

Acute Homeostatic Proliferation of Naive CD8⁺ T Cells depends on CD62L/L-selectin mediated Homing to Intact Peripheral Lymph nodes

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Introduction: Adoptive transfer of naïve CD8⁺ T cells into lymphopenic recipients results in spontaneous proliferation and partial activation of T cells, a phenomenon termed acute homeostatic proliferation (HP). HP T cells show a "memory phenotype" characterised by CD44^{bright}, CD62L^{bright}, Ly6C^{bright} surface marker expression. HP has been shown to prevent T cell tolerance, reverse T cell anergy and support T cell-mediated tumor control in vivo. **Results:** The anatomic site of HP, however, is still under debate. Based on the observation that T cells undergoing HP retain the CD62L^{bright} phenotype, we examined in more detail the idea of HP occurring in secondary lymphoid structures. HP did not occur in Lymphotoxin (LT)ab^{-/-} mice, indicating indeed the necessity for intact structures of this organ compartment. Impaired HP from CD62L gene-deficient T cells narrowed the compartment where HP occurs further down towards the peripheral lymph nodes. To formerly prove these observations we inhibited the T cell lymph node egression which, indeed, prevented the occurrence of homeostatically proliferating T cells within spleen and other compartments. Using the MC57-SIY tumor model, we found that the HP-mediated control of tumor growth depends on CD62L expression on the tumor specific T cells. **Conclusions:** Transferring our results toward clinical approaches, e.g. adoptive T cell therapies, our data implicate the need for a transfer of CD62L^{bright} memory phenotype-like T cells to gain sustained anti-tumor immune responses.

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Tissue inhibitor of metalloproteinase-1 colocalizes with CD63 on CD34⁺ STEM and progenitor cells and inhibits migration towards SDF-1

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Introduction: Recently it has been shown that TIMP-1 protein forms a complex with the tetraspanin CD63 and β_1 -integrin VLA-4 in the immortalized human breast epithelial cell line MCF10A. As we found mRNA expression of TIMP-1, CD63 and VLA-4 in our gene array data sets of CD34⁺ human hematopoietic stem and progenitor cells, we expected tetraspanins and integrins to play a role in TIMP-1 signaling in CD34⁺ hematopoietic stem cells (HSCs). Here, we show the colocalization and functional interaction of TIMP-1 with CD63 and the integrin VLA-4 forming a receptor complex on G-CSF mobilized HSCs. **Methods:** For immunoprecipitation of CD63, immunomagnetically selected CD34⁺ cell lysates were used. CD63 content of the precipitates was then analyzed by western blotting. Confocal microscopy was performed with CD34⁺ cells labeled on coverslips using antibodies to CD63 and to TIMP-1. For analysis of downstream targets of the receptor complex, cells incubated with TIMP-1 for 1 to 4 hours and, following permeabilization, were stained with antibodies against activated extracellular-signal regulated kinase (pERK) and subjected to flow cytometry. For functional correlation of the receptor complex formation, transwell migration assays were performed following overnight incubation with TIMP-1. **Results:** In this study, we could show colocalization of TIMP-1 and CD63 by immunoprecipitation experiments and confocal laser microscopy. Confocal laser microscopy showed a polarized accumulation of TIMP-1 and CD63 in the plasma membrane of CD34⁺ stem and progenitor cells. TIMP-1 stimulation activates MAPK signaling as shown by increased phosphorylation of ERK amount after 3h. Migratory capacities of CD34⁺ cells are impaired after TIMP-1 incubation, suggesting that the TIMP-1 – CD63 colocalization modulates the functional status of VLA-4. **Conclusions:** In summary, we could show that colocalization

of TIMP-1 and CD63 on CD34⁺ cells is associated with impaired migratory capacities.

