</th>
<th>NYHA stage III</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median NT-proBNP ng/l</td>
<td>10.391 (663–216.187)</td>
<td>32 / 24 pts</td>
</tr>
<tr>
<td>Median cTNT pg/ml</td>
<td>0.1 (0.04–0.21)</td>
<td>44 pts</td>
</tr>
<tr>
<td>Median dFLC mg/l</td>
<td>217 (17–14.269)</td>
<td>3 / 1–8</td>
</tr>
<tr>
<td>Median number of bd cycles</td>
<td>3 (1–8)</td>
<td>24 pts (22 of cardiac failure)</td>
</tr>
<tr>
<td>Death during bd</td>
<td>8 / 6 pts</td>
<td>Non-hematol tox NCI grade 3 / 4</td>
</tr>
<tr>
<td>Hem tox NCI grade 3 and 4</td>
<td>9 pts (all thrombocytopenia)</td>
<td></td>
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</tbody>
</table>

**Patients and methods:** We retrospectively evaluated a cohort of 56 AL patients, who were classified as Mayo stage III cardiac amyloidosis based on their NT-proBNP / troponin T or high-sensitivity TNT values. 24 patients were previously treated with a melphalan based regimen. Bd was administered twice weekly, using a bortezomib dosis of 1.0 mg/m² and a dexamethasone dose of 16 mg per week.

**Results:** Median NT-proBNP level was 10.391 ng/l, median cTNT level 0.1 pg/ml. Twenty-four out pts died during bd treatment. Median observation of patients is 7 months after start of bd. Median overall survival (OS) is 9 months (mo). Thirty-five patients died; among them 24 (43%) during ongoing bd therapy. Median OS of the first line therapy group was 7 mo and 13 mo in the relapsed group. Hematologic remission was achieved in 31/56 patients (3 CR, 28 PR). Grade 3 and 4 non-hematologic toxicity included polyneuropathy (n=4), cardiac decompensation (n=4), pneumonia (n=2), viral infection (n=2), renal failure (n=4), muscle weakness (n=1), elevated liver enzymes (n=1) and ileus (n=1). Therapy was stopped before the planned 6–8 cycles in 20 patients after a median of 3 (range 1–4) cycles, in 11 cases due to toxicity, in 7 cases due to lacking hematologic response and in 2 cases due to the patient’s choice. The therapy is still ongoing in 3 pts at the time of reporting.

**Discussion:** In this study we assessed whether patients with advanced cardiac amyloidosis benefit from bortezomib / dexamethasone therapy. We observed a high rate of early deaths in the untreated group reflecting the far advanced cardiac damage. Therefore, every patient <60 years with high NT-proBNP and cardiac failure stage NYHA 3 might be evaluated for high-urgent cardiac transplant if he has no further organ involvement.

**Conclusion:** The treatment of AL patients with far advanced cardiac disease remains a major challenge despite administration of highly effective drugs as Bortezomib and requires close supervision by an experienced hematologist.

**Disclosure:** Stefan Schönland: Financing of Scientific Research: Vorträge Janssen
Utte Hegenbart: Financing of Scientific Research: Vorträge Janssen

**P492**

**Bendamustine in advanced multiple myeloma previously treated with bortezomib and lenalidomide containing regimen**

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**Introduction:** In recent years treatment of multiple myeloma has substantially been improved through the introduction of novel substances, likethalidomide, lenalidomide and bortezomib, the use of autologous stem cell transplantation (ASCT) and supportive measures. Nevertheless, patients do relapse and therapy of the intensively pretreated and mostly older myeloma patients remains an ongoing challenge. Bendamustine can be employed in patients with restricted kidney function or peripheral neuropathy and plays an important role in the treatment of indolent Non-Hodgkin-Lymphoma. In contrast, there is only little data concerning the efficacy of bendamustine in heavily pretreated multiple myeloma patients.

**Methods:** In our retrospective, single institution analysis we evaluated multiple myeloma patients previously treated with IMiDs, i.e. thalidomide and/or lenalidomide and bortezomib.

**Results:** We evaluated 15 patients with a median age of 72.5 years (range 52 to 81) who had received a median of 7 (range 2 to 11) treatment regimen. All patients had received a treatment containing an IMiD and bortezomib alone or in distinct combinations. The median number of bendamustine cycles administered was 4.5 (range 2 to 8) with a dose between 60 to 90 mg/qm (day 1+2). The overall response rate was 46 % (0 % CR, 46 % PR). 31 % of the individuals...
achieved a SD and 23 % had progressive disease. The median PFS was 6 months (range 1 to 14). Side effects included mainly hematologic toxicity. Specific data of these patients and of patients from another large center will be presented.

Conclusions: In these intensively pretreated patients bendamustine appears to be a promising treatment option that warrants further exploration.

Disclosure: No conflict of interest disclosed.

P493
Update from the myeloma registry of the University Hospital Graz — impact of new drugs on disease management in the transplant setting

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Introduction: Although multiple myeloma (MM) remains an incurable disease, outcome for patients has improved substantially. High-dose therapy with autologous stem cell transplantation (ASCT) was already introduced in the 1980’s, remains an important treatment option for selected patients and is routinely performed in our department.

Methods: Our registry was initiated in 2008. Purpose was to determine MM treatment modalities and to assess the outcome and safety under real-life conditions. This update covers a period of more than 4 years and 151 patients cared for by the hospital’s outpatient department, with initial diagnoses extending back to 1990. Here we report on various aspects pertaining to therapeutic management with focus on stem cell transplant (SCT). Analysis of the dataset was performed by means of descriptive and exploratory methods.

Results: 41% of patients (62/151) were transplanted (autologous or allogeneic) at least once during the course of their disease. The median age at diagnosis was 55.8 years for the transplant group, while it was 69.3 years for non-SCT patients. The portion of females was higher (ASCT: 55%, non-ASCT: 54%). The disease of SCT patients was more advanced at initiation of therapy (ISS III: 74% vs. 41%). A single transplant was performed in 43 patients (69% of all transplanted patients), tandem transplant in 12 and triple SCT in 7 patients. Median time from MM diagnosis to first SCT was 235 days. Induction regimens were VAD or PAD in 94% of cases. The most common conditioning regimen was MEL 200 (53/62). Only 5 patients underwent allogeneic transplant. 42% of transplanted patients received maintenance therapy consisting of interferon, thalidomide or bortezomib. Duration was often limited due to poor tolerability. Generally, number of SCTs seems to decrease.

Conclusions: SCT is a well established treatment option for our patients. The recent introduction of new drugs such as thalidomide, bortezomib or lenalidomide has substantially altered the disease management and has also influenced timing and frequency of transplants. Number of SCTs seems to decrease. Maintenance therapy is needed in a substantial number of SCT patients and might be applied more frequently when more tolerable drugs for long-term use will be available.

Disclosure: Siegfried Sormann: Advisory Role: Consultingtätigkeit Werner Linkesch: No conflict of interest disclosed.

P494
Influence of novel agents on disease progression in patients with multiple myeloma who have received high-dose therapy with stem cell transplantation

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The treatment of Multiple Myeloma has experienced dramatic changes during the recent decade. In particular high-dose therapy followed by autologous stem cell transplantation has proven beneficial and recently potent novel agents such as bortezomib, thalidomide and lenalidomide have been introduced. These agents show impressive remission rates and have demonstrated superiority in progression and overall survival benefit when used in different scenarios, especially in patients without the option of high dose therapy. We wondered if in a real life population in patients receiving HDT a further benefit of novel agents is detectable compared to patients who received a high-dose therapy only.

In this analysis 103 patients with multiple myeloma who have received a high-dose therapy and autologous stem cell transplantation during 1994 to 2009 in a single center institution were included. Patients were excluded if they received an allogeneic SCT. The median age at first diagnosis was 53 years (28–96). 35% (n=36) were female, 65% (n=67) were male. For the purpose of this analysis patients were separated in 2 groups: one including patients who received novel agents at any time during treatment (n=41) and the other without receiving novel agents at any time (n=62). We analyzed in particular the median overall survival for the total population and for each group separately to be able to compare the results of these two groups with each other. We analyzed several influence parameters, such as renal function, ISS, stage, type of induction therapy, LDH and subtype of immunoglobulin. With all precautions of a retrospective analysis we could demonstrate in almost all subgroups a clear superiority for patients who received novel agents during the therapy.

In the entire cohort our results demonstrate a benefit in median overall survival (86 vs 52 months), with a 5 year survival rate (66% vs 40%). Results of subgroups will be demonstrated separately. In addition, our data show promising data for the early use of bortezomib as part of induction therapy, as these patients experience a 88% 5-year survival in comparison to patients with VAD or ID induction therapy only.

In this single center based experience we could find a benefit of the additional use of novel agents in patients receiving HDT, independent of the application time point in a real life patient population.

Disclosure: No conflict of interest disclosed.

P495
High-dose-Melphalan vs. Busulfan-Melphalan in patients with recurrent multiple myeloma after previous autologous stem cell transplantation

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Introduction: Patients with relapsed multiple myeloma (MM) after previous autologous stem cell transplantation (ASCT) have a poor prognosis. Possible treatment options in these patients are conventional salvage therapy, allogeneic SCT and autologous retransplantation (Re-ASCT). The goal of this retrospective analysis was to investigate the impact of the conditioning regimens high-dose-Melphalan (HD-Mel) vs. Basulfan-Melphalan (Bu-Mel) on response, toxicity and survival after Re-ASCT.

Methods: From July 2004 to September 2011, 34 MM patients (14 male/20 female, median age 65 years, range 44–74) with relapse after ASCT underwent Re-ASCT at Klinikum Chemnitz. 26 patients (76%) experienced 1st, 3 patients (9%) 2nd and one patient (3%) 3rd relapse. Four patients (12%) had reached a stable disease (SD) after previous ASCT. Pretreatment consisted of a median of 39 months (20–71) and all had been extensively pretreated with various drugs.

Results: The disease progression rate (32% vs. 33%) and overall response (87% vs. 91%). No significant differences were seen in the patient with scleromyxedema during bortezomib-based chemotherapy and side effects of this analysis patients were separated in 2 groups: one including patients who received novel agents at any time during treatment (n=41) and the other without receiving novel agents at any time (n=62). We analyzed in particular the median overall survival for the total population and for each group separately to be able to compare the results of these two groups with each other. We analyzed several influence parameters, such as renal function, ISS, stage, type of induction therapy, LDH and subtype of immunoglobulin. With all precautions of a retrospective analysis we could demonstrate in almost all subgroups a clear superiority for patients who received novel agents during the therapy. The median overall survival for the total population and for each group separately to be able to compare the results of these two groups with each other. We analyzed several influence parameters, such as renal function, ISS, stage, type of induction therapy, LDH and subtype of immunoglobulin. With all precautions of a retrospective analysis we could demonstrate in almost all subgroups a clear superiority for patients who received novel agents during the therapy. In the entire cohort our results demonstrate a benefit in median overall survival (86 vs 52 months), with a 5 year survival rate (66% vs 40%). Results of subgroups will be demonstrated separately. In addition, our data show promising data for the early use of bortezomib as part of induction therapy, as these patients experience a 88% 5-year survival in comparison to patients with VAD or ID induction therapy only.

In this single center based experience we could find a benefit of the additional use of novel agents in patients receiving HDT, independent of the application time point in a real life patient population.

Disclosure: No conflict of interest disclosed.
Frequency of occurrence of varicella zoster virus (VZV) and other complicating VZV/herpes infections (e.g. VZV-encephalitis, disseminated VZV-infection, and conus-cauda-syndrome) under lenalidomide treatment in multiple myeloma patients and assessment of potential risk factors

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Introduction: Varicella zoster (VZV) infection in multiple myeloma (MM) has been described to occur in 0–4% of patients (pts), most likely due to the underlying MM, progressive disease (PD) and/or immune surveillance defects. Under bortezomib-chemotherapy (CTX) protocols, a higher rate of VZV infections (>10%) has been observed, this advocating VZV prophylaxis (e.g. aciclovir). The frequency of VZV and other complicating VZV/herpes-infections (e.g. VZV-encephalitis [VZVE], disseminated VZV-infection [d-VZV-i] or conus-cauda-syndrome [CCS]) under novel agent therapies, e.g. lenalidomide, has not been assessed systematically in MM.

Methods: We analyzed VZV, VZV-E, d-VZV-i and CCS-frequencies in lenalidomide-treated MM pts, consecutively seen and treated in our department between 1997–2011. Via our electronic tumor documentation system, pt- and MM-characteristics, lenalidomide-demographics and possible VZV-risks were determined.

Results: In 93 MM pts, lenalidomide had been given with a median dose of 10 mg (10–25), mostly within 21-d-schedules and for a median duration of 6 months (1–13). Pts’ median age was 64 years (46–81). 7/93 acquired typical VZV-infections, and VZV-E, d-VZV-i (DD: Steffen-Johnson-Syndrome [SJS]) and CCS were found in 1 pt each. The frequency of VZV and other complicating VZV/herpes-infections was 11% (10/93). These VZV, VZV-E, d-VZV-i (DD: SJS). CCS infections led to lenalidomide discontinuation in all pts. Potential risk factors were numerous: males seemed predominantly affected (m:f = 7:3). In all pts, the MM disease was substantial with a median of 39 months (20–71) and all had been extensively pretreated with median therapy lines of 4 (1–6). Concomitant dexamethasone had been given in 7/10, myeloma-PD was present in 5/10, prior bortezomib-CTX had been applied in 4, advanced age (>70 years was noted in 3, previous VZV-infections had occurred in 2, RAD or ASZT or allo-SCT had been applied in 2, 7 and 2, respectively, and in 1/10, a secondary neoplasm was concurrently detected with VZV-infection. In 6/10 pts with lymphocyte-subpopulation analysis, all showed suppressed T4/T8-ratios (<1) and 5/10 had decreased T4-counts <0.4/µl.

Conclusions: Due to these VZV-, VZV-E-, d-VZV-i (DD: SJS), CCS-frequencies under lenalidomide, we have instituted an aciclovir-prophylaxis into our CTX protocols and will prospectively assess VZV-frequencies within the following months. These subsequent results will be presented at the meeting.

Disclosure: No conflict of interest disclosed.

P497
Skin manifestations of selected rare monoclonal gammapathies
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Introduction: Differing in strength of their relationship, dermal manifestations associated with monoclonal immunoglobulin range from diseases with direct connection to paraproteinemia on one side to disorders with anecdotal associations with monoclonal component on the other. In this review we present our experience with diagnostics and treatment of 5 rare skin manifestations closely connected to the presence of monoclonal immunoglobulin.

Methods: Two male patients with Schnitzler syndrome (rare combination of chronic urticaria, monoclonal IgM immunoglobulin, elevated markers of inflammatory, fevers and skeletal pathologies with radiological correlation); one female patient with IgA monoclonal gamopathy of unknown significance later transformed into multiple myeloma and dermal signs of subcutaneous pustular dermatosis type of pemphigus (autoimmune vesiculobullous eruptions, where monoclonal immunoglobulin binds to autologous antigens in the epidermis); one female and one male patient with IgG multiple myeloma associated scleromyxedema and scleredema, respectively (excessive collagen and mucin depositions in the dermis leading to doughy induration of the skin) and one male patient with IgG multiple myeloma associated necrobiotic xanthogranuloma (chronic granulomatous disease with indurated yellowish to red-orange lesions) were included in this retrospective study. Skin infiltrates of necrobiotic xanthogranuloma showed increased tracer (fluorodeoxyglucose) uptake on positron emission tomography imaging.

Results: Complete dermal remissions were achieved in both patients with Schnitzler syndrome using anakinra (recombinant human interleukin-1 receptor antagonist), in our patient with IgA pemphigus, for which a regimen with bortezomib and then, when the disease relapsed, chemotherapy with lenalidomide were successfully chosen, as well as in the patient with scleredema, who was indicated for bortezomib-based treatment. Partial cutaneous remissions were seen in the patient with scleromyxedema during bortezomib-based chemotherapy and in the patient with necrobiotic xanthogranuloma after palliative chemotherapy with cyclophosphamide and prednisone completed with irradiation of the painful lesions.

Conclusions: We suggest screening for monoclonal immunoglobulin in cases of dermal lesions resistant to standard therapies and when positive, a therapy targeted to removal of monoclonal component should be considered as para-proteinemia may be the cause of dermal involvement.

Disclosure: No conflict of interest disclosed.

Posterdiskussion
Myelodysplastische Syndrome

P498
Are chromosomal aberrations equally distributed in phenotypically defined cellular subsets of patients with myelodysplastic syndromes?
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Introduction: Many patients (pts) with myelodysplastic syndromes (MDS) display characteristic morphological and cytogenetic features in the bone marrow (BM). Besides, flow cytometry (FCM) has become a valuable tool in the diagnostics of MDS and allows for the distinct separation and identification of aberrant antigen expression (e.g. CD56 on myeloid progenitors). It is still unknown, whether phenotypically defined aberrant cells are predominantly part of the malignant clone.

Methods: BM samples of 22 pts (IPSS low/int-1=12, int-2/high=10) with MDS and a variety of cytogenetic aberrations (del(5q) n=18; del(7q) n=7; +8 n=2; -Y n=1) were analyzed by FCM using 8-colour staining. CD45 expression and side scatter were used to differentiate between early progenitor cells, granulocytes or erythropoiesis. Then, myeloid progenitor cells (myPC) were sorted as CD117+CD34+ and according to their CD56 expression. MDS specific FISH analysis of FCM-defined subpopulations was performed as an interphase (iFISH) with Abbott Molecular assays and interpreted by 3 investigators.

Results: The percentage of aberrant iFISH+ cells was slightly higher in myPC (CD117+CD34+) than in matured granulocytes (CD13+CD16+) (53% vs. 45%, p=0.031). Interestingly, myPC with aberrant CD56+ expression showed a higher percentage of aberrant iFISH+ cells than CD56-myPC (CD56+ vs. CD56− with 66% vs. 45%, p=0.075). The analysis revealed no difference in iFISH in cells between the maturing granulocytic subpopulations. CD10−granulopoiesis, which accounts for rather immature/dysplastic cells, displayed

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a higher percentage of aberrant iFISH+ cells than CD10+ (49% vs. 28%, ns). The differences in the erythropoiesis appeared not to be significant.

Table 1.

<table>
<thead>
<tr>
<th>Sorted subpopulations</th>
<th>Median percentage of aberrant iFISH (range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Myeloid progenitors CD117+CD34+CD56+</td>
<td>66% (41%–85%)</td>
</tr>
<tr>
<td>Myeloid progenitors CD117+CD34+CD56-</td>
<td>45% (31%–81%)</td>
</tr>
<tr>
<td>Myeloid progenitors CD117+CD34+</td>
<td>53% (0%–97%)</td>
</tr>
<tr>
<td>Granulocytes CD13+CD16+</td>
<td>40% (0%–88%)</td>
</tr>
<tr>
<td>Granulocytes CD13-CD16-</td>
<td>34% (0%–89%)</td>
</tr>
<tr>
<td>Granulocytes CD13CD16+</td>
<td>45% (0%–80%)</td>
</tr>
<tr>
<td>Granulocytes CD10-</td>
<td>49% (0%–85%)</td>
</tr>
<tr>
<td>Granulocytes CD10+</td>
<td>28% (0%–78%)</td>
</tr>
</tbody>
</table>

Conclusions: MDS-specific dysplastic bone marrow cells as defined by FCM do not seem to preferentially harbour the cytogenetically defined malignant aberration, which suggests that they represent a continuum of MDS subclones at different stages of development.

Disclosure: No conflict of interest disclosed.

P499
Prognostic parameters for patients with low risk MDS

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The prognosis of patients with malignancies is primarily influenced by the burden of tumour cells, which in patients with myelodysplastic syndromes (MDS) is the mediullary blast counts. Karyotypes are considered as a major prognostic parameters as well. This is reflected by prognostic scores using elevated mediullary blast count and dismal karyotypes as major parameters associated with impaired survival and elevated risk of AML evolution. Patients with RCUD, RARS, RMCD and MDS with del(5q) per definition have a limited mediullary blast count of less than 5% and therefore classic prognostic scoring systems may not be suitable tools. In this study we are aiming at a better description of prognostic parameters in these types of MDS.

Based on 2114 patients who were diagnosed as RCUD, RARS, RMCD and MDS with del(5q) in the MDS registry Düsseldorfer were followed up until December 31th 2011 and analysed with regard to prognosis taking into account disease related prognostic parameter i.e. mediullary blast count, karyo-type, multilineage dysplasia, cell counts, LDH, and transfusion need. Patients who received allogegenic stem cell transplantation were excluded from the analysis.

Results: 203 patients with RCUD, 229 with RARS, 1436 with RMCD and 156 with MDS del(5q) were analysed. Using univariate analysis, multilineage dysplasia as defined by WHO, high risk karyotype as defined by IPSS, Hemoglobin value <10g/dL, Platelet count <100x10^9/l, elevated LDH, transfusion need at diagnosis were associated with a worse outcome. Percentage of mediullary blasts was analyzed as category variable with 0, 1, 2, 3, or 4%. There was no difference in median survival and risk of AML evolution between 0, 1 and 2 % mediullary blasts, whereas patients with 3 or 4% mediullary blasts did worse (median survival 40 vs 34 months, p = 0.00005). In a multivariate analyze, hemoglobin <10g/dl and platelets <100x10^9/ml (p = 0.00005), mediullary blast count of 3 or 4% (p = 0.0001) as well as multilineage dysplasia (p = 0.01) were independent prognostic parameters. If karyotype findings were entered into the multivariate regression model as well, the prognostic meaning of high risk karyotype exceeded the other parameters (p = 0.00005).

In conclusion, the mediullary blast count of 3 or 4 versus <3% even in “non-lastic” MDS types is a discriminator for prognosis, and therefore should be assessed as exact as possible and could be used in prognostic scoring systems in the future.

Disclosure: No conflict of interest disclosed.

P500
Diagnostic procedures and treatment of patients with Myelodysplastic Syndromes (MDS) of low and intermediate-1 risk. Results of the outpatient MDS registry

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A registry has been established in order to describe diagnostic procedures, treatment and course of disease of patients (pts) with MDS treated in the community outpatient setting (hospital and private practice). The structure and aim of the registry has been presented previously (DGHÖ 2011).

This year we report on the first reports of the subgroup of IPSS low- and intermediate-1 (int-1) risk pts.

Since July 2009 784 newly diagnosed patients from 63 institutions were included in the registry. In April 2012 755 were evaluable with a median follow up of 18,1 month. 398 (77%) patients had a low risk or int-1 risk MDS. 91/398 (23%) patients received a MDS-related therapy within the first three month after diagnosis, whereas for 307 (77%) patients a watch-and-wait strategy was adopted. 341 from 398 (86%) reached the 3 month control level. 116/341 patients (34%) reached at least one transfusion. In total 113 patients received 594 units of packed red cells and 13 patients received 94 units of platelets. Further analysis of these patients, including the diagnostic procedures (e.g. histology, cytology, chromosomal analyses) and the different treatments (e.g. watch-and-wait only, iron chelation, epigenetic treatment, cytoreductive treatment, allogenic stem cell transplantation) will be presented.

