Freie Vorträge
Sarkome

V797

Molecular analysis with high density SNP arrays and quantitative real-time PCR in Desmoid tumors identifies new molecular candidate lesions

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Introduction: Aggressive fibromatoses (AF; desmoid tumors) are clonal, neoplastic proliferations of connective tissues. Tyrosine kinase inhibitors (TKIs) such as imatinib can be effective in controlling disease in subgroups of these patients (Mace, Cancer, 2002). However little is known about the molecular mechanisms of tumorigenesis, risk of relapse and sensitivity against TKIs. Therefore we carried out a molecular analysis with quantitative reverse transcriptase PCR (QRT- PCR) and high density SNP array analysis, in order to elucidate new molecular mechanisms underlying these tumors.
Methods: 11 patients with AF (8 females, 3 males; median age 34) were included in this study. Patient's treatment included surgery (n = 11), radiotherapy (n = 6), antihormonal/anti-inflammatory treatment (n = 2), chemotherapy (n = 3) and imatinib therapy (n = 2). Total RNA and genomic DNA were extracted from the resected specimen for molecular analysis. A QRT-PCR assay was used for expression analysis of TKI targets such as platelet-derived growth factor receptor alpha (PDGFRA), platelet-derived growth factor receptor beta (PDGFRB) and stem cell growth factor receptor (c-KIT). High resolution copy number analysis was carried out with 8 samples using Genome-Wide Human SNP 6.0 arrays (Affymetrix, Santa Clara, CA, USA).
Results: The comparison of PDGFRA, PDGFRB and C-KIT expression in normal tissues vs. desmoids tumor tissues showed no higher ratios in AF tumor samples. High density SNP array analysis revealed several genomic lesions: In single samples numerical aberrations were found on chromosome (chr.) 20 and 6 with either trisomy 20 and monosomy 6 or heterozygous deletions of chr. 5q, chr. 13q and chr. 10p. Putative sub-microscopic duplications were discovered on chr. 17q, 7p and 8p. These contained the coding regions for human epidermal growth factor receptor 2 (HER2), epidermal growth factor receptor (EGFR) and runt-related transcription factor 1 gene (RUNX1T1) as possible new candidate genes in the molecular biology of AF.
Conclusion: We describe a comprehensive genomic profiling of AF tumors. Thereby genomic alterations point to candidate genes, which could be factors in addition to known aberrations. However, our data, may help to provide a better understanding of the molecular mechanisms underlying the development of AF.

Disclosure: No conflict of interest disclosed.

V798

Inhibition of the Ras/Raf/MAPK-pathway in GIST: Therapeutic potential?

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Introduction: Gastrointestinal stromal tumors (GIST) are characterized by activating mutations of the receptor tyrosine kinases KIT or PDGFRA. KIT-inhibition by the small molecule inhibitor imatinib (IM) yields long lasting clinical responses. However, most patients eventually develop IM-resistance and the heterogeneity of secondary resistance mutations impedes the development of direct KIT-inhibitory drugs. Targeting KIT-dependent signaling pathways may therefore represent an alternative therapeutic strategy. Here we sought to evaluate the therapeutic potential of Ras/Raf/MAPK-signaling pathway inhibition in GIST.
Methods: The farnesyl-transferase (FT) inhibitor FTI-277 and the MEK1/2 inhibitor PD0325901 (PD) were evaluated alone and in combination with IM, NVP-BEZ235 (PI3K-/mTOR-Inhibitor) and RAD001 (mTOR-inhibitor) in IM-sensitive (GIST-T1 and GIST882) and IM-resistant cell lines (GIST48B and GIST430). Biological consequences were determined by cell viability assays and immunoblotting (IB) for KIT-related signaling pathways. Proapoptotic and antiproliferative effects were studied using cytometry-based annexin V/7-AAD staining and BrdU-assays.
Results: FTI-277 failed to inhibit N-ras processing and MEK activation at therapeutically relevant doses and no cytotoxicity could be observed. Treatment with PD resulted in complete MAPK-inhibition at 10nM in IM-sensitive (GIST-T1, GIST882) and at .5 to 1μM in GIST430 and GIST48B. MAPK-inhibition was accompanied by a strong reduction of the ETV1 transcription factor. Moderate inhibition of cell viability was seen following treatment with PD at 1μM (6 days) in all cell lines (GIST48B:60%, GIST430:45%, GIST-T1:82%, GIST882:47%). Time course experiments using 1μM PD showed complete inhibition of MAPK after 10 minutes. PD alone had little proapoptotic effects at 1μM (2 days) while antiproliferative effects were more pronounced. In contrast, combination of PD (1μM) with .1μM NVP-BEZ235 or .1μM RAD001 showed substantial additive effects in cell viability assays in all KIT-positive cell lines but not in KIT-negative GIST48B.
Conclusions: While FT-inhibition did not show therapeutic effects, PD0325901 effectively shut down MAPK-activity and showed antiproliferative effects independent of the KIT-mutational status. Additive antiproliferative and proapoptotic effects were seen in combination with PI3K inhibitors. Future studies should validate these findings in vivo.

Disclosure: No conflict of interest disclosed.

V799

Prognosis and treatment variables in primary and secondary angiosarcomas

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Background: Angiosarcoma (AS) can be divided into primary (PAS) and secondary angiosarcoma (SAS) the latter occurring after prior radiation therapy. The objective was to compare clinicopathologic factors for both groups.
Methods: AS cases of the Sarcoma Center North were identified. In a retrospective analysis, patient characteristics, treatment modality, and survival were determined and compared for both types of AS, PAS and SAS.
Results: Fourty patients (pts) with PAS and 24 pts with SAS were identified, overall representing 5.7% of pts in the database. Patients with PAS were younger at time of diagnosis than pts with SAS (median age 52 years vs 65 years, respectively). The proportion of female patients was 57.5 % for PA, but 95.8 % for SA. The distribution of cancer types was uneven, with AS of the breast accounting for 17.5% of PAS, but 87.5% of SAS. For the majority of PAS (63.2%), tumor size was ≥ 5 cm, as opposed to only 8.3% of SAS with tumor size ≥ 5 cm. At the time of diagnosis of the angiosarcoma, 42.5% of PAS had metastasized, but only 8.3% of SAS. All pts with SAS had previously received radiation therapy with a median time from radiation to diagnosis of SAS of 6.9 years (range, 0.7-24.8). The proportion of pts treated with surgery was the same for both groups with 79 %, however, more pts with PAS received chemotherapy. 35.5% of PAS were treated with radiotherapy and 13.3% received radiochemotherapy. 23.5% of SAS pts received a second course of radiation as part of their SAS treatment. While only half of the PAS relapses (53.6%) were local, 88.9% of SAS developed a local relapse. Median PFS was 11 months for PAS and 17 months for SAS (OR: 1.45; CI95%, 0.71-2.96; p=0.31). Median overall survival (OS) was 18 months for PAS and 36 months for SAS (OR: 1.74; CI95%, 0.85 – 3.59; p=0.13). Both PFS and OS (n.s.) were higher in pts with R0 compared to R1/2 resection, particularly for breast-AS (p=.01). Metastases at time of diagnosis lowered PFS (n.s.) and OS (p=.02). Non-breast AS had a lower PFS (p=.01) and trend for inferior OS (p=.09).
Conclusions: As expected**,** PAS and SAS were heterogeneous in their clinical behaviour . PAS consisted mostly of non-breast AS in both males and females, while SAS were primarily located in the breast of younger female pts. SAS

were detected at an earlier stage than PAS. Overall the prognosis is limited and early detection in a localized stage is warranted.

Disclosure: No conflict of interest disclosed.

V800

Interim analysis of a cooperative registry to optimize neoadjuvant treatment for large size, high grade non-rhabdomyo- soft tissue sarcoma (NRSTS) following R0/I-resection (IAWS-2)

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Introduction: The role of adjunctive anthracycline and ifosfamide based combination chemotherapy prior to or after resection in the treatment of adult and childhood ´so-called´ NRSTS continues to be controversial. In order to examine whether concomitant chemotherapy with doxorubicin (DOXO) and ifosfamide (IFO) and radiation (RTX) improves disease-free survival for patients with resected, large (>5cm), high grade (G2/3) adult-type NRSTS compared to surgery alone (and RTX if indicated/applicable) a multicenter register was launched.
Methods: Patients (pts) with locally advanced (stage III) or locally recurrent NRSTS were treated with IFO (3g/sqm for 3 days)-DOXO (60mg/sqm, day 1) x 3 cycles – IFO (3g/sqm for 2 days) x 2 cycles + RTX 50.4 Gy* – IFO-DOXO x 1 (*if indicated/applicable, otherwise IFO-DOXO x 5 cycles) prior to surgery. Key inclusion criteria were age ≤65 years at date of biopsy, histopathologically confirmed high grade NRSTS according FNCLCC, size >5cm, no evidence of metastatic disease, no previous cytotoxic or radiation treatment. This interim analysis evaluates the pCR rate.
Results: 55 pts with locally advanced NRSTS were included, but 5 were not eligible. Histologies were pleomorphic sarcoma, NOS (N=20), liposarcoma (11), synovial sarcoma (5), leiomyosarcoma (4), myxofribrosarcoma (4), MPNST (2), other (4). Median age was 49 yrs (range, 19-62). Other pts characteristics: male/female ratio was 1.3; grade 2 (18), grade 3 (30), median size 9 cm (range, 5-23); localisation: extremity 33, central 7, head and neck 5, retroperitoneal 3, girdle 2. Fourty pts are currently evaluable: 34 completed treatment and surgery. Five pts stopped treatment due to toxicity and 1 pt due to progressive disease. Pathological assessment according to Salzer-Kuntschik was performed in 37 pts: 14 pts achieved complete regression (grade 1) and 9 pts grades 2 and 3 (< 10% vital tumor). Response rate by RECIST from pts who completed therapy was: 2 CR (5 %), 12 PR (32%), 16 SD (43%) and 7 PD (19%). Pathological response did not correlate with response by RECIST since pts with pCR still had radiological disease. Dose intensity of DOXO and IFO was 89% and 81%, respectively.
Conclusions: 62% of all patients responded well to concomitant IFO-DOXO + RTX (regression grades 1-3, Salzer-Kuntschik). These preliminary results in terms of pathologic response suggest that the combination of neoadjuvant chemo- and radiotherapy may have a role in selected pts with high risk NRSTS.

Disclosure: No conflict of interest disclosed.

V801

Further analysis of the randomized EORTC-ESHO intergroup trial (NCI-00003052) in high-risk soft tissue sarcoma (STS)

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Introduction: Regional hyperthermia (RHT) improves significantly the anti-tumor efficacy of neo-adjuvant chemotherapy (N-AC) in patients (pts) with extremity (E) and non-extremity (NE) tumors in terms of tumor response, local progression-free (LPFS), and disease-free (DFS) survival (Issels et al. Lancet Oncol 2010). Especially in NE-STS there is ongoing debate on the benefit of AC alone after macroscopically complete (R0/R1) surgical resection (CSR) where postoperative radiotherapy is limited. In addition, for all STS the predictive value of histological grade (grade 2 vs 3) in terms of treatment related outcome remains unclear. Therefore we analyzed both questions based on the results of our prospective phase 3 trial.
Methods: In this study macroscopically CSR was defined as tumor-free margins ≥ 1 cm (R0) or < 1 cm (R1). Grade was assessed according to the FNCLCC system. For the impact of CRS or grade, survival rates (LPFS and DFS) were estimated using the Kaplan-Meyer-Method and compared by the log-rank test. Multivariate analyses were carried out by the Cox regression model including the stratification variables (primary, recurrence, prior surgery, anatomic site).
Results: Among 341 pts, 192 (56,3%) represented the stratified subgroup of NE (81% pelvis, abdomen; 19% others). 149 pts with CSR (43 pts: R2, or no surgery) received N-AC +/- RHT (76 vs. 73 pts). Compared to N-AC alone, LPFS (HR: 0.60 CI95: 0.37-0.97; p=0.034) and DFS (HR: 0.65 CI95: 0.44-0.96; p=0.031) was significantly in favor to N-AC plus RHT (LPFS: median duration > 100 months, at 2 years: 70%; and 5 years: 54%). Testing the predictive value of grade among all evaluable pts (339), 161 pts with grade 2 and 178 pts with grade 3 received N-AC +/- RHT (grade 2: 84 vs 77 pts; grade 3: 84 vs 94 pts). LPFS (HR: 0.54 CI: 0.31-0.94; p=0.026) and DFS (HR: 0.63 CI: 0.41-0.97; p=0.034) were significantly in favour to N-AC plus RHT in grade 2 pts and to much lesser extent in grade 3 pts (LPFS: HR 0.67 CI: 0.41-1.09; p=0.104 and DFS: HR 0.74 CI 0.51-1.07; p=0.110).
Conclusions: In pts with NE-STS receiving N-AC plus RHT complete surgical resection (R0/R1) does not abrogate the significant benefit of RHT. FNCLCC grade 2 high-risk STS preferentially benefit from N-AC but only if RHT is added.

Disclosure: No conflict of interest disclosed.

V802

Trabectedin in heavily pretreated and elderly metastatic soft tissue sarcomas – a single center experience

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Introduction: Trabectedin (T) has mostly been studied in metastatic L-sarcomas (leiomyosarcoma / LMS; liposarcoma/LPS). Only limited data are available in other sarcoma subtypes, heavily pretreated and elderly patients. We therefore conducted a retrospective analysis of patients receiving T in the sarcoma clinic of the West German Cancer Center (WTZ).
Methods: Between 2003 and 2010 94 patients were treated with T at 1.5mg/m² as 24h continuous infusion every 3 weeks. Progression-free survival (PFS) was determined from first administration of T to disease progression. Stable disease was defined according RECIST. Clinical benefit rate (CBR) was defined as percentage of patients achieving partial remission (PR) or stable disease. Covariates were histology, age, pretreatment, duration of treatment, dose reduction, response and toxicity.
Results: The median age at time of first T was 53 years (range: 19-83) with 56% male and 44% female patients. Histologies included 22 LMS, 22 LPS, 12 pleomorphic sarcomas, 11 synovial sarcomas, seven rhabdomyosarcomas (RMS), and 21 other sarcoma subtypes. L-sarcomas received T in 4th-line (median) with a median PFS of 5 months and a CBR of 52%. Long lasting remissions were observed in 7th line treatment. In this subgroup, no difference in PFS was found in patients receiving T in 1st/2nd line treatment versus those treated in 3rd or higher lines (p=0.64, t-test). PR was only seen in six L-sarcomas patients. In patients with non-L-sarcomas CBR was 34% (n=17) with a median PFS of 6 months. 3/7 RMS experienced prolonged stabilization of up to 8 months. In the elderly population (>65 years; n=22) T was given in 3rd line (median). Dose reductions were necessary in 3 elderly patients, no treatment discontinuations were necessary. 72% of all patients received T in 3rd line or higher. In these patients, an increased incidence of CTC grade 1-2 hematological toxicity was observed. In this non-trial setting, port-associated complications (paravasation) were more frequent (13%) than other continuous infusions administered in our center.
Conclusions: Approximately 50% of patients with L-sarcomas derive clinical benefit from T regardless of the extent of pretreatment. Moreover, significant activity is found in patients with non-L-sarcomas including RMS. Administration of T on an outpatient basis is well tolerated in elderly and heavily pretreated patients. The increased incidence of port complications merits further investigation

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Freie Vorträge
Tumor-/Zellbiologie II

V803

Aberrant simultaneous ERK and CDK2 kinase activity in cancer

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Genome wide analyses of regulatory elements have identified successive waves of coherent transcriptional programs controlling cell cycle progression. Each of these modules induces its successor and, at the same time, inactivates the key kinases active in the antecedent phase of the cycle. In an initial signaling module, the RAF-MEK-ERK pathway is central for phosphorylation of the tumor suppressor Rb (retinoblastoma), and subsequent cell cycle entry. CDK2 is the driving force and guardian of the consecutive S phase where DNA replication occurs. After having initiated CDK2 activity, ERK is inactived and non-inducible (Pouyssegur 2003). We here show that simultaneous rather than serial activation of the canonical ERK and CDK2 pathways is a recurrent aberrant signaling motif indicative for malignant transformation. This phenomenon was observed in leukemic blasts and also in cancer cells of epithelial and mesenchymal provenience, but not in healthy or regenerating tissues using flow cytometry and confocal microscopy techniques and phospho-epitope specific antibodies. To further visualize this "forbidden ERK/CDK2 signal combination", we developed a membrane permeable biosensor comprising the optimal peptide substrates for both, ERK and CDK kinases, which enables detection of simultaneous ERK and CDK2 activity on single cell level. In healthy cells, single but never dual phosphorylation of the biosensor was detected, thus confirming the serial and mutually exclusive piloting of ERK and CDK2 signaling pathways. In stark contrast, dual phosphorylation of the biosensor peptide was observed in malignant cells of leukemic and epithelial origins. Thus, simultaneous and therefore aberrant recruitment of ERK and CDK2 cell cycle elements in transformed but not healthy cells discloses malignancy. Our data do not add to the increasing complexity of genetic insults associated with malignant transformation. Rather, we extend our previous observation (Stuhler 2010) that violations of the orderly recruitment of common signaling pathways are indicative for a malignant phenotype which can be detected on the basis of aberrant spatiotemporal organization of essential cell signaling nodes. Based on the structural information provided by our template peptide, we further envisage a new class of cancer therapeutics where a nontoxic prodrug is converted into a tumoricidal substance exclusively in malignant cells after simultaneous double-phosphorylation by aberrantly active kinases.

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Helmut Salih: Honoraria: Patentrechte.

V804

DNA damage activates a spatially distinct late cytoplasmic cell-cycle checkpoint network controlled by MK2-mediated RNA stabilization

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Introduction: Following genotoxic stress, cells activate a complex kinase-based signaling network to arrest the cell cycle and initiate DNA repair. p53-defective tumor cells rewire their checkpoint response and become dependent on the p38MAPK/MK2 pathway for survival after DNA damage, despite a functional ATR/Chk1 checkpoint pathway.
Methods: We used functional genetics to dissect the contributions of Chk1 and MK2 to checkpoint control.
Results: Using RNAi technology, we demonstrate that nuclear Chk1 kinase activity is critical to initiate a functional G(2)/M checkpoint, while cytoplasmic MK2 kinase activity is critical to maintain a prolonged checkpoint response through a mechanism involving posttranscriptional mRNA stabilization. In response to DNA damage, the p38MAPK/MK2 complex shuttles from the nucleus to the cytoplasmic compartment where MK2 directly phosphorylates the RNA-binding protein hnRNPA0, to stabilize Gadd45α mRNA, while p38MAPK phosphorylates and releases the RNA-binding translational inhibitor TIAR. In addition, MK2 phosphorylates the poly-(A)-ribonuclease PARN, blocking Gadd45α mRNA degradation. Lastly, we show that Gadd45α functions within a positive feedback loop, sustaining the MK2-dependent cytoplasmic sequestration of Cdc25B/C to block mitotic entry in the presence of unrepaired DNA damage.
Conclusions: Our findings demonstrate a critical role for the MK2 pathway in the posttranscriptional regulation of gene expression as part of the DNA damage response in cancer cells. Furthermore, our data commend the p38MAPK/MK2 pathway as a prime drug target for chemo-sensitizing therapeutic regimens.

Disclosure: No conflict of interest disclosed.

V805

Bortezomib primes neuroblastoma cells for TRAIL-induced apoptosis by linking the death receptor to the mitochondrial pathway

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Purpose: The prognosis of children with advanced stage neuroblastoma remains poor despite aggressive treatment protocols, highlighting the urgent demand for new treatment strategies. In the present study, we investigated the potential of the proteasome inhibitor Bortezomib to augment TRAIL-induced apoptosis in neuroblastoma.
Methods: The effect of Bortezomib on TRAIL-induced apoptosis and signaling pathways was analyzed in neuroblastoma cell lines, primary neuroblastoma cultures and in an in vivo model of neuroblastoma.
Results: Bortezomib synergistically cooperates with TRAIL to induce apoptosis and to reduce colony formation of neuroblastoma cells (combination index 0.5). Mechanistic studies reveal that Bortezomib profoundly enhances TRAIL-induced cleavage of Bid into tBid, accumulation of tBid in the cytosol and its insertion into mitochondrial membranes, pointing to a concerted effect on Bid cleavage (triggered by TRAIL) and stabilization of tBid (conferred by Bortezomib), which links the death receptor to the mitochondrial pathway. Additionally, Bortezomib increases expression of p53 and Noxa. All these changes lead to increased activation of Bax and Bak, loss of the mitochondrial membrane potential, cytochrome c release, caspase activation and caspase-dependent apoptosis upon treatment with Bortezomib and TRAIL. Knockdown of either Bid, Noxa or p53 significantly delays the kinetic of Bortezomib- and TRAIL-induced apoptosis, whereas it does not confer longterm protection. By comparison, overexpression of Bcl-2, which simultaneously antagonizes tBid and p53, significantly inhibits Bortezomib- and TRAIL-induced apoptosis and even rescues clonogenic survival. Also, Bortezomib and TRAIL act in concert to trigger caspase-3 activation and apoptosis in a panel of patients' derived primary neuroblastoma cultures, underscoring the clinical relevance of these findings. Importantly, Bortezomib and TRAIL cooperate to suppress tumor growth in an in vivo model of neuroblastoma.
Conclusions: Bortezomib represents a promising new approach to prime neuroblastoma cells towards TRAIL-induced apoptosis. This study provides the rationale for future clinical studies with Bortezomib and TRAIL in neuroblastoma.