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Comprehensive validation of genome-wide CpG island methylation profiles for two human leukemia cell lines

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Methylation of CpG islands is associated with transcriptional repression and, in cancer, leads to the abnormal silencing of tumor suppressor genes. We have previously developed a genome wide methylation profiling assay based on a recombinant, antibody-like MBD-Fc fusion protein that allows methyl-CpG specific fractionation of DNA and the detection of CpG methylation independent of chemical DNA modification using bisulfite or methylation-sensitive restriction. Here, we present an in depth comparison of comparative methylation data obtained with an optimized MCIp/hybridization procedure and quantitative methylation data obtained by base-specific cleavage of bisulfite amplification products and Matrix-Assisted Laser Desorption/Ionization Time-Of-Flight Mass Spectrometry (MALDI-TOF MS). Comparative MCIp methylation profiles of appr. 23 000 CpG islands were obtained from two myeloid leukemia cell lines using 2 μ g of genomic DNA. A set of 140 genes (1300 amplicons covering approximately 13500 individual CpG dinucleotides) that were selected based on the array results were analyzed by MALDI-TOF MS. The comparison of both techniques shows an excellent correlation between bisulfite and MCIp data sets. Our comprehensive validation study shows that robust methylation profiles can be obtained with as little as 2 μ g of genomic DNA and demonstrates the high sensitivity and reproducibility of the MCIp approach.

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Large-scale expansion of CD3-CD56⁺ NK cells under GMP-compatible conditions results in highly increased antibody-dependent and natural cytotoxicity

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Introduction: Natural Killer (NK) cells not only show a high cytotoxic activity against tumour cells but are also known to be the major effector population of antibody dependent cellular cytotoxicity (ADCC). The use of monoclonal antibodies is widely growing and their efficacy could be increased by addition of high numbers of effectors cells. i.e. autologous NK cells. As numbers of NK cells in regular lymphapheresis are usually low, we aimed to develop a clinical GMP-compatible NK cell expansion protocol for subsequent combined use of monoclonal antibodies and IL-2 activated CD3-CD56⁺ NK cells. **Methods:** PBMC were obtained of the peripheral blood of healthy donors (n = 8) and patients with chronic lymphocytic leukaemia (CLL) (n=3) by CD56⁺ enrichment using magnetically labeled bead technology. NK cells were taken in culture in SCGM medium supplemented with 10% AB serum, Interleukin (IL)-2 (500 IU/ml; day 1-21) and IL-15 (10 U/ml; day 15-21). From day 1 to 5 OKT-3 (10 ng/ml) was added to the medium in order to deplete contaminating CD3+CD56⁺ NK T cells. Culture was performed for 21 days with a final analysis for NK cell purity and natural cytotoxicity against K562 cell line using calcein cytotoxicity assay and CD107a flow cytometry for assessment of NK cell degranulation. ADCC was analysed by using the anti CD20-antibody Rituximab and the CD20⁺ lymphoma cell line Raji. **Results:** CD3-CD56⁺ cells expanded 14 to 40-fold after the 21-day expansion period. Expansion of NK cells from patients with CLL was lower compared to healthy donors; however 20fold expansion was achieved. The mean NK cell purity was 75% (range 35-95%) with a viability of over 90% with NKT cells being the major contaminating cell population. The content of CD3+CD56⁺ T cells was below 5%. The expanded NK cells from healthy donors as well as from CLL patients showed a very strong lytic activity against the MHC-I deficient K562 cells compared to freshly isolated NK cells (67% vs. 38%). Phenotypic analysis

showed that expanded cells preserve high CD16 expression. Analysis of ADCC using anti-CD20 antibodies resulted in a highly increased lysis of up to 70% of the target cells. **Conclusions:** After a 21-day period, expansion and enrichment of NK cells using GMP-compatible components is feasible. The availability of high numbers of activated NK cells with highly increased lytic activity may comprise a treatment option for patients with haematological malignancies for which monoclonal antibodies are available, i.e. CD20 positive lymphomas/CLL.