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Disclosure: No conflict of interest disclosed.

P501
Clinical responses to alemtuzumab in six patients with low-risk myelodysplastic syndromes and aplastic anemia

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Introduction: A newer treatment approach for lower-risk MDS patients is borrowed from the chronic lymphatic leukemia standard treatment: alemtuzumab, a humanized monoclonal antibody that recognizes CD52 presented on lymphocytes and monocytes. It is regularly used in the treatment of CLL, and in conditioning regimens for SCT. Preliminary results showed clinical effects of alemtuzumab in a subset of lower-risk MDS patients who were candidates for immunosuppressive treatment with ATG/CSA. Here we present our experiences with alemtuzumab in patients with lower-risk MDS and aplastic anemia.

Methods: We treated 5 male patients with MDS (RCMD) and one female patient with aplastic anemia with alemtuzumab. The median age was 71 years (range 49–79). All MDS patients were classified as IPSS int-1, had a hypoplastic bone marrow and were transfusion dependent for red blood cells and/or platelets. 3 patients had a normal karyotype. 3 had single aberrations. One patient with a normal karyotype showed a DNMT3A-Mutation, another one with a trisomy 8 showed a p53-mutation. 4 Patients have been pretreated including ATG/CSA, CSA alone and Eculizumab. 5 patients were treated with alemtuzumab 10 mg/d.i.v for 10 days and one patient received the same dose
s.c. for 10 days. All patients received a standard prophylaxis including steroids before alemtuzumab and an infectious prophylaxis with valaciclovir, cotrimoxazol in 3 cases and one patient received ciprofloxacine.

Results: Treatment responses were classified according to the IWG-criteria. 2 patients had a partial remission with transfusion independence. Both patients had a normal karyotype, whereas one of them was the patient with the hypoplastic MDS. Clinical response is now observed for a median duration of 3.5 months (range: 1–12 months). The patient with the DMT3a mutation as well as the patient with trisomy 8 and p53-mutation showed no hematological improvement. All of the non-responding patients remained stable in their peripheral blood counts. Adverse events, especially infections were not observed in any patient.

Conclusions: In summary, monotherapy with alemtuzumab in lower-risk MDS patients is effective and safe. The treatment is well tolerated; especially in comparison to ATG, fewer side effects are reported with comparable response rates. In our patient cohort treated with alemtuzumab, we found a response in two third of the patients. Alemtuzumab should be considered as a treatment option in low risk MDS.

Disclosure: No conflict of interest disclosed.

PS02
Complications of 5-azacytidine: Three cases of severe colitis in a single center cohort
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Introduction: The diagnosis of high risk myelodysplastic syndrome (MDS) results in a significant loss of lifetime in almost all patients. 5-azacytidine (5-AZA) has shown a relevant benefit in terms of overall survival (OS) and the side effect profile of this drug seems favorable compared to conventional chemotherapy. However, rare side effects may have been overlooked up to now.

Methods: Here we report a relevant incidence of hemorrhagic colitis in our single center cohort of 95 patients consecutively treated with subcutaneous 5-AZA between 2007 and 2011 in our tertiary cancer center within the Austrian Azacytidine Registry.

Results: Basic characteristics of the cohort have been presented in abstract form before (Pleyer et al.). 3 of 95 patients (3,1%) developed severe colitis after a median of 1 month (range 1–2 months) of 5-AZA treatment. The median age was 81 years and 2 of the 3 patients were male. All had MDS with a high risk profile and were treated with a median absolute dose of 140mg (100–150) for 7 days. One patient was diagnosed with a severe hemorrhagic colitis at the end of the third cycle. The patient’s status deteriorated rapidly and after the diagnosis of colonic perforation colostomy had to be performed. Unfortunately, the patient developed a renal failure and died despite dialysis. Two other patients developed hemorrhagic colitis during or shortly after the first cycle of 5-azacytidine. After mesalazin treatment 5-AZA was continued in both cases after the resolution of the symptoms without further occurrence of colitis.

Conclusions: To summarize we observed an incidence of 3 cases per 66.8 person-years (4491 cases per 100,000 person-years) in our cohort resulting in a much higher incidence compared with the incidence rates of colitis in the general population or in patients with older age and comorbidities (373 cases per 100,000 person-years in patients older than 65 years with known COPD). The reason for this relatively high incidence of colitis in our cohort is not clear. The reexposition in 2 patients without an adverse effect of 5-AZA makes a direct toxic effect on the mucosa or endothelial tissue very unlikely and we discuss whether the combination of anemia and constipation due to 5-HT(3) receptor antagonist and severe illness can explain the 3 cases of ischemic colitis. However, increased awareness for this complication in patients treated with 5-AZA should be warranted.

Disclosure: No conflict of interest disclosed.

PS04
Efficacy of lenalidomide in multiple myeloma and myelodysplastic syndrome with deletion of 5q in a patient with pre-existing MGUS
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Patients with monoclonal gammopathy of undetermined significance (MGUS) have a higher risk for the development of secondary cancers like multiple myeloma (MM) and myelodysplastic syndrome (MDS)/acute myeloid leukaemia. We report a case of a 71-year-old man initially suffering from MGUS of the IgG lambda subtype, diagnosed in 2001 which remained stable over years with absence of monoclonal proteinuria. Because of successive development of anemia with hemoglobin levels <9g/dL, leucopenia with levels about 3.000/L and thrombocytopenia with a platelet count of 100.000/L, bone marrow was taken in March 2009 which established the diagnosis of a MDS 5q (IPSS intermediate-I). The medullary plasma cell count was normal and serum IgG-paraprotein was slightly increased to 22g/L (7–16g/L). The patient was transfusion dependent for packed red blood cells (RBC) with a transfusion frequency of 2 RBC every 10 to 14 days. In 2010 iron chelation with deferasirox was initiated due to secondary iron overload. In June 2010 MGUS progressed to MM stage II according to ISS with first occurrence of
Bence-Jones proteinuria of 0.52g/L, increasing β2-microglobulin (02-MG) of 4.8mg/L and progressing renal insufficiency. Based on the known excellent effects in MDS 5q and MM lenalidomide was initiated in a dose of 10mg per day for 21 days and dexamethasone at a weekly dose of 40mg. Beside short interruptions due to haematological toxicities (thrombocytopenia) and infections lenalidomide could be continued for currently more than 21 months. Dexamethasone was discontinued due to infectious complications. The patient achieved an ongoing transfusion independence after about one month of treatment. Bone marrow taken 14 months after start of treatment showed a complete cytogenetic response concerning the deletion of 5q and a plasma cell infiltration below 5%. Currently the serum IgG paraprotein is 12g/L, β2-MG dropped to 3.8mg/L while in MRI the bone marrow signal alterations remain stable. No new osteolytic lesions were detectable. In contrast to the development of MM in MGUS patients, the subsequent occurrence of MDS after diagnosis of MGUS is infrequent. However, the biological association of MDS and MGUS is not sufficiently understood, but the non-treatment related occurrence support the pathogenetic role of pre-existing alterations of the stem cells. We further show the feasibility of lenalidomide as a well tolerable treatment option in MDS 5q with co-existing MM.

Disclosure: No conflict of interest disclosed.

P506
Chemotherapy in resectable sarcomas with complex karyotypes – a single center retrospective analysis

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Background: Patients (pts) with large, deep and high grade (G2 and G3) soft tissue sarcomas (UICC stage III) have a considerable risk of relapse. A large meta-analysis has shown a small survival benefit for doxorubicin and ifosfamide-containing chemotherapies but the heterogeneity of data fuels debate and does not allow a general recommendation in favor of chemotherapy. We have retrospectively analyzed the outcome of patients (pts) with localized sarcomas known to have complex karyotypes and poor prognosis.

Methods: An analysis of our institutional database (n=1269) identified 172 pts with localized undifferentiated pleomorphic sarcomas (UUPS; n=98), leiomyosarcomas (LMS; n=53), myofibrosarcomas (n=14), and other subtypes (n=7) with UICC stage III. Of those, 81 patients received either neoadjuvant (n=32; NCT) or adjuvant chemotherapy (n=49; ACT) or both (n=3). Treatment consisted mostly of 5 cycles of a combination of doxorubicin (60mg/m²) and ifosfamide (10g/m²). Progression-free survival (PFS) and overall survival (OS) were calculated from date of surgery.

Results: Primary tumors were localized in the extremity (44%), the trunk (50%) and other locations (6%). The group consisted of 51% male (n=88) and 49% female (n=84) pts. Median age for control, NCT and ACT groups were 56y, 52y and 57y. The median follow-up was 39 months. Median metastasis-free survival was 16mo, 35mo and was not reached for control, NCT and ACT (p=0.0001). The 5-year metastasis-free survival was 22%, 37% and 54% for control, NCT and ACT. Median OS was 53mo, 58mo and was not reached for control, NCT and ACT. 5-year OS rates were 49%, 54% and 75% for control, NCT and ACT (p=0.003 for control vs SCT). Median OS was 53mo and 102mo for control versus chemotherapy (pre and/or post-op; p=0.0418). Covariates such as tumor size, grade, resection status, distribution of histological subtypes, and dose intensity will be presented.

Conclusions: While this non-randomized, retrospective analysis may have a strong selection bias, it underlines the dismal prognosis of patients with stage III soft tissue sarcomas and complex karyotypes and the need for additional systemic treatments. Dose-intensive chemotherapy is feasible in experienced centers and our data suggest a beneficial role for adjuvant combination chemotherapy in this subgroup of high risk patients.

Disclosure: No conflict of interest disclosed.

P507
Phase II randomized clinical trial of Pazopanib alone and Pazopanib plus Gemcitabine in relapsed or metastatic soft tissue sarcoma. The PAPAGEMO trial

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Introduction: Soft tissue sarcomas (STS) are malignant tumors of connective tissue. The annual incidence is around 2–3/100.000. In refractory STS patients only few cytostatic drugs have been proven to be active and in general without curative or even a long-lasting effect. Pazopanib is an orally available, 2nd

Disclosure: No conflict of interest disclosed.
Abstracts

Rapid clinical benefit of pazopanib in a patient with progressive chemorefractory rhabdomyosarcoma

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Introduction: Rhabdomyosarcoma (RMS) is a rare, aggressive tumor mainly arising in children. The state of the art treatment of RMS should employ a risk adapted combined modality approach resulting in 5-year overall survival rate of only 6% in STS it is a challenging goal of the PAPAGEMO trial to determine the benefit of new and targeted drugs in patients with relapsed or metastatic disease by the STS.

Methods: Since its initiation in October 2011 one third of the patients have been enrolled. Preliminary data demonstrate the feasibility of the treatment. The primary endpoint is the progression-free survival rate (PFSR) after 12 weeks. A PFSR at 12 weeks of 40% is supposed to be reached by the monotherapy arm. In the drug combination arm treatment should reach a PFSR at 12 weeks of 60% or higher to prove superior drug activity. The study has 60% power at a 5% significance level to test the hypothesis.

Results: Since its initiation in October 2011 one third of the patients have been enrolled. Preliminary data demonstrate the feasibility of the treatment. The primary endpoint is the progression-free survival rate (PFSR) after 12 weeks. A PFSR at 12 weeks of 40% is supposed to be reached by the monotherapy arm. In the drug combination arm treatment should reach a PFSR at 12 weeks of 60% or higher to prove superior drug activity. The study has 60% power at a 5% significance level to test the hypothesis.

Conclusions: Because of the bad 5-year overall survival rate of only 6% in STS it is a challenging goal of the PAPAGEMO trial to determine the benefit of new and targeted drugs in patients with relapsed or metastatic disease.

Disclosure: No conflict of interest disclosed.

Vascular leiomyosarcoma mimicking a thrombosis of the iliac vein

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Introduction: Vascular leiomyosarcoma (VLS) is a rare type of soft tissue sarcoma. VLS often has an aggressive course with regional lymph nodes or other distant metastases. Furthermore there is a high recurrence rate in defiance to aggressive treatment including wide resection or amputation (about 70% after primary resection). So far neoadjuvant or adjuvant chemotherapeutic approaches have not been proven to be beneficial. Response to chemotherapy in metastatic disease is also often described as poor and only of short duration.

Methods: We report a case of a 21-year old man who was admitted to our hospital because of a tumor in the right axilla with infiltration of the pectoralis and deltoid muscles. The biopsy showed ES with regional metastatic lymph nodes. Because of the infiltration of the thoracic plexus and the aspect, that the tumor had close contact to the A. and V. axillaris, we started a systemic therapy after a multidisciplinary discussion of the case. In this high-risk situation another primary amputation and dissection of the regional lymph nodes would have left the patient with a high probability of a relapsing course of the disease.

Results: After one cycle of Gem and Docetaxel we did not reach the PFSR and we noticed a small decrease of tumor mass (from 13x13x10 cm size to 11x9,3x 9,6 cm) and a significant reduced tracer accumulation (from SUVmax 7.2 to SUVmax 3.7). That shows that a PET scan is better for checking early response as CT or MRI. Because of this result and the good clinical condition we did another 2 cycles of chemotherapy and in the last PET/MRT-scan scan we saw a PR with a significant decrease of size (10x 8,5x 4,8cm) and tracer accumulation (SUVmax 2,2) fortunately.

Conclusions: Combination chemotherapy with gemcitabine and docetaxel could be a promising regimen in advanced ES. More published data about Gem/Docetaxel in ES is needed.

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and tumor thrombus. FDG-PET-CT is used for staging and response monitoring in several tumor entities. With these new imaging tools a wide range of incidental findings including thrombosis are detected. But only a few studies are published about CT or PET-CT for differentiation between tumor thrombosis and VTE.

We report a case of a 44 years old man, diagnosed with a venous thrombosis of the v. iliaca communis as initial manifestation of a vascular LMS. After progression of the thrombosis with additional lung artery embolism a tumor search including FDG-PET-CT was initiated. Only a linear pattern with increased FDG-uptake along the course of the v. iliaca communis to v. cava inferior was detected. Finally it was considered as an organized bland thrombus. No other tumors with or without FDG-uptake were detectable.

During another hospital admission due to a fall (four months later), a large pelvic tumor with infiltration of the plexus sacralis and lumbalis was detected, together with tumor thrombus from v.v. Iliaca to v. cava inferior and tumor growth in a iliaca communis. Furthermore metastases of the lung and bone (left acetabulum and os ilium) were seen. A biopsy of the pelvic tumor was performed and a leiomyosarcoma could be diagnosed. After 5 cycles of chemotherapy with doxorubicin and dacarbazine the patient had a stable disease with a non-significant reduction of tumor volume.

Vascular LMS or LMS of the v. iliaca are rare tumors often diagnosed in advance stage or initially misdiagnosed as venous thrombosis. However, despite initial surgery vascular LMS show a high recurrence rate. Better understanding of differences of imaging results between tumor thrombosis and bland venous thrombosis can help to distinguish from each other. FDG-PET-CT could be a promising tool for the diagnosis of tumor thrombi. However, more studies with numerous cases are warranted to define clear characteristics for differentiation.

Disclosure: No conflict of interest disclosed.

**P511**
Progression-free interval of 7 months by Thalidomide treatment in refractory metastatic chordoma of the sacrum

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**Introduction:** Chordoma is a rare neoplasm probably derived from undifferentiated notochordal remnants. It usually occurs in the skull base, the spine or the sacrum. Complete resection and postoperative external-beam radiotherapy are the therapy of choice for chordomas of the sacrococcygeal region. Nevertheless, therapy is challenging. Recurrence is common due to an impossible en-bloc resection and relapses show only very limited response to re-radiation therapy and further surgery. Conventional chemotherapy likewise has proven to be ineffective. With regard to a formerly case report by Chay et al we report on an elective treatment with thalidomide as a single agent in advanced chordoma.

**Case report:** In July 2002 a 53-year old male patient was diagnosed with chordoma of the mobile spine and the sacrum. He underwent surgical excision with questionable R0-resection and postoperative radiation therapy. Since July 2005 the patient suffered several relapses followed by re-operations and re-radiations. In July 2011 during palliative radiation therapy a further disease progression occurred with pulmonal metastases and affection of the spinal canal. In September 2011 the patient was referred to our oncological ambulance and we started a therapy with Thalidomide at a dose of 50 mg daily. Until March 2012 we carried out 4-weekly follow-ups including regular CT-scans showing a stable disease without any further progression.

**Conclusions:** Conventional therapeutic regimens like radiotherapy and surgery failed in disease control. Under treatment with Thalidomide as a single agent a progression-free interval of 7 months could be achieved with therapy being ongoing. Thalidomide recommends itself as a potentially efficacious therapy option in treatment of advanced chordoma.

**List of literature:**
Methods: In this retrospective study, we evaluated the outcome of patients with primary GBM, aged 265 years, treated in our institution during the period of recruitment for the NGS study (2003–2009), to which our site contributed 35 patients (NGS group). The primary endpoint was overall survival. The study population of 70 patients, 32 women and 35 men, aged 65 to 83 years, median 71 years, was divided into two groups: the NGS group consisted of 35 patients with 13 patients in the standard radiation therapy arm with 60 Gy, 12 patients in the hypofractionated radiation therapy arm with 34 Gy and 10 patients in the TMZ arm. The control group (35 patients) consisted in 23 fit elderly patients in the RCT arm who were treated with standard radiochemotherapy (RCT) like younger GBM patients and 12 frail patients who mostly started radiotherapy with 60 Gy but did not receive chemotherapy (nonRCT arm).

Results: 31 of the 70 patients underwent gross total resection (44%), 21 patients had subtotal resection (30%) whereas 18 patients underwent biopsy (26%).

The median overall survival in the three study arms of the NGS group in particular was 6.0 months in the 60 Gy arm, 7.0 months in the hypofractionated 34 Gy arm and 10.0 months in the TMZ arm (p = 0.012). The median overall survival in the RCT arm was 21.0 months vs. 3.0 months in the nonRCT arm (p = 0.0001).

Karnofsky scores were evaluated in three months interval. The median time to the loss of functional independence (K1 60%) was >6 months in RCT patients, 6 months in the NGS group and less than three months in nonRCT arm.

No grade 3 or 4 toxicities were documented in the 60 Gy and 34 Gy arm of the NGS group. In the TMZ arm 2 of 10 patients (20%) suffered from grade 3 or 4 thrombocytopenia.

In the RCT arm grade 3 haematologic toxicity (thrombocytopenia and leukopenia) occurred in 2 of 23 patients (8.7%) and in one patient of the nonRCT arm (8.3%), probably due to dexamethasone.

Conclusion: This retrospective single center experience shows the wide variety of outcomes in elderly patients with GBM and underlines the need for individualized, geriatric assessment based therapy planning, performance and follow up.

Disclosure: No conflict of interest disclosed.

PS15

An analysis of toxicity in patients with recurrent and/or metastatic squamous cell carcinoma of the head and neck (R/M SCCHN) receiving cetuximab, fluorouracil and cisplatin alone or with docetaxel in a phase II clinical trial (CeFCD)

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Introduction: The introduction of therapies targeting the epidermal growth factor receptor (EGFR) has improved prognosis in SCCHN patients. Therapeutic options were further expanded by taxoids, in particular docetaxel. This study was planned to assess the toxicity and efficacy of docetaxel in combination with cetuximab, cisplatin and fluorouracil (5-FU) for patients with R/M disease.

Methods: This ongoing, national, phase II, open-label, randomized study is enrolling patients with R/M SCCHN, not suitable for local therapy and ECOG performance status 0–1. Patients receive cetuximab (initial dose 400 mg/m², then 250 mg/m² weekly) plus a maximum of 6 cycles of docetaxel and cisplatin (40mg/m², day 1+8) and 5-FU (2000 mg/m², day 1+8 as continuous infusion for 48h) or cetuximab (as in group A), cisplatin (100 mg/m², day 1) and 5-FU (1000 mg/m², day 1–4 as continuous infusion). Cetuximab is administered until progression or intolerability. The primary endpoint is progression-free survival. Secondary endpoints include overall survival, response rate, quality of life and toxicity. This study includes multiple planned interim analyses. The current toxicity analysis included the first 40 of approximately 180 planned patients.

Results: Of 40 patients enrolled, SAE-reporting with grade III/IV toxicities was more frequent in the experimental arm with docetaxel. The toxicity interim analysis revealed considerably higher hematologic and non-hematologic, especially gastrointestinal (diarrhoea) toxicities in the experimental arm as compared to the standard arm.

Conclusions: After the first interim toxicity analysis of the first 40 patients, a substantial amendment was conducted. The dose of cisplatin was reduced from 40 to 30 mg/m² and the dose of 5-FU from 2000 to 1000 mg/m², maintaining the weekly administrations, and maintaining the full dose intensity for docetaxel and cetuximab. Enrollment for the second interim toxicity analysis with 70 patients enrolled is estimated for July 2012. Complete toxicity data will be presented at the DGOB meeting.

Disclosure: Maren Knödler: No conflict of interest disclosed.

P516
Rapid clinical benefit of sunitinib in a patient with progressive chemorefractory thymic carcinoma
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Introduction: Thymic carcinoma (TC) is a rare, aggressive tumor with poor prognosis. Current treatment regimens result in a median survival of only 2 years. Sunitinib is a multi-targeted tyrosine kinase and VEGFR inhibitor. It has shown benefit in various cancers. We report a case of major clinical benefit achieved with sunitinib in a 70 year-old female patient with metastasized TC after failure of conventional chemotherapy.

Methods: The patient was diagnosed with metastasized thymic carcinoma in 02/2011. First line carboplatin and paclitaxel (CP) therapy was initiated. Four cycles CP from 02 until 05/2011 produced a mixed response. After 3 further cycles CP from 05 until 07/2012 progression occurred. Therapy was switched to capcitabine and gemcitabine (CG). After 5 cycles (CG) from 08 until 12/2012 progression and rapid clinical aggravation took place. (B-symptoms, high laboratory parameters). We decided on a therapy with sunitinib based upon single case reports.

Results: Treatment with sunitinib was started with 25 mg daily (28 d cycles) due to the impaired liver function. General condition improved within few days from ECOG 3-4 to ECOG 0. Laboratory findings improved as well: LDH dropped from 1842 to 375 U/L (normal range <240 U/L), GGT dropped from 1441 to 295 U/L (<60 U/L), Bilirubin decreased from 5.8 to 0.6 mg/dL (<1 mg/dL) and CRP decreased from 275 to 40 mg/L (<5 mg/dL). No significant side effects were observed. After 2 cycles of sunitinib staging showed tumor regression and central necrosis of primary and pulmonary metastases and stable hepatic masses. During the 3rd cycle, however, general condition and laboratory parameters worsened again and treatment was stopped after 3 cycles because of lack of further treatment benefit.

Conclusion: Our case shows that sunitinib is feasible and well tolerated in TC. Clinical and radiographic responses were observed. Before sunitinib treatment the patients condition had been quickly deteriorating. The clinical benefit for our patient was rapid indicating that sunitinib can block key proliferative pathways of TC. However, in this case the disease was too aggressive and advanced for a maintained therapeutic effect. We conclude that sunitinib has clinical activity in TC and is a potential therapeutic option to ameliorate clinical symptoms in aggressive disease. Predictive parameters for more durable responses have to be investigated. This case report warrants further clinical investigation in prospective trials.