Disclosure: No conflict of interest disclosed.

V806

A molecular signature of telomere dysfunction in tumor cells

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Telomere maintenance is essential for the immortal growth of tumor cells. Loss of telomere function activates a DNA damage response which ultimately limits cell division and is associated with replicative senescence. Therefore, the identification of factors associated with telomere dysfunction may help to develop biomarkers for ageing and for a telomerase-based tumor therapy. An efficient method to induce telomere dysfunction in human cells is the retroviral overexpression of a dominant-negative (DN) mutant of hTERT. In former proteomic analyses of various DN-hTERT clones of different tumor cell lines (lung, prostate, breast, colon) by SELDI-TOF-MS, MS/MS and immunoblotting, we identified discrete biomarkers related to telomere dysfunction and replicative senescence including core histones, specific keratins, and S100A6 (Zimmermann et al. 2009 Proteomics 9, 521-534; Zimmermann et al. 2009 Proteomics 9, 5175-5187). A more quantitative proteomics approach was applied to such clones of the HCT-116 cell line by isotope-coded protein labeling and nanoflow HPLC MS/MS. MS data analysis revealed a list of more than 50 potential biomarkers of telomere dysfunction in tumor cells including many of the proteins identified in our former studies. The proteome data was backed up by a comprehensive microarray gene expression analysis in HCT-116 clones with different genetic backgrounds (wild type, $p53^{-/-}$ and $p21^{-/-}$). Genedata Expressionist analysis using stringent significance criteria (regulation score > 2.0, q-value < 0.01) revealed 389 differentially expressed genes in wild type, 192 in $p53^{-/-}$ and 218 in $p21^{-/-}$ cells comparing DN-hTERT and control clones.
Recently, emerging evidence points to a crucial role in cellular senescence for the 'senescence-messaging secretome' or SMS as a stage for cross-talk between senescent cells and their environment. In this line, we began to investigate the secreted proteins in the culture medium from DN-hTERT clones of HCT-116 cells. Preliminary MS analyses point to a distinctive 10-protein signature of the senescing cells in comparison to their control counterparts.
Current studies are directed at validation of such markers in telomere-damaged cells from telomerase knockout mice and patients with CLL. In summary, loss of telomere function appears to result in distinct protein and gene expression signatures which potentially could serve as biomarkers of replicative senescence and guide telomerase-based therapeutic approaches.

Disclosure: No conflict of interest disclosed.

V807

Targeting of tumor-associated angiogenic tip and stalk cells with immunoliposomes (ILs) directed against VEGFR-2 and VEGFR-3

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Introduction: Angiogenesis is a key process in tumor progression. Angiogenic sprouting involves specification of subpopulations of endothelial cells into tip cells that respond to guidance cues, and stalk cells that proliferate to form the vascular network. While angiogenic stalk cells mainly express VEGFR-2, the tip cells express VEGFR-3. Up to now, therapeutic approaches have mainly been aimed against VEGFR-2 activity. However, it remains unclear to which extend VEGFR-2 expressing stalk and VEGFR-3 expressing tip cells contribute to tumor-associated angiogenesis in vivo.
Methods: New technologies based on liposomes allow for the transport of pharmacological compounds to selected cells in vivo. In our lab we have established a method of transportation based on immunoliposomes (ILs), i.e., liposomes coated with antibodies. DC101 anti-mouse VEGFR-2 and AFL4 anti-mouse VEGFR-3 antibodies were utilized to target liposomes to VEGFR-2 or -3 expressing endothelial cells in vivo. Using this technology, we are able to selectively deplete VEGFR-2 or VEGFR-3 expressing cells from the angiogenic endothelial cell pool and thereby study the functional role of these cell populations.
Results: In this study we have investigated doxorubicine-containing ILs coated with anti-VEGFR-2 or anti-VEGFR-3 antibodies in two transgenic mouse models (the Rip1Tag2 model of human insulinoma and the MMTV-PyMT model of human breast cancer) and one xenograft model (the human colon cancer cell line HT29 in nude mice). In all three models, doxorubicin-containing ILs against VEGFR-2- or VEGFR-3-positive cells effectively suppressed tumor growth. Both were more powerful than untargeted liposomes containing the same dose of doxorubicin (PLD). Combined anti-VEGFR-2 and -3 therapy was significantly more potent than targeting anti-VEGFR-2 expressing endothelial cells alone. In the Rip1Tag2 model, tumor burden after 2 weeks of therapy was 14.4 ± 7.9 mm^3 (mean ± standard deviation) in the empty liposome group, 5.4 ± 4.7 mm^3 in the untargeted liposomal doxorubicin group, 2.1 ± 2.5 mm^3 in the anti-VEGFR-2 ILs group, 1.1 ± 1.5 mm^3 in the anti-VEGFR-3 ILs and 0.3 ± 0.4 mm^3 in the combined anti-VEGFR-2 and -3 ILs group.
Conclusion: Delivery of cytotoxic drugs to different angiogenic tumor-associated cells is feasible and may be more promising than focusing on a single entity of endothelial cells.

Disclosure: No conflict of interest disclosed.

V808

RANKL is expressed by AML and CLL cells and impairs NK cell-mediated immune surveillance

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Introduction: NK cells play an important role in tumor immunosurveillance, especially in leukemia. Their reactivity is governed by various activating and inhibitory molecules expressed by their target cells including several members of the TNF family. The TNF family member RANKL and its receptors RANK and OPG are key regulators of bone remodelling, but recently have also been shown to influence progression of hematopoietic malignancies (Sordillo et al., 2003). Recently we reported that NK cells express RANK while acute myeloid leukemia (AML) and chronic lymphocytic leukemia (CLL) cells can express RANKL, and that RANK signaling impairs the reactivity of NK cells (Schmiedel et al., 2010). Here we extended our antecedent studies and report on the immunomodulatory consequences of signaling via RANKL into leukemia cells.
Methods: RANK and RANKL expression was analyzed by FACS and RT-PCR. NK cell reactivity was studied by cytotoxicity assays and determination of granule mobilization. Cytokine levels were measured by ELISA.
Results: Leukemic cells of AML (n=60) and CLL (n=54) patients were found to express RANKL mRNA and surface protein in 70% and 100% of the investigated cases, respectively. Signaling via RANKL into the leukemia cells mediated the release of immunoregulatory cytokines like TNF, IL-6, IL-8 and IL-10. The factors released by leukemia cells upon RANKL signaling impaired the anti-leukemia reactivity of NK cells, but, beyond their direct inhibitory effect also induced upregulation of the inhibitory RANK receptor on NK cells. In line, significantly increased RANK expression was observed by ex vivo analyses of NK cells from leukemia patients as compared to healthy controls.
Conclusions: Upon interaction with RANK, bidirectional signaling via RANKL not only cause transduction of inhibitory signals in NK cells, but also induces the release of immunosuppressive factors by leukemia cells. The latter contribute to inhibition of NK reactivity by directly suppressing NK function and by increasing NK cell RANK expression thereby closing a "vicious cycle of immune evasion". Our data suggest that therapeutic modulation of the RANK/RANKL system e.g. with Denosumab/AMG162, which is approved for treatment of osteolysis, may hold promise to reinforce NK reactivity against hematopoietic malignancies.

Disclosure: No conflict of interest disclosed.

Freie Vorträge
Gerinnung

V811

Transfusion of cryopreserved autologous platelets to overcome HLA alloimmunization in patients with hematological malignancies

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Alloimmunization to platelet antigens occurs in up to 25% of mainly female patients with hematologic disorders receiving intensive chemotherapies. It leads to platelet transfusion refractoriness, increased bleeding risk and often compromises further treatment. Once alloimmunization is determined, HLA-matched platelets improve platelet count increments. However, in patients with rare HLA phenotypes, the identification of HLA matched platelets may be difficult. In this situation, transfusion of autologous platelets is a clinical option, but cryopreservation of platelets has been notoriously difficult. Here, we present the clinical experience with such platelet transfusions in patients with hematological malignancies. The platelets were collected after recovery from chemotherapy. Per patient, 2.5 (1-5) platelet aphereses were performed collecting 298 (190-598) ml per apheresis resulting in 6 (4-8) concentrates. All platelet concentrates were irradiated with 50 Gy, cryopreserved with 10% DMSO and RPMI-1640 without Phenol Red as freezing medium. Platelets were frozen by computer-controlled rate freezing and afterwards kept in liquid nitrogen. For transfusion, platelet concentrates were thawed at bedside in a warming bath (37°C) and transfused immediately without removal of DMSO. 8 patients received 32 cryopreserved autologous platelet transfusions. There was no DMSO toxicity after infusion. All patients were female and received intensive chemotherapies for acute leukemias (7) or NHL (1). The median age at diagnosis was 51 (range 30-69) years and the patients had 2 (1-4) prior pregnancies. Alloimmunization occurred 23 (15-458) days after diagnosis with a panel reactivity of 66 (14-86)%. The platelets were transfused 26 (9-133) days after cryopreservation. The platelet count before transfusion was 6 (2-16) G/L and the platelets were transfused prophylactically (28) or therapeutically (4). The median number of platelets per concentrate was 1.7 (0.8-2.9) $x10^{11}$ and the 1-hour corrected count increment (1h-CCI) 5.5 (-1.8-20.2). The 1h-CCI was negatively correlated with the CRP levels (Pearson correlation -0.500, p=0.004). Currently, we are performing functional assays with the cryopreserved platelets, which will be presented at the meeting. In conclusion, transfusion of cryopreserved autologous platelets is a feasible alternative to HLA-matched platelets offering an important clinical option for patients with HLA alloimmunization and rare HLA phenotypes.

Disclosure: No conflict of interest disclosed.

V812

Quality of Life (QOL) in nonsplenectomized Immune Thrombocytopenia (ITP) patients receiving romiplostim or Standard of Care (SOC)

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Introduction: The TPO-mimetic romiplostim is approved for the treatment of adult chronic ITP. We compared QOL between medical SOC- and romiplostim-treated pts and subgroups of interest.
Methods: Non-splenectomized ITP pts were randomized (2:1) to weekly romiplostim or SOC, open label for 52 wks. QOL was assessed at baseline and every 12 wks using the ITP-PAQ (10 scales scored 0-100). Mean and change scores from baseline were computed and differences between treatment groups and subgroups assessed. Previous research shows 8–12 points reflect the minimal important difference (MID) for clinically meaningful changes.
Results: 234 pts were randomized (romiplostim: 157, SOC: 77). At baseline, no QOL differences were found. At wk 52, change scores for both groups showed improvements that exceeded the MID, except Fatigue and Activity (SOC arm). Compared to SOC, the romiplostim group showed significantly greater improvements from baseline for all scales except Fatigue. No differences exceeded the MID. Among subgroups with favorable clinical outcomes, significantly greater improvements were seen in the romiplostim vs SOC group in many scales. These exceeded the MID once (Activity: 13 point greater improvement in pts with no platelet count ≤20 x 10^9/L).
Conclusions: Non-splenectomized ITP pts receiving romiplostim had greater improvements in QOL than pts receiving SOC. Improvements were also evident in subgroups of pts with favorable clinical outcomes.

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Table 1. Change in QOL Scores From Baseline to Wk 52 (for Abstract V812)

ITP-PAQ Scale*	Overall (N=234)		No blood transfusions or rescue medications (n=173)		No bleeding ≥ grade 2 (n=199)		No platelet count ≤ 20 x 10^9/L (n=228)	
Mean (±SE) change from baseline score	SOC	Romiplostim	SOC	Romiplostim	SOC	Romiplostim	SOC	Romiplostim
Symptoms	13 (2)	16 (2)†	12 (3)	15 (3)‡	13 (2)	18 (2)‡	15 (3)	18 (2)
Fatigue	10 (3)	11 (3)	5 (5)	9 (5)†	11 (4)	14 (4)	10 (4)	16 (4)
Bother	13 (3)	17 (3)†	8 (4)	10 (4)†	11 (3)	16 (3)†	18 (3)	22 (3)†
Activity	8 (4)	17 (4)†	7 (5)	12 (5)†	6 (4)	15 (4)†	12 (4)	25 (4)†
Psychological	16 (3)	19 (3)†	18 (4)	19 (4)†	19 (3)	21 (3)	19 (3)	23 (3)
Fear	9 (2)	14 (2)†	7 (3)	10 (3)†	9 (2)	15 (2)‡	11 (2)	16 (2)†
Overall QOL	15 (4)	16 (4)‡	12 (4)	17 (4)†	16 (4)	17 (4)	20 (5)	21 (5)
Social QOL	6 (3)	10 (2)†	6 (3)	10 (3)‡	6 (3)	11 (3)†	7 (3)	11 (3)†

*Statistical models could not be computed for Women´s Reproductive Health and Work QOL; n values vary with scale.
Statisically siginificant differences between treatment groups: †p≤0.05, ‡p≤0.001

V813

Effect of platelet transfusion on plasma CD40L and on CD40L release capacity in the peripheral blood

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Background: Soluble CD40L (sCD40L) is proven to have various effects on the adaptive immune system. Platelet derived cytokines, like sCD40L, play an important role in the development of adverse transfusion reactions associated with the administration of platelet products. In the present study we determined sCD40L concentration and release capacity in thrombocytopenic patients before and after receiving a platelet transfusion.
Study Design and Methods: The study included eight patients suffering from chemotherapy-induced thrombocytopenia. sCD40L levels were measured in plasma and in serum samples of the patients before and after platelet administration as well as in the respective platelet apheresis concentrates. sCD40L concentrations were determined by an ELISA-Kit. Sixteen healthy blood donors served as a control group.
Results: In platelet apheresis concentrates, elevated sCD40L levels (2,678 ± 129 pg/ml) were observed in comparison to plasma sCD40L levels in controls (238.4 ± 35.3 pg/ml). sCD40L plasma concentration of thrombocytopenic patients was lowered to 92.5 ± 18.2 pg/ml before transfusion and increased to 208.6 ± 25.4 pg/ml after platelet administration. In parallel, the total sCD40L release capacity raised from 235.2 ± 62.3 pg/ml before to 912.4 ± 185.7 pg/ml after platelet transfusion.
Conclusions: In thrombocytopenic patients, sCD40L levels were clearly influenced by platelet transfusions: In the stored platelet apheresis concentrates, an accumulation of sCD40L was found leading to a normalization of sCD40L plasma concentration in the patients immediately after transfusion. Additionally, the sCD40L release capacity was enhanced by platelet administration dependent on the increase in platelet count.

Disclosure: No conflict of interest disclosed.

V814

Successful rituximab treatment of refractory immune thrombocytopenia during pregnancy

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Immune thrombocytopenia (ITP) is mediated by autoantibodies resulting in accelerated platelet destruction. ITP clinically manifests in bleeding and can present with minor symptoms as petechiae, but may also lead to severe intracranial hemorrhage. ITP with severe thrombocytopenia < 50.000/microL may occur in women during pregnancy representing a challenge in terms of management and treatment. If corticosteroids and IVIG (intravenous immunglobulin) are not successful, administration of rituximab may be considered. However, since the IgG-antibody rituximab potentially crosses the placenta, binding to fetal peripheral B-lymphocytes may subsequently cause immunosuppression.
The following case report describes the effects of rituximab given to a pregnant woman with ITP after corticosteroids and IVIG had failed to durably increase the platelet count. Furthermore, reduction of corticosteroids was necessary owing to fetal macrosomy. Rituximab treatment was initiated in the 25th week of pregnancy and was given weekly four times (375 mg/m^2) leading to a transient increase in maternal platelet count. There occurred no bleeding complications during and after primary cesarean section. In the mother platelet count was already normalized three days post partum.
In the neonate, rituximab caused complete B-lymphocyte depletion. B-lymphocyte count was normalized at two months after delivery, whereas immunoglobulin levels were still inadequate at the age of 11 months. However, there occurred no complications related to infection in the neonate.
The clinical course indicates that rituximab treatment during pregnancy is feasible. Administration of rituximab during pregnancy should be carefully considered for selected cases.

Disclosure: No conflict of interest disclosed.

V815

Immature platelet fraction in clinical routine

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Introduction: New blood cell counters enable us to evaluate the immature peripheral blood cell fractions, which may give a hint to underlying cause of peripheral cytopenia and function as early predictor of hematopoietic recovery.
Method: We serially measured the IPF% using the fully automated Sysmex XE-2100 blood cell counter in 30 patients with hematologic or oncologic diseases and various causes of thrombocytopenia on a routine basis. IPF and platelet counts were correlated with the clinical courses of the diseases. In addition, we measured the IPF fraction in 40 patients with known or suspected hematologic diseases at the time-point of bone marrow investigation and correlated it with the pathohistological diagnosis.
Results: The analysis is ongoing at the time-point of abstract submission. Based on the results we will present our estimation of the clinical value of IPF assessment in our patient cohort. Most interesting courses will be presented graphically.
Conclusion: Simultaneous IPF measurement on the fully automated Sysmex blood cell count device is a quick and reliable tool to estimate the recovering ability of the bone marrow in peripheral blood. Larger studies are certainly needed to assess the potential and predictive value of this method to spare certain patients from bone marrow investigation or guide the need for blood cell transfusions. The data collection and analysis is still ongoing. Clinically relevant findings and statistically analysis will be presented at the meeting.

Disclosure: No conflict of interest disclosed.

V816

A role of Toll-like receptor mediated Signals in Neutrophils in the pathogenesis of the anti-phospholipid syndrome

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Introduction: The anti-phospholipid syndrome (APS) is a systemic autoimmune disease characterized by an adaptive immune response against self phospholipid (PL)--binding proteins and clinically by recurrent thrombotic or thrombembolic events and/or fetal losses. Although APS is considered as an autoantibody-mediated disease, there is growing evidence that anti-phospholipid antibodies (aPL) are necessary but not sufficient for the clinical manifestations of the syndrome. Mediators of the innate immunity are increasingly recognized to be additionally involved in both the thrombogenic effects and fetal losses. In fact, complement activation and proinflammatory cytokines have been shown to be involved in fetal loss in an experimental model. Beyond this, a role for neutrophil activation by the tissue factor/Factor VIIa/PAR2 axis have been demonstrated to play an additional role. Finally, microbial infections have been reported to act as triggers for the production of autoantibodies cross-reacting with PL-binding proteins as well as inflammatory stimuli that potentiate the aPL thrombogenic effect. Altogether, these findings suggest a role for the innate immunity in APS pathogenesis. Toll-like receptors (TLR) directly recognize microbial structures and most of them are expressed in neutrophils. TLR triggering induces prompt inflammatory responses and mediate functional activation in immune effector cells. There is evidence that aPL, and in particular anti-beta(2) glycoprotein I antibodies, may activate endothelial cells and monocytes through TLR-4-dependent signalling. Whether or not TLR in conjuction with aPL may behave similarly in neutrophils is not known and is the focus of this project.
Methods: We used purified neutrophils from healthy donors and stimulated them in the presence or absence of the human monoclonal aPL HL5B or HL7G and a TLR4 agonists (LPS) monitoring neutrophil effector functions, such as the oxidative burst, phagocytic activity, degranulation, L-selectin shedding, and survival.
Results: aPL alone were only able to induce minor activation of PMN effector functions at high concentrations. However, in the additional presence of LPS the activation threshold was marked lower indicating a synergistic activation pathway of aPL and microbial products also in PMN.
Conclusions: PMN may be important innate immune cells that contribute to the pathophysiology of APS. These results may help to develop new treatment options for patients in the future.

Disclosure: No conflict of interest disclosed.

Freie Vorträge
Multiples Myelom (klinisch)

V817

Bortezomib before and after high-dose chemotherapy improves the survival in patients with newly diagnosed multiple myeloma and renal insufficiency – a subgroup analysis of the prospective randomised GMMG HD4/ HOVON 65 study

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Introduction: Renal impairment in patients with multiple myeloma (MM) is correlated with an inferior prognosis. We wanted to investigate the role of renal function at baseline on prognosis in a large randomized study comparing a bortezomib-containing induction regimen (PAD) with standard VAD followed by HDM and maintenance with either thalidomide or bortezomib.
Methods: 833 patients were randomly assigned to 3 cycles of standard VAD (arm A) or PAD (Arm B); PAD was dosed as bortezomib 1.3 mg/m^2, days 1,4,8,11, doxorubicin 9 mg/m^2, days 1-4, dexamethasone 40 mg, days 1-4, 9-12, 17-20). Patients received one (HOVON) or two (GMMG) cycles of high-dose melphalan (HDM) 200 mg/m^2 with ASCT. Maintenance consisted of thalidomide (T): 50 mg daily (arm A) or bortezomib (B): 1.3 mg/m^2, 2-weekly (arm B) for 2 years. For this analysis patients were grouped according to renal function at baseline with creatinine up to 2 mg/dl (176 µmol/l, n=664) or > 2 mg/dl (176 µmol/l, n=78). Frequencies were compared by logistic regression, survival data by Cox PH regression. The analysis was intention-to-treat, with PFS censored for patients treated with allo-SCT (n=46).
Results: Patients with elevated creatinine had a higher proportion of high-risk features such as ISS stage 3 or tdel 17p. 78% of patients with creatinine < = 2 mg/dl received at least 1 HDM and 59% proceeded to maintenance. For creatinine > 2 mg/dl this was the case for 65% and 49% respectively. Progression-free survival (PFS) was significantly influenced by renal function (p< 0.001): In the VAD arm patients with creatinine < = 2 mg/dl had a 3yr PFS of 44% compared to 12% for creatinine > 2 mg/dl. In the PAD arm 3yr PFS was 48% and 49% respectively. Overall survival at 3 yrs in the VAD arm was 75% for patients with creatinine < = 2 mg/dl versus 32% with creatinine > 2 mg/dl, while in the PAD arm the corresponding rates were 78 and 72% (p< 0.001).
Discussion: Our results confirm experience in relapsed MM and show for the first time that using bortezomib both before and after HDM in newly diagnosed MM markedly improves overall and progression-free survival in patients with elevated creatinine > 2mg/dl, getting very close to the results in patients with creatinine < 2 mg/dl. We conclude that combining HDM with a bortezomib-containing induction- and maintenance regimen has the potential

to overcome the negative prognostic impact of an impaired renal function in patients with newly diagnosed MM.