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Heterogeneity of Human Mesenchymal Stromal Cells – Comparison of Isolation Methods Using Density Gradient Centrifugation or Red Blood Cell Lysis

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Introduction: Human mesenchymal stromal cells (MSC) raise high hopes for tissue engineering and clinical therapy but their use is hampered by the lack of standardized isolation methods and specific molecular markers. The initial steps of MSC isolation commonly involve density fractionation of mononuclear cells (MNC). However this is difficult to standardize especially under good manufacturing practices (GMP) conditions and the composition of subpopulations might be affected already by the initial steps of cell preparation. **Methods:** In this study we compared 1) isolation of human MSC by density gradient centrifugation on Ficoll, 2) red blood cell (RBC) lysis with ammonium chloride or 3) seeding of untreated bone marrow (BM) aspirate. The same amounts of BM aspirate were initially used and differences in the number and morphology of fibroblastic colony forming units (CFU-F) were determined as well as immunophenotype and differentiation potential. **Results:** With RBC lysis the number of CFU-F was slightly higher than by Ficoll procedure (median of 494 to 448 CFU-F per ml) and the number of larger colonies containing 50 – 250 cells after 1 week were significantly higher (29% ± 15%; 15% ± 14%, p = 0.014). This might result from the higher number of remaining platelets that provide growth factors. In contrast the erythrocyte fraction of untreated whole blood samples impaired CFU-F growth. All cell preparations demonstrated a similar immunophenotype (CD13⁺, CD29⁺, CD31⁺, CD34⁺, CD44⁺, CD45⁺, CD73⁺, CD90⁺, CD105⁺, CD146⁺, CD166⁺ and CD184⁺) and displayed adipogenic and osteogenic differentiation potential. The heterogeneous composition of subpopulations is reflected by morphological differences in the initial CFU-F. The three isolation methods did not affect the composition with regard to growth pattern (disperse versus tight) or cellular morphology (elongated versus round). **Discussion:** This study demonstrates that RBC lysis is an efficient method for isolation of MSC. This technique is faster and more standardized than the commonly used methods using density gradient centrifugation and it does not affect the heterogeneous composition of MSC preparations. Therefore, RBC lysis might facilitate use of MSC for clinical application.

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Triggering functionality of a human gp100 specific single chain TCR by partial murinisation

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Introduction: Adoptive T-cell transfer has proven efficiency for patients suffering from malignancies. In T-cell receptor (TCR) gene therapy, tumorantigen-specific TCRs are introduced into human T-cells in vitro and transferred into the

tumor patient to eradicate malignant cells. The transfer of double chain (dc)TCRs into human T-cells contains the risk of pairing of the introduced TCR α and β chains with the endogenous TCR chains, potentially leading to autoimmunity. To prevent this, a 3-domain single chain (sc)TCR specific for the gp100 tumor antigen was constructed by linking the variable V α - to the V β /C β -domains. To supplement the missing constant C α -domain, a truncated TCR α consisting of the C α domain and preceded by the TCR α signal peptide was generated and coexpressed. **Results:** After retroviral transduction of the scTCR/C α constructs into human T-cells, expression, cytotoxicity and cytokine secretion was strongly reduced compared to T-cells transduced with a gp100 specific dcTCR. Murinization of the C β -domain and coexpression with the murine C α construct restored expression and functionality comparable to that of the dcTCR. Since murine elements could lead to rejection of TCR transduced T-cells in vivo, altering subdomains or only single amino acids in the human constant TCR chains to trigger function is desirable. Initially, the non-homologous residues downstream the interchain disulfid-bond in C α and C β were changed to the corresponding amino acids of murine constant domains in human sc TCR/C α . T-cells that were transduced with a scTCR which was partially murinized in this way did not reveal functionality. In contrast, T-cells expressing a scTCR/C α which was murinized in the ectodomain upstream the disulfid-bond revealed cytotoxicity, albeit not to the extent of T-cells transduced with a wildtype or a C α /C β -murinized scTCR. This points to the importance of murine ectodomains. Subsequently, four amino acids in the human C β ectodomain were exchanged to the corresponding basic amino acids present in the murine C β . Two of the basic residues may interact dominantly with the CD3 complex whereas the other two may influence the interchain interaction of TCR α and β . The exchange of certain, but not all, of these amino acids resulted in a moderate increase in cytokine secretion and cytotoxicity. Currently, we are investigating if an additional exchange of the potential interaction partners of these residues in C α might improve the observed effect.