Disclosure: No conflict of interest disclosed.

P517
Position of nuclear medicine imaging in selected rare oncological diagnoses
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Introduction: Positron emission tomography (PET) alone or in combination with computed tomography (PET/CT) and traditional bone scintigraphy are well-established imaging modalities in diagnostics and treatment response monitoring of malignant diseases. Herein, focusing on 4 rare oncological entities, we discuss the benefit of nuclear medicine while emphasizing its significance to clinical practice.
Results: We developed and verified new methods for therapy response monitoring in pulmonary Langerhans cell histiocytosis and Erdheim-Chester disease using SUVmaxLungs/SUVmaxLiver and SUVmaxLesion/SUVmaxLiver indexes, respectively. In a patient with Castleman disease, through a series of PET/CT examinations during thalidomide- and lenalidomide-based regimens, gradual regression of enlarged lymph nodes as well as reduction of their pathological glucose accumulation were documented. In Schnitzer syndrome, traditional bone scintigraphy proved superior to PET imaging in finding areas of abnormal bone metabolism.

Conclusions: Nuclear medicine modalities significantly contribute to the diagnostic and therapeutic process of the aforementioned diseases and further investigation of their exact role in this field is therefore suggested. Measuring SUVmaxLungs(or Lesion)/SUVmaxLiver values and their time-trend monitoring represent simple, noninvasive screening tools.

Disclosure: No conflict of interest disclosed.

Wissenschaftliches Symposium Genetik und Epigenetik bei AML

VS24
Clinical impact of genetic features in Acute Myeloid Leukemia (AML)

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Introduction: In recent years, research in molecular genetics has major contribution on deciphering the molecular pathogenesis of acute myeloid leukemia (AML). Gene mutations or deregulated expression of genes or sets of genes now allow us to explore the enormous diversity that is seen among cytogenetically defined subsets of the disease, in particular the large subset of cytogenetically normal AML. Based on these findings there has been increasing translation of genetic diagnostics into the clinic; in the current World Health Organization (WHO) classification more than two thirds of AML cases are categorized on the basis of their underlying genetic defect, in part defining distinct clinicopathologic entities. Moreover, cytogenetic and molecular genetic changes have been shown to be among the most powerful prognostic but also predictive markers. Finally, novel therapies are being developed that target some of these genetic and epigenetic changes, such as use of tyrosine kinase inhibitors (TKIs) and demethylating agents.

Methods and Results: In this session we will report on the clinical relevance of genetic alterations in AML, in particular, how these molecular markers can be integrated into the clinical management of AML. The first part of this review is devoted to biomarkers that have already entered clinical practice and affect diagnosis and also guidance to standard or investigational therapy such as NPM1, FLT3, and CEBPA. In the second part, selected markers will be discussed that are still under investigation. These biomarkers include mutations in IDH1, IDH2, DNMT3A, TET2, ASXL1, and TP53 genes.

Conclusions: Progress in characterizing the molecular pathogenesis of AML has been enormous, and translation of these findings into the clinical setting has been increasing over the last years. Nevertheless, given the broad molecular heterogeneity of the disease, large patient cohorts have to be analyzed entirely for all markers to evaluate their interaction and their precise prognostic and predictive value. Furthermore, it will be of prime importance to study these markers in the context of novel therapies to identify genetically defined patient subgroups that benefit from a particular treatment approach.

Disclosure: No conflict of interest disclosed.

Wissenschaftliches Symposium Aggressive Lymphome – neue Entwicklungen

VS25
Novel strategies in the treatment of diffuse large B-cell lymphoma

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Diffuse large B cell lymphoma (DLBCL) represents the most common type of malignant lymphoma and accounts for approximately 30–40% of all cases. DLBCL is heterogeneous with respect to morphology, biology, clinical presentation and outcome. This view was supported by gene expression profiling studies that have distinguished three molecular subtypes of DLBCL, termed germinal center B cell-like (GCB) DLBCL, activated B cell-like (ABC) DLBCL, and primary mediastinal B cell lymphoma (PMBL). These subtypes differ not only with respect to the expression of thousands of genes, but also utilize different oncogenic pathways and have significantly different survival rates following therapy. GCB DLBCL and PMBL patients respond relatively favorably to conventional treatment. In contrast, ABC DLBCL represents the least curable subtype and less than 50% of patients can be cured. Thus, to further improve outcome, novel therapeutic strategies are critically warranted. The B-cell receptor signaling pathway, the nuclear factor-kappa B pathway, BCL6 as well as the anti-apoptotic BCL2 family member MCL1 might represent novel targets that can be utilized therapeutically in subsets of DLBCL patients.

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Burkitt lymphoma pathogenesis

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Burkitt lymphoma (BL), a germinal center (GC) B cell derived tumor, is characterized by c-MYC (MYC) translocations and mutations resulting in its deregulation. Whereas MYC over-expression has been extensively studied, secondary and tertiary genetic alterations involved in MYC-driven tumorigenesis are less defined.

We predicted activation of phosphoinositide-3-kinase (PI3K), a major survival determinant in mature B cells, as additional transforming event in BL, and generated a mouse model by targeting MYC expression together with PI3K pathway activation into GC B cells. The resulting tumors faithfully model human BL with respect to the expression of typical markers and accumulation of tertiary mutations. By identifying PI3K pathway activation as key element for the malignant transformation of MYC expressing GC B cells in the mouse and demonstrating PI3K pathway activation in human BL, we establish a framework of Burkitt lymphoma pathogenesis and offer a pre-clinical model for the evaluation of new therapeutic strategies.

Disclosure: No conflict of interest disclosed.

CMV-replication after allogeneic stem cell transplantation is associated with a GVHD-independent reduced relapse risk in lymphoma

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Background: We have previously showed that a human CMV-reactivation after allogeneic HSCT is associated with a reduced risk for leukaemic relapse in patients with AML. Further, experimental data in a lymphoma mice model reported from Erlach et al. showed that coinfection with murine CMV revealed a strong anti-lymphoma effect by induction of apoptosis in lymphoma cells and improving rate of overall survival in mice.

Methods: The anti-lymphoma effect of early CMV replication was evaluated in 92 consecutive patients (median age [years]: 45, 18–70) with indolent (n = 12), aggressive B or T cell (67), or mantle cell (n = 13) NHL, who received transplants from unrelated (n = 67) or related (n = 27) donors.

Results: Most study-patients (87%) had received at least 3 chemotherapy lines and 59% also an autograft prior to HSCT. The HCT-CI were 0–2 in 71 pts (77%) and 3+ in 21 pts (23%). Myeloablative preparative regimen was applied in 81 pts (88%) while 11 pts (12%) received aRIC. Seventy % of pts (n=65) were at risk for CMV reactivation based on either patient or donor pretransplant CMV serostatus. CMV replication as detected by pp65 antigenemia assay occurred in 36 pts (39%) at a median of day +39 (range 23–84) after HSCT. Taking all relevant competing risk factors into account, the cumulative incidence (CI) of progression-free survival (PFS) at 10 years after alloSCT was 58% (95% confidence limit [95%-CL]: 35–51) in 56 patients without compared to 76% (95%-CL: 69–81) in patients with pp65-antigenemia (p=0.039). A substantial and independent anti-lymphoma effect of CMV replication was confirmed by multivariate analysis for pp65-antigenemia (hazard ratio [HR]: 0.2, 95%-CL: 0.1–0.4, p<0.0001) including parameters as donor, conditioning intensity, disease status at alloSCT, and sex mismatch. In contrast, VEGF was elevated in steroid sensitive GvHD (p=0.039). A substantial and independent anti-lymphoma effect of CMV replication was confirmed by multivariate analysis for pp65-antigenemia (hazard ratio [HR]: 0.2, 95%-CL: 0.1–0.4, p<0.0001) including parameters as grades II-IV acute GvHD, chronic GvHD, disease stage, chemorefractory, previous chemotherapy lines. The anti-lymphoma effect was detectable across all lymphoma subsets and was most pronounced in patients with chemotherapy refractory disease, whereas the use of anti-lymphocyte globulin for GVHD prophylaxis totally abrogated the HCMV induced anti-lymphoma effect. However, overall survival rate did not differ in both groups (52% for pts with CMV-replication versus 51% without n.s.).

Conclusions: This is the first report which demonstrates a strong and independent effect of early CMV replication on the PFS in pts with lymphoma. This effect deserves further and more comprehensive studies with regard to its clinical relevance and the underlying anti-lymphoma mechanisms.

Disclosure: No conflict of interest disclosed.

Clinical grade depletion of naïve T cells using CD45RA MicroBeads

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Introduction: We and others have previously demonstrated that naïve T cells contain a much higher frequency of alloreactive precursors compared to memory T cells. This study aims at the development of a good manufacturing practice (GMP) procedure for depleting naïve CD45RA+ T cells from donor lymphocyte infusion (DLI) products, as a new approach to primary graft-versus-host disease (GVHD) prophylaxis.

Methods: Leukapheresis products from healthy donors (n=6) were depleted from CD45RA+ cells under clean room conditions, using CliniMACS®...
CD45RA MicroBeads. Two fractions (i.e. unseparated, CD45RA-RA) were analyzed by phenotypic and functional assays.

**Results:** CD45RA depletion eliminated >99.9% of CD45RA+ cells (median 4.4 log depletion). The CD45RA− subset contained a higher proportion of CD4+ T cells than the untouched leukapheresis (median 17.5 vs. 11.6%). In contrast, CD8+ T cells and CD4+CD25Foxp3+ regulatory T cells were reduced in the CD45RA− fraction (6.8 vs. 13.9% and 1.3 vs. 1.8%). CD16+CD56+ NK cells and CD19+B cells were almost completely eliminated (0.1 vs. 7.7% and 0 vs. 13.9%), whereas CD14+ monocytes and CD15+ granulocytes were enriched in the CD45RA− fraction (40.9 vs. 23.1% and 7.9 vs. 1.6%). CD4+ and CD8+ T cells in the CD45RA− subset showed a memory phenotype (entirely CD45RO−, low expression of CD62L and CCR7, intermediate expression of CD27 and CD28). In IFN-gamma ELISPOT assays, persistent reactivity to cytokine-megalovirus, Epstein-Barr virus, candida, and aspergillus antigens could be demonstrated in the CD45RA− subset. In allogeneic mixed lymphocyte reaction (MLR) assays, alloreactive CD8+ T cells were strongly reduced (around 1-log) upon CD45RA depletion, even in clinically inapplicable (10 out of 10) HLA mismatch settings. In contrast to our previous *in vitro* work (single HLA mismatch), alloreactive CD4+ precursors appeared in similar quantities in both fractions when applying 10 out of 10 HLA mismatch MLR.

**Conclusion:** Clinical grade depletion of CD45RA+ cells from entire leukapheresis products is feasible and highly efficient using GMP CD45RA MicroBeads. The CD45RA− target fraction contains memory T cells that show preserved reactivity to common viral and fungal pathogens. T cell mediated alloreactivity appears to be reduced in the CD45RA− fraction, with apparently stronger efficacy in the CD8 compared to the CD4 subset. Our data pave the way for clinical trials that investigate if CD45RA-depletion of DLI can reduce GvHD.

**Disclosure:** No conflict of interest disclosed.

**V534**

**Evaluation of EBV monitoring after allogeneic stem cell transplantation supports therapeutic rather than preemptive rituximab administration for PTLD control:** Implications from a single center analysis


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**Introduction:** Epstein-Barr virus (EBV) associated post-transplant lymphoproliferative disease (PTLD) is a major complication of allogeneic stem cell transplantation (SCT). Risk factors for EBV PTLD are use of mismatched and unrelated donors and/or anti-thymocyte globulin (ATG). Regular monitoring for EBV viremia is a recommended strategy for PTLD prevention if risk factors are present. The purpose of this retrospective analysis was to evaluate the efficacy of this policy which was adopted by our center in 2010.

**Patients and methods:** From April 2010 onwards, all EBV-positive adult patients receiving a transplant from an unrelated and/or HLA-mismatched donor and/or ATG were routinely monitored by EBV PCR. Samples were collected weekly until at least day +100 after SCT or until the discontinuation of systemic immunosuppression. Preemptive therapy with rituximab was initiated based on individual decision, taking into account the viral load and the dynamics of the EBV viremia, as well as the clinical presentation of the patient.

**Results:** Between April 2010 and April 2012, 174 patients were screened in total. In 37 patients (21%) EBV viremia was detected. 11/37 patients (30%) had <1,000 copies/ml; 18/37 (49%) had <10,000 copies/ml; 6/37 (16%) had <100,000 copies/ml and 2/37 (5%) had >100,000 copies/ml. EBV viremia lasted for 4 weeks in 14/37 (38%) patients, between 5–12 weeks in 9/37 (24%) patients and more than 12 weeks in 14/37 (38%) patients. Subset analysis revealed that patients who had received rituximab therapy prior to SCT developed EBV viremia less frequently (2/31 patients [6%] vs. 35/143 patients [24%]; p=0.028). There was only one single patient who developed PTLD, which clinically presented as a tonsillar mass. EBV copy number at PTLD onset was relatively low with <3,000 copies/ml. Four cycles of rituximab (375 mg/m2 weekly) resulted in successful resolution of EBV viremia and complete remission of the affected tonsil.

**Conclusions:** Routine EBV monitoring effectively detects EBV reactivation after SCT. However, kinetics and levels of EBV viremia in this high-risk population were not clearly correlated with PTLD, which occurred in only <3% of viremic patients. Accordingly, this data questions the recommended strategy of preemptive rituximab administration but rather supports its therapeutic use. Rituximab treatment prior to SCT may reduce the risk of post-transplant EBV reactivation.

**Disclosure:** No conflict of interest disclosed.

**V535**

**Steroid refractory GVHD but not grade 3–4 therapy sensitive GVHD is associated with an increased risk of mortality and endothelial damage**


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Graft-versus-host disease (GVHD) is the major complication of allogeneic stem cell transplantation (alloSCT) and its therapy-resistant form causes significant morbidity and mortality. The pathomechanism of steroid resistance is currently not completely understood; however, we have recently suggested that endothelial dysfunction seems to play an important role (Luft et al., Blood 2011). The aim of this study was to validate in a large cohort of patients that steroid refractory acute GVHD is associated with endothelial stress.

**Patients and methods:** For this retrospective study, 393 patients were eligible who had undergone alloSCT between 09/2001 and 08/2010 at our institution. Serum levels of endothelial stress markers (Angiopoietin-2: Ang-2, soluble Thrombomodulin: sTM, IL-8 and VEGF) were compared between patients with no GVHD (n=221), grade 1–2 (n=103), steroid sensitive grade 3–4 (n=27) and steroid refractory grade 3–4 (n=41) GVHD.

**Results:** Landmark analyses at days +50 and +100 after alloSCT showed that NRM was dramatically high in the steroid refractory group but was equivalently low in the no GVHD-, sensitive grade 1–2 – and grade 3–4 – groups (p<0.0001, hazard ratio 21). ANG2, an endothelial-derived hormone that determines endothelial vulnerability, was significantly elevated in patients with steroid-refractory grade 3–4 GVHD compared to patients with steroid-sensitive grade 3–4 GVHD. sTM and IL-8 are markers that indicate endothelial stress. Steroid refractory patients showed stronger rises in IL-8 (day 50: p=0.006; day 100: p=0.005) and sTM (day 50: p=0.04; day 100: p=0.006) levels post alloSCT than sensitive grade 3–4 GVHD patients. High levels of sTM, IL-8, Ang-2 were significantly associated with increased NRM rates (day +50: IL8 p=0.006, sTM p=0.0008, Ang-2 p=0.0001; day +100: IL8 p=0.0007, day +100 sTM p=0.0001, Ang-2: p=0.05); even after multivariate adjustment for donor, conditioning intensity, disease status at alloSCT, and sex mismatch. In contrast, VEGF was elevated in steroid sensitive grade 3–4 GVHD (p<0.05) patients at day 100 but did impact on NRM rates.

**Disclosure:** No conflict of interest disclosed.

**Fig. 1. for abstract V535**
Abstracts

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Oefner, P.

Peter Oefner: No conflict of interest disclosed.

Disclosure:

changes in antimicrobial peptides. Recently observed GvHD dependent suppression of Paneth cells and associated by antibiotic decontamination but suggests additional suppression of the diver-

Results:

received matched unrelated donor transplants. Urine samples were collected at hematological malignancies, the median age at SCT was 47 ± 2 yrs; of the 63 tryptophan metabolite processed by colonic bacteria, to 63 patients in the

GvHD is still poorly characterized. Metabolomic analysis of serum or urine

in patients receiving allogeneic stem cell transplantation:

Hollender: as a marker for intestinal bacterial diversity

in patients receiving allogeneic stem cell transplantation:

Impact of antibiotic treatment and intestinal GvHD

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Introduction: The role of the bacterial microflora in the pathophysiology of GvHD is still poorly characterized. Metabolomic analysis of serum or urine metabolites processed by bacterial enzymes now allows indirect assessment of bacterial load and diversity in patients.

Methods: We applied metabolic analysis of urinary indoxyl sulfate (IS), a tryptophan metabolite processed by colonic bacteria, to 63 patients in the course of allogeneic stem cell transplantation. Patients suffered from various hematological malignancies, the median age at SCT was 47 ± 2 yrs; of the 63 patients, 20 received HLA identical sibling transplants, while the remaining 43 received matched unrelated donor transplants. Urine samples were collected at regular time intervals from d -10 until d+90 following transplantation and cyropreserved until analysis by liquid chromatography-tandem mass spectrometry. IS levels were correlated with the use of antibiotics for intestinal decontamination or treatment of infections and with the development of intestinal GvHD.

Results: With initiation of decontamination, urinary IS levels dropped from 48±7 µmol mmol-1 creatinine to 4.1 ± 2 on day 7 and remained low until day 21. Thereafter, a gradual recovery was observed, and pretransplant levels ware reached on day 90 in patients with uneventful courses. Patients developing intestinal GvHD had significantly lower levels even in the aplastic period during decontamination, and levels remained low after engraftment (patients without intestinal GvHD 35±7 vs 9±6 in patients with intestinal GVHD, p=0.02). In multivariate analysis, decontamination and GvHD were independent risk factors for low urinary IS levels.

Conclusion: Our study demonstrates expected suppression of colonic bacteria by antibiotic decontamination but suggests additional suppression of the diversity of the intestinal flora by GvHD. Our observation may be related to the recently observed GvHD dependent suppression of Paneth cells and associated changes in antimicrobial peptides.


Freie Vorträge

Multiples Myelom I (experimentell und klinisch)

V537

Constitutive activation of gp130 induces multiple myeloma in a novel mouse model

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The interleukin-6 (IL-6/gp130/Stat has been implicated in the pathogenesis of multiple myeloma (MM). However, other signaling pathways as well as contact to stromal cells have been demonstrated to render MM cells IL-6-independent. In order to study the role of constitutively activated gp130 in vivo, we ectopically expressed a constitutively dimerized gp130 construct (L-gp130) in murine bone marrow cells derived from Balb/c mice prior to syngeneic transplantation. These mice developed MM with a relatively short latency resulting in a median survival of seven months. In contrast, median survival of the control groups (wildtype gp130 and empty vector) was not reached without any signs of MM. Notably, L-gp130-induced MM was serially transplantable with a rapid onset of the disease and a median survival of 80 days. These results provide evidence for a MM-initiating cell compartment. Analysis of the bone marrow and other lymphatic tissue revealed accumulation of CD138-positive MM cells. These plasma cells were light chain-restricted strongly indicating monoclonal origin. These data was further substantiated by monoclonal paraprotein as determined by serum electrophoresis. Using conventional X-ray as well as CT-scan, we detected osteolytic lesions and bone destruction, a major clinical hallmark of human MM. Interestingly, PET scan was able to visualize MM bone marrow infiltration and response to anti-myeloma treatment including bortezomib. Myc translocation occurs late in the course of the human disease and is frequently observed in murine MM. We hypothesized that gp130-dependent signaling and Myc overexpression might be cooperating oncogenic events during myeloma progression. We therefore used bone marrow cells derived from Eµ-Myc for the L-gp130-dependent MM transplantation model. Strikingly, in these recipient mice, we observed a rapid onset of MM revealing CD138-positive cells with plasmablastic morphology. Further biochemical analysis of these MM cells showed a robust activation of Stat3 and members of the bcl-family. In conclusion, we generated a novel gp130-dependent MM mouse model characterized by monoclonal plasma cells, short latency, evidence of a MM-initiating cell compartment and osteolytic lesions. Further studies demonstrated that constitutive gp130 signaling not only drives or facilitates MM development but also induces plasmablastic differentiation in the context with Myc overexpression.

Disclosure: No conflict of interest disclosed.
Development of a novel pharmacological Hsp70 inhibitor with anti-myeloma activity

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Introduction: Increasing experimental evidence suggests that the molecular chaperones heat shock proteins 70 (Hsp70) might represent potential anti-cancer targets. Thus, we have recently shown that Hsp72 and Hsp73 are frequently overexpressed in multiple myeloma (MM), sustain Hsp90-chaperone function and critically contribute to survival of MM cells. However, currently only a limited number of Hsp70 inhibitors are available, while efficient and selective pharmacological agents are almost completely missing. Therefore, we aimed to develop novel, more efficient pharmacological Hsp70 inhibitors for anti-MM therapy.

Methods: Starting from structural information about the Hsp70 protein, a computer-aided molecular drug design approach was employed to characterize chemical properties of the Hsp70 interdomain region in order to perform a structure-guided synthesis of a library of derivatives. These compounds were further tested for potential anti-Hsp70 and anti-MM effects.

Results: The search resulted in the discovery of a compound, which caused molecular effects that were similar to those of Hsp70/siRNAs, such as down-regulation of well-defined Hsp70/90-dependent client proteins, nuclear translocation of the apoptosis-inducing factor, and activation of the caspases 9 and 3. Importantly, the lethal effect of the novel anti-Hsp70 compound was strongly enhanced by concomitant Hsp90 inhibition in MM cell lines as well as in primary MM cell without showing a significant toxicity on non-malignant B cells.

Conclusion: Here we demonstrate substantial anti-MM activity of the first Hsp70 inhibitor that targets the functionally critical interdomain interface. Our study might provide the rationale for a therapeutic approach that combines Hsp70 and Hsp90 inhibitors.

Disclosure: No conflict of interest disclosed.

Detection of clonotypic B cells with individual immunoglobulin ligands reveals the rarity of this cellular population in patients with multiple myeloma

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Introduction: Circulating B cells expressing the same immunoglobulin rearrangement as the malignant plasma cell clone, so called clonotypic B cells, have been proposed to possess stem cell like properties in multiple myeloma. It has been suggested that such cells could feed the malignant plasma cell compartment in disease initiation and progression. Their abundance de-scribed in the literature – up to one fourth of peripheral mononuclear cells based on indirect calculation methods – has added to the biological significance attributed to this B cell population in myeloma patients. We developed an innovative approach to label clonotypic cells by individually designed immunoglobulin ligands to physically detect and directly quantify this interesting B cell population.