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Hartmut Goldschmidt: Financing of Scientific Research: Janssen, Celgene; Expert Testimony: Janssen, Celgene.

V818

Metascoring and gene expression profiling in multiple myeloma

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Background: Multiple myeloma is characterized by molecular heterogeneity transmitting to survival ranging from several months to over 15 years. Gene expression profiling allows assessment of biological entities, risk, and targets. Its translation into clinical routine alongside conventional prognostic factors has been prevented by lack of appropriated reporting tools and the integration with other prognostic factors into a single risk stratification (metascoring).
Methods: We present here a non-commercial open source software-framework developed in the open source language R (GEP-report) containing a graphic user interphase based on Gtk2. Affymetrix microarray raw-data and a documentation-by-value strategy to directly apply thresholds and grouping-algorithms from a reference cohort of 262 myeloma patients are used. Gene expression-based and conventional prognostic factors are integrated within one risk-stratification (HM-metascore) and the final report is created as a PDF-file.
Results: The GEP-report comprises i) quality control, ii) test of sample identity, iii) biological classifications of multiple myeloma, iv) risk stratification, v) assessment of target-genes, and vi) integration of expression-based and clinical risk factors within one metascore. This HM-metascore sums over the weighted factors gene-expression based risk-assessment (UAMS-, IFM-score), proliferation, ISS-stage, t(4;14), and expression of prognostic target-genes (AURKA, IGF1R) for which clinical grade inhibitors exist. It delineates three significantly different groups of 13.1, 72.1 and 14.7% of patients with a 6-year survival of 89.3, 60.6 and 18.6%, respectively.
Conclusion: GEP-reporting allows prospective assessment of target gene expression and integration of current prognostic factors within one risk stratification (metascoring), being customizable regarding novel parameters or other cancer entities.

Disclosure: No conflict of interest disclosed.

V819

Anti-EGFR antibody Cetuximab in refractory or relapsed multiple myeloma: Final results of a phase II clinical trial

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Introduction: Cetuximab (Erbitux©) is an anti-epidermal growth factor receptor (EGFR) antibody. EGFR is also expressed on multiple myeloma (MM) plasma cells and bone marrow stromal cells (BMSC). Recently, the inhibition of EGFR by small molecule inhibitors has been shown to induce apoptosis in primary myeloma cells revealing a synergistic effect with dexamethasone. Therefore, the anti-EGFR antibody cetuximab might be of clinical benefit in the treatment of MM, especially in combination with dexamethasone. Here we show the final results of the first clinical trial with an anti-EGFR antibody in MM.
Methods: Cetuximab once weekly was administered to patients with refractory or relapsed MM who had previously received at least one line of prior treatment and were not eligible to undergo autologous stem cell transplantation. Dexamethasone 20 mg on day 1-3 of each cycle was added starting week 5 in case of tumor progression or week 9 if no partial response (PR) or complete response (CR) was achieved with cetuximab alone. Planned treatment duration was 16 weeks (primary endpoint).
Results: Fifteen patients have been enrolled in total. Seven patients were treated for a minimum of 16 weeks and 5 of those patients received cetuximab for at least 28 weeks. One patient received cetuximab treatment as single agent for more than one year. Thrombocytopenia, hyponatremia and acneiform rash were the most common CTC grade 3 or 4 side effects. Acneiform rash CTC grade 1 occurred in all patients and 3 patients suffered from acneiform rash CTC grade 3.
After 16 weeks (primary endpoint) cetuximab in combination with dexamethasone induced 3 responses (2 minimal responses (MR) and 1 partial response (PR)) and led to stable disease (SD) in 3 patients, cetuximab as single agent led to SD in 1 patient. Five of the 13 patients included did not receive the planned 16 weeks of treatment due to progressive disease (PD). Six patients were treated more than 16 weeks: 1 patient received cetuximab as single agent and had SD more than one year and 5 patients continued treatment with cetuximab and dexamethasone in combination. There was 1 PD after 21 weeks and 2 SDs and 2 MRs after 28 weeks in this cohort.
Conclusions: Cetuximab is feasible and safe in MM patients. It demonstrated efficacy in highly pre-treated patients, especially in combination with dexamethasone. Because of its favourable side effect profile it should be evaluated in clinical trials in combination with other compounds.

Disclosure: No conflict of interest disclosed.

V820

Bortezomib in combination with Bendamustine and Prednisone in the treatment of patients with newly diagnosed/untreated Multiple Myeloma and Light Chain induced renal failure

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Introduction: Renal failure represents a main complication of Multiple Myeloma (MM) in many patients at the time of diagnosis. These patients are at increased risk for infections and have a significantly worse prognosis. Small phase I/II studies suggest that treatment with chemotherapy and/or new substances results in recovery of renal function in up to 25%. Due to the limited regenerative ability of renal tubular cells, the window of opportunity for reversible damage is small making an immediate and highly active treatment strategy mandatory. Bortezomib as well as bendamustine are potent drugs for the treatment of MM. Bortezomib is known to induce CR. Bortezomib and Bendamustine can be administered at the full approved dose and schedule in patients with impaired renal function.
Methods: Between June 2006 and April 2011, 16 patients (median age 66; range 46 – 86 years) with newly diagnosed/untreated MM and disease associated renal failure (Creatinine clearance < 35 ml/min and 8 on dialysis) were treated with bendamustine 60 mg/qm day 1 and 2, prednisone 100 mg on day 1, 2, 4, 8 and 11, and bortezomib 1,3 mg/qm on day 1, 4, 8 and 11 (BPV). Cycles were repeated every 21 days until maximum response or progressive disease. MM response was assessed using EBMT criteria modified to include near complete remission (nCR) and very good partial remission (VGPR).
Results: 14 patients (88%) responded after at least one cycle of chemotherapy with three CR, five nCR, three VGPR, and three PR. One patient had a stable disease and one patient had a progress. With a median follow up of 17 months, PFS at 12 months was 68 %. The median number of the BPV-treatment was 2 (1-5) cycles. Five of the 14 responding patients showed a rapid decrease of the

myeloma protein and reached the best reponse after the first cycle and four after the second cycle.
Six patients showed a complete remission of the kidney function (Creatinine clearance > 60 ml/min) and five patients a partial remission (Creatinine clearance > 30 ml/min). Transient grade 3 – 4 neutropenia was reported in one patient, and grade 3 – 4 thrombocytopenia occurred in five patients. One patient had a new grade 3 polyneuropathy.
Summary: These results indicate that the combination of bortezomib, bendamustine and prednisone is effective and tolerated in patients with newly diagnosed/untreated MM with renal failure.

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Dietger Niederwieser: No conflict of interest disclosed.

V821

Association between CD86-I179V polymorphism and response to bortezomib based treatment in multiple myeloma patients

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Introduction: Multiple myeloma (MM) treatment has significantly improved within the last decade. Bortezomib based regimens have replaced the former chemotherapeutic standards VAD (vincristine/adriamycine/dexamethasone) and MP (melphalane/prednisone). Although, response rates of bortezomib are promising, treatment failure is observed in numerous cases. Furthermore, the depth of therapy response after induction therapy has been shown to be of important value for the further course of the disease but observed stringent complete (sCR), complete (CR) and very good partial remission (VGPR) rates lie only between 10 to 50%. Predictive molecular markers for response are currently lacking. Therefore, we aimed to analyze the association between CD86 polymorphisms and occurrence of VGPR/CR/sCR upon bortezomib treatment.
Methods: 105 pts were included in this retrospective analysis. 51 patients were treated with bortezomib +/- dexamethasone and 72 pts received VAD. Of notice, 18 pts received both regimens during the course of the disease. Clinical data were obtained from a prospectively administered data base. Response was evaluated according to the IMWG criteria. All patients signed an informed consent for genetic analyses. DNA was extracted from bone marrow aspirates. Genotyping was performed using RFLP techniques. Primary endpoint was to analyze the association between the CD86-I179V polymorphism and response. Hereby, pts were divided into good responders (VGPR/CR/sCR) and poor responders including partial response, stable and progressive disease.
Results: Median age of the study population was 61 years (range 40 to 79). In median bortezomib was administered in 2nd-line (range 1st to 4th-line) and VAD in 1st-line (range 1st to 3rd-line). VGPR/CR/sCR was observed in 22% of bortezomib treated pts and in 26% of VAD treated pts. CD86-I179V was associated with occurrence of VGPR/CR/sCR with 50% in pts. with at least one variant 179V allele compared to 17% in wildtype 179I/I pts (p=0.059). Multivariate logistic regression analyses identified the presence of the variant CD86-179V allele as an independent predictor of good response with an OR of 7.5 [95%CI (1.1;50.7); p=0.039]. No significant association was observed between in VAD treated pts.
Conclusions: These data suggest that CD86 polymorphisms might be associated with bortezomib efficacy in MM. Further investigation of this gene in translational MM research within larger cohorts is warranted.

Disclosure: No conflict of interest disclosed.

V822

Monoclonal gammopathy of undetermined significance and solid tumor development in the Heinz Nixdorf Recall Study

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Background: Monoclonal gammopathy of undetermined significance (MGUS) increases the risk of lymphoproliferative diseases (LPD). Recent studies also suggest an increased risk of relatives of myeloma and MGUS cases to develop solid tumors. To determine an increased risk of solid tumor development in MGUS cases, we analyzed data of the ongoing epidemiologic Heinz Nixdorf Recall Study.
Methods: The Heinz Nixdorf Recall Study cohort comprises 4814 men and women aged 45-75 from 3 adjacent cities located in the German Ruhr area. Subjects were randomly selected from statutory lists of residence and gave informed consent. MGUS cases were determined from baseline serum samples using standard serum electrophoresis combined with immunofixation electrophoresis employing pentavalent antisera (Hydragel 12 IF, Penta-Kit, Sebia, Germany). Incident cancer diagnoses from death certificates and self-reported from annual questionnaires were validated by reviewing medical records and coded using ICD-10 codes. LPD (ICD-10: C81-C91) and non-melanoma skin cancer (ICD-10: C44) were excluded for this analysis. Poisson regression was used to estimate a risk ratio (RR) of incident solid tumors occurring between baseline and 5-year follow-up with regard to MGUS status at baseline adjusted for sex and age.
Results: 165 MGUS cases were identified at baseline among 4702 screened samples (prevalence 3.5%, 95%-confidence interval (CI) 3.0 – 4.1). Median age of MGUS cases was 63 years (range 47-75), 103 (62%) were male. 351 self-reported solid tumors were documented at baseline in 308 individuals with a median age of 65 years (range 45-75) and 161 (46%) with male gender. 244 validated incident tumors were reported in 234 individuals during 21.586 person-years of follow-up between baseline and 5-year follow-up. The crude RR of solid tumor development during 5-year of follow-up in individuals with MGUS at baseline was 1.90 (95%-confidence interval (CI) 1.06-3.42). The age- and sex-adjusted RR was 1.45 (95%-CI 0.83-2.55).
Conclusion: These results from a prospective cohort study may indicate an increased risk of solid tumor development in MGUS cases. Current analyses focus on differences in the occurrence of specific tumor entities with regard to MGUS status. Together with the continuing follow-up, this will help to further clarify our results.

Disclosure: No conflict of interest disclosed.

Freie Vorträge
Stammzellen I

V823

The F-Box protein NIPA regulates the hematopoietic stem cell pool

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Hematopoietic stem cells (HSCs) are characterized by their ability to self-renewal and multilineage differentiation. Since mostly HSCs exist in a quiescent state re-entry into cell cycle is essential for their regeneration and differentiation and the expression of numerous cell cycle regulators must be tightly controlled. We previously characterized NIPA (Nuclear Interaction Partner of ALK) as a F-Box protein that defines an oscillating ubiquitin E3 ligase targeting nuclear cyclin B1 in interphase thus contributing to the timing of mitotic entry.Using NIPA knockout mice we investigated the effect of NIPA loss on hematopoiesis. Peripheral blood (PB) counts revealed no apparent difference between NIPA-/-

and wildtype (wt) mice. In contrast, FACS analyses of bone marrow (BM) showed significantly decreased numbers of lin-Sca1+cKit+ (LSK) cells in NIPA deficient animals. The absolute LSK number was fourfold lower in NIPA-/- than in wt BM (20.000 vs. 80.000). Moreover the primitive HSC population of CD150+ CD34- cells within LSKs is greatly reduced in NIPA-/- mice compared to control BM (600 vs. 1300). To examine efficient activation of this population to self-renew in response to myeloid depression, we treated mice with the cytotoxic drug (5-FU) four days before BM harvest. As expected, fraction of CD150+CD34-LSKs in wt mice rose four- to fivefold, interestingly NIPA deficient animals failed to compensate to 5-FU depression (p=0.047). Using competitive BM transplantation assays (TX) CD45.2+ NIPA-/- or NIPA+/+ BM cells were mixed with CD45.1+ wt BM cells and transplanted into lethally irradiated CD45.2+ recipient mice. 30 days after TX, FACS analyses of PB showed reduced numbers of NIPA-/- cells in comparison to NIPA+/+ BM recipient mice. This result was even more severe with aging of transplanted mice pointing to a profound defect in repopulation capacity of NIPA deficient HSCs.Taken together our results demonstrate an essential role of NIPA in regulating the HSC pool as a regulator of self-renewal, cycle capacity and HSC expansion.

Disclosure: No conflict of interest disclosed.

V824

Deficiency of the *evi-1* gene in zebrafish strongly impairs embryonic myelopoiesis and hematopoietic stem cell development

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Introduction: The Evi-1 locus was originally identified as a common site of retroviral integration in murine myeloid tumors. Over the last years, Evi-1 evolved as one of the most potent oncogenenes associated with human and murine myeloid leukemia. More recently, involvement of Evi-1 in embryonic hematopoiesis has been shown in knockout mice, yet the precise role of Evi-1 in this context remains elusive.
Methods: Hematopoietic development was analyzed in zebrafish embryos by *in situ* hybridization (ISH) for early hematopoietic markers. Loss-of-function studies were performed by injection of morpholino oligonucleotides inhibiting pre-mRNA splicing. Rescue experiments were performed by co-injection of *gata2* mRNA. Possible interactions of *evi-1* with the NOTCH pathway were analyzed by ISH with several NOTCH pathway components in morphants and DAPT-treated embryos.
Results: We here show that *evi-1* and *scl are* co-expressed in the posterior blood islands at early developmental stages. Morpholino mediated *evi-1* knockdown induces severely reduced numbers of circulating blood cells and hemorrhages. ISH reveals strongly impaired formation of myeloid embryonic cells as well as *runx1+/cmyb+* hematopoietic stem cells (HSC) and *rag1+* lymphoid cells in *evi-1* morphants versus control fish. Co-injection of *gata2* mRNA was able to rescue the impaired myeloid phenotype. Since promoter studies have identified the NOTCH pathway component *jag2* as an *evi-1* target gene, we are investigating possible interactions of *evi-1* with the NOTCH pathway, which has been previously implicated in HSC development. First results show reduced expression of several NOTCH pathway components in *evi-1* morphants versus control fish, while normal *evi-1* expression was detected in mind-bomb and DAPT-treated embryos, suggesting *evi-1* as a possible upstream regulator of NOTCH in this system.
Conclusion: Our data demonstrate that *evi-1* plays a role during zebrafish blood development, regulating embryonic myelopoiesis as well as HSC development through interactions with *gata2* and possibly the NOTCH pathway. Currently ongoing experiments are further functionally exploring the interactions between NOTCH and *evi-1* in the context of myelopoiesis and embryonic HSC development.

Disclosure: No conflict of interest disclosed.

V825

Mobilization mechanisms of human primary precursor-B-ALL cells in an *in vivo* model system by the CXCR4-antagonist AMD3100 and by catecholamines

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Introduction: Leukemia stem cells (LSC), similar to their normal counterparts (HSC), are well protected by adhesion to their niche in the bone marrow. Mobilization of LSC to the circulation might render them vulnerable to anti-leukemia therapy. The aim of this study was to explore mechanisms of leukemia mobilization from the BM with mobilizing agents like AMD3100 (AMD) in a pre-clinical immune deficient mouse model.
Methodology**:** Immunodeficient mice were engrafted with the childhood pre-B-ALL leukemic cell line G2 and with primary childhood precursor-B-ALL cells from 4 patients with up to 100% of transplanted mice being engrafted. Engraftment was without prior irradiation, thereby leading to a more physiological model of human leukemias.
Results: Treatment with AMD lead to a significant mobilization of all transplanted leukemias with a mobilization level of between 3 – 8 times above baseline. Next, we examined the role of SDF-1 release by AMD. It could already be shown, that AMD3100 releases SDF-1 in healthy mice from the bone marrow to the peripheral blood, resulting in progenitor cell mobilization (Dar et al. Leukemia 2011). In the experiments reported here, inhibition of SDF-1 action with neutralizing CXCR4 antibodies abrogated AMD-induced leukemia mobilization. Recently we also demonstrated catecholamine receptor expression on hematopoietic stem and progenitor cells and of mobilization of these cells by catecholamines (Spiegel et al. Nat. Immunol. 2007). We showed now that the G2 cell line and all 4 examined precursor-B-ALL samples express the catecholamine receptors D3, D5 and beta-2. Treatment with high doses of epinephrine alone led to leukemia mobilization *in vivo* similar to AMD treatment. Lower doses of norepinephrine in combination with AMD increased leukemia mobilization up to 20 times above baseline.
Conclusions: We could demonstrate the applicability of an *in vivo* xenotransplantation system of primary human precursor-B-ALL cells for research into leukemia cell mobilization. These leukemic cells can be mobilized efficiently by the CXCR4 antagonist AMD3100 and synergistically by cytecholamines. The AMD-induced mobilization mechanism is most likely via secretion of SDF-1. This mobilization approach could be potentially used for future mobilization protocols of leukemia in combination with established chemotherapy to improve eradication of minimal residual disease of leukemia.

Disclosure: No conflict of interest disclosed.

V826

Regulatory networks of hematopoietic stem cells and their micro-environment

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Hematopoietic stem cells (HSC) are thought to be regulated by extracellular cues from the 'niche,' which trigger downstream signal transduction cascades within the HSC. Current studies have, so far, not resulted in comprehensive understanding of the signaling networks dictating HSC fate.
In the present work, theoretical systems-biology and experimental hematology approaches are combined to determine the role of the niche in orchestrating

HSC epigenetic machinery and the cell cycle. Thus, time-course gene expression analysis of co-cultured Lin-Sca1+cKit+ (LSK) and HSC-supportive UG26-1B6 stromal cells was performed. Microarray results were independently confirmed by RT-qPCR, demonstrating ≥80% agreement for the selected candidate genes. HSC activity and functionality was confirmed by colony forming cell (CFC) assay and in vivo transplantation assay. Search space reduction using clustering analysis suggests that the most intense molecular cross-talk between LSK and stromal cells occurs during the first 24 h of co-culture. In LSK cells, gene function enrichment analysis revealed up-regulation of transcripts associated with cell adhesion and migration, TGFβ signaling, metabolism, as well as MAPK-regulated cell proliferation. A the same time, epigenetic regulators mediating gene silencing were among the down-modulated transcripts. Interestingly, similar analysis in stromal cells demonstrated molecular signatures also involved in cell adhesion, migration and proliferation, as well as Wnt, TGFβ and mTOR signaling.
In both, LSK and stromal cells, among the most significantly up-regulated transcripts was an ECM-associated TGFβ and Wnt signaling intermediate. By integrating gene expression data with various sources of prior knowledge (e.g., PPI and pathway databases, semantic text mining) an in silico hypothesis was generated predicting the putative role of this factor in HSC mobilization by regulating the G0/G1 phase transition of the cell cycle and the epigenetic modifications accompanying it. Current work is focused on the experimental validation and further refinement of the network.

Disclosure: No conflict of interest disclosed.