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Orexins play a functional role in CD34⁺ STEM and progenitor cell adhesion and differentiation

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Introduction: The hypothalamic peptides orexin A (OxA) and orexin B (OxB) have been initially identified as regulators of sleep, wakefulness and feeding. By screening of gene array data sets we found expression of their receptors orexin receptor 1 (OxR1) and orexin receptor 2 (OxR2) on CD34⁺ hematopoietic stem and progenitor cells. Upon stimulation of these receptors with OxA and OxB we found a G-protein-mediated decrease of intracellular cAMP concentrations. **Methods:** We assessed the OxR signaling after stimulation with their ligands OxA and OxB alone, in combination with the selective OxR1 antagonist SB-334867 or the antagonist alone by measurement of the intracellular calcium content as well as the amount of phosphorylated ERK (pERK) by flowcytometry-based assays. Further functional assays include clonogenic assays, long-term culture initiating cell (LTC-IC) assays, ELISA, apoptosis and proliferation assays as well as adhesion and transwell migration assays. **Results:** OxA and OxB stimulation leads to a decreased intracellular cAMP and calcium concentration suggesting association of OxR with inhibitory G-proteins rather than stimulatory G-proteins. A higher amount of pERK after 4h stimulation with OxA and OxB indicated activation of the MAPK/ERK pathway which declines already after 6 and 8h of continuous stimulation. Functionally, overnight incubation of CD34⁺ cells with OxA or OxB, respectively, showed impaired adhesive capacities whereas no influence could be seen on chemotaxis of the stem- and progenitor cells. Of note, orexin stimulated cells seem to bear less burst forming units erythroid (BFU-E) and colony forming units erythroid (CFU-E) and a lower proportion of colony-forming units granulocyte (CFU-G), granulocyte-macrophage (CFU-GM) and macrophage (CFU-M) as compared to unstimulated cells or those treated with the antagonist. In contrast the multipotent conlony forming units granulocyte, erythrocyte, megakaryocyte, macrophage (CFU-GEMM) were increased as confirmed by an increased LTC-IC frequency in orexin stimulated cells. To adress the question, if orexins are delivered in a paracrine fashion or produced locally, we compared the concentration of OxA in serum of peripheral blood and bone marrow of patients and healthy stem cell donors and found a at least 1.5 fold increased concentration in the bone marrow serum, thus point-

ing towards an OxA production by niche-forming tissues. **Conclusion:** OXR1 and OXR2 exert functionally relevant effects upon stimulation with OxA and OxB in hematopoietic stem and progenitor cells.

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VIP loaded nanoformulated protamin based particles target VPAC receptors, – a new tool for tumor targeting

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Vasoactive intestinal peptide (VIP) can target tumor cells, it binds to specific G-protein coupled receptors. The peptide-receptor-complexes transport conjugated chemical groups (e.g. therapeutic substances & fluorochromes) into the cell. The clinical use for tumor detection via peptides is hampered by i) the peptides rapid degradation resulting in a short half-life, and ii) the possible vasoreactive hypotonic reactions induced by the peptide. Protamin based nanoparticles (proticles) as pharmacophor for VIP conjugates can overcome the particular shortcomings. They could serve as dispersible substance depot, protect the peptide from enzymatic degradation, and ameliorate the hypotonic reaction by sustained substance release. Furthermore we speculated that the VIP loaded proticles might be targeted as a whole due to the peptide receptor interaction, and hence accumulate therapeutic and diagnostic moieties in front of the target cell. To investigate the proticle's potential to target tumor cells, we used stained proticles loaded with VIP-Cy3. Fluorescence correlation spectroscopy (FCS) revealed the stability of proticles in dilutions and the internalization of VIP-Cy3 by PANC-1 tumor cells. Because any bioactivity (e.g. artery relaxation), requires the peptide internalization, we observed artery relaxation to measure the dynamics of VIP release in a non fluorescent assay. With confocal laser scanning microscopy we observed that proticles accumulate in front of the target cell, while the peptide-receptor-complex transfers the conjugate to the cytoplasm. With double fluorescent labelled VIP loaded proticles we directly observed the target kinetics of proticles towards the cancer cell and the release and uptake of the VIP-conjugate. We conclude that VIP can mediate the proticle-targeting towards the VIP-receptors. While the peptide improves the proticle targeting, the nano-formulated proticle can improve the VIP mediated conjugate delivery via the receptor.