Methods: Paraproteins or recombinantly expressed myeloma idiotypies were used as targets for the selection of immunoglobulin ligands from random peptide phage display libraries. The bacteriophage ligands were used to stain peripheral and bone marrow B cells as assessed by immunofluorescence and flow cytometry.

Results: As proof-of-principle two monoclonal B cell systems were used to establish the detection of B cells with individual immunoglobulin ligands. Phage selected on the recombinantly expressed immunoglobulins of both control cellular systems bound specifically to the parental cells as analyzed by immunofluorescence and flow cytometry. A spiking assay was used to determine the sensitivity of the detection system, which ranged around 0.1%. After having established the detection approach, we selected individual ligands on the idiotypic immunoglobulins derived from 15 myeloma patients. The selected ligands were subsequently used to screen the peripheral blood and bone marrow of the respective patients for the presence of clonotypic B cells. We found that in the vast majority of investigated patients, clonotypic B cells are a very rare event constituting less than 0.1% of peripheral blood mononuclear cells. This contradicts clearly previous PCR-based indirect quantitative estimates.

Conclusion: Our new detection approach may pave the way to experimentally address some of the ap-parent inconsistencies and controversies regarding the clinical role of clonotypic B cells in multiple myeloma in the future.

Disclosure: No conflict of interest disclosed.
Lenalidomide combined with Bendamustine, Prednisolone (RBP) in patients with refractory or relapsed multiple myeloma. Final results of a phase I clinical trial -OSHO – #077

Pönisch, W.1, Heyn, S.1, Wagner, I.1, Mohren, M.2, Hoffmann, F.A.3, Lange, T.1, Schmalfeld, M.1, Zehnfeld, T.1, Schwarzer, A.4, Winkelmann, C.7, Edelmann, T.1, Hebenstreit, K.1, Al-Ali, H.K.1, Jäkel, N.1, Niederwieser, D.1, Ostdeutsche Studiengruppe für Hämatologie und Onkologie (OSHO)

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Introduction: While the role of lenalidomide mono therapy in the treatment of relapsed/refractory patients with multiple myeloma (MM) is established, combination therapies with lenalidomide are still under investigation. In the current trial, combination therapy of bendamustine, lenalidomine and prednisolone (RBP) was tested for feasibility and safety in patients with advanced MM.

Patients and methods: In this phase I part of the trial the dosing of lenalidomide in combination with bendamustine and prednisolone was examined. The first cohort of patients received a starting dose of 10 mg/d d1–21 lenalidomide, 60 mg/m² d1–2 bendamustine and 100 mg/d d1–4 prednisolone. Escalation steps in the next cohorts included 15, 20 and 25 mg of lenalidomide followed by an escalation step of 75 mg/m² bendamustine. 3 (to -6) patients were enrolled at each dose level and the first two cycles were evaluated for maximum tolerable dose (MTD). Patients received RBP in 4-week cycles for a maximum of 8 cycles in order to evaluate efficacy. Patients with stable or responding disease following 8 cycles of RBP received single-agent oral lenalidomide 10 mg once daily on days 1–21 of each 28-day cycle as maintenance.

Results: 21 patients (3 at the first three dose levels and 6 at the last two dose levels) have been enrolled in this phase I study and all patients have completed at least 2 cycles.

Two of the 21 patients developed dose-limiting hematotoxicity as defined by an ANC <1.0 x 10⁹/l with fever for > 3 days or an ANC <0.5 x 10⁹/l for > 7 days or platelet count <25 x 10⁹/l for > 3 days: One patient in the dose level with 60 mg/m² bendamustine and 25 mg lenalidomide and one patient in the last dose level with 75 mg/m² bendamustine and 25 mg lenalidomide. The study finished with 75 mg/m² for bendamustine and 25 mg for lenalidomide without reaching MTD. 16 patients (76%) responded after at least two cycles of RBP with 1 CR, 1 nCR, 4 VGPR and 10 PR. Three patients experienced a MR and two a SD/PR. After median observation time for surviving patients of 16 months, PFS at 18 months was 48% and OS was 64%.

Conclusions: RBP with a dose of 25 mg lenalidomide d 1–21 and 75 mg/m² bendamustine d 1–2 is well tolerated in patients with advanced MM. The study is ongoing and 20 additional patients will be enrolled in the phase II study at the last dose level to better evaluate toxicity and clinical activity.


Fig. 1. Six1-overexpressing cells.

Fig. 2. Control cells.
containing plasmid and the control plasmid. For invasion assays, Six1 was knocked down in Panc1 and BxPC3 cell lines by siRNA.

**Results:** Six1 expression is significantly increased in ductal pancreatic cancer in contrast to benign pancreatic ducts (p<0.001). Six1 overexpression in ACRBII 515 cells led to increased colony formation in soft agar, reduced apoptosis after gemcitabine treatment, a more mesenchymal phenotype (figure attached) and increased growth. Knockdown of Six1 in Panc1 and BxPC3 cells led to reduced invasion.

**Conclusion:** Six1 is an important player in the development and progression of ductal pancreatic cancer. In accordance with the findings in other cancer types, Six1 could serve as a relevant target in the treatment of pancreatic and other cancers.

**Disclosure:** No conflict of interest disclosed.

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**Impact of hand-foot skin reaction (HFS) on treatment efficacy in patients (pts) receiving capcitabine/gemcitabine + erlotinib for pancreatic cancer (PC): a subgroup analysis from the AIO-PK0104 randomized, cross-over phase III trial in advanced PC**


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**Introduction:** AIO-PK 0104 investigated the efficacy and safety of gemcitabine/erlotinib (GE) followed by capcitabine (C) vs. C/E followed by G. The present subgroup analysis evaluated the correlation between C-associated skin toxicity and outcome parameters in PC.

**Methods:** Within this multicenter phase III trial, pts with confirmed advanced PC were randomly assigned to 1st-line treatment with either C (2,000 mg/m², d1–14 q 21) plus E (150 mg/d, arm A) or G (1,000 mg/m² over 30 min weekly x 7, then d1, 8, 15 q 28) plus E (150 mg/d, arm B). A cross-over to either G (arm A) or C (arm B) was performed after treatment failure (e.g. disease progression or unacceptable toxicity). Time to treatment failure after 1st- and 2nd-line therapy (TTF2) was the primary study endpoint. Treatment-related skin toxicity was evaluated separately for each treatment arm/each regimen based on NCI-CTCv2.

**Results:** Of 281 randomized pts, data on skin toxicity were available for 255 pts. Of those, 43 (17%) had locally advanced disease, 212 (83%) had metastatic disease and 138 (54%) received second-line therapy with either G or C. For the 75 pts (29%) with a HFS any grade documented at any time during the treatment strategy, TTF2 and OS both were significantly prolonged compared to pts without HFS (7.4 vs 4.0 months, HR 0.61, 95%CI 0.47–0.81, p=0.001 and 9.7 vs 5.5 months, HR 0.64, 95%CI 0.48–0.86, p=0.002, respectively). Considering HFS during 1st-line treatment in 123 pts within the CE arm, these results could be confirmed for the 47 pts (38%) with a documented HFS of any grade (TTF2: 7.6 vs 3.2 months, HR 0.46, 95%CI 0.32–0.67, p<0.001; OS: 10.2 vs 4.4 months, HR 0.52, 95%CI 0.35–0.76, p=0.001). In pts receiving 1st-line treatment with GE (n=132) no difference in outcome was observed for pts with (n=13) or without (n=119) HFS of any grade (TTF2: 5.7 vs 4.2 months, HR 0.71, 95%CI 0.43–1.37, p=0.375; OS: 8.4 vs 6.6 months, HR 0.81, 95%CI 0.44–1.51, p=0.505).

**Conclusions:** The current subgroup analysis of AIO-PK0104 supports the assumption of a correlation between HFS in PC pts treated with capcitabine or gemcitabine/erlotinib and efficacy endpoints like TTF2 and OS. A capcitabine-associated HFS thus might be predictive for efficacy in patients with advanced PC.

**Disclosure:** Michael Haas: Other Financial Relationships: Roche Pharma Volker Heinemann: Advisory Role: Roche Diagnostics; Financing of Scientific Research: Roche Diagnostics; Other Financial Relationships: Roche Diagnostics
Hepatocellular carcinoma (HCC) is the fifth most common cancer and it is the third leading cause of cancer-related deaths worldwide. For the development of novel therapeutics, functional in vivo models are highly valuable. Orthotopically growing xenografts in immunodeficient mice reflect well the clinical situation. In the present study sensitivity towards the multikinase inhibitor sorafenib of patient-derived HCC xenografts LXIF 575 implanted orthotopically into NMRI nude mice was investigated.

Cancer cells were injected into the murine liver. 3 days after inoculation of 5 Mio. tumor cells, mice were subdivided into two groups, and treatment was started either with vehicle (PBS, 10 ml/kg/d, po, days 3–36) or sorafenib (50 mg/kg/d, po, days 3–36). Tumor growth was monitored via a) fluorescence-based in vivo imaging (IVI, using CF750 labeled anti-human CD10 antibody) once weekly, b) survival time, c) determination of liver weight and d) histological examination of murine liver tissue. IVI pictures were merged with x-ray for an optimal localization of the fluorescent tissue. Antitumoral and antimetastatic capacity was determined by comparing the fluorescent intensity for primary tumors and metastasized organs over the time. Data were compared to a mock-injected control group.

9 days after implantation, LXIF 575-derived tumors could be detected in the liver of 14 out of 17 animals via IVI. Lymphnode metastases (6/6 control mice and 4/8 sorafenib-treated mice) could be determined 16 days after tumor cell inoculation. Sorafenib showed a strong antitumoral and antimetastatic activity, reducing tumor load to 79% of the control on day 23. Metastasis rate was reduced to 50% on day 16. Liver weight was reduced from 3.4g ± 1.2 to 1.2g ± 0.6 g, and median overall survival prolonged from 31 to 37 days by treatment with sorafenib. Tolerability of the test compound was very high, without any marked body weight losses or drug related toxicities could be observed.

Thus, patient-derived liver cancer xenograft implanted orthotopically into nude mice is a valuable in vivo model for HCC exhibiting high reproducibility closely mimicking the clinical situation. Collection of whole-body IVI data proved to be a time- and animal-saving analysis that allows to monitor tumor growth accurately. Further investigations will optimize the very promising antitumor activity of sorafenib with focus on the synergistic effect of sorafenib with additional well-established HCC therapeutics.

Disclosure: No conflict of interest disclosed.

V546

Interim analysis of overall survival per subgroups in the prospective, non-interventional INSIGHT study in patients with hepatocellular carcinoma treated with Sorafenib

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Background: The efficacy of Sorafenib in patients (pts) with hepatocellular carcinoma (HCC) has been proven in randomized, controlled trials. INSIGHT is a prospective, non-interventional study, conducted in Germany and Austria in pts with HCC. The objectives of this study are the evaluation of safety and efficacy under practice conditions in both hospitals and private practices and reflect current standard of care. Enrollment into INSIGHT is NOT restricted to any particular tumor stage. Recruitment into the study is ongoing.

Disclosure: No conflict of interest disclosed.

Table 1.

<table>
<thead>
<tr>
<th>Patients recruited n, Male n (%)</th>
<th>618, 528 (85)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ECOG PS, n (%), 0, 1, 2</td>
<td>203 (33); 310 (50); 98 (16)</td>
</tr>
<tr>
<td>BCLC-Stage n (%), A, B, C, D</td>
<td>80 (13); 149 (24); 319 (52); 12 (2)</td>
</tr>
<tr>
<td>Child Pugh Stage, n (%), A, B, C</td>
<td>264 (43); 99 (16); 12 (2); 243 (39)</td>
</tr>
<tr>
<td>mOS total population (Events n = 164)</td>
<td>17,1 month</td>
</tr>
<tr>
<td>mPFS total population (Events n = 408)</td>
<td>4,1 month</td>
</tr>
<tr>
<td>mOS according to BCLC A, B, C, D</td>
<td>29,2; 19,6; 14,5; 4,0 month</td>
</tr>
<tr>
<td>mOS according to Child Pugh A, B, C</td>
<td>n.r.; 7,2; 4,0 month</td>
</tr>
<tr>
<td>mOS Child Pugh B: 7, 8, 9 points</td>
<td>11,5; 9,5; 2,5 month</td>
</tr>
<tr>
<td>mOS Duration of therapy&gt;24 weeks (n = 176); &gt;40 weeks (n = 91)</td>
<td>19,8; 26,7</td>
</tr>
<tr>
<td>n.r.-not reached</td>
<td></td>
</tr>
</tbody>
</table>

Methods: The second interim analysis (data cut-off 23 FEB 2012) evaluated overall survival and safety data in subgroups. All patients with HCC were observed for the duration of their sorafenib therapy. In addition to baseline data the performance status, tumor status (clinical and/or radiological), time to progression and overall survival time are documented. Documentation of adverse events comprises relationship with drug, seriousness, grade (CTCAE version 3.0), and outcome.

Results: Until the data cut-off 623 pts have been enrolled; 618 of which are evaluable for safety and efficacy analyses. The table summarises major base-line characteristics together with median overall survival (mOS) data for relevant subpopulations.

Conclusions: Results of mOS in pts with HCC treated in clinical practice in hospitals and private practices confirm the efficacy of Sorafenib as reported in randomized controlled trials. It gives important further insight into pts with Child B/C cirrhosis and BCLC stage A/B.


G Gerken: No conflict of interest disclosed.

V547

Tivantinib (ARQ 197) vs Placebo in patients with hepatocellular Carcinoma (HCC) who failed one systemic therapy: results of a randomized controlled phase 2 trial


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Introduction: Tivantinib (T) is a selective, oral inhibitor of c-Met, a tyrosine kinase receptor involved in tumor cell migration, invasion, proliferation and...
angio genesis. T has shown promising results in HCC in phase I studies as monotherapy and in combination with sorafenib.

Methods: This multi-center RCT enrolled patients (pts) with unresectable HCC, failure to 1 systemic therapy, ECOG PS ≤2. Child-Pugh A. Pts were randomized 2:1 to oral T (360 mg (TA) and 240 mg bid (TB)) or placebo (P). Pts were stratified by PS and vascular invasion (VI). Response was evaluated using RECIST 1.1, by CT/MRI every 6 weeks. Crossover to open-label T was in the intent-to-treat (ITT) population by central radiology review. Other endpoints included DCR, PFS, OS, efficacy in Met+ (Met >2+) in ≥50% of tumor cells by IHC pts, and safety.

Results: 107 pts were enrolled, 71 received T (TA: 38, TB: 33). Pts Main characteristics of pts on T vs P were: 82% vs 78% male; median age 70y vs 68y; PS 0.58% vs 58%; VI 31% vs 36%; Met+ 22 vs 15%; HCV+ 51% vs 39%; HBV+ 23% vs 14%. TA was reduced to TB in all pts due to ≥3 neutropenia rate. Major advantage of TA vs P was observed in Met+ pts, with median TTP: 2.7 vs 1.4 months (HR 0.43, P=0.03); PFS: 2.2 vs 1.4 months (HR 0.45, P=0.02); DCR: 50% vs 20%. A preliminary OS trend (HR 0.47) favoring T is being followed for updates. In ITT, T vs P results are: TTP: 1.6 vs 1.4 months (HR 0.64, P=0.04); PFS: 1.5 vs 1.4 months (HR 0.67, P=0.08); DCR: 95% (CI) 44% (31-56%) vs 31% (16-48%). The most common AEs in T were asthenia (26.8%), neutropenia (25.4%), low appetite (25.4%); the most common drug-related AEs were neutropenia (25.4%), anemia (15.5%). The most frequent drug-related serious AE was neutropenic sepsis (4.2%). Efficacy was independent of dose with less frequent ≥3 neutropenia in TB (21.1% vs 6.1%).

Conclusions: T was shown to be effective as second-line therapy in HCC when compared to P. This advantage is more evident in Met+ pts. The safety profile of T at 240 mg BID was manageable.

Disclosure: No conflict of interest disclosed.

Freie Vorträge
Immuntherapie I (experimentell und klinisch)

V548 Cyclin-A1: A novel cancer-testis antigen in acute myeloid leukemia stem cells
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The direct cytotoxic effect after allogeneic stem cell transplantation (HSCT) is essential for the elimination of leukemic stem cells (LSC) in intermediate or high risk patients with acute myeloid leukemia (AML). A potentially more specific and less toxic alternative to HSCT is the targeted T-cell therapy. However, this strategy requires the identification of immunogenic and selectively expressed proteins. Aim of the study was the identification of such a protein, which can act as leukemia-associated antigen.

To screen for candidate antigens, microarray expression data of LSCs hematopoietic cell subpopulations and healthy tissues were analyzed. Cyclin-A1 was identified as a gene with suitable expression pattern. Its expression was confirmed by a second microarray data set, quantitative real-time PCR, immunofluorescence and FACS. T-cell clones were generated by stimulating T-cells with dendritic cells and monocytes pulsed with a cyclin-A1 peptide library or single peptides. HLA restrictions of epitopes were determined by stimulation of T-cell clones with different lymphoblastic cell lines of known HLA type. Cytotoxicity of T-cells was tested by chromium release and caspase-3 cleavage assays.

Cyclin-A1 was identified both on mRNA and protein level as gene with selective expression in AML cells including the stem cell compartment of more than 50% of the AML patients analyzed. No significant expression was observed in healthy tissues except in testis. Cyclin-A1 promotes AML cell proliferation and survival, and has been shown to be leukemogenic in mice without being essential for normal development except male fertility. Therefore it provides all features of a suitable T-cell antigen. We isolated cytotoxic T-cells from several donors reactive against a multitude of cyclin-A1 oligopeptides. The minimal immunogenic sequence of eight MHC class I epitopes restricted for at least three different HLA alleles was determined. T-cells against two A*02:01 restricted epitopes showed in vitro toxicity against a cyclin-A1 expressing cell line, and thus endogenous processing and presenting of the respective epitopes. Furthermore, cyclin-A1-specific T-cells showed HLA-dependent killing of primary blasts.

In conclusion, cyclin-A1 is the first prototypic leukemia-testis-antigen to be expressed in AML stem cells. Its pro-oncogenic activity, the high expression levels, and the high number of immunogenic epitopes make it a viable target for T-cell based therapy strategies in AML.

Philip Greenberg: Honoraria: Ein Patent wurde angemeldet

V549 Targeting leukemia using allo-HLA-DQ and allo-HLA-DP specific CD4+ T cells
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Introduction: In allogeneic hematopoietic stem-cell transplantation (allo-HSCT), patient and donor are usually matched for HLA class I (A/B/C) and class II molecules (DRB1/DQB1). However, the HLA-DPB1 locus is still ignored in donor selection. Retrospective studies have shown that single mismatches at DQB1 and distinct DPB1 alleles do not adversely affect the outcome of allo-HSCT. It is also known that HLA class II is mainly expressed on hematopoietic cells under non-inflammatory conditions. Thus CD4+ donor T cells recognizing patient-derived DQB1 or permissive DPB1 mismatch alleles may primarily target leukemic and hematopoietic cells, while sparing non-hematopoietic tissues.

Methods: Mature monocyte-derived dendritic cells of healthy donors were transfected with in vitro transfected RNA coding for single DQ/DP mismatch alleles by electroporation. The allo-HLA expressing dendritic cells were used to simulate autologous naive CD4+ CD45RA+ T-cells in mixed lymphocyte reactions (MLR) in vitro (n=6). Resulting DQ-/DP-specific T cells were analyzed for allorreactivity by IFN-γ ELISPOT and chromium-release assays. The target cell panel included HLA class II typed primary acute myeloid leukemia (AML) blasts (n=24), fibroblasts (FB) (n=21), and keratinocytes (KC) (n=14). In addition, HLA class II expression was analyzed on hematopoietic and non-hematopoietic cells by flow cytometry.

Results: Rapidly expanding MLR clones showed specific IFN-γ production and cytotoxicity only against those targets that carried the DQ or DP allele used for T cell priming. Reactivity could be blocked by DQ-/DP-specific and CD4-specific monoclonal antibodies. While AML blasts were recognized independent of pre-incubating them with IFN-γ, recognition of FB and KC required IFN-γ pre-treatment. HLA class II was not detected on primary FB, KC, and normal kidney cells (each n=10), but at significant levels on primary AML blasts and B-cell lines (each n=10). Expression levels followed the hierarchy: DQ>DR>DQ. Up-regulation of HLA class II expression was observed on all cell types after pre-incubation with IFN-γ, but not after addition of TNF-α, IL-1β and IL-6.

Conclusions: Our approach appears suitable for generating allo-DQ-/DP specific CD4+ T cell lines that eliminate AML blasts while sparing non-hematopoietic cells under non-inflammatory conditions. It may be of potential use in adoptive immunotherapy of allo-HSCT patients who express single HLA-DQ or permissive HLA-DP mismatch alleles.

Disclosure: No conflict of interest disclosed.
V550
Reduced alloreactivity of human memory versus naive CD8+ T cells in vitro as well as in vivo: Defining the optimal target-population for TCR-RNA based immunotherapy

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Adoptive transfer of tumor/virus-specific T lymphocytes is a promising strategy for treating cancer and viral diseases. However, the approach is not feasible if specific T cells are absent or their numbers are too low in patients and donors. Grafting T lymphocytes with T-cell receptors (TCR) allows the redirection of non-reactive T cells into tumor- or virus-specific effectors for a broad range of antigens. One rapid approach for transfer of TCR is the electroporation of T lymphocytes with in vitro transcribed RNA, which results in potent effector functions to specific targets for up to one week. Due to the transient expression of the introduced RNA, a potential study protocol would have to include the weekly administration of TCR redirected T cells. However, in an allogeneic setting this approach might be hampered by the induction of serious alloreactivity through the repeated transfer of polyclonal donor T cells. Considering these limitations, we generated TCR transfected naive (T(N)) and memory (T(M)) CD8 T-cell populations, using a CMVpp65-specific TCR as a model antigen receptor. Although both T(N) and T(M) subsets showed comparable level of TCR expression upon RNA transfection, CD8 T(M) cells mediated superior cytotoxicity against CMV-infected fibroblasts. In addition, we analyzed alloreactivity of CD8 T(N) and T(M) subsets against HLA-mismatched targets in IFNγ ELispot and cytotoxicity assays. As expected, alloreactivity was mainly mediated by CD8 T(N) cells of naive phenotype. To investigate the relevance of these findings for a clinical application, we used a mouse model that allows the analysis of T-cell alloreactivity directed against human hematopoiesis. For this, we reconstituted NOD/SCID/IL2Rγc-/- mice with human CD34+ stem cells and adoptively transferred them with CD8 T(N) and T(M) populations previously stimulated against cells of the stem cell donor. In line with the in vitro data, T(N) cells mediated stronger alloreactivity toward donor hematopoiesis than T(M) cells did. This was shown by a significant decrease of human CD45+ hematopoietic cells and B-cells in spleen and bone marrow of the mice. Moreover, in vivo proliferation of adoptively transferred T cells was mainly detected in mice treated with T(N) cells. Our data show that memory CD8 T-cell populations mediate decreased alloreactivity in vitro as well as in vivo. This observation along with strong effector function upon TCR transfer makes memory CD8 T cells promising tools for adoptive immunotherapy.

Disclosure: No conflict of interest disclosed.