V827

Leukemia stem cell surogates of patients with ALL are sensitive towards treatment with the novel agent TRAIL

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Tumor necrosis factor (TNF)-related apoptosis-inducing ligand (TRAIL) is a member of the TNF family and a promising future cytotoxic drug for cancer treatment. Here, we studied the effect of TRAIL on patient-derived acute lymphoblastic leukemia (ALL) stem cell surrogates and its therapeutic effect in an *in vivo* ALL-xenograft model.
Frequency of leukemia-initiating cells (LICs) was determined by repopulation assays with limiting dilution analysis. Fresh xenograft ALL-cells were treated with and without TRAIL *in vitro* and were transplanted into 25 mice per treatment group in different absolute cell numbers. After 3 months, engraftment was determined in all mice by FACS and immunohistochemistry of bone marrow, spleen and liver, and LIC frequencies were calculated. Preclinical *in vivo* experiments were performed treating mice injected with patient-derived ALL-cells.
Limiting dilution assay showed that in vitro incubation with TRAIL resulted in a significant decrease in frequency of LICs in all 3 ALL primary samples tested, corresponding to an induction of 75 up to more than 99% apoptosis in these cells.
In vivo, TRAIL treatment significantly delayed the onset of leukemia, prolonged the survival of mice engrafted with patient-derived ALL cells and even cured a subset of mice from leukemia.
Our data show that TRAIL can highly reduce engraftment rates and thus induce apoptosis in ALL-stem cell surrogates. Furthermore, when TRAIL was given to mice injected with ALL cells, it could significantly delay the onset of disease and prolong survival. TRAIL might therefore represent an interesting molecule for treatment of ALL.

Disclosure: No conflict of interest disclosed.

V828

MCAM/CD146 expression on human mesenchymal stromal cells influences proliferation and maintenance of hematopoietic stem and progenitor cells during ex vivo expansion

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Multipotent human mesenchymal stromal cells (hMSC) are known to support the growth of hematopoietic stem and progenitor cells (HSPC) during ex vivo co-culture. Besides various growth factors and chemokines secreted by MSC, the direct cellular interaction of MSC and HSPC via adhesion molecules and membrane-bound growth factors has been shown to be of relevance for the in-vitro supportiveness of MSC. During co-culture, the direct invasion of HSPC underneath the MSC monolayer results in the formation of so called 'cobblestone' areas detectable in-phase contrast microscopy. Recently, MCAM (Melanoma Cell Adhesion Molecule/CD146) was introduced as a novel marker for MSC defining them as osteoprogenitor cells with enhanced self-renewal properties and the capacity to support HSPC (Sacchetti et al., 2007).
In this study, we investigated the impact of MCAM expression on hMSC on the proliferation and maintenance of HSPC in co-culture during ex vivo expansion. Therefore, we established primary hMSC stably expressing shRNA targeting MCAM or overexpressing MCAM using lentiviral gene transfer. Hematopoietic support was examined by co-culture of CD34+ hematopoietic stem cells with MCAM-depleted or MCAM-overexpressing hMSC, respectively.
After 7 days of ex vivo expansion, CD34+ HSPC in co-culture with MCAM-overexpressing hMSC as feeder layer maintained a primitive immunophenotype (CD34+, CD133+), whereas CD34+ cells kept on a MCAM-depleted monolayer showed strongly decreased CD34 and CD133 expression compared to CD34+ HSPC kept on control monolayer. Furthermore, co-culture with MCAM-depleted hMSC resulted in enhanced proliferation of HSPC, as monitored by CFSE staining, whereas HSPC on MCAM-overexpressing hMSC showed less numbers of cell divisions. Long-term culture-initiating cell assay (LTC-IC) with MCAM-overexpressing hMSC as feeder layer displayed a higher number of cobblestone-area forming cell (CAFC) and also an increased number of secondary colony-forming cells (CFC). In concurrence, MCAM-depleted hMSC enabled much lower CAFC and secondary colony formation.
In conclusion, these results indicate that MCAM expression plays an important role in regulating HSPC proliferation and maintenance not only in-vivo but also during ex vivo expansion.

Disclosure: No conflict of interest disclosed.

Posterdiskussion
Kolon-/Rektumkarzinom

P829

Common single nucleotide polymorphisms in the genes of integrin α-2 and β-3 subunits are not associated with overall survival in rectal cancer patients

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Introduction: Integrin receptors are heterodimeric plasma membrane proteins which consist of α and β subunits. 18 α- and 8 β-subunits combine to at least 25 integral proteins which mediate the attachment and interaction of cells to

the extracellular matrix (ECM) and to other cells as well as signal transduction from the ECM to the cell. Therefore, they have been suggested as central players for tumor development and progression. Several polymorphisms, two in integrin α 2 (ITGA2 807C>T and 1648G>A) and one in β 3 (ITGB3 176T>C), affect the levels, structure and possibly the function of these integral membrane proteins. In this study we tried to analyze the role of the genetic variants in these integrins for overall survival in rectal cancer.
Methods: 159 patients with histologically confirmed rectal cancer were included in this investigation. Genomic DNA was extracted from peripheral blood and ITGA2 and ITGB3 polymorphisms were analyzed by a fluorogenic exonculease assay (TaqMan™) and analyzed as a scatter plot.
Results: 5 patients had to be excluded because of missing values. From the remaining 154 patients, 35 (23%) died during a follow-up of maximum 10 years (Mean follow-up time 58 ± 34 months, median 55 months). In a cox regression model including the different integrin genotypes, age at diagnosis, tumor grade, tumor size, stage according to the AJCC, number of lymphnodes evaluated, number of pathologic lymph nodes and administration of adjuvant 5-FU containing chemotherapy we were not able to detect a correlation between these polymorphisms and overall survival. The relative risk (RR) for the ITGA2 807C>T SNP was 1.045 (95% CI: 0.736-1.483, p = 0.807), for the ITGA2 1648G>A SNP 1.182 (95% CI: 0.628-2.223, p = 0.604) and for the ITGB3 176T>C SNP 0.764 (95% CI: 0.477-1.222, p = 0.261).
Conclusions: Polymorphic genetic variations of integrins are not associated with overall survival in caucasian rectal cancer patients.

Disclosure: No conflict of interest disclosed.

P830

Exploring the cost-effectiveness of targeted therapy with cetuximab vs bevacizumab in Germany in patients with KRAS wild-type colorectal cancer presenting with initially unresectable metastases limited to the liver

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Introduction: The role of targeted therapies in colorectal cancer has recently been discussed widely. In patients with metastases limited to the liver (LLD), effective therapies with monoclonal antibodies as add-on to chemotherapy may facilitate metastasis resection and improve long-term survival. This present analysis explores the cost-effectiveness of bevacizumab and cetuximab in the treatment of patients with colorectal cancer presenting with initially unresectable liver metastases of the Kirsten rat sarcoma viral oncogene homolog (KRAS) wild type, from the perspective of the German statutory health insurance.
Methods: The health economic analysis applies an indirect comparison incorporating publicly available data on bevacizumab and cetuximab treatment outcomes using evidence synthesis techniques. In a second step, the data is extrapolated from the follow-up duration of the clinical trials to a longer time horizon of up to 10 years. As a result, costs and health outcomes are then based on the modeled patient pathways. Delphi panel methods were used for some assumptions when evidence was missing. Probabilistic sensitivity analyses and extensive scenario analyses were applied to test for uncertainty around input parameters and assumptions.
Results: For the metastatic colorectal cancer LLD population with KRAS wild-type genotype, mean estimated overall survival was 37.7 months for first-line treatment with cetuximab plus FOLFIRI and 30.4 months for bevacizumab plus FOLFOX. Corresponding discounted survival estimates were 2.88 life-years with cetuximab plus FOLFIRI versus 2.38 life-years with bevacizumab plus FOLFOX, yielding an average gain of 0.50 discounted life-years for cetuximab plus FOLFIRI?. The incremental cost-effectiveness ratio of cetuximab plus FOLFIRI versus bevacizumab plus FOLFOX was €15,020 per life-year gained in the base case (with a 95% confidence interval from the probabilistic sensitivity analysis of €3,806-24,660). Results were robust in different scenario analyses as well as in the probabilistic sensitivity analysis.
Conclusions: First-line treatment with cetuximab plus FOLFIRI offers a cost-effective treatment option versus bevacizumab plus FOLFOX for the mCRC LLD population with KRAS wild-type genotype in Germany. Hence, KRAS testing should be performed on all presenting cases of metastatic colorectal cancer to ensure access to this treatment option.

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Thomas Mittendorf: Expert Testimony: Die Studie wurde von Merck Serono GmbH, Darmstadt, gesponsert. Die Forschungsförderung war nicht beschränkt und bezog sich auf die Studiendurchführung und die Erstellung des Posters.

P831

Selective PI3K inhibition in wild type and *PIK3CA* mutated human gastrointestinal cancer

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Introduction: New targeted agents like antibodies or small molecules against tyrosine kinases clearly expanded the standard therapy in oncology. However, tumor resistance is still a challenge, particularly often induced by mutations in growth-related signalling cascades. 20% and 10% of all patients with human colorectal and gastric cancer carry PI3K mutations, respectively and do not react to receptor blocking therapies. Recently, selective tyrosine kinase inhibitors have been generated which block the PI3K signalling pathway in tumor cells. So far, their therapeutical role for human gastrointestinal cancers has not yet been clarified.
Methods: To define the inhibitory and pro-apoptotic effects of the two PI3K inhibitors BEZ235 and BKM120 three human colon cancer (HT-29, HCT-116, DLD-1) and three gastric cancer cell lines (NCI-n87, AGS, MKN-45) with different mutation status of the *PIK3CA* were used. First, viability, apoptosis and caspase assays were performed during incubation with the inhibitors alone or combined with different cytotoxic agents. Second, molecular consequences for cell cycle and the signalling pathways were analysed by defining the protein levels by FACS and Western blot.
Results: Both PI3K inhibitors BEZ235 and BKM120 induced concentration dependently a significant reduction in viability and an increase in apoptotic cell death, while the mutated cells reacted more sensitive to the treatment. BKM120 had a higher efficiency than the dual PI3K/mTOR inhibitor BEZ235. In addition, the single agent BEZ235 caused a G1 arrest in tumor cells. In contrast, BKM120 induced a G2 shift in all gastrointestinal cancer cell lines. There was a clearly down regulation in the protein levels of the AKT pathway and for BEZ235 an additional inhibition of the mTOR (via p70S6K) pathway. Furthermore, BEZ235 caused synergistic induction of apoptosis combined with irinotecan in colon cancer cells. Combinations with 5-fluoruracil and the two substances induced additive apoptotic effects. Human gastric cancer cells were less sensitive to BEZ235 and BKM120.
Conclusion: In general, we found higher pro-apoptotic effects for all cell lines and in special cases a better response of restistant mutant cells. Our data support the clinical development of these PI3K inhibitors BEZ235 and BKM120 as potential targeting agents for patients with different wild type or mutated gastrointestinal cancer cells.

Disclosure: No conflict of interest disclosed.

P832

Influence of KRAS status of colorectal cancer liver metastases in patients receiving neoadjuvant chemotherapy including Bevacizumab prior liver resection

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Introduction: The prognostic value of KRAS and BRAF mutation on recurrence-free survival in patients with colorectal cancer liver metastases (CLM) receiving neoadjuvant chemotherapy including bevacizumab prior liver resection is unclear.
Methods: KRAS and BRAF status of resected CLM (2005-2010) from 56 resectable patients of 3 prospective studies investigating neoadjuvant chemotherapy including Bevacizumab was retrospectively assessed. Mutations were correlated to recurrence-free and overall survival. Only patients with remaining vital tumor cells in the resected specimens and those without progression were analysed (PD excluded from resection, overall < 5%). Patients received 3 neoadjuvant and 3 adjuvant cycles of Oxaliplatin-containing regimens.
Results: Of 56 patients, 13 showed KRAS mutation (mt), whereas BRAF mutation was not detected in a single patient. Radiological response assessment according to RECIST to neoadjuvant chemotherapy including Bevacizumab revealed partial response (PR) in 49 and stable disease (SD) in 7 patients. Median Fong score of all 56 resected patients was 3 (range, 1-5). Although responses were improved compared to historicals, KRAS mt patients were less likely to have PR than patients with KRAS wild-type (wt) tumors but was not statistically significant (p=0.335). Recurrence-free survival trended to be better in KRAS wt patients (1-year 51.2%, 3-years 33.9%, 4-years 29%) compared to mt patients (1-year 38.5%, 3-years 15.4%, 4-years 15.4%), but this was also not statistically significant (log rank, p=0.093). Overall survival was significantly better in KRAS wt patients (1-year 97.4%, 3-years 88.6%, 4-years 71.8%) compared to mt (1-year 100%, 3-years 43.3%, 4-years 43.3%) (log rank, p=0.034).
Conclusions: The addition of Bevacizumab to Oxaliplatin-based chemotherapy improved response rates irrespective of KRAS status. Primary high response to Oxaliplatin-based chemotherapy including Bevacizumab is especially desirable in mt patients as an anti-EGFR-targeted therapy cannot be alternatively offered which seems to be correlated with a prolongation of the overall survival.

Disclosure: No conflict of interest disclosed.

P833

Pattern of human epidermal growth factor receptor 2 (HER2) expression might predict response to treatment with trastuzumab. Report of a case with colorectal cancer (CRC)

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Introduction: Overexpression as a result of gene amplification of HER2 occurs in several epithelial malignancies, with a reported rate of 3-6% in CRC. Overexpression of HER2 might theoretically render CRC patients eligible for treatment with trastuzumab (T), a monoclonal anti HER2 antibody. However, HER2 expression is mostly heterogenous within CRC as measured by immunohistochemistry (IHC) or amplification by fluorescence in situ hybridization (FISH). Furthermore, HER2 expression levels might differ between primary tumor (PT), local lymph node or distant metastases.
Case report: A 69-year-old male subject was diagnosed with adenocarcinoma of the colon and synchronous pulmonary metastases in November 2006. After surgery for PT systemic treatment with FOLFIRI and bevacizumab (bev) was administered for 6 months, resulting in partial response (PR), followed by maintenance with capecitabine for 11 months and two-staged resection of pulmonary metastases in September/November 2008. Upon pulmonary recurrence in May 2009 FOLFIRI with bev was restarted, resulting again in PR after 2 months of treatment. Following complete treatment discontinuation for 2 months (because of hyperthyroidism-induced atrial fibrillation) disease progressed and 5FU/LV combined with bev was resumed in November 2009 with progressive disease diagnosed after 6 months of treatment. At this point, further molecular analyses were performed in order to determine possible salvage treatments. KRAS-sequencing revealed wild type. FISH analysis revealed high-level HER2 amplification in PT, lymph node and pulmonary metastases. To rule out HER2 heterogeneity, all tissue blocks with tumor (n=12) were stained by IHC (HercepTest, DAKO) revealing an exceptionally homogenous HER2 positivity (3+). During two months of paused systemic therapy pulmonary metastases progressed, without new lesions or local/general symptoms. After obtaining fully informed consent weekly treatment with T alone was initiated in August 2010. After 3 months of T with no related adverse events, besides cytokine release syndrome during loading dose, PET/CT revealed PR. Treatment with single agent T is currently ongoing.
Conclusion: This case suggests that a homogenous pattern of HER2 positivity might predict response to single agent T, likely not limited to CRC.

Disclosure: Alexander Stein: No conflict of interest disclosed.
Carsten Bokemeyer: Expert Testimony: Roche.

P834

Overcoming cetuximab resistance in *KRAS* mutant colorectal carcinoma through HSP90 inhibition

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Elevated epidermal growth factor receptor (EGFR) expression is present in 60-80% of metastatic colorectal carcinomas (CRC) and determines poor prognosis. EGFR represents a validated therapeutic target; however, response rates to cetuximab, an anti-EGFR antibody, remain low in unselected patients. *KRAS* mutations occur in ~30% of CRC and predict for resistance to anti-EGFR therapy. Due to its function in stabilizing multiple proteins that promote cancer cell survival, including known RAS effectors such as components of the PI3K-AKT and MAPK pathways, the chaperone HSP90 has emerged as promising cancer drug target, and several HSP90 inhibitors are currently under clinical investigation. Recent preclinical studies have suggested that *KRAS* mutant (*KRAS*mut) lung cancer cells are particularly sensitive to HSP90 inhibition, but it is unknown whether this observation can be extrapolated to other cancers. In this study, we evaluated whether HSP90 inhibition can overcome cetuximab resistance in human CRC cells. We first treated a panel of CRC cell lines with increasing cetuximab concentrations. Cetuximab reduced cell viability and proliferation in *KRAS* wildtype (*KRAS*wt) cell lines that express EGFR (IC50, 500-1500 nM), whereas *KRAS*mut HCT-116 cells were unaffected with concentrations up to 1500 nM, mirroring previous observations in patients. Treatment of *KRAS*mut HCT-116 cells and *KRAS*wt Caco-2 cells with PU-H71, an optimized, water-soluble HSP90 inhibitor, demonstrated selective sensitivity of HCT-116 cells, as determined by MTS assay (IC50, 50 nM). Incubation of HCT-116 cells with both cetuximab and PU-H71 had an additive effect. Depletion of two known RAS effectors, AKT1 and RAF1, required high PU-H71 concentrations (>100 nM), indicating that the genotype-selective effect of PU-H71 may be accounted for by degradation of other HSP90 clients. Together, these data show that (1) *KRAS*wt CRC cell lines are sensitive to anti-EGFR treatment in vitro; (2) HSP90 inhibitors are preferentially toxic to *KRAS*mut CRC cancer cell lines; and (3) addition of PU-H71 may sensitize *KRAS*mut CRC cancer cells to cetuximab treatment. The mechanism underlying the sensitivity of *KRAS*mut cancer cells to HSP90 inhibition requires further investigation.

Disclosure: No conflict of interest disclosed.

P835

Stem cell marker cancer testis antigen (CT 45) expression in colorectal cancer: An immunhistochemical study of 704 patients

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Introduction: A subset of colorectal cancer derived from stem cells. Cancer testis antigen (CT45) is expressed in immature gonocytes, spermatogonia and also in germ cell tumors. There are only limited data about CT 45 expression in colorectal cancer and no data if the expression in tumor cells has an influence on the clinical course of the disease.
Methods: We investigated immunohistochemically embedded tissue from 704 patients with a monoclonal antibody against CT 45 (Ki-A10). The percentage of CT45 expressing cells was quantified according to the following classification: negative, 0 to 25%, 25 to 50%, 50 to 75% and more than 75% positive tumor cells.
Results: The majority of cases were negative (n=633). 71 tumor specimens were CT45 positive. The Kaplan Meier analysis revealed no significant difference (p=0.11) in the clinical outcome of CT 45 positive and negative cases.
Conclusions: Cancer testis antigen (CT 45) is expressed in a subset of colorectal cancer. The CT 45 expression is no prognostic factor for the clinical outcome of the disease, but it might have clinical implication for therapy decision of stem cell derived cancer.

Disclosure: No conflict of interest disclosed.

P836

Proliferation associated protein expression of Ki-67 and Repp 86 in colorectal cancer: High Ki-67 index is prognostic factor for clinical outcome

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Introduction: Proliferation indices are important prognostic factors in for the clinical outcome of patients with cancer. The clinical relevance of Ki-67 Expression in colorectal cancer is discussed controversial in the literature. We investigated whether the expression of Ki-67 and repp86 (restrictedly expressed proliferation-associated protein 86 kDa), a new proliferation specific marker expressed in cell cycle phases G2, S, and M, but not in G1, correlates to with the clinical course in patients with colorectal cancer.
Methods: Biopsy specimens from 359 untreated patients (184 men, 175 women) with colorectal cancer were investigated immunohistochemically with the monoclonal antibody against Ki-67 (Ki-S5) and repp86 (Ki-S2).
Results: Ki-67 expression had a range from 9.4% to 94.2% with a median of 57% and a mean of 57%. Patients with >50% Ki-67 expression had a median overall survival time that was undefined, compared to with 43 months for patients with < 50% positive cells. Kaplan-Meier analysis revealed a significant difference in the overall survival time between patients with very high (>50%) and low (< 50%) Ki-67 expression (p< 0.0001) in the tumor cells. Repp 86 expression had a range of 7.6% to61% with a median of 29% and a mean of 29%. Kaplan-Meier analysis showed no significant difference in the overall survival time between patients with high or low Repp86 expression.
Conclusions: Based on these findings, high expression of Ki-67 is a positive prognostic factor in patients with colorectal cancer.

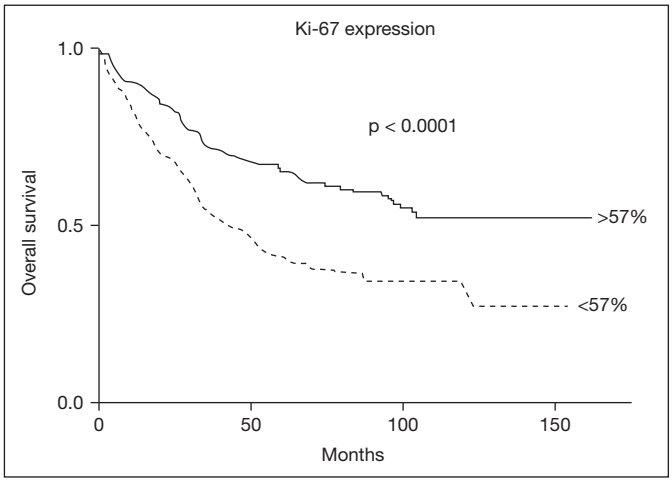

Fig. 1. Ki-67 OS

Disclosure: No conflict of interest disclosed.