The Austrian Nano-Initiative co-financed this work as part of the Nano-Health project (no. 0200). This work was co-financed by the European commission (grant No. QLK3-CT-2002-02031). By the the medical science fond of the major of Vienna (# 2528), and by the Austrian National Bank (grant no. 12264).

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A Peptide Epitope Derived from the Cancer Testis Antigen HOM-MEL-40/SSX2 with Bispecificity for CD4+ and CD8+ T cells

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Introduction: Antigen-derived HLA class I-restricted peptides can generate specific CD8+ T-cell responses in vivo and are therefore often used in peptide vaccine protocols for patients with cancer. However, only occasional objective clinical responses have been reported suggesting the necessity of CD4+ T-cell help and possibly antibodies for the induction of effective anti-tumor immunity in vivo. The SSX2 gene encodes the cancer-testis antigen HOM-MEL-40/SSX2 frequently expressed in melanomas and breast cancers, but also in other tumors. Both humoral and cellular immune responses against SSX-2

have been described making SSX2 an attractive candidate for vaccine trials. **Methods:** SYFPEITHI algorithm was used to predict five pentadecamer peptides with a high binding probability for six selected HLA-DRB1 subtypes (*0101, *0301, *0401, *0701, *1101, *1501) which are prevalent in the Caucasian population. **Results:** Using peripheral blood cells of 9 breast cancer patients and 5 healthy controls the HOM-MEL-40/SSX2-derived peptide p101-111 was identified as an epitope with bispecificity for both CD4+ helper and cytotoxic CD8+ T cells. Our study shows that SSX2/p101-111 simultaneously induced tumor antigen-specific CD8 and CD4 responses in vitro including cytotoxic activity against SSX2 expressing tumor cells. **Conclusions:** This bispecific peptide represents an attractive vaccine candidate for the induction of effective tumor immunity in vitro and in vivo.

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New strategies for cancer research via a platform for interdisciplinary human life tissue donation

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Background: Pre-clinical research on human material increases the chances of successful drug development. Currently there is no clear regulation of good practice for the use of human tissue for pharmacological research. Interdisciplinary collaboration has higher chances to produce exploitable results. Because human tissue is frequently disposed by the pathologist, the interdisciplinary collaboration of surgeons, pathologists and basic researchers could enable the use of this tissue for in vitro tests on human tissue without any risk for the patient. We aimed to establish guidelines for "good practice tissue donation" to regulate the interdisciplinary collaboration from "bed to bench" but also to avoid the threat of patient claims for tissue ownership. **Methods:** Ethical and legal experts were consulted in the process of the platform development. A data bank structure to facilitate the efficient data exchange between clinical and basic researchers, which respects legal principles and current law (e.g. patient privacy) was developed. Project Management tools were introduced to build up the transfer logistics and to enable efficient communication between clinical and basic researchers. **Results:** We suggest the use of an informed consensus statement reviewed by the relevant ethic commission to allow the patient to give up ownership related rights on the tissue. We established communication pathways and tested them successfully at the operative level. We developed and now present an "operational map" to summarize the potential and the activities establishing of such a platform. **Conclusion:** The operational map proved to be administrable and executable. It shows the platform's potential to improve interdisciplinary biomedical research. Typical reasons for project failure are lack of communication at the executive and/or at the project-governance level. In case of successful platform installation and operation it creates new potential for successful collaborations between clinicians, pathologists, and preclinical researchers.

The Austrian Nano-Initiative co-financed this work as part of the Nano-Health project (no. 0200). This work was co-financed by the European commission (grant No. QLK3-CT-2002-02031). By the the medical science fond of the major of Vienna (# 2528), and by the Austrian National Bank (grant no. 12264).