V552
Fusion proteins of the NKP30 ligand B7-H6 and CD20 antibody-fragments induce NK cell-mediated lysis of lymphomas

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Introduction: The activating surface receptor NKP30 plays an important role in controlling the cytotoxicity of natural killer (NK) cells and contributes to recognition and elimination of emerging tumors. NKP30 recognizes the induced-self molecule B7-H6 which is expressed by a variety of different tumors but not by healthy tissues. Thus, increasing the cell surface density of B7-H6 on malignant cells may represent an attractive approach to promote NK cell cytotoxicity. In order to attract NK cells against lymphomas a recombinant immunoligand comprising a CD20 single-chain fragment variable (scFv) and B7-H6 was generated and characterized.

Methods: A human CD20 scFv was genetically fused to the extracellular domain of B7-H6. The resulting immunoligand B7-H6:CD20 was expressed in mammalian cells and purified by affinity-chromatography. Binding abilities were analyzed by flow cytometry. Cytotoxic properties of B7-H6:CD20 were determined in 14C release assays. The ability of B7-H6:CD20 to trigger NK cell effector functions was assessed by analyzing the expression of the activation marker CD69, surface exposure of the degragation marker CD107a, and intracellular staining for immunomodulatory cytokines using flow cytometry.

Results: B7-H6:CD20 showed bispecific properties as reflected by its ability to simultaneously bind to the CD20 antigen and to the NKP30 receptor. B7-H6:CD20 bound to CD20-positive lymphoma cells activated NK cells resulting in enhanced NK cell degranulation and cytokine production. Consequently, the immunoligand B7-H6:CD20 induced NK cell mediated killing of lymphoma-derived cell lines as well as freshly isolated tumor cells from chronic lymphocytic leukemia or lymphoma patients. B7-H6:CD20 was active at nanomolar concentrations in a strictly antigen-specific manner and required interaction with both CD20 and NKP30. Remarkably, NK cell cytotoxicity was further augmented by concomitant activation of natural killer group 2 member D (NKG2D) or the Fcy receptor IIIa. Thus, B7-H6:CD20 acted synergistically with the similarly designed immunoligand ULBP2:CD20 engaging the NKG2D receptor and the CD20 antibody rituximab.

Conclusions: B7-H6:CD20 represents the first biologic agent recruiting NK cells in an NKP30-dependent manner. The observed cytotoxic abilities by B7-H6:CD20 as single agent and in combination provide proof of concept that...
NKp30 engagement may represent an innovative strategy to enhance anti-tumoral NK cell cytotoxicity.

**Disclosure:** Christian Kellner: No conflict of interest disclosed. Matthias Peipp: Advisory Role: Consultancy: Gennab

**V553**

**CD30-targeted therapy with brentuximab vedotin combined with donor lymphocyte infusions for relapsed Hodgkin lymphoma following allogeneic stem cell transplantation induces tumor specific immunity and sustained clinical remission**

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**Introduction:** Relapsed Hodgkin lymphoma (HL) remains a therapeutic challenge. Allogeneic stem cell transplantation (alloSCT) is a setting in which the graft-versus-lymphoma (GvL) effect can lead to immune mediated tumor control. We hypothesized that treatment with brentuximab vedotin would be a promising approach for relapsed HL after alloSCT that selectively targets the lymphoma cells and enhances the GvL response by the induction of immunogenic cell death. Additionally, the combination of this targeted therapy with DLI would have the potential to further increase the GvL effect.

**Methods:** Four patients with relapsed HL after alloSCT were consecutively treated from March 2011 to March 2012 with brentuximab vedotin and DLI in an alternating regimen. Evaluation of treatment response was performed clinically and every 2–3 months with computed tomography scans (CT) following established criteria. Moreover, metabolic changes were analyzed by FDG-PET/CT scans. To assess the induction of a GvL effect, we developed an in vitro method using co-cultures of patient PBMCs (pre and post treatment) and well characterized CD30+ HL cell lines and controls as surrogate targets.

**Results:** All four patients showed marked clinical and metabolic responses with a median duration of disease control of at least 349 days (range 259–366) after treatment initiation which is still ongoing in three patients. Sensory polyneuropathy and mild thrombopenia were the most common side effects. Prior to treatment, we could not detect significant tumor-specific T cell reactivity. In contrast, a significant increase of HL-specific T cell activation could be observed in vitro in all patients after treatment. Reactive HL-specific T cells mainly co-expressed CD161 and CD4 suggesting a TH17 like phenotype.

**Conclusion:** Taken together, we present evidence that CD30-targeted Hodgkin lymphoma therapy with brentuximab vedotin and DLI induces sustained clinical responses and tumor-specific immunity in an allogeneic setting which warrants further investigation.

**Disclosure:** No conflict of interest disclosed.

**V554**

**Characteristics of patients with limited metastatic gastric cancer in the FLOT3 trial**

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**Background:** A prospective trial of the AIO (FLOT3) categorized pts with metastatic gastric cancer into pts with limited and those with extensive disease. Pts with limited disease (LD) showed a favorable outcome after preoperative FLOT chemotherapy in regard to progression-free and overall survival. The goal of this analysis was to characterize this subset in detail.

**Methods:** Using a predefined algorithm, pts with untreated gastric cancer were prospectively stratified into 3 groups: operable (OD), limited metastatic (LD), or extensive metastatic (ED) disease and treated with FLOT chemotherapy. LD was defined as: distant intra-abdominal lymph node metastases only or/and a maximum of 1 organ involved, normal serum alkaline phosphatase, <5 liver lesions, no visible carcinoatosis (peritoneum or pleura), and ECOG ≤ 1. Pts with LD received 8 cycles FLOT with surgery allowed for complete macroscopic resection.

**Results:** 60 of 252 pts included had LD. LD pts had distant lymph nodes only (41%), liver (22%), lung (17%), localized peritoneal involvement (7%), or other metastases (13%). Median OS was 22.9 vs. 10.7 months in pts with LD vs. extensive disease (ED), respectively (HR 0.37; 95% CI, 0.25–0.56; P=0.001). LD was the strongest predictor of OS in the multivariate analysis including all single determinants of LD status (p=0.002). In the LD, surgical resection was conducted in 62% of pts (R0-resection rate 81%). Within the LD arm, operated pts had better outcome than non-operated pts (median OS 31.3 vs. 15.9 months; p=0.004). Irrespective of surgery, pts with lymph node only involvement had the best outcome, while pts with liver metastases the worst. ALL LD subgroups had a favorable outcome compared with the corresponding ED subgroups (s. table).

**Conclusions:** This analysis characterizes a subset of pts with limited metastatic gastric cancer who have a favorable outcome and shows that this was true for all subgroups of the pts with limited disease based on metastases localization.

**Disclosure:** No conflict of interest disclosed.

**V555**

**Feasibility of perioperative chemotherapy with infusional 5-FU, leucovorin, and oxaliplatin with or without docetaxel, in elderly patients with locally advanced esophagogastric cancer**

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**Introduction:** The aim of this study was to determine tolerability and feasibility of perioperative chemotherapy in elderly, potentially operable esophagogastric cancer patients.
Methods: Patients aged 65 or older with locally advanced esophageogastric adenocarcinoma were randomized to perioperative chemotherapy consisting of four preoperative and four postoperative cycles of infusional 5-FU, leucovorin, and oxaliplatin (FLO) without or with docetaxel 50mg/m² (FLOT), every 2 weeks.

Results: 44 patients with a median age of 70 years were randomized (FLO, 22; FLOT, 22). Thirty-eight (86.4%) patients completed four cycles of perioperative chemotherapy (FLO, 20; FLOT, 18) and 32 (72.7%) proceeded to surgery (FLO, 17; FLOT, 15), with 90.6% R0 resections (FLO, 88.2%; FLOT, 93.3%). FLOT was associated with significantly more neutropenia (P<0.001), leukopenia (P<0.001), stomatitis (P=0.02) and nausea (P=0.02) and a higher rate of patients experiencing a ≥210-points deterioration of EORTC Qol. global health status scores (FLOT, 54%; FLOT 18%; p=0.045) at 8 weeks. No perioperative mortality was observed. Postoperative morbidity was observed in 46.9% of patients (FLO, 35.3%; FLOT, 60%) and 20 (62.5%) were able to undergo postoperative chemotherapy (FLO, 64.7%; FLOT, 60%). In the ITT population, median overall survival was not reached and median disease-free survival was 17.3 months. Compared with the FLO group, the FLOT group had a higher response rate (51.9% vs. 18.2%; p=0.02) and a trend towards an improved median disease free survival (21.1 vs. 12.0 months; p=0.09).

Conclusion: Age alone is not a contra-indication for the selection of patients for perioperative chemotherapy, since perioperative mortality and morbidity and resection rates are comparable to younger patients.

Disclosure: Claudia Pauligk: Expert Testimony: Sanofi-Aventis
Salah-Eddin Al-Batran: Expert Testimony: Roche Diagnostics

V557 Clinical utility of circulating tumor cell analysis in adenocarcinoma patients

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Introduction: Despite advances in cancer treatment, therapy resistance and metastasis continue to pose major problems in its clinical management. Therefore, the present study aimed to develop an immunomagnetic/RT-PCR assay and assess its clinical value for the detection of circulating tumor cells (CTCs) in peripheral blood (PB) of adenocarcinoma cancer patients.

Methods: CTCs were analysed in 54 colorectal, 44 gastric and 34 pancreatic adenocarcinoma patients before systemic therapy and in 40 healthy controls, through immunomagnetic enrichment, using the antibodies BM7 (mucin 1) and VU119 (EpCAM), followed by real-time RT-PCR analysis of the genes KRT19, MUC1, EP CAM, CLACAMS and BIRC5. Progression-free survival (PFS) was estimated by Kaplan-Meier method and compared between groups according to CTCs detection (positive vs negative) by log-rank test.

Results: The assay showed 100% specificity, as none of the healthy controls were CTC positive. Baseline CTCs were detected in 68.5% of colorectal, 50.0% of gastric and 47.1% of pancreatic adenocarcinoma patients. The presence of CTCs in colorectal, gastric and pancreatic adenocarcinoma patients, before the beginning of a new line of systemic therapy, was significantly associated with a shorter progression-free survival time (log-rank P<0.0001, P<0.0001 and P=0.001, respectively).

Conclusions: The results suggest that baseline CTC detection is an independent predictor of PFS in adenocarcinoma patients. Identification of CTCs using a multimarker panel may give information about the genetic properties of the tumor cells and promote the creation of tailored therapy regimes.

Disclosure: No conflict of interest disclosed.

V558 Aryl-heteroaryl-maleimides – new selective inhibitors for treating gastrointestinal cancers

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Introduction: Aryl-heteroaryl-maleimides (also called Moguntiones) are new innovative, synthetic designed small molecules, which are a combination of three natural products. These patent-protected kinase inhibitors were invented by the Institute of Pharmacy and our Departments of Internal Medicine, Mainz. The substances displayed a new generation of targeted inhibitors for tumour progression, angiogenesis suppression and resistance blockade.

Methods: The substances were analysed by kinase assays and HET-CAM as well as in vitro in four human colon cancer (HT-29, HCT-116, DLD-1, SW480) and two gastric cancer cell lines (MKN-45, AGS) for RNA and protein expression levels (RT-PCR, Western blot, ELISA, FACS). Different viability and apoptosis assays were performed in the tumour cells, which were incubated with Moguntiones and different cytostatic drugs. Additionally, intracellular signalling pathways were analysed. In vitro data were further verified in a human xenograft NOD/SCID mouse model.

Results: Moguntiones showed clear anti-angiogenic effects in HET-CAM assays and inhibitory activities in IC50 kinase assays. After generating additional substances with little structural changes and better biological effects, these Moguntiones alone induced apoptosis only in higher concentrations (>10μM). Furthermore, stronger synergistic effects for induction of apoptosis
were observed in lower concentrations (<10μM) in combination with classical cytostatic drugs like irinotecan. These effects could not be seen in HUVEC. The signalling pathways AKT, GSK3β or FAK were totally inhibited in incubated tumour cells. The in vivo mouse model again showed significant reductions in tumour growth and tumour weight. Even more, comparable suppressive effects of Moguntinones in KRAS, BRAF and PI3KCA-mutated colon and gastric cancer cell models were identified.

**Conclusions:** Our in vitro and in vivo data clearly showed significant pro-apoptotic, anti-angiogenic and anti-proliferative effects of Moguntinones in different human gastrointestinal cancer cells. The experiments argue for a high potency of these substances to complement standard combinations and to overcome possible tumour resistance mechanisms. Thus, the consortium aims to develop these substances in clinical phase I studies.

**Disclosure:** No conflict of interest disclosed.

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**Plenarsitzung**

**Best Abstract**

**V561**

Cancer specific support in the catchment area of the Tumorregister Munich: Distress, acceptance, knowledge & use of psychooncological facilities by patients with colorectal cancer. Results of a representative study with country-town comparison

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**Introduction:** Psychooncological studies about distress usually take place during primary care and lack of representativeness. Little is known about distress and needs of patients when back in their familiar environment. In our study this absence of representativeness could be avoided by the recruitment procedure and by the cooperation with the Tumour Register of Munich (TRM). The following questions were investigated:

- What is the prevalence of distress, depression and anxiety in patients with colorectal cancer?
- How is the acceptance, knowledge and use of psychooncological facilities in the neighborhood?
- Which offers of psychooncological treatment can be found in the region?
- Which differences can be observed between city regions and rural regions?

**Method:** Patients with a colorectal tumour were recruited by their hospital surgeon and received 3 months after inclusion a questionnaire concerning socio-demographic and medical information, acceptance and knowledge about psychooncological facilities as well as psychosocial distress, depression and anxiety. We adjusted our sample for age and sex according to the data of the TRM, so that the resulting sample was representative for the catchment area of the TRM. At the same time we carried out an internet search recording all psychooncological facilities in the catchment area of the TRM.

**Results:** N=534 patients. Mean age was 68.9 years (SD=11.33). 27.6% of the patients presented metastases. 50.8% received chemotherapy. 26% of the sample presented distress. 12.4% of the patients had elevated anxiety and 14.8% elevated depression scores. 52% of the sample did not know any psychosocial support facility. Only 1.2% of the patients made use of support. 55.8% answered that they would accept or probably/perhaps accept support. Predictive factors for psycho-social distress were not talking to the general practitioner, psychotherapy in the past and chemotherapy. Predictive factors for acceptance were psychotherapy in the past and distress. In our sample patients from rural regions were better informed than patients from the city. The outpatient care situation showed that 10% of the patients did not have a psycho-social support in the vicinity (20 km) of home. Outpatient psychooncological support and resident haemato-oncologists showed the strongest undersupply in rural regions.

**Conclusions:** There is an important gap between distress, acceptance, knowledge and use of support facilities.

**Disclosure:** No conflict of interest disclosed.

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**V562**

GILT phase III study: Concomitant radiotherapy (RT) with Oral Vinorelbine (NVBo) plus Cisplatin (P) followed by consolidation (C) with NVBo+p plus BSC or BSC alone in stage (st) III NSCLC

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**Introduction:** Concurrent chemoradiotherapy (CT-RT) is standard in inoperable stage III NSCLC. Trials with consolidation chemotherapy (C) after CT-RT show encouraging but discordant results. This phase III assessed C in inoperable stage III NSCLC.

<table>
<thead>
<tr>
<th>Table: for abstract V562</th>
<th>Randomisation (N=201)</th>
<th>CT+BSN=96</th>
<th>BSCN=105</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male (%)</td>
<td>71.0</td>
<td>71.9</td>
<td>71.4</td>
<td>0.96</td>
</tr>
<tr>
<td>Median age (y. [range])</td>
<td>60.3 [33.9–75.7]</td>
<td>60.3 [34.1–75.9]</td>
<td>59.5 [40.4–75.1]</td>
<td>0.96</td>
</tr>
<tr>
<td>Squamous/adeno (%)</td>
<td>53.0/36.2</td>
<td>54.2/36.5</td>
<td>52.4/37.1</td>
<td>0.96</td>
</tr>
<tr>
<td>St IIA/IIIB (%)</td>
<td>17.6/82.4</td>
<td>208/792</td>
<td>190/81.0</td>
<td>0.96</td>
</tr>
<tr>
<td>ORR ITT (%) [95% CI] Squamous/adeno (%)</td>
<td>55.6 [49.5–61.5]</td>
<td>60.5; 54.5</td>
<td>29.2 [20.4–39.4]</td>
<td>24.8 [16.8–34.2]</td>
</tr>
<tr>
<td>DCR ITT/eval (%) (n eval=242/76/89)</td>
<td>78.5; 36.0</td>
<td>66.7/84.2</td>
<td>56.2/66.3</td>
<td>p=0.12 / p=0.0084</td>
</tr>
<tr>
<td>Median time PFS (m)</td>
<td>3.0</td>
<td>2.9</td>
<td>0.63</td>
<td></td>
</tr>
<tr>
<td>Median OS (m)</td>
<td>6.4 [5.0–8.7]</td>
<td>5.5 [3.8–7.4]</td>
<td>p=0.87</td>
<td></td>
</tr>
<tr>
<td>2/4year survival (%)</td>
<td>41.6/25.3</td>
<td>41.7 / 21.4</td>
<td>0.87</td>
<td></td>
</tr>
</tbody>
</table>
Methods: Patients (pts), received NVBo 50 mg/m² D1,8,15 + P 20 mg/m² D1-4 2xq4w + RT (66 Gy / 33 Fr). C for OR+SD pts: NVBo 60-80 mg/m² D1,8,15 + P 80 mg/m² D1 2xq3w + BSC (Arm A) or BSC (Arm B). PFS was the primary endpoint.

Results: From 07/05 to 05/09, 279 pts received CT+RT and 201 (72%) were randomized to CT+BSC or BSC as C. Toxicity G3-4 (%) CT+RT / C (Arm A/B): anaemia 3.2/3.1,1; thrombopenia (G3) 2.5/1.20; neutropenia (N) 11.2/22.10; febrile N 1.4/0.0; nausea (G3) 5/2.30; vomiting (G3) 2.3/2.50; anorexia 3.6/0.0; dyspnoea 0.4/0.00; MI 0.4/0.0; sens. neuropathy (G3) 0.4/0.00; DVT (G3) 1.1/1.20; purl. embolism 1.1/0.00; fatigue 3.3/1.20; pneumonitis/pneumonia 2.0/0.3; esophagitis related events 12.9/3.10; RT pain 2.2; RT skin injury (G3) 0.4; 5 toxic deaths.

Conclusions: These results show a high efficacy of concurrent NVBo+P+RT (OR 55.6%; DCR 78.5%) with low tox. The DCR (eval) is significantly improved by C with NVBo+ P in eval pts (p=0.0084). Lang toxicity was not enhanced. However no survival advantage for C was achieved so far.

Disclosure: Rudolf Huber: Expert Testimony: Pierre Fabre
Michael Flentje: Expert Testimony: Pierre Fabre

V564 Favorable remission control by allogeneic stem cell transplantation in NPM1 positive intermediate-risk AML: Donor versus no-donor analysis of 302 patients from the SAL AML-2003 trial

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Introduction: A mutated Nucleophosmin-1 gene (NPM1+) in acute myeloid leukemia (AML) is associated with a favorable prognosis, particularly in the absence of an FLT3-ITD mutation (FLT3-ITD-). In order to assess the value of allogeneic stem cell transplantation (allo SCT) in these patients, we compared the clinical course of 302 NPM1+ intermediate-risk (IR) AML patients eligible for allo SCT in a donor versus no-donor analysis.

Patients and methods: Patients with intermediate-risk AML, aged 18-60 years, and treated in the AML 2003 trial of the Study Alliance Leukemia (SAL) were analyzed. According to the strategy of the trial, IR patients should have received an allo SCT as consolidation if an HLA-identical-sibling donor was available. Patients with no available sibling donor received cytarabine-based consolidation or autologous SCT. In order to avoid selection bias, we compared outcomes depending on the availability of a suitable donor in a donor-no-donor analysis.

Results: Of 1182 patients enrolled between 2003 and 2009, 375 were NPM1+ (32%), and 302 patients were eligible for the donor vs. no-donor analysis, 237 patients had a normal karyotype (79%). A donor was identified for 78 patients (26%), of whom 56 actually received allo SCT as first consolidation (72%). The no-donor group consisted of 224 patients. Demographic and disease characteristics were equally distributed between the two groups. Median follow up was 47 months (3.9 years). The 3-year RFS in the donor and no-donor groups was 72% and 47%, respectively (p=0.002). The OS in the donor and no-donor groups was 71% versus 60% at 3 years, and 71% versus 55% at 5 years (p=0.089). In multivariate analyses, the presence of a donor as a prerequisite for allo SCT retained its statistically significant favorable influence on RFS (HR=0.53). In patients with normal karyotype and NPM1+/FLT3-ITD- (n=141), the 3-year RFS in the donor and no-donor groups was 88% and 54%, respectively (p=0.001).
V565

Early dose intensification with autologous stem cell transplantation results in improved disease control in patients with PTCL,NOS,AITL and ALCL

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Introduction: Since no standard therapy exists, CHOP-like therapies are commonly used as therapy for T-NHL. The impact of early dose intensification with high-dose therapy and autologous stem cell transplantation (ASCT) is less well defined.

Methods: We present retrospective longterm follow up data from a single center using response adapted early dose intensification and first line ASCT in patients with T-NHL.

Results: From 12/1986-07/2009 a total of 113 patients were treated. The median age was 56 years (range: 18–90), the diagnoses included: PTCL,NOS (n = 46), AITL (n = 25), ALC,ALK negativ (n = 26), ALC,ALK positive (n = 7), and others (n = 11). Initial chemotherapy included anthracycline based/CHOP-like regimens in most of the cases. If no complete remission (CR) could be achieved by CHOP-like induction, early intensification, mainly with VPE/VCPE (etoposide 50 mg/m², etoposide 500 mg/m², cisplatin 50 mg/m², ifosfamide 4 g/m²) or cyclophosphamide 1350 mg/m² or DHAP regimens, and ASCT after BEAM conditioning (66.6% of all ASCT) was initiated. 5-year overall survival (OS) of the entire group was 53.1% (median follow-up: 59.7 months). 5y-OS were 62.8% for patients with PTCL,NOS, 45.1% (AITL) and 48.6% (ALC,ALK neg.). In PTCL,NOS patients 31/46 (68.9%) experienced a CR1 after primary therapy. Of those 31 patients, CR was achieved after CHOP-like induction in 18 and after intensification in additional 3 cases, while the remaining 10 patients experienced CR only after ASCT. 5-year-relapse rate (RR) for patients in CR was significantly worse for patients not undergoing ASCT (68.0% v. 15.6%, p = 0.0046) with relapses occurring up to 4 years after completion of standard dose therapy. Nevertheless, primary therapy with ASCT did not result in significantly improved 5y-OS compared to patients without ASCT (64.6% v. 60.1%), mainly due to longterm disease control with second line ASCT in some patients relapsed after standard therapy. Similar results were observed in patients with ALC,ALK neg.: 5y-OS with/without primary ASCT was 60.8% v. 38.5% (p = 0.64); cumulative 5y RR for patients in CR1 was 11.1% v. 40.0% (p = 0.26). In contrast, in AITL patients treatment with ASCT resulted in a significantly improved 5y-OS (87.5% v. 21.8%, p = 0.01).