P837

Minichromosome maintenance protein 6 (MCM 6) expression in colorectal cancer: A proliferationmarker and a prognostic factor for clinical outcome

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Introduction: MCM6 is one of six proteins of the MCM family which are involved in the initiation of DNA replication and is a marker of proliferating cells. Proliferation markers and their expression levels as prognostic factors are discussed controversial in colorectal cancer.
Methods: We investigated paraffin embedded tissue from 570 patients with colorectal cancer with stage 1 to 4 immunohistochemically with a monoclonal antibody against MCM6. 500 tumor cells were counted and the percentage of positive cells was calculated. Overall survival time was calculated from the date of diagnosis until death or loss to follow-up evaluation. Univariate survival analysis was computed by means of the Kaplan-Meier method and significance levels were assessed by means of the log-rank test.
Results: The percentage of MCM6 expressing tumor cells ranged from 27,6% to 97.0%, with a mean of 82.8%. A high MCM6 expression level of more than 80% positive cells was associated with a significantly longer overall survival time (130,5 months) compared to patients with a low MCM6 expression level of less than 80% (58,7 months, p=0.0016).
Conclusions: Immunohistochemical detection of MCM6 seems to be a promising marker for predicting the outcome in patients with colorectal cancer.

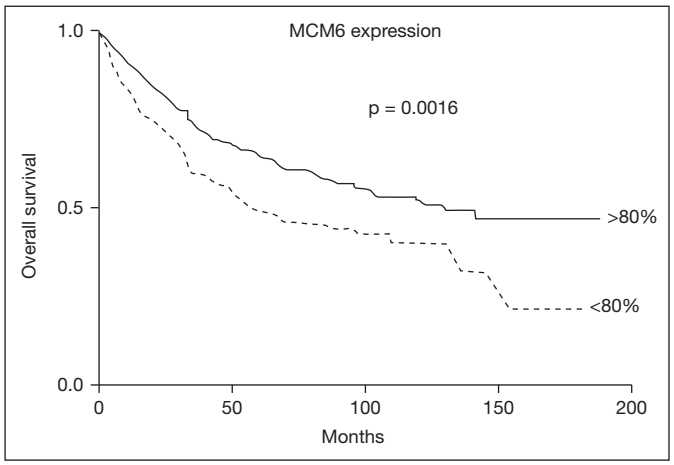

Fig. 1. MCM 6 OS

Disclosure: No conflict of interest disclosed.

P838

Topoisomerase II alpha expression in colorectal cancer: A clinicopathological investigation of 430 tumor specimens

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Introduction: Topoisomerase IIα is an enzyme that is needed whenever uncoiling of DNA is necessary during the cell cycle. The enzyme is a marker of cell proliferation. We analyzed the expression of topo IIα in relation to the clinical outcome in patients with colorectal cancer.
Methods: Biopsy specimens from 430 untreated patients were investigated immunohistochemically with monoclonal antibodies against topo IIα (Ki-S4).

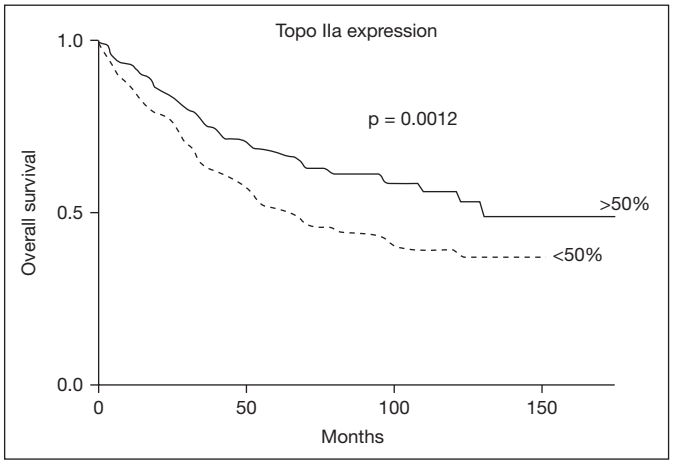

Fig. 1. Topo OS

Results: Patients with low (< 50%) topo IIα expression had a median overall survival time of 66.1 months, compared to 130.5 months for patients with high (>50%) topo IIα expression. The Kaplan-Meier analysis showed a significant difference in the overall survival time related to the percentage of topo IIα (p= 0.0012) positive tumor cells.
Conclusions: Patient with colorectal cancer and high expression level of topo IIα have a superior clinical outcome compared to patients with low expression levels.

Disclosure: No conflict of interest disclosed.

P839

Vanishing bile duct syndrom in a colon cancer patient after 2 months treatment with Capecitabine and Bevacizumab

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Introduction: Vanishing bile duct syndrome is characterised by selective damage of intrahepatic bile ducts by either toxic agents, circulation deficits, autoimmune or idiopathic reasons or hereditary disorders. It may result in restitutio ad integrum or persisting liver damage or even liver failure.
Methods: We report a case of a patient suffering from recurrent colon cancer with abdominal lymph nodes who developed vanishing bile duct syndrome under therapy with Capecitabine and Bevacizumab. Preexisting long term comedikation of the patient included Ramipril, Allopurinol and Metoprolol.
Results: From the given comedication, single cases have been reported for medications with Allopurinol and Ramipril but not for Metoprolol. Capecitabine can cause hyperbilirubinemia and elevation of liver enzymes, and Bevacizumab can cause arterial thromboembolism in about 3,8% of patients which could result in selective bile duct damage since intrahepatic bile ducts have a blood supply predominantly by the hepatic arteries. Thus, the definitive cause of the patients liver failure remains unclear, but a toxic side effect of the medication seems probable. Patient developed septicemia in the course of biliary damage and died. No autopsy was perfomed. Autoimmune disease and viral hepatitis were excluded.
Conclusion: This case reports a rare but possibly deadly complication of therapy in a patient with colorectal cancer. With Allopurinol, Ramipril, Capecitabine and Bevacizumab as possibly related agents, a clear identification of the causative agent could not be done, and the event may also be provoked by cumulativ toxicity of several agents.

Disclosure: No conflict of interest disclosed.

P840

Does baseline level or response of carcinoembryonic antigen (CEA) predict survival in patients with metastatic colorectal cancer (CRC) treated with chemotherapy and bevacizumab?

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Introduction: Elevated serum CEA level is a poor prognostic factor for both early stage and metastatic colorectal cancer. CEA monitoring is recommended after curative resection to ensure early detection of recurrence and therefore a timely potential secondary curative approach. Furthermore, the American Society of Clinical Oncology currently recommends CEA for monitoring response to chemotherapy in the 2006 guidelines for the use of tumour markers in gastrointestinal cancers. About 20-25% of patients (pts) have normal CEA levels at baseline (BL) with the majority remaining normal during their course of disease. For pts with elevated CEA levels at BL several patterns are described e.g. decrease, increase, or flare. Data about use or kinetics of tumour markers in case of treatment with targeted agents are rare.
Methods: Pts treated within a community-based observational cohort study of various chemotherapy regimens with bevacizumab in Germany (Arnold et al. ASCO-GI 2010) were analyzed for correlation between CEA BL levels or response, defined as >30% decrease compared to baseline within 8-12 weeks of treatment, and progression free (PFS) or overall survival (OS).
Results: CEA levels were available from 1299 out of 1608 pts. Pts with BL CEA levels >20ng/ml (n=665; 51%) showed a significantly lower PFS (median: 9.6 vs 11.1 months; p< 0.0001) and OS (median 22.4 vs 29.9 months; p< 0.0001) compared to pts with BL CEA levels ≤ 20ng/ml (n=634). CEA response information was available in 548 pts: CEA response (>30% decrease from BL) throughout treatment was observed in 34% (n=188) of pts, and was not predictive for improved PFS or OS compared to non-responders (n=360).
Conclusion: Whereas baseline CEA level using a cut off value of 20ng/ml has a strong impact on pts prognosis, CEA response after 8-12 weeks is not a useful tool for early prediction of survival in pts with metastatic CRC treated with bevacizumab and chemotherapy.

Disclosure: Alexander Stein: No conflict of interest disclosed.
Dirk Arnold: Financing of Scientific Research: Roche; Expert Testimony: Roche.

P841

Prognostic impact of carcino embryonic antigen (CEA), carbohydrate antigen (CA 19-9), and lactate dehydrogenase (LDH) decrease in patients with metastatic colorectal cancer (mCRC) receiving a bevacizumab- or cetuximab-chemotherapy combination

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Introduction: CEA and CA 19-9 correlate with prognosis of mCRC patients (pts) with chemotherapy (chemo). LDH may reflect tumor burden and proliferation. Information from early decrease of latter parameters may provide information on successful treatment. However, no accepted thresholds are defined for percentage of decrease, and for the impact of decrease on time-to-event parameters like progression free survival (PFS) and overall survival (OS).
Methods: Medical records of 120 pts set with mCRC and treated with chemo plus bev and/or cet in a single institution were analyzed: Thresholds for CEA, CA 19-9, LDH response was defined as percentage of decrease which was seen for the "best" 35 % of patients after 6-9 weeks of treatment. According to those criteria, correlation of responding patients with progression free (PFS) or overall survival (OS) was examined using the Kaplan Meier method and compared by the log-rank test.
Results: The number of pts in the subgroups and response criteria are displayed in table 1: Distribution of marker decrease differs between cet and bev containing regimen. A CEA decrease threshold of ≥ 30 % correlated with better PFS (median 8.1 vs. 3.4 months (mth); p=0.007) and OS (21.6 vs. 10.4 mth, p= 0.045) with bev. With cet, response threshold for CEA was 45 %, and no correlation with either PFS or OS was observed. CA 19-9 response thresholds were 10 % and 40 % with bev and cet, respectively. Decrease showed a trend towards better PFS with cet, whereas no correlation was observed with bev. LDH did not present a correlation between decrease and improved PFS or OS.
Conclusion: CA 19-9 and LDH decrease seem not to have any prognostic value. Whereas a CEA decrease of ≥ 30 % after 6-9 weeks of treatment may represent an early marker for a therapeutic benefit in bev containing combinations. However, heterogeneity of chosen regimen and treatment lines may have impacted. Prospective investigations with a larger sample size are mandatory to confirm the displayed hypotheses.

Disclosure: No conflict of interest disclosed.

Table 1. (for Abstract P841)

	CEA		CA 19-9		LDH	
Total no. of pts. with elevation	73		61		79	
mab	Bev	Cet	Bev	Cet	Bev	Cet
no. of pts.	35	47	29	40	40	48
Response criteria: decrease >= %	30	45	10	40	25	35
Cases with response / no response	16 / 36	19 / 39	12 / 27	16 /32	17 / 38	13 / 40

P842

Comparison of Hypoxia Inducible Factor 1α (HIF-1α) levels in HCT-15 cell line and Human Colon Cancer Stem Cell-like Cells

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Backround: Hypoxia Inducible Factors (HIFs) are transcription factors that respond to decrease of oxygen level in the cellular environment. Three types of HIFs are known, HIF-1, HIF-2 and HIF-3. All HIFs are heterodimers consisted of α and β subunits. HIF signaling plays a central role in angiogenesis and in the regulation of human metabolism and HIF-1 specifically is responsible for cellular and systemic responses to hypoxia. HIF-1 consists of an α and a β subunit. HIF-1α is oxygen dependent and HIF-1β a constitutively-expressed aryl hydrocarbon receptor nuclear translocator (ARNT). The aim of this study was to quantify and correlate the HIF-1α levels between Human Colon Cancer Cells and Human Colon Cancer Stem Cell-like Cells (Colon CSCs) growing in normal oxygen concentration and hypoxia.
Materials and Methods: Growth curves were generated for three cultures, HCT-15, Colon CSCs growing at physiological O_2 concentration and Colon CSCs growing at absence of O_2. HIF-1α levels were quantified with both flow cytometry and Real Time PCR.
Results: Data were drawn and correlated between the three cultures. HIF-1α levels were higher in Colon CSCs than in HCT-15, both growing at physiological O_2 concentration. The HIF-1α levels of Colon CSCs growing at absence of O_2 were significantly higher from the other two cultures as it was expected.
Conclusion: HIF-1 is expressed in most of the oxygen breathing animals and is responsible for cellular and systemic responses to hypoxia. It consists of two subunits (α and β). The α subunit is oxygen dependent and was quantified in HCT-15 cells and in Colon CSCs to investigate the percentage of metabolic changes that hypoxia promotes in Colon CSCs. The results indicate that Colon CSCs, which express higher levels of HIF-1α, have a higher angiogenic and metabolic profile than Colon Cancer Cells.

Disclosure: No conflict of interest disclosed.

Posterdiskussion
Sarkome / Melanom

P843

Rehabilitation of 121 patients with melanoma. Experiences of the Reha-Klinik Bad Salzelmen

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Introduction: Management of cutaneous melanoma includes surgery and adjuvant therapies. Although the side effects of therapy can often be managed with appropriate supportive care, some patients need rehabilitation therapy because of somatic and/or psychological disturbances.
Methods: Retrospektive evaluation of signs, symptoms and outcome of patients with cutaneous melanoma treated at the Reha-Klinik Bad Salzelmen.
Results: 121 patients were treated between 2003 and 2010. The majority of patients (97%) had constitutional symptoms like fatigue, deconditioning, weight loss, or fever. 81% of the patients had psychological problems like depression, anxiety disorders or sleep disorders. 72 % of the patients had local problems in the area of surgery like lymphedema, pain, paresthesia, wound dehiscence or wound infection. Rehabilitation therapy improved most of the symptoms.
Conclusions: Rehabilitation therapy may be a helpful addition to primary therapy in selected patients with cutaneous melanoma.

Disclosure: No conflict of interest disclosed.

P844

Long term stabilisation of metastatic uveal melanoma with single agent bendamustine. A case report

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Introduction: Advanced uveal melanoma is a disease medically difficult to treat. Few drugs are considered to be active. Bendamustine, a classic, bifunctional alkylating agent has been regarded as inactive, according to a former phase 2 study in which all out of 11 patients showed progressive disease (Schmidt-Hieber, 2004). Other substances with comparable mode of action and chemical relationship are accepted as standards (dacarbacine, fotemustine), but, the remission rate is commonly low.
Methods: Case report: A 50 years old man was diagnosed with uveal melanoma of the right eye in 2006. The primary treatment was Ru brachytherapy. In 06/2009, he suffered from a relapse with metastases to both lungs. A subsequent bilateral pulmonary metastasectomy was performed in 04 and 06/2008, respectively. In second relapse in 01/2009, 2 cycles of cisplatin and dacarbazin were applied without objecive change but with very poor tolerance and therefore early termination. Due to further pulmonary progression, cytostatic treatment with bendamustine (120 mg/m² over 2 days, every 3 to 4 weeks) was initiated with acceptable toxicity profile over 10 cycles from 06/2009 to 02/2010 without any change (stable disease) over ten months. He finally died from fulminant progression due to acute hepatic and renal failure and lower venous obstruction syndrome in 05/2010.
Conclusions: Bendamustine is able to give clinical benefit to patients with otherwise pretreated and advanced metastatic uveal melanoma without producing measurable remission but with durable stabilisation. So, the benefit should be considered not only with respect to graphic control or remission, but to tolerability and stabilisation.

Disclosure: No conflict of interest disclosed.

P845

Genetic differences in preoperative specimen of osteosarcoma and their impact on response to neoadjuvant chemotherapy

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Introduction: Osteosarcomas are rare malignancies but the most common primary malignant tumors of the bone. They are characterized by the production of osteoid or immature bone. Before the introduction of chemotherapy, 80 to 90 % of patients with osteosarcoma developed metastases and died of their disease. Therefore, today all patients with osteosarcoma are treated with adjuvant chemotherapy, most receive this treatment modality in the preoperative period. Unfortunately, not all of the patients respond to neoadjuvant chemotherapy. In this analysis, we tried to search for pretherapeutic genetic differences in tumor tissue that could predict response to neoadjuvant chemotherapy (NCT).
Methods: 10 patients with histological confirmed osteosarcoma were included in this retrospective study. All underwent a standardized preoperative chemotherapy following the COSS-96 protocol. DNA was extracted from the pretherapeutic tumor biopsy via laser microdissection (LMPC). Response to chemotherapy was determined histologically from the definite surgical preparation by using the regression grading from Salzer-Kuntschnik. 5 patients had a good response to NCT (regression grade 2), the other 5 had very poor response (regression grade 5) to NCT. The extracted tumor-DNA was hybridized on 44K Oligonucleotide Arrays (Agilent). This array is able to detect loss or increase of DNA from 100 kb upward which facilitates the specification of possible involved genes
Results: All tumors that responded better to NCT (regression grade 2) showed a large genomic gain on the short arm of chromosome 12 compared to the

tumors with regression grade 5 as well as the 5 controls from healthy bone, that were tested, as well.
Conclusions: It seems that there exists genetic differences between osteosarcomas which could lead to different response to NCT but these preliminary findings have to be confirmed in a larger population.

Disclosure: No conflict of interest disclosed.

P846
MMP-9 supplied by bone marrow-derived cells does not contribute to melanoma lung metastasis

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Melanoma has a high probability of metastasizing to the lung. The matrix metalloproteinases (MMPs) are a family of proteolytic enzymes including more than24 human MMPs, that contain a zinc ion in the active site. MMPs function in physiological and pathological processes including migration, angiogenesis, and tissue repair, as well as metastasis and tumor progression. Up to now it is unclear whether MMP-9 secreted from infiltrating bone-marrow derived cells or resident cells in the lung contribute to pulmonal melanoma metastasis. Using a tail vein injection experimental lung metastasis model, MMP-9 knockout mice demonstrated a more than two-fold decrease in melanoma lung metastasis compared to wild type mice following injection with B16F10 syngeneic melanoma cells. There constitution of the bone marrow of MMP-9 knockout mice with bone marrow competent to produce MMP-9 did not recapitulate the WT phenotype of overwhelming burden of pulmonal metastasis. In contrast, wilde type mice reconstituted with the bone marrow of MMP-9 knockout mice displayed the same burden of pulmonal metastatic disease as the WT phenotype. Hence, these results demonstrate that rather stromal derived MMP-9 from resident cells contribute to melanoma lung metastasis instead of MMP-9 secreted from infiltrating bone-marrow derived cells. These results provide new insights into the influence of MMP-9 in melanoma lung metastasis and demonstrates that bone-marrow derived MMP-9 is not essential for the growth and establishment of pulmonal melanoma metastasis.

Disclosure: No conflict of interest disclosed.

P847
Whole-body-PET/MRI – a new technique for staging and treatment evaluation in patients with soft tissue sarcomas

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Introduction: Simultaneous positron emission tomography (PET) and magnetic resonance imaging (MRI) is a new imaging technique combining metabolic and cross-sectional diagnostic imaging. Up to now the only available clinical data are drawn from feasibility studies in small series of head and neck cancers and intracranial tumors. So far no data exist in evaluating soft tissue sarcomas (STS) with PET/MRI.
MRI is the recommended imaging method in most types of sarcomas. PET is of emerging importance for the management of patients with STS. The combination of MRI with metabolic PET imaging could provide an interesting approach for imaging in STS.
Methods: We examined six patients suffering from STS for staging with an Ingenuity PET/MRI system (Philips Healthcare). It combines a 3 Tesla MRI scanner and a PET scanner with time-of-flight technology. MRI and PET data are acquired sequentially in analogy to PET/CT. We report two patients with liposarcoma examined before start and after two cycles of chemotherapy with trabectedin. The 1st patient presented with local relapse of a pleomorphic liposarcoma of the oropharynx after prior resection followed by irradiation and not responding to relapse treatment with combination chemotherapy consisting of doxorubicin (Doxo) and ifosfamide (Ifo). The 2nd patient presented with a progressive multifocal myxoid liposarcoma of the pelvis after previous combination chemotherapy with Doxo/Ifo.
Results: Simultaneous PET/MRI shows a high contrast imaging without significant artefacts in all patients. The 1st patient showed a low FDG-uptake in the known lesion without metastasis prior to chemotherapy with trabectedin. In staging prior to chemotherapy in the 2nd patient, there were two lesions detectable in MRI with no or only minimal FDG-uptake.
The complete and follow up data of the two patients treated with trabectedin will reported at presentation.
Conclusion: To our knowledge we report the first patients with STS treated with trabectedin and examined with whole-body-PET/MRI. The combination of simultaneous PET and MRI is feasible also in sarcoma patients and can provide additional information in treatment monitoring and guidance in STS. For treatment monitoring with repeated PET/MRI the lower radiation exposure could also be an additional advantage. Further studies should evaluate PET/MRI as imaging method for staging and treatment evaluation in STS.

Disclosure: No conflict of interest disclosed.