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Vasoactive intestinal peptide receptor type 1 (VIP R1) targeting - a vaso-inactive marker for tumor search

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Certain tumor species like pancreas, colon, breast, prostate, lung, etc. bind and internalize the Vasoactive Intestinal Peptide (VIP), and therefore can be visualized via positron emission tomography (PET) scan. There are two high affinity receptors for VIP, i. e. VPAC-1 and VPAC-2 recognized by the peptide. The tumor detection is hampered by the vasoactivity of the peptide, the clinical use requires the administration of high doses, which leads to a generalized hypotonic reaction. This hypotony reaction limits the clinical use of VIP, it could be circumvented by using VIP analogues which target the tumor but do not relax blood vessel to the same extend as native VIP. If blood vessels preferentially express only one receptor type, then a specific analogue for this particular receptor type could overcome the inherent problems of high doses of systemically administrated VIP. Envisioning a peptide, which can be used in high doses without limiting side effects, we investigated VIP-Analogues and their vasorelaxing potential. In an established setup we investigated a VPAC-1 selective analogue on life arteries "ex vivo - in vitro" from rats and human. Rats were sacrificed and the surviving pulmonary artery was dissected and exposed to VIP-analogues under experimental condition. Human arteries where obtained from lung tissue after therapeutic lobectomy (e.g. lung cancer). In our setting native VIP relaxed the arteries from rat and human, to over 40%, while a commercially available analogue specific for VPAC-1 (Bachem, Bubendorf, CH) revealed a vasorelaxing response below 10%. We could reliably reproduce the result in rat and human tissue. Our results indicate that analogues, specific for the VIP-receptor 1 considerably and significantly reduce the limiting side-effects of tumor detection, they have promising potential for the diagnostic use in PET-Scans with VIP.

The Austrian Nano-Initiative co-financed this work as part of the Nano-Health project (no. 0200). This work was co-financed by the European commission (grant No. QLK3-CT-2002-02031). By the the medical science fonds of the major of Vienna (# 2528), and by the Austrian National Bank (grant no. 12264).

P414a

CARL – Controlled application and removal of liposomal chemotherapeutics by apheresis - a new dimension in pharmacotherapy

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Introduction: Using highly toxic drugs like chemotherapeutic agents (CTA), therapeutic success is often limited by severe side effects. Beside many efforts in drug targeting, only a small portion of a given total dosage reaches its target tissue. The predominant portion has no therapeutic value, but leads to the severe adverse effects occurring during chemotherapy. To lower the acute adverse effects of CTA, liposomes (Caelyx®) can be used as drug-delivery systems (DDS). The toxic profile of the encapsulated chemotherapeutic agent is shifted, but detoxification of drugs not reaching the tumor still remains an obstacle. Nanoscale particle based DDS like liposomes accumulate to some extent in tumor tissues due to the enhanced permeability and retention effect. But the liposomes accumulate in other tissues as well, one of the prominent tissues is the skin, where they cause hand-foot-syndrome. Extravasation into skin and paws of mice is much slower than extravasation into tumor tissue. After 24 h, cmax is reached in the tumor tissue while only about 50 % of cmax is reached in the skin, and 70 % of the initial dose is still circulating in the plasma. Thus extracorporeal elimination of liposomal chemotherapeutics after accumulation in tumor tissue may significant reduce the patients burden with toxic drug and reduce severe adverse effects occurring during chemotherapy. We present the data of the first patients treated for breastcancer in our phase I

pilot study. **Methods:** CARL-Apheresis of Caelyx by therapeutic MDF: Apheresis was performed on a Octonova Apheresis system (Diamed Medizin-technik GmbH, Germany), a filter Plasmaflow OP-02W(L) for cell separation and a filter Cascadeflow EC-50W for plasma filtration were used (both Asahi Medical Co. Ltd., Japan) after Informed consent. Apheresis was performed after 36-42h after administration of Caelyx. To allow continuous extraction of samples, three-way valves were used. Flow rate in the blood circuit was 50-75 ml/min and flow rate in the plasma circuit was 20-25 ml/min. Transmembrane pressure was not higher than 95 mbar. The filter system was equilibrated with 0,9% (w/v) NaCl prior to use. **Results:** Elimination of Caelyx in the first 5 treatments ranged from 65% to 75% of the initial dose before apheresis. The apheresis procedure did not cause release of free doxorubicin. **Conclusions:** The first treatments proof high efficiency (~70% elimination of circulating CTA) and safety. After presentation of the proof of principle at the last DGHO meeting last year we moved on and successfully started the phase I clinical trail.