Conclusion: Primary ASCT results in improved disease control in T-NHL. Nevertheless, less well understood heterogeneity of lymphoma biology might be of more importance for the treatment outcome than dose intensity.

Disclosure: No conflict of interest disclosed.

V566

Fc-engineered NKG2D-Ig fusion proteins for induction of NK cell ADCC against breast cancer

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Introduction: The monoclonal antibody Herceptin (Trastuzumab®) is approved for treatment of breast cancer displaying HER2/neu-overexpression. Its activity is mainly attributed to blocking receptor signaling. Anti-tumor antibodies may also stimulate anti-tumor immunity by inducing antibody dependent cellular cytotoxicity (ADCC), and this was previously also reported for Herceptin. Notably, only about 20% of breast cancer patients show overexpression of HER2/neu. Moreover, this antigen is also expressed on healthy cells, and application of Herceptin is associated with side effects. In contrast, ligands of activating immunoreceptor NKG2D (NKG2DL) are widely expressed on malignant cells, but generally absent on healthy tissues. We aimed to take advantage of the tumor-restricted expression of NKG2DL by using them as tumor-antigens for FC-optimized NKG2D-Ig fusion proteins targeting breast cancer cells for NK cell ADCC and compared NK reactivity against tumor cells after application of Herceptin, NKG2D-Ig fusion proteins or a combination of both.

Methods: NKG2D-Ig fusion proteins with distinct modifications in their Fc portion were generated by amino acid exchange as previously described (Lazar 2006; Armour 1999). Functional properties were evaluated in analyses of NK cell activation, degranulation and cytotoxicity in cultures with breast cancer cell lines expressing different HER2/neu levels.

Results: Compared to wildtype NKG2D-Fc (NKG2D-Fc-WT) or Herceptin, our mutants (S239DF332E and E233PL234VL84ADEG36/A327QGA330S) displayed highly enhanced (NKG2D-Fc-ADCC) and abrogated (NKG2D-Fc-KO) affinity to the NK cell FcγRIIA receptor. This resulted in lacking (NKG2D-Fc-KO) or highly enhanced (NKG2D-Fc-ADCC) activation of NK cells. In cultures of NK cells and breast cancer cells, NKG2D-Fc-KO significantly reduced NK cell reactivity due to blockade of NKG2DL-mediated activating signals, while NKG2D-Fc-WT substantially enhanced NK reactivity by induction of ADCC. Notably, the effect of our NKG2D-Fc-ADCC by far exceeded that of NKG2D-Fc-WT and, in case of HER2/neu-low targets, also that of Herceptin, resulting in potentially enhanced NK anti-tumor reactivity.

Conclusions: Fc-engineered NKG2D-Fc-ADCC fusion proteins effectively target breast cancer cells for NK anti-tumor reactivity. Based on the tumor-restricted expression of NKG2DL, Fc-modified NKG2D-Ig may constitute an attractive means for immunotherapy especially of HER2/neu-low or -negative breast cancer.

Disclosure: No conflict of interest disclosed.

V567

Treatment-related mortality in patients undergoing therapy for advanced Hodgkin’s Lymphoma: Analysis of the German Hodgkin Study Group (GHSG)

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Introduction: Patients with advanced-stage Hodgkin’s Lymphoma (HL) show a 10 year freedom from treatment failure (FFFF) of 82% and an overall survival (OS) of 86% when treated with eight cycles of BEACOPPescal (Bleomycin, Etoposid, Adriamycin, Cyclophosphamide, Vincristine, Procarbazine, Prednisone). However, treatment related mortality (TRM) is a concern.

Conclusions: Allo SCT leads to a significantly prolonged RFS in NPM1+ intermediate-risk AML patients with a pronounced effect in NPM1+/FLT3-ITD- patients. The absence of a statistically significant difference in OS is most likely due to the fact that relapsed NPM1+ patients responded well to salvage treatment, particularly to allo SCT from an unrelated donor. Our results suggest that patients with NPM1+ intermediate-risk AML and a well-matched donor benefit from allo SCT in first remission.

Disclosure: No conflict of interest disclosed.
Methods: We investigated the incidence and risk factors for TRM in a retrospective analysis of 3402 patients with advanced HL treated with BEACOPPescalated. Within the GHSG HD9, HD12 and HD15 clinical trials between 1993 and 2008.

Results: The overall TRM rate was 1.9% (64/3402). 20 of 64 (31.3%) treatment related deaths occurred during the first course of BEACOPPescalated. The median age of patients who died from TRM was 50 years. Most common causes of TRM were neutropenic infections (n=56; 87.5%). Univariate analyses of possible risk factors revealed that TRM was six times increased in patients older than 40 years, four times increased in patients with a poor performance status (ECOG ≥2 or Karnofsky ≤80) and three times increased in patients with an International Prognostic Score (IPS) of ≥2 compared with patients without these risk factors, respectively. Gender, stage of disease, B-symptoms, and other known risk factors were not associated with an increased risk of TRM. In a multivariate analysis of TRM and all known risk factors, age and poor performance status were the only significant prognostic factors of TRM. A particularly higher risk of TRM is seen in patients between 40 and 50 years of age with poor performance status as well as in patients who are 50 years and older. According to the identified risk factors age and performance status, it is possible to define a risk score for TRM ranging from zero to three. Patients with a TRM Score of ≥2 have an increased risk for TRM.

Conclusions: Based on our analysis, patients with increased risk of TRM under treatment with BEACOPPescalated can be identified. Approaches to reduce TRM in these high-risk patients include a pre-phase treatment of patients over 40 years of age, inpatient treatment of patients with risk factors at least for the first course of BEACOPPescalated, an earlier administration of G-CSF starting from day 4 of every cycle, as well as the prophylactic use of antibiotics during the chemotherapy period. These measures have currently been implemented in the GHSG ongoing trial HD18 for advanced stage HL.

Disclosure: No conflict of interest disclosed.

V568
An RNAI-based system for loss-of-function analysis: Identification of Raf1 but not BRAF as a crucial mediator of BCR-ABL-driven leukemogenesis

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Introduction: Genetic loss-of-function studies in murine tumor models have been essential in the analysis of downstream mediators of oncogenic transformation. Here we describe a versatile method allowing the efficient expression of an oncogene and simultaneous knockdown of targets of interest (TOI) from a single retroviral vector. By using this vector system, we investigated the role of Raf1 and BRAF in BCR-ABL-mediated leukemogenesis. Raf kinases are key enzymes in the activation of the MAPK/ERK cascade, one of the central pathways activated by BCR-ABL.

Results: We designed an MSCV based retrovirus encoding both BCR-ABL and miR for BRAF and Raf1 on one shared RNA transcript. This approach ensured knockdowns of 80–90% for the respective Raf protein in every BCR-ABL transduced cell. Primary bone marrow (BM) cells coexpressing Raf1 miR and BCR-ABL had a 2-fold decrease, BRAF miR an unchanged colony forming ability. We transplanted BM, transduced with retrovirus coexpressing Raf1 or BRAF miR and BCR-ABL, to mice. The onset and progression of leukemia was significantly delayed in Raf1 miR mice with only a moderate rise of white blood cell counts and prolonged overall survival. Importantly BRAF had no significant effect in this transplantation model. We could further demonstrate that Raf1 but not BRAF is necessary for BCR-ABL dependent ERK activation.

In addition, transforming capacity of hematopoietic cells mediated by BCR-ABL V569 isomorf or by imatinib-resistant BCR-ABL T315I and BCR-ABL Y253H mutants was strongly reduced upon Raf1 downregulation.

Conclusion: Here we show that Raf1 but not BRAF is an important intermediate in the BCR-ABL signaling cascade in vivo, required for the efficient activation of the MAPK/ERK pathway and transformation of hematopoietic cells. Raf1 but not BRAF is also crucial for the development of a CML-like disease in mice. Therefore, a combination of Raf1 and BCR-ABL inhibition may be a promising approach therapy of CML. Moreover the simultaneous expression of a miR and an oncogene facilitates stable TOI knockdown in every oncogene-expressing cell and eliminates the need for cell sorting or antibiotic selection. Any TOI can be stably downregulated in combination with any desired oncogene. By strongly facilitating the characterization of oncogenic signaling networks, this system should help to establish a hierarchical map of functionally relevant genes in a defined tumor context and supply new targets for drug development.

Disclosure: No conflict of interest disclosed.

V569
Bone sarcoma in patients older than 40 years. Interim results of the European Bone Over 40 Sarcoma Study (EURO-B.O.S.S.) for patients with localized completely resected tumors

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Background: EURO-B.O.S.S. is the first prospective multicenter international study for patients 41–65 year old with the following bone tumors: High-grade osteosarcoma, high-grade sarcoma not otherwise specified (NOS), fibrosarcoma, malignant fibrous histiocytoma (MFH), leiomyosarcoma, dediffereniated chondrosarcoma and angiosarcoma. Here, interim results from the Cooperative Osteosarcoma Study Group (COSS), the Italian Sarcoma Group (ISG) and the Scandinavian Sarcoma Group (SSG) are reported.

Methods: Prospective protocol for patients with standardized, age-adapted recommendations for diagnostic work up, local therapy and chemotherapy [drugs used: cisplatin, doxorubicin, ifosfamide and postoperatively in very poor responders (≥50% tumor viability) methotrexate].

Results: As of February 2011, 300 patients were registered [COSS: 125 patients (42%)]. 283 were evaluable for analysis, 215 (76%) with localized and 68 (24%) with metastatic disease. Surgical complete remission was achieved in 197/215 patients with localized disease. Of these 197, 91 (46%) were female, 106 (54%) male; median age 51 years (range 40–66); tumor sites were female, 106 (54%) male; median age 51 years (range 40–66); tumor sites
Sorafenib paradoxically activates the Ras/Raf/Erk pathway in polyclonal expanded NK cells and thereby enhances effector functions in a dose dependent manner

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**Introduction:** A potential way to improve efficacy of natural killer (NK) cell immunotherapy is the use of drugs enhancing NK cell activity. Tyrosine kinase inhibitors show promise as potential suitable candidates as they modulate signal transduction in malignant and in NK cells at the same time. It has been shown (Krusch et al. 2009), that the TKI sorafenib has inhibitory effects on NK cells when applied short term. Contrasting, we demonstrated immune enhancing activity when used longterm (Dotterweich et al. 2010). As cytokine production, degranulation marker expression and cytotoxicity were enhanced after pre-treatment and amplified by target cell contact, the data were suggestive for transient rebound effects or induction of a lower threshold for NK cell activation by sorafenib. To further investigate this phenomenon we performed Western analysis for phosphorylation status of major sorafenib’s targets and compared its effects with those of a specific c-Raf inhibitor.

**Methods:** Ex vivo expanded polyclonal NK cells from healthy human blood donors were incubated with clinically relevant doses of sorafenib (0.1; 1; 3 µg/ml) or with the c-Raf inhibitor ZM336372 (0.05; 0.5; 1.5 µg/ml) for either 24 h or during their expansion period. K562 and Daudi cells were used as target cells. Functional outcomes assessed included cytokine production (IFNγ/ TNFα), degranulation marker expression (CD107a) and cytotoxic activity. The phosphorylation status of c-Raf and Erk1/2 was evaluated by Western analysis.

**Results:** Sorafenib and ZM336372 activated NK cells and enhanced their effector functions in a dose dependent manner when the NK cells were pre-treated before target cell contact. These effects were further enhanced when followed by co-incubation with target cells indicating lack of exhaustion. In line with these results we observed dose-dependent enhancement of c-Raf and Erk1/2 phosphorylation in sorafenib or ZM336372 pre-treated NK cells. In contrast, application of the drugs only during the functional assays led to NK cell inhibition as described before.

**Conclusions:** Paradoxically, sorafenib and ZM336372 both lead to increased Ras/Raf/Erk pathway activity in NK cells when used longterm, thus leading to enhanced effector functions. There were no signs for exhaustion as the enhancement was further amplified when pre-treated NK cells were exposed to target cells. Our data provides a functional rationale to develop the use of sorafenib as adjuvant in NK cell-based immunotherapies.

**Disclosure:** Julian Lohmeyer: No conflict of interest disclosed.
Ruth Seggewiss-Bernhardt: Financing of Scientific Research: Präsentationen für BMS

**Wissenschaftliches Symposium Stammzellbiologie**

V571

**Leukemic stem cells in acute myeloid leukemia**

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There is now compelling evidence that many tumors, once believed to be a homogeneous mass of abnormally proliferative cells, comprise a heterogeneous population of transformed cells resembling the hierarchically organized populations in the corresponding tissue. At the top of this tumor hierarchy are the cancer stem cells (CSCs) which are critical for tumor growth and maintenance as they constantly replenish the tumor bulk and are believed to be resistant to most conventional chemotherapies. The first evidence for both malignant hierarchy and CSCs came from studies on acute myeloid leukemia (AML) followed by mounting evidence in other types of cancer. More recent data have questioned that the CSC theory is valid for all tumors demonstrating that the frequency of CSCs depend on the tumor entity and the in vivo model used. However, just recently several groups have confirmed that acute myeloid leukemia obeys the CSC model and is maintained by subpopulation of cancer stem cells (or leukemic stem cells) which reside mostly in the CD34+ leukemic compartment, but partly also in the CD34+ compartment dependent on the underlying genetics of this disease. The concept that AML is driven by a subpopulation of leukemic stem cells (LSC) has clear biological but also clinical implications as only efficient targeting of the LSC pool would eradicate the disease and would prevent relapse from surviving LSCs. In contrast successful debulking by e.g. conventional chemotherapy would induce clinical remission, but would result in relapse triggered by LSC derived leukemic re-growth. Therefore cellular and molecular characterization of LSCs is one of the key goals in leukemia research nowadays, in particular identifying novel genetic drivers or tumor suppressors at the level of LSCs. The most recent aspects of the leukemic stem cell concept will be discussed in the talk.

**Disclosure:** No conflict of interest disclosed.

**Wissenschaftliches Symposium**

Debatte 2: Therapie des Rektumkarzinoms

V576

**Endoscopic ultrasound in rectal cancer**

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Accurate staging of rectal cancer is essential for selecting patients who may undergo local endoscopic or surgical procedures or sphincter-preserving surgery and for identifying those who might benefit from neoadjuvant therapy. Performing transrectal endoscopic ultrasound (EUS) is strongly recommended (power of evidence 2b, strong consensus) in the current S3-Guideline (2008) for staging of rectal cancer. Whereas usually EUS is done with rigid instruments, we prefer to use flexible echoendoscopes that are able to traverse a stenotic tumor and perform fine needle aspiration (FNA).

EUS is superior for T-staging of rectal cancer (in comparison to CT and MRI), in several studies diagnostic accuracy is reported in 80–95%, in daily practice 85% can be achieved provided an experienced endosonographer is available. Nodal involvement in rectal cancer (N stage) can not be sufficiently diagnosed by any imaging modality; the accuracy of EUS has been suggested in 70–75% (in a large study 65%), the main reason for not visualizing all metastatic lymph nodes is that 45% of them are smaller than 5 mm in size. In early T stages with suspected nodal disease FNA is useful. The extent of tumor involvement of the mesorectal fascia defining the circumferential resection margin, which has important prognostic implications, cannot be sufficiently estimated by EUS, it should be diagnosed using MRI.

EUS plays a very important role in early detection of recurrent rectal cancer which often develops extraluminally. Despite the difficulties in differentiating postoperative or post-radiation changes FNA can prove tumor recurrence. Even though there is no general agreement we think that patients with a locally advanced tumor or a local excision should undergo an aggressive surveillance including EUS.

**Disclosure:** No conflict of interest disclosed.

V577

**Which imaging in the staging of rectal cancer. Why to prefer the magnetic resonance imaging (MRI)?**

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**Methods, results:** Contrast enema, percutaneous ultrasound are not helpful, CT is a tool to rule out metastatic disease and complications, endoscopy can help in the decision between T1 and T2. Endoscopic ultrasound is well established to describe the tumor infiltration in the rectal wall, limitations are the acoustic penetration and the individual experience in this imaging. Problems for the MRI (specificity 52–82%) is the differentiation between T1 and T2. Endoscopic ultrasound is well established to describe the tumor infiltration in the rectal wall, limitations are the acoustic penetration and the individual experience in this imaging. Problems for the MRI (specificity 52–82%) is the differentiation between T1 and T2. In these cases the endosonography is superior (specificity 80–90%), but MRI will
top the ultrasound in the demonstration of T3 and T4. Also MRI is the best imaging of visualize the mesorectal Fascie. Introduced by Heald, surgeons know, that total mesorectal excision is the important step in surgery to avoid tumour recurrence and to improve and prolongate lifetime. Furthermore in the Mercury study, preoperative demonstration of the circumferential resection margin (CRM) was reached by MRI with a specificity of 92%. Diagnostic of lymph nodes is not bad (sensitivity and specificity 60–80%) but also not perfect in all features, especially tumor infiltration of small lymph nodes are not detected. Important in the technique are T2w images with high resolution, contrast medium (Gadolinium) might be helpful in some cases. Although magnetic resonance imaging with endorectal coil might be better in special questions, the imaging with body array coils is more comfortable, less expensive, better in high grade tumor stenosis, permits a great field of view (FOV).

Some authors have reported that the use of apparent diffusion improves the discrimination between malignant and benign lymph nodes. Sequential determination of fluorodesoxyglucose uptake at positron emission tomography, in combination with computed tomography or magnetic resonance imaging, is useful to differentiate responding from nonresponding tumors during and at the end of radio chemotherapy. However radionuclide techniques are expensive and their limitation in spatial resolution needs the combination with cross sectional imaging, further studies will verify the part of PET in combination with the MRI.

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Wissenschaftliches Symposium Bebedeutung der Versorgungsforschung

V580

What are the benefits of health services research?
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Health services research is a growing topic in the health system. Health services research describes, analyses and interprets the real world of health care in the population. It concentrates on the performance of outcomes of health care, eg. therapies, diagnostic measures and on the performance of structures of the health system, eg. design and implementation of health care services. Despite the growing impact of health services research for health policy the discussion of health services research within the classical clinical specific fields are widely missed.

The beneficiaries of health services research are all the stakeholders in health services: health politicians, health insurance funds, institutions in the health systems, pharmaceutical industry, clinicians and patients. All of them have there specific perspectives, time lines and criteria of interpretation. For the health insurance the benefit of every research will be evaluated whether or not it can be the basis of improvements for health of patients in relation to harms. The classical clinical trials analyze only the efficacy of new drugs or other health measures. The actual discussion asks whether the efficacy outcomes of clinical trials, eg. mortality, survival are sufficient for final decisions about allocation in the health system. Following these arguments we will need additional outcome research in real world situations, especially to evaluate the effectiveness. Health services research is extremely at risk to be misused in the statement of grounds for political or economic decisions in the health systems.

This paper will introduce into the perspective of an integrative multidisciplinary health services research. The benefits of health services research will be demonstrated by classical and new examples. But also some methodological problems and pitfalls will be discussed.


Disclosure: No conflict of interest disclosed.

Wissenschaftliches Symposium Aggressive Non-Hodgkin-Lymphome

V585

R-CHOP14 or R-CHOP21 as a standard for DLBCL? Approaches for improvement

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Since the invention of rituximab the prognosis of diffuse large B-cell lymphoma (DLBCL) has improved considerably. However, up to 50% of patients will eventually relapse, depending on the international prognostic score (IPS) risk group. Patients in highest risk group tend relapse early, within the first year after the treatment started. These patients have a very poor outcome with salvage therapies, even with stem-cell transplantation. First line treatment has to be improved. One strategy is to intensify the induction treatment. Dose-dense therapy keeps intervals between the drugs as short as possible to avoid regrowth of lymphoma cells during the treatment intervals. Dose-dense regimens as R-ACVBP, R-EPOCH, and R-CHOP/IMVP-Dexa will be discussed. R-CHOP14 is equally effective as R-CHOP21 without higher toxicity in elderly patients with DLBCL. In male patients rituximab results in lower serum levels and intensifying dosing early on may improve the results. Another strategy to deepen the remission is high-dose therapy with stem-cell support. However, high-dose therapy with stem-cell support takes time and will probably not solve the problem of primary resistance. Targeted substances combined with chemotherapy are promising. However, bevacizumab and bortezomib caused severe toxicities when combined with R-CHOP. Monoclonal antibodies as ofatumomab or obinutuzumab and radiolotope conjugated antibodies currently are studied in phase 3 trials. For the lower IPS risk groups late relapse may be a problem. Maintenance treatment strategies with rituximab or lenalidomide are on the way and results are eagerly awaited. For patients in the favorable prognostic IPS group results are excellent and omission of late toxicities are important. Cardiac
toxicity will be a problem for cured patients with DLBCL, several years after treatment. The role of liposomal encapsulated doxorubicin will be presented. There are several approaches for improvement of outcome in DLBCL. Deepening the response by high-dose therapy and stem-cell support. Attack resistant cells by new or targeted drugs. Dose-dense therapy may deepen the response and obviate resistance. Finally maintain a remission may be another way for improvement. However, there will be no improvement as long as patients are not included in clinical trials. After the lecture participants will have an overview of new and promising treatment strategies and pro’s and con’s of R-CHOP21 and R-CHOP14.

Disclosures: Michael Fridrik: Advisory Role: Cephalon, Roche; Financing of Scientific Research: Amgen, Cephalon, Roche, Novartis, Janssen, Ratiopharm; Expert Testimony: Roche, Janssen

Freie Vorträge

Acute myeloische Leukämie III (experimentell und klinisch)

V587

KIR Haplotype B donors but not KIR-ligand mismatch result in a reduced risk of relapse of AML patients after haploidentical hematopoietic stem cell transplantation using reduced intensity conditioning and a CD3/CD19 depleted graft

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Introduction: Natural killer (NK) cell alloreactivity after allogeneic hematopoietic cell transplantation (HCT) is, among others, influenced by the interaction of killer-cell immunglobulin-like receptors (KIRs) on donor NK cells with human leukocyte antigen (HLA) class I ligands on recipient cells. Recently, a positive influence of KIR haplotype B versus haplotype A donors on outcome HLA-matched allogeneic HCT was demonstrated (Cooley et al., Blood 2010). Previously, Ruggeri et al. (Science 2002) reported the positive influence of KIR-ligand mismatch (MM) on outcome of haploidentical HCT (HHCT). Patients and methods: We investigated the influence of the donor KIR haplotype and KIR-ligand MM on relapse of AML in patients receiving HHCT after reduced intensity conditioning and graft CD3/CD19 depletion. 34 adults with acute myeloid leukemia (median age 46 years) were evaluated. Patients were “high risk” because of relapse (n=12), prior HCT (n=9), refractory disease (n=7) or cytogenetics (n=6). At HCT, 16 patients were in complete remission and 18 in partial remission. 15 KIR genes were studied by real-time PCR as described (Vilches et al., Tissue Antigens 2007, Alves et al., Tissue Antigens 2009) and donors were assigned the A/A or B/B haplotype. Patients and donors were HLA-typed by high-resolution molecular methods.