P848
Necrotizing granulomatous disease in patients receiving imatinib mesylate treatment for gastrointestinal stromal tumors (GIST)

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Introduction: Imatinib mesylate (IM) represents the standard treatment for patients with *BCR-ABL*-positive chronic myelogenous leukemia (CML) and is the first line adjuvant and palliative treatment modality for those with disseminated or inoperable gastrointestinal stromal tumor (GIST). IM is not known to be associated with an elevated risk for tuberculosis, since only five patients have been reported to date who developed tuberculosis during or after IM treatment for CML (n=4) and GIST (n=1).
Methods: We describe here 3 patients (45-79 yrs of age) with GIST who developed necrotizing (caseating) granulomatous disease during IM treatment. Mean duration of treatment with Imatinib was 22 (range 9-49) months.
Results: Enlarged lymph nodes with increased metabolism in 18-fluorodeoxyglucose-positron emission tomography (FDG-PET)-CT -scans were detected and resected in 3 GIST patients under treatment with IM. Affected sites were subcarinal/ mediastinal (1), mediastinal/ supraclavicular (1) and periparotideal cervical (1) lymph nodes. Histologic examination revealed necrotizing granulomatous disease suggestive of infection with M. tuberculosis or sarcoidosis. Sputum examination for acid fast bacilli was negative in all patients and DNA was negative for M. tuberculosis and other mycobacteria in two cases. In one GIST patient M. tuberculosis was detected by PCR in the lymph node,who was then successfully treated by antituberculous agents. The other patients received no antituberculous therapy and showed no evidence of active tuberculosis during follow up. White blood cell and total lymphocyte counts remained within the normal range throughout treatment with IM. Further testing did not show any differences either in the distribution of lymphocyte subpopulations including regulatory T cells or in the function of immune cells.
Conclusions: Our observations underline the importance to resect enlarged or metabolic active lymph nodes developing during IM treatment for timely diagnosis and appropriate treatment of these rare complications. Extensive microbiologic workup including PCR for mycobacteria is necessary. For those lesions being negative for M. tuberculosis by PCR , a “watch and wait”- strategy appears to be safe. More studies are needed to clarify the potential causal relationship between IM treatment and granulomatous disease and the pathogenesis of lesions being negative for mycobacteria.

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P849

Development of xenograft-based in vivo models of gastrointestinal stromal tumors

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Indroduction**:** Gastrointestinal stromal tumors (GIST) are driven by activating mutations of c-KIT and can be effectively treated with Imatinib (IM). Despite long lasting remissions to IM the majority of patients eventually progress with poor prognosis. Novel treatment strategies are being tested preclinically, however, to allow prioritization of drugs for future translation, representative in vivo models are needed. Therefore we aimed to study tumorigenicity of IM-sensitive (IM-sens) and IM-resistant (IM-res) GIST cell lines and cells from surgical GIST specimen.

Methods: Cell lines comprised GIST882 (K642E, IM-sens), GIST-T1 (del558-614, IM-sens), GIST48 (V560D/D820A) and GIST 48B (V560D, KIT-neg). Cells were injected subcutaneously into the flanks of nude mice. Engraftment and growth were evaluated twice weekly by caliper measurement. Tumors were sequenced for KIT mutations, analyzed by immunohistochemistry (IHC) and western blot for analyses of morphology and KIT-expression. Tumor-bearing mice were also treated with IM to generate and validate an IM-control group.

Results: The engraftment rate of IM-sens cell lines was 100% and visible tumors were detected after a median latency of 14 (T1) and 35 days (GIST882) from time of injection. Subsequent transplantations allowed generation of homogenous treatment groups. IM-res cell lines were not tumorigenic. Four patient-derived cell suspensions have yet been tested (P1:del554-559/N822K; P2:del557-558; P3:V560D/C809V; P4: V559D) with an engraftment rate of 75%. Tumor growth was seen 3 to 10 weeks after the time of injection. Histopathological and IHC evaluation of xenografts revealed highly proliferative tumors (Ki67>90%) in cell-line derived tumors with a morphology resembling high grade sarcomas. Tumors retained DOG1 expression as well as activation of KIT and KIT-depending signaling pathways. Notably, treatment with IM resulted in tumor shrinkage and a loss of KI-67 expression but cells remained completely vital. Tumor-derived xenografts showed slower tumor growth but were morphologically non-distinguishable from typical primary GIST.

Conclusion: We have established a reliable xenograft-model of IM-sensitive GIST which can be used for evaluation of novel treatments in GIST. The model may be particularly useful to evaluate treatment strategies that improve the apoptotic response to treatment. While available IM-res cell lines do not grow in nude mice, tumor derived xenografts may serve as valuable substitute.

Disclosure: No conflict of interest disclosed.

P850

Safety and efficacy of nonpegylated liposomal doxorubicin plus ifosfamide versus conventional doxorubicin plus ifosfamide in locally advanced or metastatic soft tissue sarcoma: full analysis of a randomized phase II trial

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Introduction: Conventional anthracyclines are active against locally advanced or metastatic soft tissue sarcoma (STS), but cardiotoxicity related to the cumulative dose may limit their use. Non pegylated liposomal doxorubicin (NPLD, Myocet®) is reported to have almost no cardiotoxicity. The trial was undertaken to investigate, whether NPLD improves the therapeutic index of doxorubicin by reducing cardiotoxicity, while providing comparable antitumor efficacy, when used in combination with ifosfamide as first-line therapy for locally advanced or metastatic STS.

Methods: 26 patients with STS were randomized to receive 6 cycles of 75 mg/m^2 NPLD or conventional doxorubicin in combination with 5000 mg/m^2 ifosfamide, every 3 weeks. Antitumor efficacy was assessed by objective tumor response rates (RECIST criteria), time to progression and overall survival. Cardiotoxicity was defined by reduction in left-ventricular ejection fraction, assessed by echocardiography or congestive heart failure.

Results: Antitumor efficacy of NPLD versus conventional doxorubicin was comparable so far: disease control rate, 55 % versus 58 %; so far median time to progression 5.9 versus 5.9 months and median survival 14.3 versus 13.8 months. The most common grade 3 or 4 adverse events in the NPLD and conventional doxorubicin groups were anaemia (13 % and 25 %), leukopenia (38 % and 42 %), and thrombocytopenia (13 % and 3 %). Sepsis occurred in no patient in the NPLD group and in 3 patients in the conventional group. Common non-hematologic adverse events were hypokalemia and gastrointestinal symptoms. In both there were no acute or sub-acute anthracycline-induced cardiotoxicities**.**

Conclusions: As compared with conventional doxorubicin, NPLD provides comparable antitumor efficacy and consistent safety profile when given as first-line treatment in patients with locally advanced or metastatic STS. Full data of delayed cardiotoxicities and survival of all patients will be presented at the meeting.

Disclosure: No conflict of interest disclosed.

P851

Successful treatment of metastatic embryonal Rhabdomyosarcoma in an adult using an adapted multimodality treatment protocol for children

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Introduction: Rhabdomyosarcoma is an aggressive soft-tissue sarcoma of childhood. It is less frequent in adults. The tumor cells arise from undifferentiated mesenchymal cells. Children are usually treated with multidisciplinary protocols that include chemotherapy. The cure rate is reported to reach up to 70% in localised situations. Generally, in adults, outcome to therapy is much less optimistic. We here report on a patient with metastatic embryonal rhabdomyosarcoma who was treated using a multimodality therapy regimen based on the cooperative soft tissue sarcoma study group (CWS).

Patient: A 44-year-old male patient was transferred to our hospital in November 2010. He initially presented with a huge tumour on the right thigh. Histologically, a biopsy from the tumor showed embryonal rhabdomyosarcoma. We treated in accordance to the CWS protocol with one course of ifosfamide, actinomycin D and vincristine (IAV). This was followed by whole brain irradiation and localised radiation of thoracic vertebra 5 to 7. Then, one course of carboplatin, epirubicin and vincristine (CEV) followed by one course of ifosfamide, etoposide and vincristine was administered. Re-staging showed a 40% decrease of the thigh tumour and regression of pulmonary metastasis. Then IAV was repeated followed by CEV without epirubicin with simultaneous radiation of the right thigh. Infectious complications and progressive cachexia necessitated cessation of intensive chemotherapy. Oral maintenance chemotherapy (trofosfamid with alternating idarubicin/etoposide) was then initiated.

Results: The patient responded to multimodality treatment (chemotherapy and radiation) based on the CWS protocol. The patient developed various complications so that intensive chemotherapy had to be stopped. Instead, oral maintenance therapy was initiated. We are currently planning a total of eight courses of maintenance therapy.

Discussion: In our hands, multimodal therapy was effective but also toxic in this adult patient with metastatic embryonal rhabdomyosarcoma. Some case reports have demonstrated good prognosis for adult patients (Ferrari et. al 2003) while others have shown poor outcome for adults when compared with results in children (Hulse et al. 2006).

Conclusions: Our experience suggests that multimodality protocols may be effective but also toxic in treatment of adults with embryonal rhabdomyosar-

coma. The crucial point is to adjust intensity of therapy to balance efficacy and toxicity.

Disclosure: No conflict of interest disclosed.

P852

Angiosarcomas of the adulthood – a large single center analysis

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Introduction: Angiosarcomas (AS) represent a rare sarcoma subtype with aggressive clinical behaviour regardless of treatment modalities used. AS respond to various novel therapies but little is known about the impact on overall survival in these patients.
Methods: We retrospectively analyzed our institutional database for clinical characteristics and survival of angiosarcomas treated at the West German Cancer Center.
Results: A database query resulted in 44 patients with a diagnosis of AS. With a total of 1044 sarcoma patients AS represented 4.2% of all sarcomas and 6.5% of all non-GIST sarcomas. The primary locations were chest (31%, including chest wall, cardiac and pulmonary AS), abdomen (31%), extremity (13%), head and neck (11%). 29% were stage III and 44% of patients were stage IV at time of diagnosis. 61% of patients received neoadjuvant or adjuvant treatment (including radiotherapy, chemotherapy or isolated limb perfusion). 37% of patients received chemotherapy as adjuvant treatment mostly consisting of doxorubicin and ifosfamide (70%) as well as paclitaxel (30%) containing regimen. Relapse rate after adjuvant treatment was 83%. More than 50% of patients received chemotherapy for metastatic disease, comprising doxorubicin, ifosfamide, paclitaxel, gemcitabine, and other drugs (such as VEGFRi) with promising clinical responses for several combination regimens.
66% of patients received a 2nd line therapy and 55% more than 2 lines of treatment. 15% of patients received more than 3 lines of chemotherapy. Using multimodal approaches (including metastasectomy) and sequential chemotherapies long term survival was observed in selected patients. A patient with isolated brain metastasis who underwent multimodal treatment including metastasectomy remains free of disease for 6 years. Median overall survival of all patients was 15 months, 13 and 12 months for stage III and IV. 5 year OS for all patients was only 20%.
Summary: Despite novel therapeutic strategies, AS remain a particularly aggressive sarcoma subtype with a poor prognosis regardless of stage. Clinical responses to systemic therapies are frequently observed but despite multimodal treatment approaches long-term survival is seen only in a small subset of patients. Novel chemotherapeutic drugs and drug combinations may yield responses albeit of short duration. Novel treatment strategies are therefore urgently needed for angiosarcomas in all tumor stages.

Disclosure: No conflict of interest disclosed.

P853

The BH3-mimetic ABT-263 enhances the apoptotic response of GIST cells to KIT-inhibitory treatments

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Introduction: Imatinib (IM) treatment of gastrointestinal stromal tumors (GIST) results in long-lasting growth arrest and tumor shrinkage in the majority of patients. However, low pCR rates suggest a moderate proapoptotic effect of IM. IM-induced apoptosis is executed via the intrinsic pathway of caspase activation, which is regulated by pro- and antiapoptotic members of the BCL-2 protein family. Here we have studied expression levels of BCL-2 proteins and the therapeutic potential of BH3-mimetics in GIST cell lines.
Methods: We used IM sensitive and resistant GIST cell lines to evaluate the BH-3 mimetic ABT-263 (ABT), alone and in combination with inhibitors (IM, 17-AAG, nutlin-3 and LBH589) that have therapeutic activity in GIST models. Cell viability was measured using SRB assays; induction of apoptosis was measured by annexin-V/7AAG staining and activation of caspase 3/7. Expression of BCL-2 proteins and caspase-dependent protein cleavage was detected by immunoblotting (IB). Tissue micro arrays (TMAs) were used to determine the expression levels of BCL-2 proteins in patients.
Results: Expression of BCL-2 proteins varied in the cell lines examined and GIST48B exhibited high levels of proapoptotic BIM (15-fold compared to GIST48). In SRB assays ABT alone displayed IC50s between 500nM (GIST48B) and 10μM (GIST48). In IM-sensitive GIST882 combination of IM 1μM with of ABT 100nM led to a significant increase of PARP cleavage (15-fold, compared to 9- and 1.5-fold for IM and ABT alone) as measured by IB. Synergistic effects were also seen in cytometric assays (42% apoptotic cells for combinational treatment compared to 16% and 1% for respective single treatment). Treatment with the MDM2 antagonist nutlin-3 led to an increase of proapoptotic PUMA. Combining ABT with nutlin-3 in GIST48, GIST430 and GIST48B led to an 11-19-fold activation of caspase 3/7, as compared to nutlin-3 (1- and 4-fold) or ABT alone (each 2-fold). TMA analyses revealed overexpression of anti-apoptotic proteins in a substantial number of patients.
Conclusions: Antiapoptotic proteins are commonly overexpressed in both untreated and IM-resistant GIST. BH3-mimetics show moderate activity in GIST but may substantially enhance the apoptotic response of other KIT-inhibitory drugs. Our findings support a potential therapeutic value of BH3-mimetics in GIST. Future studies aim to validate these findings in vivo to identify the most promising drug combination for translation into a clinical trial.

Disclosure: No conflict of interest disclosed.

Posterdiskussion
Supportive Therapie

P854

Quality of life during intravenous immune globulin substitution therapy

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Introduction: Patients with symptomatic immune globulin-deficiency frequently suffer from infections impairing different aspects of their quality of life (QL). No data are available how intravenous immune globulin therapy (IgG-therapy) influences QL and how the burden of this therapy is judged by the patients who receive treatment in a community based oncology group practice.
Methods: QL was assessed prospectively using a standardized interview in patients during IgG-therapy between 01/2009-11/2010. Patients were interviewed before and every 3-4 weeks during therapy. Patients, who had started

their IgG-therapy before the commencement of this project, had to recall their QL before IgG-therapy by memory.
Results: 66 patients (28 male, 38 female) with a median age of 65.5 (19-93) were interviewed. 12 patients were started newly on IgG-therapy during the project, 54 patients were interviewed within a chronic substitution therapy. The mean number of interviews per patient was 9 (1-16). 29% suffered from a primary immune globulin-deficiency and 71% from a secondary immune globulin-deficiency. Patients reported significant mean improvements concerning physical complaints 0.4-0.8 (scale 0-5), daily living 0.7-1.2 (scale 0-5), social life 0.5-0.7 (scale 0-5), mental state 0.1-0.4 (scale 0-4) and general satisfaction 0.2-0.6 (scale 0-4). Success of therapy is judged by the patients with a mean score of 7.8 (scale 0-10). The burden of therapy is judged with a mean score of 1.8 (scale 1-5).
Conclusions: Intravenous immune globulin-therapy applied in a community based oncology group practice improves physical complaints, daily living, social life, mental state and general satisfaction from the patient´s point of view. The general burden of therapy is considered low.

Disclosure: No conflict of interest disclosed.

P855

Management of xerostomia (X) with Xerotin®, a ready-to-use artificial saliva: the German clinical experience

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Damage to the salivary glands during and following radiation (RT) and chemotherapy (CT) for cancer results in xerostomia (X). X leads to severe and long-term oral disorders with a considerable physical and emotional impact and patients' decreased nutritional intake and weight loss. As a consequence continuity of cancer therapy can be disrupted. Xerotin® is a ready-to-use artificial saliva which provides relief from dry mouth. This physiological spray, without propellants, is pH neutral, gluten-and saccharose free and without animal derivatives. Clinical use and patient acceptance of Xerotin® were assessed in 6 german cancer centres according to local clinical practice. Changes in salivation, difficulties in speaking and swallowing, oral discomfort, dysgeusia, impairment of quality of life and Xerotin® acceptability were assessed using visual analogue scales (VAS). 63 patients [39 males/24 females, mean age: 60.5 years, 58 with cancer history, 41 with documented surgeries including 5 with surgery on salivary glands, treated with RT (n=58)/ CT (n=42)], used Xerotin® on average 4 times/day over a median 35 day period. Impaired salivation, difficulties in speaking and swallowing, oral discomfort, dysgeusia and impaired quality of life were significantly improved under Xerotin® (VAS scores improved respectively by 31%, 18%, 33%, 35%, 28% and 16% versus baseline values). An improvement in the ability to eat and drink was documented respectively in 38% and 37% patients treated with Xerotin®. One third of patients early discontinued Xerotin® treatment because of the improvement of their oral condition while worsening of xerostomia was reported in only 1 patient. No adverse drug reaction related to Xerotin® was reported. Xerotin® represents a valuable therapeutic option in the management of xerostomia in patients undergoing radio/chemotherapy.

Disclosure: No conflict of interest disclosed.

P856

Prospective multicenter evaluation of an aspergillus PCR assay and a galactomannan ELISA in bronchoalveolar lavage samples of patients with hematologic malignancies for diagnosing pulmonary aspergillosis

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Background: Diagnosing pulmonary aspergillosis (PA) remains a clinical challenge and difficult issue in patients (pts) with hematologic malignancies. Testing bronchoalveolar lavage (BAL) samples with polymerase chain reaction (PCR) and galactomannan (GM) assays may be more promising than testing blood specimens. Data from prospective studies are still lacking. The BAL cutoff for GM in this setting has not been defined yet.
Methods: Using a PCR assay and a commercially available GM ELISA we prospectively examined BAL samples from 87 pts at high risk of PA in a multicenter clinical trial. Of 78 (90%) evaluable pts, 28 pts had proven or probable disease, all other were classified as controls. We evaluated GM optical density (OD) cutoff levels of 0.5 and 1.0.
Results: The statistical analysis yielded the following findings for sensitivity (sens), specificity (spec), positive predictive value (PPV) and negative prospective value (NPV) for different BAL OD cutoff levels.

Table 1. BAL OD ciutoff > 0.5

Positivity defined by	GM	PCR	GM or PCR	GM and PCR
Sens	0.82	0.61	0.85	0.58
Spec	0.94	0.88	0.84	1.0
PPV	0.88	0.74	0.75	1.0
NPV	0.91	0.80	0.84	0.80

Table 2. BAL OD ciutoff > 1.0

Positivity defined by	GM	PCR	GM or PCR	GM and PCR
Sens	0.46.	0.67	0.86	0.55
Spec	1.0	0.82	0.83	1.0
PPV	1.0	0.61	0.63	1.0
NNP	0.77	0.87	0.92	0.77

Conclusions: In this study GM, which by itself defines a probable diagnosis, was evaluated as a diagnostic tool. Therefore the significance of GM may be overestimated while PCR testing may be underestimated.
Both, lowering the GM OD cutoff for BAL samples to 0.5 and additional PCR testing improves sensitivity. In addition the combination shows a higher specificity and PPV rates in BAL samples. Positivity for both GM and PCR in BAL makes a pulmonary aspergillosis highly likely. From a clinical point of view the combination of BAL GM and PCR testing is therefore recommended.

Disclosure: Mark Reinwald: No conflict of interest disclosed.
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P857

A single centre experience of treatment outcomes during three antimycotic prophylaxis periods in patients with AML: Itraconazole versus posaconazole versus no prophylaxis

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Introduction: After twelve years of itraconazole prophylaxis (group ITRA, 400 mg/d oral solution + loading dose 800 mg/d capsules d1-7) in patients with AML and neutropenia after myelosuppressive chemotherapy we stopped antimycotic prophylaxis during the first half of 2007 (group NO) to verify the continued usefulness of our prophylaxis. In the second half of 2007 we initiated posaconazole prophylaxis (group POSA, 3x200 mg/d) as the marketing approval for this indication had been obtained. The aim of the present study was to evaluate the incidence of invasive fungal infection (IFI), the time to IFI and overall survival (OS) during these three periods in a "real-life" setting.
Methods: Outcomes of all induction and consolidation courses in patients with newly diagnosed AML were retrospectively evaluated using a standard questionnaire. Modified EORTC/MSG criteria of 2008 for IFI were applied: A positive PCR-result for *Aspergillus spp.* in bronchoalveolar lavage was also defined as probable IFI. For better comparison only data from 2005 to 2009 were evaluated.
Results: In total, 239 courses in 124 patients receiving chemotherapy according to standard protocols were evaluated. Baseline characteristics were not different between the groups except for male sex. Antifungal treatment was started in 43/123 (35%) ITRA courses, 7/17 (41%) NO courses, and 22/99 (22%) POSA courses. A proven or probable IFI was observed in 10 (8.1%, 95%CI 4-14%) ITRA courses, in 3 (17.6%, 95%CI 4-43%) NO courses and in 4 (4.0%, 95%CI 1-10%) POSA courses, p=0.107. Two patients with IFI (20%) in ITRA courses died, and none in NO and POSA courses, p=0.452. Median time to IFI (calculated from start of chemotherapy to first diagnosis of IFI) was 22 days (d) in ITRA, 20d in NO and 12d in POSA, p=0.341. Mean OS at d100 after initiation of chemotherapy was not significantly different between the groups: 84d in patients with ITRA (95%CI 77-91d) vs. 93d in NO (95%CI 80-106d) and 95d in patients with POSA (95%CI 89-100d), p=0.05.
Conclusions: A higher incidence of IFI, although not significant, could be detected in the NO prophylaxis group. This did not lead to a difference in OS between the groups, especially for POSA vs. NO. Nevertheless, the absolute risk reduction of IFI with POSA vs. NO of 13.6% in our hospital and a published trial demonstrating reduced mortality with POSA have prompted us to continue treatment of patients with AML with POSA prophylaxis.