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Proteomic characterization of primary human endothelial cells of different tissues at various functional states

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Introduction: The vascular endothelium is involved in a variety of processes such as coagulation, hemostasis, inflammation, atherosclerosis, angiogenesis and dissemination of cancer cells. Mounting evidence suggests a distinct difference between endothelial cells of various tissue origins and activation states detectable by mRNA protein profiling. **Methods:** Endothelial cells (EC) from liver, prostate and large intestine were isolated using magnetic beads coated with antibodies against CD31. EC from umbilical cords where isolated by flushing the umbilical vein with Dispase. Endothelial origin of isolated cells was confirmed by FACS analysis (expression of CD31, CD34, CD54, expression of CD62E after treatment with TNF- α no expression of podoplanin and CD106). Cells where cultured in Endothelial Basal Medium-2 (EBM-2) supplemented with growth factors VEGF, FGF-2, EGF, IGF, and 20% fetal calf serum. Cellular response was triggered by 24 hours incubation with 10U/ml TNF-alpha and 100U/ml IL-1beta in protein and growth factor free EBM-2(MV). Supernatant and cytosol was collected and subjected to 1D-PAGE to preseparate proteins prior to mass spectrometry. Proteins where identified by MS/MS fragmentation analysis of tryptic digests with an iontrap mass spectrometer. MS/MS data were interpreted by the Spectrum Mill MS Proteomics Workbench software (Version A.03.03, Agilent) and searched against the SwissProt Database. Mass data were organized and searched for potential biomarkers using the CPL/MUW database developed in our lab. **Results:** We identified 1240 proteins in the cytosol and 442 proteins in the secretome of EC, encompassing cytoskeletal proteins, proteins involved in motility and plasticity, signal transduction, transcription, translation, regulation of apoptosis, coagulation, endocytosis, antigen presentation, detoxification and various metabolisms (lipides, amino acids, carbohydrates and purines/pyrimidines). Cytokines, chemokines, growth factors, proteases, protease inhibitors and extracellular matrix proteins were identified in the secretome. **Conclusions:** EC express a protein profile highly typical for their tissue origin and functional state. With the aid shotgun proteomics and the CPL/MUW-database, we were able to identify indicative protein clusters, which may be useful for the identification of biomarkers as well as therapeutic targets.

Roscovitin up-regulates p53 protein and induces apoptosis in human HeLaS₃ cervix carcinoma cells

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Introduction: Exposure of human HeLaS₃ cervix carcinoma cells to high doses of conventional cytostatic drugs e.g. cisplatin (CP) strongly inhibits their proliferation. However, most cytostatic agents are genotoxic and may generate a secondary malignancy. Therefore, therapeutic strategy using alternative, not cytotoxic drugs would be beneficial. Inhibition of cyclin-dependent kinases (CDKs) by pharmacological inhibitors became recently a promising therapeutic option. Roscovitin (ROSC), a selective CDK inhibitor, efficiently targets human malignant cells. ROSC induces cell cycle arrest and apoptosis in human MCF-7 breast cancer cells^{1,2}. ROSC also activates p53 protein². Activation of p53 tumor suppressor protein is essential for induction of apoptosis in MCF-7 cells. **Methods:** The number of viable cells was determined using CellTiterLumiGlo Assay, DNA concentration in single cells was measured by flow cytometry, the potential of mitochondrial membrane by flow cytometry, expression and activity status of cell cycle regulators by immunoblotting. **Results:** Considering the fact that in HeLaS₃ cells wt p53 is inactivated by the action of HPV-encoded E6 oncoprotein, we addressed the question whether ROSC would be able to reactivate p53 protein in them. Their exposure to ROSC for 24h induced cell cycle arrest at G₂/M and reduced the number of viable cells. Unlike CP, ROSC in the used doses did not induce DNA damage and was not directly cytotoxic. Despite lack of detectable DNA lesions, ROSC activated wt p53 protein. The increase of p53 levels was attributable to the ROSC-mediated protein stabilization. Further analyses revealed that ROSC induces site-specific phosphorylation of p53 protein at Ser46. After longer exposure, ROSC induced apoptosis in HeLaS₃ cells. **Conclusions:** These results indicate that therapy of HeLaS₃ cells by ROSC could offer an advantage over that by CP due to its increased selectivity and markedly reduced risk of generation of a secondary cancer.