Results: Of the 34 donors, 9 had KIR haplotype A and 25 KIR haplotype B. A KIR ligand MM was found in 23 of 34 patients. Patients grafted with a KIR haplotype A donor were more likely to relapse (HR=9.1 [CI=1.8–47.1], p=0.01), whereas patients who received a graft from a KIR haplotype B donor had a 9.1-fold lower risk of relapse. This resulted in a trend in the Kaplan-Meier estimated event free (EFS) and overall survival (OS) at 3 years of 35 % for KIR haplotype B and 11 % for KIR haplotype A (EFS: HR=1.8 [CI=0.7–4.8], p=0.21; OS:HR=2.2 [CI=0.8–6.0], p=0.12). However, evaluating the same patient cohort by the missing ligand model, KIR-ligand MM was associated with higher risk of relapse (HR=3.4 [CI=0.9–13.0], p=0.08) and OS at 3 years was 17 % with KIR-ligand MM and 53 % without KIR-ligand MM (OS: HR=2.2 [CI=0.9–4.9], p=0.07; EFS: HR=2.2 [CI=1.0–5.0], p=0.62). Therefore only the KIR haplotype B but not a KIR-ligand MM was associated with a significantly reduced risk of relapse.

Conclusion: We conclude from our results that a donor KIR haplotype B should be favored as donor for HHCT using RIC and CD3/CD19 depletion in patients with AML.

Disclosure: No conflict of interest disclosed.

V588

T cells are required for the CMV-induced anti-leukemic effect in patients with acute myeloid leukemia patients after transplant

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Introduction: We have previously showed that a CMV reactivation after T cell repleted allogeneic HSCT is associated with a reduced risk for leukemic relapse in patients (pts) with AML.

Methods: Here, we first evaluated in a cohort of 64 pts the effect of a CMV reactivation on the cumulative relapse incidence (CIR) after in-vivo T cell depleted transplant using Campath®, and secondly, the influence of a CMV reactivation on CIR in pts with KIR ligand incompatibilities in a 2nd cohort of pts (n=100) transplanted from HLA-mismatched (-MM) sibling (SIB) or unrelated donors (URD).

Results: In the first cohort (n=64) pts after myeloablative T cell depleted transplantation using Campath® (50mg or 100mg) did not benefit from a CMV-reactivation in regard of CIR. The 5-year CIR for patients with CMV replication (n=23) after transplant was 57% vs 51% (n.s.) in pts (n=41) in whom a CMV replication was not detected. Pts were transplanted in 1. CR (n=18), 2. CR (n=21) or beyond (n=25) from HLA-identical SIB (n=18) or URD (n=32) or MM donors (n=14). CMV status of recipient (R) or donors (D) were in 25% R−D+, 8% R+/D−, 25% D+/R− and 42% R+/D+. 5-year OS was statistically not different in both groups. In the 2nd cohort of 100 AML-pts transplanted from HLA-MM URD (n=96) or SIB (n=4), a documented CMV reactivation was associated with a reduced 5-y CIR of 17% (95% CL: 14–20) compared to 44% (95% CL: 34–54) (p=0.04). GVHD prophylaxis was performed with MTX: CSA with or without ATG (n=40(30–60mg)). Pts were transplanted in 1. CR (n=40), 2. CR (n=32) or beyond (n=28) from a donor with one HLA-MM (n=85) or 2 HLA-MM (n=15). Although CIR ligand expression is reported to be influenced by a CMV-reactivation, here the KIR ligand status had no influence on the CIR. Pts with (n=23) or without KIR ligand incompatibility (n=19) towards their donor benefit both from a CMV reactivation. In multivariate analysis CMV including all important factors which may have influence on the CIR, replicative status was confirmed as an independent predictor of relapse (HR: 0.12, 95% CL: 0.015–0.95, p<0.04) together with chronic GVHD (HR: 0.18, 95% CL: 0.74–0.79, p<0.018). The reduced risk for CIR translated into superior OS estimates at 3 yrs for pts with CMV replication (n.s. OS: 63 % vs. 48 %). In conclusion, this report confirms the strong and independent effect of early CMV replication on the leukemic relapse risk in pts with AML after HSCT.

Disclosure: No conflict of interest disclosed.
V589

Stem cell transplantation can provide durable disease control in Blastic plasmacytoid dendritic cell neoplasia (BPDC): A retrospective study from the European Group for Blood and Marrow Transplantation (EBMT)


European Group for Blood and Marrow Transplantation (EBMT)

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Blastic plasmacytoid dendritic cell neoplasm (BPDC) is a rare hematopoietic malignancy involving the skin, bone marrow and lymph nodes. The overall prognosis of BPDCN is dismal but anecdotal long-term remissions have been reported in patients who received early allogeneic stem cell transplantation (alloSCT). We therefore performed a retrospective analysis of patients identified in the EBMT registry in order to evaluate the impact of stem cell transplantation for BPDC. Diagnosis was confirmed by central review.

Results: Overall, 139 patients could be identified in the database (alloSCT 100, autoSCT 39). Of 74 patients for whom histology reports were obtained, central reviews confirmed the diagnosis of BPDC in 39 patients (34 alloSCT, 5 autoSCT). The 34 patients who had undergone alloSCT had a median age of 41 years (range: 10–70 years), were transplanted from a related (n=11) or unrelated donor (n=23) and had been treated with a reduced intensity conditioning regimen (RIC, n=9) or myeloablative conditioning (MAC, n=25). Nineteen of 34 patients died before transplantation. Of the patients who underwent alloSCT, 14/34 (41%) had a complete remission (CR) of BPDC, 7/34 (21%) had a partial remission (PR), 3/34 (9%) died the first 30 days after transplantation, 2/34 (6%) died because of disease progression before CR could be documented after transplantation, 11/34 (32%) died because of toxicities or infections, and 1/34 (3%) died because of other complications. Overall, 32% of patients transplanted in CR1 of whom 27% (8/30) died because of disease progression before CR could be documented after transplantation, and 7% died because of toxicities or infections. The median follow-up time of 28 months (range: 4–77+ months), 6/34 (18%) relapsed of whom 4 died due to disease progression and 1 patient lived in the absence of relapse. No relapse occurred later than 27 months after transplantation. Median disease free survival (DFS) was 15 months (range: 4–77+ months and median overall survival (OS) was 22 months (range: 8–77+ months). However, long-term remissions of up to 77 months after alloSCT could be observed. Patients allografted in CR1 tended to have a superior DFS (p=0.119 and OS (p=0.057). Patients who developed chronic graft versus host disease did not have a better disease control. In contrast MAC was associated with a better OS (p=0.005). Median age in the autoSCT group was 47 years (range: 14–62 years). Three of 5 patients were transplanted in CR1 of whom 1 patient relapsed after 8 months, 1 patient experienced treatment related mortality and 1 patient remained in CR for 28 months. The 2 remaining patients had more advanced disease at autoSCT and relapsed 4 and 8 months thereafter. Conclusion: AlloSCT is effective in BPDC and might provide curative potential in this otherwise incurable disease, especially when performed in CR1. However, it remains to be shown by prospective studies if the potential benefit of alloSCT in BPDC is largely due to conditioning intensity, or if there is a relevant contribution of graft-versus-leukemia activity.

Disclosure: No conflict of interest disclosed.

V589

Functional analysis of EF413001 as a differentially regulated candidate gene in Acute Promyelocytic Leukemia (APL) and hematopoietic stem cells

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Introduction: In a previous study using high density SNP arrays to search for new genomic alterations in acute promyelocytic leukemia (APL) we identified a recurrent micro-deletion on the chromosomal subband 1q31.3 in 14% of APL patients (Nowak et al. Genes Chromosomes and Cancer 2012). These deletions harbour the microRNAs mir181a/b and the transcript EF413001. In order to elucidate a potential pathogenetic role of these genes in APL we investigated their differential expression profiles in hematopoietic cells and in vitro differentiation assays.

Methods: SYBRGreen based quantitative gene expression analysis of EF413001 was carried out in leukemic blasts of APL patients (n=45) as well as CD34+ (n=29), granulocytes (n=11), mononuclear peripheral blood cells (MPBC; n=35) and unselected bone marrow cells (n=11) of voluntary healthy donors as well as in ATRA treated Nb4 cells. Precursor and mature mir181a/b quantitative gene expression was determined using the miScript PCR System (Qiagen). Differentiation of ATRA treated NB4 cells was assessed using the nitroblue tetrazolium reduction assay and Pappenheim staining.

Results: Compared to a baseline expression level in unselected healthy bone marrow cells the expression of EF413001 in leukemic blasts of APL patients (n=45) as well as CD34+ (n=29), granulocytes (n=11), mononuclear peripheral blood cells (MPBC; n=35) and unselected bone marrow cells (n=11) of voluntary healthy donors as well as in ATRA treated Nb4 cells. Precursor and mature mir181a/b quantitative gene expression was determined using the miScript PCR System (Qiagen). Differentiation of ATRA treated NB4 cells was assessed using the nitroblue tetrazolium reduction assay and Pappenheim staining.

Conclusion: EF413001, a previously unknown transcript coded on the chromosomal subband 1q31.3 is differentially expressed in hematopoietic progenitor cells in dependency of their differentiation status and treatment with the differentiation inducing agent ATRA. Our results suggest a stem cell specific role of EF413001 with a functional role in granulocyte differentiation. Further analysis such as in vitro overexpression of EF413001 in APL cells coupled with ATRA treatment and establishment of a gene expression profile during in vitro differentiation of healthy CD34+ cells are underway to demonstrate and characterize the role of EF413001 in haematopoiesis and the pathogenesis of APL.

Disclosure: No conflict of interest disclosed.

V591

Molecular risk stratification: Superior outcome among patients with acute promyelocytic leukemia and low BAALC expression

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Introduction: Acute promyelocytic leukemia (APL) is a distinct entity of acute myeloid leukemia characterized by the translocation t(15;17) (PML-RARα) fusion protein. ATRA based treatment regimens lead to long term survival in approximately 75% of cases. In APL no additional molecular markers have been established for risk stratification as yet. However, risk stratification of patients according to biologic or molecular criteria would most...
probably improve current treatment modalities. The gene BAALC has been shown to be of prognostic relevance in other acute myeloid leukemias as well as in T acute lymphoblastic leukemia.

**Methods:** Bone marrow mononuclear cells were retrospectively evaluated in 86 APL patients after informed consent. All patients were treated in the German AMLCG study. The treatment consisted of simultaneous ATRA and double induction (TAD/HAM), one cycle of consolidation chemotherapy (TAD) and 3 years monthly maintenance chemotherapy. Expression of BAALC expression was analyzed by multiplex reverse transcriptase quantitativa- real-time PCR (qRT-PCR) in triplicates on a LightCycler 480 (Roche, Mannheim, Germany). Glucose-6-phosphate-isomerase (GPI) served as a housekeeping gene for normalization. BAALC expression groups were defined as follows: BAALC expression lower than the 25th percentile (BAALC<sub>25</sub>) and higher than the 25th percentile (BAALC>25). Time to complete remission, relapse free survival (RFS) and overall survival (OS) were calculated using the Kaplan-Meier method and a log-rank test was used to compare differences between the groups (p<0.05).

**Results:** The OS of all patients was 66% at 10 years, whereas patients who achieved a CR had an OS of 75%. In univariate as well as in multivariate analysis low BAALC expression was significantly associated with a superior OS. Patients in the BAALC<sub>25</sub> group showed an OS at 10 years of 87% as compared to 66% in the BAALC>25 group (p=0.019). In addition, this difference was even more pronounced in treatment responders (92% vs 70%; p=0.035). Moreover, RFS in the BAALC<sub>25</sub> group was significantly higher at 10 years as compared to the BAALC>25 group (92% vs. 59%; p<0.0067). Time to complete remission was not correlated to BAALC expression levels.

**Conclusion:** Low BAALC expression in APL patients is significantly associ- ated with improved outcome with a longer OS and RFS. BAALC should be prospectively evaluated in patients with APL as a new molecular marker for risk stratification.

**Disclosure:** No conflict of interest disclosed.
V594

Immunotoxin HM1.24-ETA\(^\text{1}\) shows potent anti-plasacytoma activity

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Introduction:

While stem cell transplantation and the ‘new drugs’ have significantly improved longterm outcome of multiple myeloma, there is still a need for antibody-based targeted therapies. HM1.24 (CD37) represents a surface molecule that is overexpressed on malignant plasma cells. Since it is efficiently internalized, it may represent an opportunity for the strategy of myeloma-directed immunooconrects. Here, the characterization of a novel single-chain immunotoxin, HM1.24-ETA\(^\text{1}\), and its \textit{in vitro} and \textit{in vivo} activity is presented.

Methods:

HM1.24-ETA\(^\text{1}\) was generated by genetic fusion of an HM1.24-specific single-chain Fv antibody and a truncated variant of \textit{Pseudomonas aeruginosa} exotoxin A (ETA). The immunotoxin was expressed in \textit{E. coli} and purified to homogeneity by affinity chromatography. Specific induction of apoptosis was measured by annexin V-Propidium iodide staining and PARP cleavage. Anti-proliferative effects were analysed by MTT and 3H-thymidine assays. The \textit{in vivo} activity of HM1.24-ETA\(^\text{1}\) was anaalyzed in the INA-6 xenograft model.

Results:

HM1.24-ETA\(^\text{1}\) efficiently inhibited growth of several plasmacytoma cell lines (INA-6, RPMI8226, U266). Half maximal growth inhibition was observed at low nanomolar concentrations. Target cell killing occurred via induction of apoptosis and was antigen-specific, because an excess of unconjugated parental antibody completely blocked the cytotoxic effect. Importantly, HM1.24-ETA\(^\text{1}\) efficiently triggered apoptosis (up to 80% annexin V-positive cells) of freshly isolated tumor cells from 5/5 myeloma patients. HM1.24-ETA\(^\text{1}\) was also effective against INA-6 cells in co-culture experiments with bone marrow stromal cells. Establishment of plasma cell tumors in INA-6 xenografted mice was efficiently abrogated by treatment with HM1.24-ETA\(^\text{1}\) immunotoxin (p<0.04).

Conclusions:

Efficient killing of malignant plasma cells by the HM1.24-ETA\(^\text{1}\) immunotoxin \textit{in vitro} and \textit{in vivo} demonstrates, that CD37, the HM1.24 antigen, is a promising target structure for immunotherapy of multiple myeloma.

Disclosure:

No conflict of interest disclosed.

V595

PR671, the first proteasome inhibitor that selectively inhibits the bortezomib/carfilzomib insensitive \(\beta_2\) proteasome subunits, overcomes bortezomib/carfilzomib resistance in myeloma cells in vitro

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Introduction:

Resistance of myeloma against bortezomib or carfilzomib will remain a major clinical problem also in the future. Both drugs inhibit the \(\beta_5\) (chymotryptic), and to a lesser extent the \(\beta_1\) (caspase-like) catalytic sites of the proteasome, but not the \(\beta_2\) (tryptic) active sites. However, inhibition of \(\beta_2\) activity may be required to achieve maximum cytotoxic activity of proteasome inhibitor. In addition, bortezomib/carfilzomib-resistance is accompanied by upregulation of \(\beta_2\) activity, suggesting that \(\beta_2\) activity may counteract the cytotoxic activity of bortezomib/carfilzomib.

Methods:

We report the development and initial preclinical characterization of PR671, the first \(\beta_2\)-selective proteasome inhibitor available for preclinical testing.

Results:

PR 671 was derived from a library of rationally designed peptide-vinylsulfones. It selectively inhibits the \(\beta_2/\beta_2\) (tryptic) proteasome activity at low micromolar concentrations in viable cells, both in bortezomib-sensitive and bortezomib-adapted myeloma cell lines and primary myeloma cells. PR671 alone resulted in the induction of ER stress and the accumulation of poly-ubiquitinated protein, but not cytotoxicity, demonstrating that proper \(\beta_2\) proteasome activity is not required for myeloma cell survival, if normal \(\beta_1/\beta_5\) proteasome activity is present. However, the combination of PR671 with agents that target the \(\beta_5\) proteasome activity (bortezomib, carfilzomib, or the \(\beta_5\)-selective proteasome inhibitor PR523 developed by us) resulted in markedly increased cytotoxic activity, compared to \(\beta_5\) proteasome inhibition alone, both in cell lines and primary myeloma cells. To assess whether additional inhibition of the \(\beta_2\) proteasome activity by PR671 would also overcome bortezomib/carfilzomib-resistance, we used bortezomib-adapted myeloma cell lines that were bortezomib- and carfilzomib-resistant (AMO-1a), and bortezomib-resistant primary myeloma cells isolated from patients with clinical bortezomib resistance. We observed that PR671 re-sensitized AMO-1a cells to bortezomib- or carfilzomib treatment. Likewise, PR671 in combination with bortezomib overcame bortezomib-resistance in primary myeloma cell samples.

Conclusion:

We here provide the first characterization of the preclinical activity of a \(\beta_2\)-selective proteasome inhibitor in myeloma cells, and demonstrate that additional \(\beta_2\) inhibition by PR671 in combination with bortezomib or carfilzomib can overcome bortezomib- or carfilzomib resistance of myeloma cells in vitro.

Disclosure:

No conflict of interest disclosed.

V596

The role of the E3 Ubiquitin Ligase SCF\(^{\text{Fbxo3}}\) in multiple myeloma

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Introduction:

The multiple myeloma is principally characterized by genomic instability and high clinical response to proteasome inhibitors like bortezomib. Upon DNA damage the 26S proteasome accounts for the degradation of previously ubiquitlated proteins. F-box proteins (FBP) mediate the substrate specificity of SCF (Skp1/Cul1/F-box) E3 ubiquitin ligases. However, specific aberrations in the ubiquitin proteasome system (UPS), which potentially display a play a powerful therapeutic target in multiple myeloma, have not been widely described.

Methods:

We carried out a proteome wide mass spectrometric screen for interactors of the FBP Fbxo3. Binding was verified by immune precipitation and western blotting. Downstream effects of Fbxo3 were evaluated by overexpression or siRNA-mediated knockdown. Direct and indirect immunofluorescence assessed localisation of Fbxo3 and its interactors. DNA damage was induced by doxorubicin or etoposide, proteasome inhibition by MG132. Analysis of cycling or apoptotic cells was supported by FACS (BrDU, Annexin-P). Experiments were performed in HEK 293T, HeLa, CoSt7 and U2OS cells.

Results:

Fbxo3 is an orphan F-box protein that is overexpressed in multiple myeloma according to CGH array data. Parp1 and its small apoptotic fragment (Parp_p24) were identified as direct interactors of Fbxo3. Ubiquitylation of Parp_p24 demonstrated that these two proteins co-bind to the DNA and colocalize with Parp1 and Parp_p24. Fbxo3 overexpression was reversed with proteasomal inhibition, however less so with Fbxo3 knockdown. Upon DNA damage Fbxo3 was observed to translocate to the nucleus, bind to the DNA and colocalize with Parp1 and Parp_p24. Fbxo3 overexpressing cells proved to be less sensitive to DNA damage: Cos7 cells transiently transfected with Fbxo3 showed accelerated clearance of yH2AX foci. According to cleaved caspase levels and Annexin-P FACS, apoptosis was significantly reduced in Fbxo3 overexpressing cells.

Conclusions:

We here identify a new pathogenetic and one of the first specifically deregulated UPS mechanisms in multiple myeloma. We show that the E3 ubiquitin ligase SCF\(^{\text{Fbxo3}}\) ubiquitylates and degrades the small apoptotic fragment of Parp1 that is known to block DNA access and DNA damage repair through Parp1. This seems to allow an increased Parp1 function and determine a survival advantage and oncogenic factor in Multiple Myeloma cells.

Disclosure:

No conflict of interest disclosed.
The feed-forward loop between YB-1 and MYC is essential for Multiple Myeloma cell survival

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Introduction: The potentially oncogenic Y-box Binding protein-1 (YB-1) is a transcription factor belonging to the evolutionarily highly conserved family of cold-shock proteins. YB-1 binds to DNA as well as RNA and fulfills pleiotropic cellular functions, including the regulation of proteins involved in cellular growth, survival and stress response. YB-1 is strongly expressed in the cytoplasm of primary Multiple Myeloma (MM) samples and MM cell lines compared to normal plasma cells. A functional role has been suggested for YB-1 assisted MYC mRNA translation in MM involving a mutation in the MYC IRES.

Methods: We performed bidirectional sequencing of the MYC IRES of CD138-positive cells from 88 primary patient samples. Furthermore, we used primary mouse plasma cell tumors (PCTs) and different human MM cell lines (HMCLs) to perform YB-1 immunoprecipitation and gene expression arrays. Immunohistochemical analysis of YB-1 and MYC were done in primary samples and the function of YB-1 and MYC in MM was analysed by protein knock-down in HMCLs, density gradient centrifugation, real-time quantitative PCR, Western Blot and FACS analysis of mitochondrial membrane potential and caspase activation.

Results: We show that YB-1 mediated translation of MYC mRNA occurs independently of the reported IRES mutation, as neither HMCLs nor MM patient material were positive for the mutation. YB-1 immunoprecipitated revealed a novel set of potentially YB-1 regulated mRNAs in the cytoplasm of mouse PCTs and MM cell lines and confirmed MYC as an YB-1 target. We show for the first time that YB-1 co-expresses with MYC in malignant plasma cells and describe a novel oncogenic circuit involving the two proteins in regulating HMCL survival. YB-1 knock-down in HMCLs reduced both MYC protein levels and MYC mRNA in the polosomal fraction, providing a mechanism by which YB-1 controls MYC translation. Loss of either YB-1 or MYC induces apoptosis in HMCLs. MYC transcription of YB-1 is demonstrated in HMCLs as MYC knock-down resulted in reduced YB-1 protein and mRNA levels. Furthermore, we show that activation of MYC in non-malignant mouse embryonic fibroblasts (MEFs) increased YB-1 mRNA, clearly indicating that MYC drives YB-1 transcription.

Conclusions: We demonstrate for the first time that these two proteins co-regulate each other via combined transcriptional/translation activity establishing their pivotal role in MM cell survival.

Disclosure: No conflict of interest disclosed.

DEPTOR expression associates with mTOR specific microRNAs and characterizes genetic subgroups in Multiple Myeloma

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Introduction: Multiple myeloma (MM) is characterized by frequent and complex genomic abnormalities. In the majority of cases the PI3K/Akt/mTOR pathway is activated promoting growth, progression and resistance to therapy. Recently, an mTOR interacting protein named DEPTOR was found to be highly expressed exclusively in MM (Peterson et al. Cell 2009). Importantly, knockdown of DEPTOR results in prevention of MM cell growth and apopto-

sis making it an attractive potential therapeutical target. Therefore, we aimed to determine if DEPTOR expression associates with clinically relevant subgroups. In addition, to investigate additional regulatory layers we determined key microRNAs (miRNAs) downstream of DEPTOR expression.

Methods: DEPTOR expression was measured by quantitative real-time RT-PCR (TagMan) using CD138 purified plasma cells from 175 patients with MM. DEPTOR copy numbers were measured and normalized to GUSB (internal control) copy numbers. In 38 patients miRNA expression levels were analyzed using Agilent miRNA-Chips to measure miRNAs associated with DEPTOR expression. Additionally, all patients were characterized by a comprehensive set of FISH probes for the presence of recurring cytogenetic abnormalities.