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Marie von Lilienfeld-Toal: Advisory Role: MSD Sharp & Dohme GmbH; Financing of Scientific Research: MSD Sharp & Dohme GmbH; Expert Testimony: MSD Sharp & Dohme GmbH

P858

Significance of current antifungal therapy on PCR based investigation of bronchoalveolar lavage samples for diagnosis of pulmonary aspergillosis in patients with hematologic malignancies

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Background: Pulmonary aspergillosis (PA) remains a major cause for morbidity and mortality in patients with hematologic malignancies. This emphasizes the need to improve non culture based methods for detection of invasive aspergillosis (IA), particularly as the diagnosis of IA is rarely based on positive culture yield. Analyzing bronchoalveolar lavage (BAL) samples with polymerase chain reaction (PCR) is promising, however, little is known about the effect of current antifungal drugs on the performance of this diagnostic tool.
Methods: BAL samples from 213 patients (pts) at high risk of PA were retrospectively analyzed using a validated and published nested PCR assay; 207 pts (97%) were evaluable. According to EORTC/MSG 2008 criteria 6 patients were classified as having proven disease, 22 patients were classified as probable, 157 patients were classified as possible while 22 pts did not fulfil the EORTC criteria for at least possible PA.
Antifungal treatment in probable or proven pts consisted of amphotericin B formulations, voriconazole, itraconazole or caspofungin. Median number of administered antifungal regimes were 2 (range 0-4) prior to BAL sampling.
Results: The results for sensitivity, specificity, positive predictive value (PPV) and negative prospective value (NPV) rates for pts with probable or proven disease are shown in the table.

Table 1.

Classification according to EORTC/MSG 2008 criteria	Proven (n = 6)		Probable (n = 22)	
No of antifungals prior to BAL sampling	≤ 1 antifungal	> 1 antifungal	≤ 1 antifungal	> 1 antifungal
Sensitivity	1.0	0.25	0.58	0.27
Specificity	0.98	0.97	0.97	0.97
PPV	0.5	0.33	0.78	0.6
NPV	1.0	0.96	0.94	0.91

Conclusions: Sensitivity and PPV rate of PCR testing decreased in patients receiving more than one novel antifungal drug prior to BAL sampling, whereas the test specificity and NPV rate remained high. Drawback of this study is the small number of proven or probable patients. Our data suggest the crucial need to investigate BAL samples as early as possible in case of suspected PA, especially in the current clinical setting of effective mold-active treatment with novel antifungals. A prospective clinical trial to confirm these findings is warranted.

Disclosure: No conflict of interest disclosed.

P859

Nutrition assessment at admission: Simple tools to predict outcome in oncology and hematology patients

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Problems: Malnutrition in oncology and haematology patients (pts) is linked to higher morbidity and mortality. To ameliorate support possibilities, we determined the nutritional status in 200 consecutive pts at admission for a hospital stay (T1) in our department, mainly to continue or start chemotherapy (89%) and six months later (T2).
Methods: The 120 male and 80 female pts with a median age of 59 years had haematological (n=84) or oncological (n=116) malignancies. Following parameters were assessed to define the nutritional status: height (m), actual weight (kg), usual weight (weight 6 months earlier), BMI (kg/m^2), "Subjective Global Assessment" (SGA), upper arm circumference (cm; UAC), triceps skinfold (mm; TSF), Bioelectric Impedance Analysis (BIA), CRP (mg/dl) and serum-albumin (g/dl). Further all three SGA groups were evaluated for socioeconomic status.
Results: All pts had a significant weight loss in the 6 months prior to admission ($p < = 0.001$) but had stable weights in the following observational 6 months (p=n.s.). According to the SGA 90 pts were classified malnourished

[41%; moderately 23% (SGA B); severe 18% (SGA C)]. Further in BIA the important phase angle alpha was in median significantly lower in the SGA B&C groups ($p < .001$) compared to the SGA A group. UAC and TSF were only decreased in SGA C pts. According the socio-economic status no differences could be detected between the three SGA groups. During the observational time of 6 months we did not observe any difference in malnourished and not malnourished pts according infection, haemorrhage, thrombosis and organ-failure morbidity. But due to the underlying disease significantly more pts died in the SGA B&C groups in the 6 months follow up. These pts had significantly less serum-albumin and higher CRP ($p< 0.001$) at T1.
Conclusion: Prevalence of malnutrition is 41% in unselected pts admitted for therapy. Serum-albumin, CRP, the phase angle in the BIA diagnostic and the SGA score are the most important tools for nutritional status evaluation in tumour pts regarding outcome. Prospective evaluation of nutritional counseling / support in the different groups should be evaluated.

Disclosure: No conflict of interest disclosed.

P860

Microbiological spectra are changing over time and differ between patients with and without hematological diseases

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Background: Knowledge of microbiological data is important for calculated antibiotic therapy in hematological patients. Thus, it is crucial to know if their microbiological spectrum differs from that of other patients.
Patients and Methods: In a retrospective study, we analyzed microbiological isolates obtained from patients of the Department of Hematology/Oncology (HD) and of all other departments (OD) at our institution between 1992 and 2009. The frequency of isolates from 1992-2000 were compared to those from 2001-2009.
Results: In total, 603,944 pathogens were isolated, of whom 21,431 (3.5%) were obtained from HD. Compared to OD, in HD the most frequent isolates were from blood cultures (43.2% *vs.* 15.8%; $p< 0.001$). $Gram^+$ bacteria predominated. In HD specimens, the proportion of $Gram^+$ bacteria was significantly higher than in OD specimens (67.4% *vs.* 58.4%). Anaerobic bacteria were found less frequently in HD than in OD samples (0.6% *vs.* 1.0%; $p=0.02$). No difference was seen for yeasts. In HD, *S. aureus* (4.9% *vs.* 9.6%; $p< 0.001$), *Enterobacteriaceae* (12.4% *vs.* 19.3%; $p< 0.001$), and *Pseudomonadaceae* (2.6% *vs.* 5.6%; $p< 0.001$) were significantly less frequent than in OD, while coagulase negative staphylococci (CNS; 31.6% *vs.* 19.6%; $p< 0.001$) and streptococci (16.2% *vs.* 12.0%; $p=0.01$) were more frequent. Comparing both time periods, an increase in $Gram^+$ bacteria was seen in both HD (+9.0%; $p=0.002$) and OD (+2.7%; $p=0.002$), while no change was found for both $Gram^-$ bacteria and anaerobes. The frequency of yeasts was significantly reduced over time in HD (-6.0%; $p=0.006$), while it remained unchanged in OD. A significant increase in *S. aureus* was observed in both HD and OD (+2.7% and 3.1%, respectively), while streptococci increased in OD only (+3.8%). In contrast, the frequency of CNS, streptococci, and *Enterobacteriaceae* remained stable in HD, while in OD the frequency of *S. aureus* (+3.1%; $p< 0.001$) and streptococci (+3.8%; $p< 0.001$) was increasing, while CNS (-3.4%; $p< 0.001$) and *Enterobacteriaceae* (-3.3%; $p=0.009$) decreased. No difference over time was seen for both enterococci and *Pseudomonadaceae*.
Conclusions: At our institution, the microbiological spectrum differed only slightly between HD and OD specimens. The largest differences were seen for CNS and *Enterobacteriaceae*. Over time, the frequency of $Gram^+$ isolates was increasing. According to our data, calculated antibiotic regimens for HD patients should provide adequate activity against $Gram^+$ bacteria.

Disclosure: No conflict of interest disclosed.

P861

Detection of human herpesvirus-6 DNA in the gastrointestinal tract of patients after allogeneic stem cell transplantation

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Introduction: In 40% of patients after allogeneic stem cell transplantation (allo-SCT) DNA of human herpesvirus-6 (HHV-6) can be detected by PCR in peripheral blood samples. Clinical features of HHV-6 infection include fever, rash, delayed engraftment, encephalitis and gastrointestinal symptoms. However, the significance of HHV-6 in gastrointestinal complications after allo-SCT remains unclear.
Methods: In this study 50 consecutive patients after allogeneic stem cell transplantation with severe vomiting or diarrhea were retrospectively analyzed. 102 biopsies obtained by colonoscopy or endoscopy of the upper gastrointestinal tract were histologically analyzed for signs of GvHD and by qualitative polymerase chain reaction (PCR) for viral DNA of HHV-6 and other virus of the herpes family. Samples were collected after onset of symptoms as well as after antiviral therapy.
Results: DNA of HHV-6 was detected in 38 of 75 initial samples (51%) and in 19 of 27 follow-up biopsies (70%). In the presence of acute GvHD, HHV-6 DNA was detected in 20/37 (54%) biopsies compared to 18/38 (47%) biopsies without signs of acute GvHD. At the time of the first endoscopic examination most biopsies were obtained while patients received antiviral prophylaxis with aciclovir (51/75) or antiviral therapy with intravenous aciclovir, ganciclovir or foscarnet (19/75). After detection of HHV-6 nine patients received antiviral therapy. As antivirals patients received either high-dose intravenous aciclovir (3x10 mg/kg, n=3), foscarnet (2x60 mg/kg, n=4) or a sequential therapy with ganciclovir (2x5 mg/kg) and foscavir (n=2). Despite high dose antiviral therapy no patient with initial positive HHV-6 PCR turned negative in follow up biopsies after treatment. No risk factor for HHV-6 detection was found by univariate analysis. There was no significant difference in overall survival between patients with or without HHV-6 DNA detection in the gastrointestinal tract.
Conclusions: HHV-6 DNA is frequently detected by PCR in biopsies from patients with gastrointestinal symptoms after allo-SCT. The detection of HHV-6 DNA had no impact on the overall survival. Moreover, antiviral therapy against HHV-6 was without effect. Thus, a positive PCR result in GI-tract samples does not necessarily reflect infection with HHV-6. Further studies are needed to determine the adequate method to detect significant HHV-6 reactivation in the gastrointestinal tract.

Disclosure: No conflict of interest disclosed.

P862

Infectious complications in patients after autologous hematopoietic stem cell transplantation: risk factor analysis of 197 therapy cycles

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Introduction: Patients (pts) undergoing autologous hematopoietic stem cell transplantation (HSCT) are at high risk for infectious complications.
Methods: From 2005 to 2008 we conducted a retrospective analysis of 157 pts undergoing HSCT. The incidence and causes of infections were statistically evaluated depending on risk factors.
Results: In total, 197 therapy cycles were evaluated. The median age was 54 (range 17-71) years with a male to female ratio of 1.9:1. Pts had following underlying diseases: multiple myeloma (MM) (48%), non hodgkin lymphoma (NHL) (30%), germ cell tumor (GCT) (16%), Hodgkin`s disease (4%) and others (2%). The median time of neutropenia < 0.5x10(9)/L was 9 days. In 182 therapy cycles an antimicrobial therapy was necessary because of febrile

events: 88 pts with fever of unknown origin, 45 pts with bacteremia (30 gram-positive, 15 gramnegative), 23 pts with pneumonia, 16 pts with clinically documented infection, 10 pts with microbiologically documented infection. 7 pts died due to infectious complications. The median time to defervescence correlated with the duration of neutropenia ($p < 0.001$) and with proven infections ($p < 0.001$). The incidence of infection was significant more common in pts older than 40 years ($p = 0.01$) and depended on the underlying disease: MM > NHL > GCT. The median time of neutropenia was significant longer in pts with NHL than in pts with MM or GCT.
Conclusions: In this study, pts with MM undergoing HSCT have a higher risk of infectious complications than pts with NHL despite a shorter median duration of neutropenia. This may be due to the presence of the humoral immune defect in MM.

Disclosure: No conflict of interest disclosed.

P863

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

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Background: In a meta-analysis by Gafter-Gvili et al. 2005 it was shown that antibiotic prophylaxis in patients with neutropenia after chemotherapy reduced the incidence of fever and the mortality rate. This was demonstrated in particular in patients with acute leukemia when a fluoroquinolone was used as prophylaxis. Therefore, we changed our antibiotic prophylaxis from TMP-SMZ/COL to the fluoroquinolone ciprofloxacin (CIP) in April 2008. The aim of the present study was to compare efficacy and duration of neutropenia during the two prophylaxis regimens from 2006 to 2010.
Methods: All chemotherapy courses in patients with AML and antibiotic prophylaxis with TMP-SMZ/COL (01/2006 – 04/2008) or with CIP (04/2008 – 06/2010) were retrospectively analyzed with a standard questionnaire. Data were analyzed using SPSS 18 and GraphPad InStat V2.05.
Results: At the moment, 315 of 331 chemotherapy courses were evaluated (129 courses with TMP-SMZ/COL, 186 courses with CIP). There was no significant difference between both groups regarding status of disease before chemotherapy, intensity of chemotherapy, application of G-CSF and patient age. Median age was 59 years (range 18-85).In the CIP group the incidence of fever was significantly lower 116/186 (62.4%, 95%CI 55-69%) vs 99/129 (76.7%, 95%CI 68-84%) during TMP-SMZ/COL, p=0.007. There was also a significant decrease in the number of microbiological documented infections (40/186 courses (21.5%, 95% CI 16-28%) in the CIP group vs. 41/129 courses (31.8%, 95% CI 24-40%) in the TMP-SMZ/COL group, p=0.049. No significant difference in the distribution of gram(+)-, gram(-)-and mixed infections could be observed, but the incidence of gram(-) infections was lower in the CIP group (3.2% vs 7,8%, p=0.0496). Although not significant, there was also a trend to a reduction in infection-related mortality in the CIP group (1.6% vs 6.2%, p=0.127). Interestingly, we also found a significant reduction in the duration of neutropenia in the CIP group, 15 days vs. 18 days, p=0.032.
Conclusion: In this update of the study, the antibiotic prophylaxis with CIP compared to TMP-SMZ/COL in neutropenic patients with AML showed a significant reduction in the incidence of fever, the incidence of gram (-)-infections and the duration of neutropenia. In contrast to the meta-analysis of Gafter-Gvili we found no difference in the infection-related mortality rate.

Disclosure: No conflict of interest disclosed.

P864

Parenteral nutrition as a risk factor for port-infections in hemato-oncological patients

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Port-systems (PS) as central venous catheters, play an important role in the treatment of patients with hemato-oncological disorders. PS are used on the one hand for the application of cytotoxic drugs and/or monoclonal antibodies and on the other hand for the administration of intravenous (i.v.) nutrition. However, infections of PS can lead to prolonged hospitalisation with an increased morbidity, requiring treatment by i.v. antibiotics and frequent removal of the PS.
We retrospectively analyzed all diagnosed PS-infections between July 2010 and April 2011 in our hemato-oncological department with the aim to detect causative micro-organisms and to analyze the clinical course of the infection and the history of parenteral nutrition of the patients as a possible risk factor.
During the stated period 10 cases of PS-infections were seen of whom 8 patients received parenteral nutrition at the time of diagnosis. Diagnostic criteria were clinical signs of infection such as fever (>38°C), inflamed skin, swelling, tenderness and/or purulent secretion in combination with two pairs of microbial growth-positive blood cultures from both the PS and peripheral vein showing the same micro-organism. In 5 cases infection was caused by coagulase-negative staphylococci (CoNS), in 4 cases by Gram-negative bacilli (GNB) and in one case by methicillin-susceptible *S. aureus* (MSSA) in combination with *Candida glabrata*. In 7 out of 10 patients the PS were removed, but intraluminal treatment with antibiotics was not attempted in all patients.
The results of our analysis suggest that parenteral nutrition could be a possible risk factor for PS-infections in hemato-oncological patients, as it was shown in other studies (Ishizuka et al., Eur Surg Res 2008; 41: 341-345). The rate of PS-removal should be decreased by use of a standardized protocol for intraluminal treatment with antibiotic-lock therapy (ALT) in the case of PS-infection which is going to be established in our department. For the prevention of PS-infections a standardized protocol for disinfection during the application of parenteral nutrition, especially in outpatients, should be used.

Disclosure: No conflict of interest disclosed.

P865

Candida kefyr caused fungal pneumonia, brain abscesses and multiorgan failure in a patient with acute myeloid leukemia

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Introduction: Candida kefyr is an uncommon but emerging fungal pathogen among immunocompromised patients. We report of a patient with acute myeloid leukemia who developed an invasive fungal infection with pneumonia, brain abscesses and consecutive multiorgan failure caused by Candia kefyr.
Case report: The patient was of German Nationality without migration background. He was diagnosed acute myeloid leukemia (M6 according to FAB classification). He received 2 cycles of cytotoxic chemotherapy (TAD-HAM) and was with the start of chemotherapy to observe special rules of behaviour like stop smoking, to eat no fresh fruits or yoghurts. The patient was not strictly compliant regarding our recommendations.
During bone marrow depression/neutropenia the patient developed a pneumonia. An antibiotic (vancomycin and imipenem) and antifungal therapy with voriconazol was started. Blood culture revealed Candida kefyr.
Brachiocephalic hemiparesis and motoric aphasia led to a MR scan. This showed hypodensic lesions that were interpreted as septic spreading.
Despite switching the antimicrobial and antifungal regimen to fosfomycine and ceftazidim combined with caspofungin neurologic symptoms worsened. The patient developed a progressive septic disease course and died of multiorgan failure within a few days.
Conclusions: This case report emphasizes the importance of concrete nutrition recommendations and the patients compliance.

Disclosure: No conflict of interest disclosed.

P866

Bisphosphonates-associated osteonecrosis of the jaws: a registry-based study

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Bisphosphonates (BP)-associated osteonecrosis of the jaws (ONJ) is a major adverse drug effect reported in cancer patients undergoing BP drug therapy. However, risk factors predisposing patients to the early development of ONJ have not been firmly established. The present study examined data of patients with BP-associated ONJ with the aim to identify these risk factors. A standardized questionnaire including medical and dental history and malignancy-related data was used to assess fifty ONJ patients (29 female and 21 males, median age 65 years) who have underwent treatment in eight institutions throughout Northeast Germany between 1993 and 2011. Patients with BP-associated ONJ were treated for the following malignancies: plasmocytoma/NHL (n=20), breast cancer (n=16), prostate cancer (n=8) and other malignancies (n=6). Three patients (6%) have received radiotherapy, 19 (38%) chemotherapy, and 28 (56%) combined radiochemotherapy, respectively. BP were administered intravenously at monthly intervals to all patients. Zoledronate (4 mg) was given to 37 patients (74%), pamidronate (90 mg) to 20 (40%), and ibandronate (6 mg) to 7 (14%), respectively. Sixteen patients underwent sequential treatment with more than one BP. The median duration of BP treatment before diagnosis of ONJ was 4.0 years (6 months to 13 years). Among potential risk factors for the development of ONJ, neither anaemia nor local infections were associated with early manifestation. In contrast, recent dental extraction was a significant predictor of ONJ. We conclude that local interventions (dental extraction) may respresent a major risk factor for the early development of BP-associated ONJ in cancer patients whereas tumor-driven risk factors (anaemia, steroid administration) may have less implication than previously thought.

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P867

Analysis of the non-interventional study with Filgrastim-Hexal®: safety profile and patient perception

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Background: Chemotherapy (CT) induced neutropenia (CIN) is a common complication in the treatment of cancer. Administration of granulocyte colony stimulating-factor (G-CSF) is able to protect against CIN. Documentation of the use of Filgrastim Hexal® will give further insight into the efficacy and safety of this biosimilar. This interim analysis of a non-interventional study of Filgrastim-Hexal® focuses on the safety profile and patient perception during first documented CT-cycle.
Methods: 500 patients are to be enrolled at 100 German sites. Patients complete a customized questionnaire focusing on self-injection and handling of the syringe. Patients characteristics were ≥18 years of age, receiving CT and being treated with Filgrastim Hexal® either prophylactically or interventionally. All patients provided signed informed consent. Patients contraindicated according to the SmPC or who have been treated with G-CSF in the current line of CT are to be excluded (www.register.germanctr.de).
Results: By 4/2011, 263 complete datasets of 344 enrolled patients were available. 69.6% of patients were being treated in an (neo)-adjuvant setting, 30.4% with palliative chemotherapy. Most patients had solid tumors (84.4%), with breast cancer being the most common (58.6%). The majority of patients received Filgrastim Hexal® for 3 cycles (70.8%) and the mean duration of treatment was 4.0 (± 1.7) days. Treatment benefit was greater for patients receiving filgrastim as primary prophylaxis compared with secondary prophylaxis or interventional therapy. Evaluation of patient questionnaires showed that the majority of patients (94%) reported no or mild pain while self-applying Filgrastim Hexal® (intensity: 0/1.5 on a10-point scale) with only minor reactions at the injection site, the occurrence of which was independent of patients' experience with self-administration. Handling of the syringe featuring an innovative needle protection system was generally assessed as easy (98%).
Conclusions: Filgrastim Hexal® in treating/preventing CIN proved to be safe and well-tolerated. Self-application using the injection system was consistently described as easy. Final data will be presented at the congress.