Results: DEPTOR expression was highly variable in the investigated MM samples (median: 0.33; range: 0.003–12.50). DEPTOR expression was significantly higher in patients presenting with translocations involving the immunoglobulin heavy-chain locus compared to patients with a hyperdiploid karyotype (p = 0.0016). In particular, high DEPTOR expression was associated with the presence of a t(14;16) (p < 0.0001) and a deletion 13q14 (p = 0.04), whereas low DEPTOR expression was associated with the presence of a gain at chromosomal band 9q34 (p = 0.0006). Recently, activation of the mTOR pathway has been shown to be under the control of miRNAs regulating tumor growth and differentiation. We detected twelve differentially expressed miRNAs (p < 0.05), 8 miRNAs positively and 4 miRNAs negatively correlated with high DEPTOR expression. Of particular interest, miRNAs targeting the mTOR pathway (miR-99a, miR-193b, miR-365) or regulating IL6-Expression (miR-365) were found to be upregulated in high DEPTOR expressing patients, indicating a regulatory loop by mTOR activation and DEPTOR expression.

Summary: High DEPTOR expression is associated with prognostic relevant genetic aberrations and specific miRNAs in MM.

Disclosure: No conflict of interest disclosed.

Freie Vorträge Psychoonkologie

V599

Family planning of hematological patients in physician-patient dialogue

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Introduction: Cancer disease and cancer treatments lead to a variety of negative side effects. Infertility can be a long-term consequence of many cancer diagnoses and treatments for all patients. This is essential for young adults who did not finish family planning. As with other possible complications of cancer treatment, oncologists have to inform the patient about the potential risk of infertility. This study examined whether the physician-patient consultation addressed the wish for a child, the risk of infertility and the offer of a fertility protection.

Methods: We interviewed patients with hematological malignancies and a prospective wish for a child with semi-structured face-to-face interviews after the completion of acute treatment. Additional information on treatment and socio-demographic data was obtained by a questionnaire. The interviews were evaluated in a content-analytic way.

Results: We included 15 women and 14 men between 21 and 43 years from eleven hospitals. Nine of the patients had already children aged between two and eight years. 17 patients talked to the attending physician about the wish for a child and possible fertility preservation, or alternatively were referred to a specialist in reproductive medicine. 12 patients can’t remember receiving any information regarding this and talked to the physicians themselves or got the information from leaflets and the internet. Fertility preservation techniques were carried out in seven women and nine men. All these nine men decided to freeze sperm before starting the treatment, six women were given hormone injections, and one woman had their eggs freeze.

Conclusions: Based on our conducted interviews we can show that nearly half of the cancer patients didn’t register the information about impairment fertility in an appropriate way during the physician-patient-dialogue and took the conversation initiative about the issue of the wish for a child and potential long
term effects themselves. In this regard one has to consider how the conversation could be improved and when the right moment is indicated. Only about half of patients received fertility preservation techniques. It thus often happens that patients did not have information about impairment fertility and a protection was too late. Therefore, the impairment fertility should be essential in the consultation as a side effect of cancer treatment and, if necessary and having enough time, initiating an advice by a specialist of reproductive medicine.

Disclosure: Diana Richter: Employment or Leadership Position: Wissenschaftliche Mitarbeiterin der Universität Leipzig; Expert Testimony: Finanzierung der Studie durch die Deutsche José Carreras Leukämie-Stiftung e. V.

Yve Stoebel-Richter: Employment or Leadership Position: Stellvertretende Abteilungsleiterin der Abteilung für Medizinische Psychologie und Medizinische Soziologie der Universität Leipzig; Expert Testimony: Finanzierung der Studie durch die Deutsche José Carreras Leukämie-Stiftung e. V.

V600
A newly structured concept of psychosocial care: Expectations, information needs and effects of a 12-hour lecture and information series “living with cancer”

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Introduction: Standards in psychosocial care (pc) for most cancer patients are still lacking and there are general deficits regarding pc access and supply. To improve pc by providing easy-access information, we created a string of lectures as a new type of educational support for patients, relatives and professionals, consisting of 6 x 2 interactive lessons targeting psychosocial issues, nutrition, sexuality, sports and coping strategies for psychological comorbidities and communication problems.

Methods: Using standardized 24 item questionnaires, we evaluated the participants’ expectations, their current need for information and support due to cancer-influenced changes in daily-life topics. Thereby, we were able to investigate whether the presented strategies were helpful in coping with cancer-related effects. In addition actual distress was evaluated using the standardised NCCN “distress thermometer”.

Results: Altogether 523/571 (92%) attendees returned the questionnaires. 50% of them were patients, 30% relatives, 17% professionals and 3% had general interest. 51% of the participants attended 4 or more lectures. The patients reported a median distress-level of 5; 68% of them had been diagnosed with cancer more than 12 months ago. 70% of the attendees stated that they need more detailed information brought them to the event. 80% were hoping to receive practical tips, 62% wanted to obtain ideas about how to cope with their current situation and 59% expected information about cancer and therapy-related effects. Overall 69% of the patients and relatives indicated that more support is needed. Nevertheless even within the group expressing need for more support, 42% have never sought professional help. Attending the program, 86% of the participants received new information about the respective topic discussed. 81% of the patients and relatives felt that the information given was helpful or partially helpful in coping. The expectations of 91% of attendees were fulfilled and 86% would definitely recommend the series.

Conclusions: Cancer patients and relatives show an unmet need for coherent and applicable information and practical tips regarding daily-life issues. Even patients with long histories of their disease still feel inadequately informed. Using an easy-access support and education program offered through low threshold lecture, attendees were able to acquire new knowledge and strategies, which help them to cope with cancer-related effects in daily life.

Disclosure: No conflict of interest disclosed.

V601
How to screen for need of psychosocial support – experience with the Hornheide Screening Instrument (HSI) in a community-based oncology center

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The majority of patients with malignant disease need psychosocial support in addition to medical treatment. Detection of patients who may require psychosocial support is difficult in routine clinical practice and preselection of patients according to urgency of individual needs may be useful to optimize allocation of dedicated support. The HSI is a seven items questionnaire established to cover both of these aspects. Here we report on our experience with this instrument in patients in a community-based oncology center. From 2010 to present all patients with malignant disease entering the oncology center were asked to complete the HSI. We retrospectively analyzed 90% of these patients including 70 patients with the poorest screening values indicating urgent need for psychosocial support and 20 patients with a low (normal) screening value declining this need. All patients had a structured phone interview to monitor their personal view and to detect accordance or discrepancies of the test result with their former and current situation. 30 of the 70 patients with poor HSI value had repeated testing during the course of one year. Patients with a poor HSI screening enjoyed enhanced awareness for their psychosocial needs by the medical staff. Most of these patients had some kind of dedicated support but only 54% had been visited by a psychooncologist during their stay in hospital. 49% of the 70 patients with poor and 55% of those with a very poor HSI screening value reported they currently had psychosocial support when the telephone interview was done. In 30 of the 70 patients with a poor HSI repeated admissions to the clinic allowed a second (n=30), third (n=22) or even fourth (n=13) testing. During the course of these repetitions HSI values improved unless disease deterioration occurred. The 20 patients with normal HSI value did not obtain special attention for possible psychono- logical needs. Interestingly 45% of these patients reported they would have liked or currently would like to have some psychosocial care.

From our results we conclude that screening for psychosocial needs by HSI can help to focus support to the patients who may need it most. In the absence of disease deterioration coping strategies may reduce the need for psychosocial help. Whether or not the wishes of a relatively high proportion of patients with a normal HSI to have psychosocial support reflects a real need is currently under investigation.

Disclosure: No conflict of interest disclosed.

V602
Health related quality of life in cancer patients – data from a large middle-aged German patient cohort

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Background: With rising numbers of cancer patients and better survival rates, cancer will become more and more a chronic disease. In this context, health related quality of life (QOL) will play an increasingly important role in these patients. As data concerning health related QOL in greater German cohorts are sparse, we conducted this analysis.

Methods: All cancer patients of our clinic undergoing an inpatient rehabilitation program between October 2007 and April 2011 completed a questionnaire concerning health related QOL (EORTC QLQ-C30, version 3.0). This questionnaire comprises 30 questions, subdivided into the scales physical, emotional, cognitive, social and role function as well as global QOL and fatigue. Each scale scores between 0 and 100 points with higher results indicating better outcomes (and worse outcomes concerning fatigue, respectively). Statistics were done in descriptive manner using means with standard deviations and 95%-confidence intervals, differences were calculated with the Mann-Whitney-Test with an α = 5%.

Abstracts
Results: 1879 cancer patients with a mean age of 57.0±11.4 years completed the questionnaire. 71% of them were female (n=1336) and 29% were male (n=543). The most frequent diagnosis was breast cancer (45%, n=845), followed by systemic hematologic malignancies (13%, n=242) and colorectal cancer (12%, n=219). Mean scores in all patients for physical, emotional, cognitive, social and role function were 66.7±20.0 [95%-confidence interval 21.3; 21.6], 50.4±28.6 [27.7; 29.5], 65.5±29.3 [29.4; 30.4], 58.3±32.0 [31.0; 33.1], and 50.4±30.9 [29.9; 31.7], respectively, mean scores for global QOL and fatigue 49.2±21.2 [21.3; 22.8] and 56.5±27.6 [26.7; 28.5], respectively. All scores were distinctly worse than scores of the German general population (90.1±16.7; 88.0±22.9; 78.7±21.0; 91.2±17.0; 91.0±19.4; 17.1±22.1, and 70.8±22.1, respectively). Age, cancer stage, kind of therapy, and partnership did not influence QOL significantly, while sex, cancer site, time window between first diagnosis and rehabilitation, body-mass-index, and origin did.

Conclusion: Health related QOL in German middle-aged cancer patients is worse than QOL in the general population. While the major burden seems to be due to the diagnosis itself, also time appears to play a role. Patients with a diagnosis of cancer therefore should receive psycho-oncological support in time accounting for the relevant clinical and sociodemographic factors.

Disclosure: No conflict of interest disclosed.

Freie Vorträge

Infektionen

V603

Epigenetic modulation of myeloid-specific CCAAT/ enhancer binding protein epsilon improves the innate immune system to clear Staphylococcus aureus infection

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Introduction: Staphylococcus aureus in community and healthcare settings commonly causes serious and potentially life-threatening infections. Widespread use of antibiotics is responsible for the emergence and rapid spread of resistant pathogens, and highlights a pressing need for development of novel antimicrobial therapies. The myeloid-specific transcription factor, CCAAT/enhancer binding protein epsilon (C/EBPe) is a critical mediator of the terminal differentiation as well as functional maturation of neutrophils.

Method: We applied in vitro as well as in vivo assays to investigate C/EBPe and its modulating effect on the clearance of S. aureus.

Results: We show that C/EBPe-knockout mice are severely affected by infection with S. aureus, and C/EBPe deficiency in neutrophils contributes significantly to the infectious phenotype. Because C/EBPe appeared essential for defense against S. aureus, we hypothesized that a pharmacologic agent that enhanced expression of C/EBPe above physiologic level might lead to therapeutic bacterial killing. Indeed, overexpression of C/EBPe could be achieved in wild-type myeloid cells by exposure to the histone-deacetylase-inhibitor, nicotinamide (vitamin B3). We show that nicotinamide increased the activity of C/EBPe, as well as select downstream antimicrobial targets like lactoferrin and cathelicidin, particularly in neutrophils. In a systemic murine infection model and in murine and human peripheral blood, nicotinamide enhanced killing of S. aureus by up to 1000-fold, but had no effect when administered to either C/EBPe-deficient mice or mice depleted of neutrophils. Notably, nicotinamide was efficacious in both prophylactic and therapeutic settings.

Conclusion: Our findings suggest that C/EBPe is an important target to boost killing of bacteria by the innate immune system. The results constitute a proof of principle that compounds exerting modulatory effects on this myeloid-specific transcription factor may be suitable candidates for antimicrobial therapeutics.

Disclosure: No conflict of interest disclosed.

V604

Nosocomial outbreak of respiratory syncytial virus infections in a hematology and stem cell transplantation unit

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Respiratory syncytial virus (RSV) is an enveloped single stranded RNA virus of the family of Paramyxoviridae. It is the single most important viral pathogen for respiratory tract infections in children under the age of two. Furthermore, RSV can also cause lower respiratory tract infections (LRTI) associated with high mortality in immunocompromised adults. From December 30th 2011 to February 27th 2012 an outbreak of nosocomial RSV infections occurred in a hematology and stem cell transplantation unit and affected a total of 40 patients. Sequence analysis revealed in nearly all cases RSV strain A, genotype GA2. 11 patients (27.5%) were asymptomatic or developed only upper respiratory tract infection. Their outcome was not different to comparable non-infected patients. 29 patients (72.5%) showed signs of LRTI on CT scan, 11 of them (37.9%) suffered a severe course of infection (i.e. intensive care unit and/or death). No difference regarding age, sex, underlying disease or disease control was noted between the two groups. However, patients with a severe course of infection had significantly lower levels of immunoglobulin G than those with a non-severe course (p=0.03). In most cases the infection resolved without sequelae. Six patients deceased (AML (2), CLL, CNS B-NHL, multiple myeloma, T-NHL), yet the exact impact of RSV on the fatal outcome is difficult to assess due to the presence of co-infections with other bacterial (e.g. Pseudomonas aeruginosa), fungal (Aspergillus fumigatus) or viral (CMV, EBV, HSV) pathogens. Systematic screening by PCR for RSV of patients and staff showed a nosocomial spread of infections by direct patient-to-patient contact. Implementation of rigorous barrier measures led to containment of the outbreak.

We showed that a highly infectious virus such as RSV can cause a rapidly spreading outbreak of nosocomial infections in a hematologic department. PCR could be established as a sensitive screening method, analysis of patient movements and bed occupancies revealed a chain of infection by patient-to-patient contact. The significance of systematic screening lies primarily in isolation of infected patients since only by rigorous barrier measures can an outbreak of this extent be contained.

Disclosure: No conflict of interest disclosed.

V605

Infectious disease of salvage therapy with everolimus in moderate and severe chronic GvHD after allogeneic stem cell transplantation

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Introduction: Infections are frequent complications of chronic graft-versus-host disease (cGvHD), determine up to 15% mortality. Recently studies show promising response in steroid refractory cGvHD with the use of the mTOR (mammalian target of rapamycin) inhibitors. Therefore we sought to investigate the incidence of infectious complications after immunosuppressive rescue therapy based on m-TOR inhibitor with everolimus.

Methods: Between 7/2006 and 6/2010, a total of 38 patients (pts) (median age 47.9 years, 23% female) received everolimus combined with either prednisolone (n=17, 44.7%) or with prednisolone and mycofenolate mofetil (n=21, 55.3%). Pts had classic cGvHD (n=23, 60.5%) or an “overlap syndrome” (n=15, 39.5%) (moderate or severe) according to the current NIH criteria. Everolimus was second-line therapy in 15 pts (39.5%), third-line in 14 pts (36.8%), and fourth-line in 8 pts (21.1%). Median duration of treatment was 11.5 months (range, 0.53–47.6).

Disclosure: No conflict of interest disclosed.
**V607 Diagnosing cerebral aspergillosis in immunocompromised patients: a retrospective evaluation of an Aspergillus PCR assay in cerebrospinal fluid samples of 63 patients**


**Background:** Cerebral invasive aspergillosis (cIA) is a fatal complication of invasive aspergillosis (IA) in immunocompromised patients. Although often suspected due to clinical signs and symptoms in combination with corresponding radiologic abnormalities, obtaining a valid diagnosis is rarely accomplished as invasive biopsy or surgical procedures are impeded by underlying neutropenia and low platelet counts. Results from cerebrospinal fluid (CSF) cultures or galactomannan usually yield negative results, underlining the need for improving diagnostics. Therefore the performance of an established Aspergillus specific nested PCR assay in CSF samples of immunocompromised patients for detection of cIA was retrospectively evaluated.

**Methods:** We investigated 133 CSF samples of 63 immunocompromised patients for whom cIA was suspected based on underlying immunosuppression and radiologic abnormalities. Twentythree CSF samples were not evaluable due to lack of DNA. Therefore 110 samples from 53 patients were included in the analysis. Eight patients were identified as having proven (n=8) cerebral aspergillosis. According to the EORTC/MSG 2008 criteria, no patient was classified as probable, the remaining 45 patients were classified as having either possible (n=19) or no cIA (n=26).

**Results:** Positive PCR signals in CSF samples were observed for 8/8 proven and 6/45 possible/no IA patients. Sensitivity respectively specificity rates of 1.0 (95% CI 0.68–1.0) and 0.87 (95% CI 0.74–0.94) were observed. The positive likelihood ratio was found to be 7.5, and the negative likelihood ratio was 0, thus leading to a diagnostic odds ratio of >200.

**Conclusions:** In our retrospective analysis of the largest number of CSF samples from patients at risk of suffering from cerebral aspergillosis up to now, a high sensitivity rate of a nested PCR assay was observed. Based on our data, PCR testing of CSF samples is suggested for patients in whom cIA is suspected, especially for those whose clinical condition forbids invasive procedures as a positive PCR result makes the presence of cIA highly likely.

**Disclosure:** No conflict of interest disclosed.
wards. Cases were reviewed and classified as proven, probable or possible (EORTC/MSG definitions). Survival was defined from the date of diagnosis and at 90 days or death.

Results: All but 10 patients had a hematological disease (mostly acute myeloid [n = 64] or lymphoid [n = 28] leukemia) and 64 (40%) were hematopoietic cell transplant (HCT) recipients. Neutropenia and receipt of corticosteroids were present in 86% and 52%, respectively. The clinical forms were disseminated disease in 74%, fungemia only in 11%, cutaneous disease in 9%, sinusits in 3%, pneumonia in 2%, and arthritis in 1%. Primary treatment with deoxycholate amphotericin B (d-AMB) was more frequent before 2000 (84% vs. 27%; p = 0.001), whereas voriconazole (40% after 2000 and none before) and combination therapy (21% after 2000 and none before) emerged in the second period. Primary therapy with a lipid formulation of AMB was used in 16% before and 9% after 2000. The median overall survival was significantly longer (195 vs. 29 days, p = 0.001) after 2000, with 90-day survival of 53% after 2000 vs. 20% before (p = 0.001). Among treated patients (n = 150), multivariate predictors of poor outcome (90-day survival) by Cox regression were persistent neutropenia (hazard ratio [HR] 3.73, 95% confidence interval [95% CI] 2.29–6.06), receipt of corticosteroids (HR 1.81, 95% CI 1.17–2.79) and primary treatment with d-AMB (HR 1.83, 95% CI 1.07–2.79), whereas primary treatment with voriconazole was protective (HR 0.36, 95% CI 0.15–0.84). Neither l-AMB nor combination therapy as primary treatment predicted the outcome.

Conclusions: The outcome of fusariosis improved in the last decade, and the improvement seems to be related to a change in primary therapy, from d-AMB to voriconazole.

Disclosure: No conflict of interest disclosed.

Freie Vorträge
Urogenital Tumoren (inkl. Prostata)

V609
Combined anti-osteoplastic, anti-inflammatory, immunomodulatory and angiostatic treatment in patients (pts) with castration-refractory prostate cancer (CRPC): Biologic features during therapy interruption and tumor progression

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Background: Therapeutic options for pts with CRPC are still limited. Combined targeting of normative functions in CRPC, i.e. the 'hallmarks' of cancer, could be a novel approach to attenuate tumor growth or to achieve tumor regression. Therefore, a phase II study was implemented in pts with CRPC to assess tumor response to combined immunomodulatory agents. Primary objective was the effect of this treatment approach on PSA-response rate in pts with CRPC.

Methods: 69 pts with histologically confirmed CRPC (criteria according to EAU guidelines) were enrolled in 11 German centers. In the core phase (lasting 6 months) and thereafter, pts were treated continuously with daily doses of imatinib mesylate, pioglitazone, etoricoxib, treosulfan and dexamethasone until PSA progression. During the study, PSA-values, ECOG performance status and QoL were continuously assessed. Pts responsive to study medication were allowed to enter the extension phase until disease progression or intolerable toxicity occurs.

Results: The core phase of this study was finished in July 2009. Two patients are currently >3.5 years on continuous treatment. At baseline the median PSA value was 45.3 ng/ml (5–3603 ng/ml) and the majority of pts had PSA doubling times of 50 to 100 days. 23 patients (37.7%) were considered as PSA responders with a confirmed PSA decline of at least 50%. During the treatment period PSA decreased from 278.9 (± 784.1) to 8.8 (±11.6) ng/ml.

Median time to PSA response and to progression as well as overall survival were not yet achieved. In some patients the therapy led to complete resolution of bone lesions in bone scan. Of the 38 non-responders, 14 pts showed a stable disease ≥ 6 months. Single responding patients with non-tumor-related surgery and discontinuation of the combined therapy had a PSA doubling time of ≥ 12 months, other patients with tumor progression responded again to hormone ablation.

Conclusions: This multi-targeted, biomodulatory approach led to an impressive response rate of 37.7%, and in single cases to long-time response at 'minimal residual disease'. In responding patients intermittent therapy seems to be feasible, and preceding response to study medication may reconstitute hormone sensitivity.

Disclosure: No conflict of interest disclosed.

V610
Prostate cancer-infiltrating B cells are clonally related

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Introduction: Chronic inflammation is implicated in the development of prostate cancer and localized stages are characterized by variable numbers of clustered stromal B cells. The B cell immunoglobulin (Ig) repertoire reflects the antigenic exposure in the neoplastic prostate gland and may therefore help to identify potential tumor-specific therapeutic targets.

Methods: We studied IgM (IgM V_H), IgG (IgG V_H) heavy chain variable region transcripts of tumor-infiltrating B cells in 4 early stage prostate cancer samples. CD20+ B cells surrounding neoplastic prostate glands were identified in all prostate cancer tissue sections by immunohistochemistry. IgM V_H and IgG V_H transcripts were amplified by RT-PCR from each tumor. PCR products were then cloned and sequenced. To exclude PCR amplification bias, 2 cDNA libraries were prepared from separate tissue sections of each tumor.

Results: 10 IgM V_H and 10 IgG V_H PCR products were sequenced from each cDNA library. Analysis of the heavy chain third complementarity determining regions (HCDR3) revealed clonally related B cells in 3 of 4 and evidence of antigen exposure in 4 of 4 prostate cancer samples. IgM and IgG B cell clones, defined by the same HCDR3 and detection in two separate cDNA libraries, were widely distributed in the prostate. In addition, B cell clones showed somatic hypermutation, Ig class switch and insertions or deletions indicating antigen experience of the immunoglobulin-expressing B cells.

Conclusions: Prostate cancer infiltrating B cells express a restricted Ig heavy chain variable region repertoire suggesting exposure and/or response to a limited number of antigens. Future studies will define the identity of the antigens stimulating the clonal expansion of these cells in prostate cancer and might reveal novel tumor antigens amenable to therapeutic targeting.

Disclosure: No conflict of interest disclosed.