Disclosure: Hans Tesch: Expert Testimony: Study supported by HEXAL AG Tanja Schaller-Kranz: Employment or Leadership Position: Employee of HEXAL AG

P868

The flow cytometric granulocyte immunofluorescence test (Flow-GIFT) in donor and patient screening is able to improve the safety of granolucyte transfusions

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Introduction: Granulocyte transfusions have been suggested in treating neutropenic patients with life-threatening bacterial and/ or fungal infections. However, the presence of leukocyte-associated antibodies can render this transfusion ineffective or cause transfusion-related acute lung injury (TRALI) when antibodies in patient and/or donor bind to the respective leucocytes. Prior to administration of granulocytes, only red blood cell crossmatch is mandatory. In this study, we describe the procedures for investigation of leukocyte-associated antibodies in donor and patients prior to granulocyte apheresis and granulocyte transfusion using the flow cytometric granulocyte immunofluorescence test (Flow-GIFT).
Methods: Between July 2009 and April 2011 we provided 112 granulocyte preparations for 31 patients. Before a donor was declared a match for granulocyte apheresis, screening for antibodies against leukocytes in both patient and donor as well as crossmatches between patients' serum and donors' leukocytes were performed using Flow-GIFT. This procedure was repeated every two weeks in order to exclude an new alloimmunization. Positive Flow-GIFT results were differentiated by simultaneous analysis of specific granulocyte antibodies assay (SASGA)
Results: All patients tolerated well the granulocyte transfusions. In one patient, anti-HLA class II and -granulocyte antibodies with unknown specificity were found. The granulocyte antibodies reacted with all selected donors. In this case, we excluded all TRALI-relevant antibodies against HNA-1a, -1b, -2a and -3a as well as lymphocyte-reactive antibodies including specific anti-HLA-class II. In a further patient, Flow-GIFT results turned positive against one donor's granulocytes after shortly more than two weeks of granulocyte administration. The specificity could not be determined. This donor was not permitted to donate granulocytes for this patient. As a result of the treatment with granulocytes all patients stabilized, only one of died due to the severity of the underlying illness, another died months later to GvHD after allogeneic stem cell transplantation.
Conclusions: Flow-GIFT is an ideal tool for the investigation of leukocyte antibodies in the supply of granulocyte preparations. Screening for leukocyte antibodies as well as the individual patient donor crossmatches should be performed to achieve safety of granulocyte transfusion.

Disclosure: No conflict of interest disclosed.

Posterdiskussion
Stammzellen

P869

Serum isolated after autologous transplantation stimulates proliferation and *in vitro* expansion of human CD34+ hematopoietic stem- and progenitor cells

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After hematopoietic stem cell transplantation (HSCT), regeneration of the hematopoietic system requires activation of the stem cell pool. So far, the mechanisms that recruit these cells into proliferation and self-renewal are scarcely understood. Here, we have addressed the question if activation of hematopoietic stem and progenitor cells (HPC) after autologous HSCT is mediated by systemically released cytokines and growth factors. Serum was taken from patients before chemotherapy, during neutropenia and after hematopoietic recovery. Subsequently, it was used as supplement for *in vitro* culture of $CD34^+$ cord blood HPC. Serum samples that were isolated during hematopoietic stress between 4 and 11 days after HSCT significantly enhanced HPC-proliferation and maintained primitive immunophenotype ($CD34^+$, $CD133^+$, $CD38^-$, $CD45^-$) over more cell divisions. The frequency of colony forming units (CFU) as well as the number of cobblestone area forming cells (CAFC) was also increased. More than 2 weeks after HSCT when hematopoietic recovery was almost completed, this stimulating effect declines to normal levels as observed with samples from before chemotherapy. Chemokine profiling revealed down-regulation of several growth factors after HSCT including platelet-derived growth factors PDGF-AA, PDGF-AB and PDGF-BB, whereas expression of monocyte chemotactic protein-1 (MCP-1) increased. Metabolomic profiling was used for identification of 46 metabolites that are currently tested for their functional relevance in HPC expansion. Taken together, these results demonstrate that systemically released factors stimulate hematopoiesis after autologous HSCT. This feedback mechanism opens new perspectives for *in vivo* stimulation of the stem cell pool.

Disclosure: No conflict of interest disclosed.

P870

CML therapy can benefit from the activation of HSCs: simulation studies of different treatment combinations

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Introduction: Complete eradication of residual disease in Imatinib treated patients with Chronic Myeloid Leukemia (CML) is a general problem that might not be resolved solely by the application of tyrosine kinase inhibitors (TKIs) such as Imatinib. The recent findings that IFNalpha induce activation of mouse hematopoietic stem cells (HSCs) stimulate the discussion of whether this effect could activate quiescent CML stem cells to make them more prone to the cytotoxic effects of TKIs and thus lead to a complete eradication of the CML clone.
Methods: We base our simulation approach on a mathematical model describing human CML as a clonal competition phenomenon between normal and malignant cells, which consistently explains short- and long-term treatment kinetics of CML patients under standard Imatinib monotherapy. We amend this model to incorporate a model description of IFNalpha activity. We apply this model to study different scenarios for potential treatment combinations and compare their predicted outcomes to the Imatinib.
Results: We demonstrate that the overall sensitivity of CML stem cells to IFNalpha activation is a crucial determinant for the benefit of a combination therapy. We furthermore show that pulsed IFNalpha together with continuous TKIs administration is the most promising strategy for a combination treatment in which the therapeutic benefit prevails adverse side effects.
Conclusion: Our modelling approach is a highly beneficial tool to quantitatively address the competition between normal and malignant hematopoiesis in treated CML patients. Based on our modelling results we derive testable predictions for different experimental settings that are required prior to the clinical implementation of combination treatments with TKIs and stem cell activating drugs.

Disclosure: No conflict of interest disclosed.

P871

Novel human bone marrow stroma cell lines from a normal donor and leukemia and lymphoma patients

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Introduction: Haematopoiesis is dependent on the interaction of a haematopoietic stem (HSC) or progenitor cell with stromal cells in the bone marrow (BM) microenvironment by cell-cell contact and soluble factors. In murine systems mesenchymal stem cells (MSC) represent a central stromal component for appropriate HSC function. Cumulative evidence suggests that in haematopoietic malignancies the BM stroma is altered in a way to support neoplastic cells rather than normal haematopoietic differentiation.
Methods: To study HSC-stroma interactions with more standardized tools, we established novel cell lines from BM stroma of two patients suffering from acute myeloid leukemia, two lymphoma patients without malignant infiltration of the BM, and from the BM of a healthy donor starting from plastic-adherent primary BM cells cultured under conditions favouring outgrowth of fibroblasts.
Results: Five independent polyclonal cell lines were established and are currently under investigation. They proliferate well in culture, two lines now for more than 50 continuous passages, and surface markers are reminiscent of MSC. Two of the cell lines have been differentiated into osteoblasts, adipocytes, and chondrocytes. In an additional cell line from one lymphoma patient expression of the immortalizing gene(s) is regulated by a Dox-responsive element allowing to expand the cells in the presence and use them for experiments in the absence of Dox, respectively. Differential expression patterns were observed for membrane-anchored and soluble molecules HLA-G, MICA, MICB, ULBP2, ILT-4 known to be implicated in the suppression of the innate and adaptive immune system.
Conclusion: These cell lines are considered as a robust and reliable *in vitro* test system to investigate mechanisms of leukemic vs. normal haematopoiesis.

Disclosure: Tobias May: Employment or Leadership Position: InSCREENeX GmbH, Inhoffenstraße 7, 38124 Braunschweig, Germany; Stock Ownership: InSCREENeX GmbH, Braunschweig, Germany; Honoraria: T. May et al filed a patent covering the immortalization technology
Bertram Opalka: No conflict of interest disclosed.

P872

Efficient methods for leukemic stem cell separation

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Leukemic stem cells (LSC) are likely to be the source for leukemic disease self-renewal and the cause for relapse of acute myeloid leukemia after treatment, and thus, a critical target for therapeutic options. It has been shown

repeatedly by xenograft models that LSC from AML patients reside mainly in CD34+CD38- compartment of leukemic blasts. Therefore, the pure and efficient separation of this population is mandatory to identify new therapeutic drugs to target LSC in different AML subtypes.
We separated this subpopulation from primary AML peripheral blood mononuclear cell (PBMC) samples with fluorescence-activated cell sorting (FACS) and magnetic-activated cell sorting (MACS) and compared the efficiency. MicroArrays were performed with GeneChip Human Genome U133 Plus 2.0 from Affymetrix to profile gene expression of LSC but also normal hematopoietic stem cells (HSC).
We separated CD34+CD38- cells from peripheral blood of 10 AML patients and 5 healthy volunteers with FACS. For the 10 primary AML samples, the ratio of the CD34+CD38- cells ranged from 0.79% to 86.2%; 1-5×10^7 PBMC were used for separation. After sorting, the purity of those AML samples increased to 88.4-98.4% while 2×10^4-3.6×10^6 cells were obtained. MACS was used to separate 2 representative samples, in which the CD34+CD38- subpopulation was rather small (sample1: 0.78%) or large (sample2: 86.1%), and was compared with FACS. In order to evaluate separation efficiency in a standardized manner, we defined the recovery rate: CD34+CD38- cell number obtained/total CD34+CD38- cell number x 100%. The total CD34+CD38- cell number was calculated according to FACS analysis. In sample1, MACS resulted in a recovery rate of 4.2-6.4% with a purity of 86.6-90.3%, which is inferior to the recovery rate of 17% and the purity of 92.1% by FACS. Sample2, MACS resulted in a recovery rate of 0.4% with a purity of 98.8%, compared to the recovery rate of 11.6% with a purity of 98.1% by FACS. When comparing the 2 methods, it occurs that the purity doesn't differ a lot, but the yield is much higher using FACS, which could be a powerful tool, when managing rare samples.
Finally, by comparing purity and yield, we showed that FACS is the preferred separation method. MicroArrays are being performed at the moment to investigate the gene expression profile for 10-15 AML patients and 5 HVs. Furthermore we will be able to define LSC in different AML subtypes through their expression profiles and compare HSC with LSC.

Disclosure: No conflict of interest disclosed.

P873

Characterization of aldehyde dehydrogenase (ALDH) expressing cells in acute myeloid leukemia (AML)

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Introduction: A high expression of the cytoplasmic enzyme ALDH is known to correlate with stem cell features in a normal hematopoietic system. In AML a population of ALDH$^+$ cells has been reported to contain higher frequencies of LTC-ICs as well as NOD/SCID-repopulating cells in comparison to the ALDH$^-$ cells. Nevertheless, data about the role of ALDH$^+$ cells as leukemia initiating cells are still controversial. In this study we are showing interesting characteristics of this stem cell population.
Methods: Mononuclear cells from bone marrow of AML patients at diagnosis and healthy donors were analyzed for the ALDH activity and the expression of CD34, CD38 and AML-associated aberrant markers by using flow cytometry. Populations of interest such as CD34$^+$ALDH$^+$, CD34$^+$ALDH$^-$ were sorted by FACS and either expanded for the FISH analysis or used for gene expression studies in order to detect chromosomal aberrations and gene mutations.
Results: ALDH expression was very heterogeneous in AML. In about 80% of patients the frequency of ALDH$^+$ cells was lower than in healthy bone marrows. These samples were thus classified as the ALDH$^-$ AML group. Other patients were characterized by a higher amount ALDH$^+$ cells and were grouped in the ALDH$^+$ AML group. The ALDH$^+$ and CD34$^+$ALDH$^+$ stem cells of the two groups showed clearly different properties. While these cells in the ALDH$^-$ group are almost free of aberrant markers, those in the ALDH$^+$ group showed a high expression of them. In both cases, the markers were largely observed in whole blasts, CD34$^+$ cells and CD34$^+$CD38$^-$ stem cells. Furthermore, sorted CD34$^+$ALDH$^+$ stem cells in the ALDH$^-$ group did not contain chromosomal aberrations such as t(8;21), trisomy 8 and MLL-rearrangement as well as mutations in Flt3 and NPM1 genes, which were abundantly detected in whole blasts and CD34$^+$ALDH$^-$ cells. In contrast, genetic disorders were observed in both subpopulations in the ALDH$^+$ group. These results are in line with our previous results, in which ALDH$^+$ cell frequency was associated inversely with the outcome of AML patients.
Conclusion: High expression of ALDH characterized two different stem cell populations in AML. In most samples, the ALDH$^+$ cells display a similar phenotype for aberrant markers like normal HSCs and an absence of the explored genetic disorders. In some samples, ALDH is expressed more frequently and correlates with leukemic features. Mechanisms, which lead to the transformation of ALDH$^+$ cells, are still under investigation.

Disclosure: No conflict of interest disclosed.

P874

Highly methylated SFFV promoter sequences prevent lentiviral transgene expression *in vivo*

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Gene functions in development, homeostasis and disease can be thoroughly analyzed using transgenic animal models. Lentiviral gene transfer is considered to be a promising technology for the generation of transgenic mice with a higher yield of transgenic offspring and less time-consumption than other protocols. We therefore evaluated lentiviral gene transfer to generate a mouse model transgenic for the SET binding protein (Setbp1) and the marker eGFP. Embryos were infected at dE0.5 by injection of lentiviral particles into the perivitelline space. The presence of lentiviral integrations in newborn F0 mice was determined by specific PCRs for Setbp1 or the Wpre element of the lentiviral construct and we assessed in 65% of 31 analyzed mice the viral integration flanks by highly sensitive LAM-PCR. Germline transmission was observed in 5 out of 9 F0 mice but no ectopic transcription and overexpression of Setbp1 or eGFP was detected in vector-positive mice. Methylation status analyses of the internal SFFV promoter (SFFVp) by bisulfite sequencing revealed that in F0 mice and in all progeny determined (n=12) all analyzed CpGs (18/18) were highly methylated. To elucidate whether the transgene itself affects epigenetic silencing of SFFVp, we transduced murine ES cells with LV.SFFV.Setbp1.IRES.eGFP or the corresponding eGFP-expressing control vector. Interestingly, eGFP expression decreased 1.8fold and 3.5fold after differentiation of ESC infected with the transgene vector and SFFV driven control vector, respectively. To investigate whether silencing of lentiviral promoter sequences is restricted to embryonic stem cells, we analyzed LV.eGFP transduced and transplanted adult bone marrow derived hematopoietic stem cells and compared the SFFVp methylation pattern of their progeny to γ-retroviral transduced hematopoiesis (n=7). Strikingly, transduced long-term hematopoiesis did not express eGFP due to consistent methylation of LV SFFVp in close proximity to CAAT- and TATA-box elements.
Here, we demonstrate that the commonly used SFFV promotor can be highly methylated with remarkable strength and frequency during development and differentiation. We conclude that lentiviral vectors using an internal SFFVp are not suitable for the generation of transgenic mice or constitutive expression studies in hematopoietic cells. The choice of promoters driving lentiviral vectors is crucial and should be considered in future gene therapy trials of the hematopoietic system.

Disclosure: No conflict of interest disclosed.

P875

The impact of aging on gene expression profiles of human hematopoietic progenitor cells

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Introduction: The regenerative potential diminishes with age and this has been ascribed to functional impairments of adult stem cells. Cells in culture undergo senescence after a certain number of cell divisions with a decreasing proliferation and differentiation potential, which might also happen *in vivo*. In this study we have analyzed the effect of aging on gene expression profiles of human hematopoietic progenitor cells (HPC).
Methods: HPC were isolated within the CD34+ cell fraction of four fresh umbilical cord bloods (0 years) or from mobilized peripheral blood of fifteen healthy donors between the age 27 and 73 years. Total RNA was extracted and gene expression profiles were analyzed by Affymetrix GeneChip technology. To identify differential gene expression that correlated with increasing age we have performed PTM using the MultiExperiment Viewer. Genes that were either up-regulated or down-regulated were further classified by GeneOntology analysis. Data were additionally verified by RT-PCR analysis of independent cord blood, young and elderly donor samples.
Results: Microarray data revealed a consistent pattern of alterations in the signature of HPC of different age. 530 genes were age-induced and 501 genes were age-repressed. Especially genes involved in metabolic processes and gene expression were age-repressed. Differentially regulated genes were associated with organelles as the nucleus and mitochondria and could be related to various types of cancer. RT-PCR results validated age associated gene expression changes observed in microarray data.
Conclusions: These studies have demonstrated that aging causes gene expression changes in human HPC. The data of differentially regulated genes build a basis for further experiments on age-specific changes and diseases in the human hematopoietic system. Further defining of specific components during the process of aging may help to develop new therapies to prohibit age-related pathophysiologies or further enhance the effects of current treatment.

Disclosure: No conflict of interest disclosed.

P876

A comprehensive model to understand the SDF-1/CXCR4 axis

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Introduction: The SDF-1/CXCR4 axis plays a crucial role in cell migration as well as for the retention of hematopoietic stem cells (HSCs) in their protective niche. For a better understanding of the nature of this axis, we performed a systematic experimental work. With this we could identify adhesion and migration patterns, internalization, and degradation of CXCR4 and surface changes in adhesion molecules.
Methods: Leukemic cell lines like Jurkat, Kasumi, HL-60, KG-1a, K562 as well as HSCs were used. Western blot and flow cytometry analyses (FC) were performed to characterize the expression of CXCR4 and adhesion molecules like Integrin-b2, ALCAM and N-Cadherin. Adhesion assays using our new developed adhesion model and transwell migration assays were carried out. Cell cycle analysis of different HSCs subpopulations (CD34+/CD38- vs. CD34+/CD38+) were performed using multicolor FC.
Results: CXCR4 surface expression varied depending on the cell line, higher levels of the receptor were found in Jurkat and Kasumi-1 cell. After incubation of Jurkat cells with SDF-1, they responded with internalization of the receptor, loss of adhesion and migration towards SDF-1 and the degradation of CXCR4. We also could observe changes in the antibody binding pattern to the adhesion molecules Integrin-b2, ALCAM and N-Cadherin, demonstrating the direct effect of cell activation by SDF-1. Nevertheless, not all cells were responding equally to the SDF-1 stimulation by losing of adhesion or migrating (either subpopulations of Jurkat, HSCs or between cell lines) and therefore we further investigated this phenomenon. Cell cycle analysis revealed that in HSCs, and Jurkat the CXCR4 surface expression is cell cycle dependent with the highest surface expression in the late S phase. Adhesion assays showed that this has functional consequences demonstrated in CD34+/CD38- HSCs, which were in the S phase and showed a higher loss of adhesion in comparison to CD34+/CD38- cells in other cell cycle phases.
Conclusion: In this work we demonstrate that migration and adhesion of leukemic cell lines and HSCs depend on their sensibility to SDF-1 and their surface expression of CXCR4. Three types of cells could be identified: cells which express surface CXCR4 and respond to SDF-1, cells which express surface CXCR4 with low or no sensibility to its agonist, and those which do not express it. From the cells which respond to SDF-1, only a small subpopulation does it in a cell cycle dependent manner.

Disclosure: Abraham Zepeda-Moreno: No conflict of interest disclosed. Anthony Ho: Advisory Role: Is on the Advisory Board of Genzyme.

P877

The ability to undergo a temporary cell cycle arrest contributes to the maintenance of the stem cell character in human multipotent stromal cells after genotoxic damage

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Background: Human multipotent stromal cells (MSC) persist throughout life in the adult organism and can be isolated after chemotherapeutic treatment as well as from elderly donors. We have previously shown a resistance of MSC for genotoxic damage which may facilitate this persistence. However the underlying mechanisms for this resistance remain unclear.
Methods: MSC were treated with cisplatin and etoposide for 24 h. Platinum accumulation was determined by atomic absorption spectroscopy. Cell cycle analyses were performed by flow cytometry, protein expression was examined by western blot. Treated MSC were analyzed for MSC characteristics using growth kinetics, cytochemistry and flow cytometry.
Results: Based on our previous work we showed that 2 µM cisplatin and 0.75 µM etoposide represented apoptosis-inducing concentrations in sensitive testicular germ cell tumor cells but resulted in no induction of apoptosis in MSC. However, 2 µM cisplatin resulted in a similar platinum accumulation in both cell types thus representing equivalent genotoxic damage leading to differential cellular response. This supported the notion that specific mechanisms confer resistance for genotoxic damage in MSC.
Treatment of MSC with these subapoptotic doses of cisplatin and etoposide resulted in a cell cycle arrest in the G2/M phase. Upon cisplatin treatment an intermediate S phase accumulation preceded the G2/M arrest.
The observed cell cycle arrest was temporary as after a lag phase treated MSC showed a reconstitution of proliferation. This reconstitution was characterized by an identical cell cycle distribution as well as similar population doubling times in treated cells (cisplatin 1.7 d, etoposide 1.6 d) and untreated controls (1.5 d). After reconstitution treated MSC showed the typical multipotent differentiation capacity and immunophenotype as characteristic for MSC.
Analysis at the time point of cell cycle arrest showed an increase in p53 and p21 as well as a decrease in cyclin B and cdk1 expression. This led to the assumption that the cell cycle arrest was induced by damage-associated p53 induction.
Conclusions: Our data suggest that a p53-dependent temporary cell cycle arrest contributes to the maintenance of the stem cell characteristics in MSC upon subapoptotic genotoxic damage. This property may contribute to the life-long persistence of the MSC population in the human adult organism. In contrast to pluripotent cells MSC seem to rely on functional p53.

Disclosure: No conflict of interest disclosed.