References

1. J. Wojciechowski et al. (2003) Rapid onset of nucleolar disintegration preceding cell cycle arrest in roscovitin-induced apoptosis of human MCF-7 breast cancer cells. *Int. J. Cancer* 106: 486-495.
2. J. Węsierska-Gądek et al. (2005) Roscovitin-induced up-regulation of p53AIP1 protein precedes the onset of apoptosis in human MCF-7 breast cancer cells. *Mol. Cancer Ther* 4: 113-124.

Interaction of the *Fusarium* Toxin Enniatin with Multidrug Resistance-Mediating ABC Transporters

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Introduction: Enniatin (ENN) is a fungal secondary metabolite, produced by the genus *Fusarium*. It possesses ionophoric, antibiotic, anthelmintic and phytoxic properties. Moreover, ENN was previously shown to exert potent cytotoxic activities against several cancer cell lines based on apoptosis induction. Thus, this mycotoxin might be of interest for the development as an anticancer drug. One of the main obstacles in chemotherapy of cancer is the multidrug resistance (MDR). Consequently, this study aimed to determine the interaction of ENN with several MDR-associated ABC transport proteins. **Methods:** For this purpose cytotoxicity studies (MTT assays) and clonogenic assays with several human cancer cell lines and their chemoresistant sublines were performed. The effect of ENN on the ABCB1 and ABCG2 transport function was tested in efflux assays using specific fluorescence substrates (Rhodamine 123, Pheophorbide A and topotecan). To gain further insights into the interaction of ENN with ABCB1 and ABCG2, crude membranes (1 µg/ml) of ABCB1- and ABCG2-overexpressing cells were photoaffinity labelled with 3-6 nM

[¹²⁵I]IAAP (2200 Ci/mmol) in the presence of ENN. Moreover, to test whether ENN treatment led to induction of ABC transporter expression, KB-3-1 cells were selected against this fusariotoxin for ~ 2 years. **Results:** Short-term cytotoxicity assays showed no impact of ABC transporter overexpression on ENN-induced cytotoxicity. In contrast, sublines overexpressing ABCB1 or ABCG2 responded less sensitive to ENN treatment in long-term clonogenic assays. Moreover, ENN potently inhibited the ABCG2- and ABCB1-mediated efflux of specific fluorescent substrates. Additionally, photoaffinity labelling experiments proofed interaction with ABCG2 and ABCB1. The selection of KB-3-1 cells against increasing concentrations of ENN led to a 2-fold ENN-resistant subline displaying upregulation of ABCC1, ABCC2 and ABCG2 but not ABCB1. **Conclusions:** Summarizing, these data demonstrate that classical MDR ABC transport proteins only weakly protect against ENN-induced cytotoxicity. Moreover, acquired ENN resistance development is negligible further suggesting its possible use in cancer therapy. Moreover, ENN potently blocks several ABC transport proteins suggesting MDR-reversing functions but also possible impacts on the pharmacological profiles of other drugs and toxins.

Gerinnung

Poster:

Blood Count Score enables to predict thromboembolic events in cancer patients – results from the Vienna Cancer and Thrombosis Study (CATS)

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Introduction: Venous thromboembolic events (VTE) are a frequent complication in cancer care. In order to identify reliable markers of risk prediction, we assessed a Blood Count Score (BCS) including platelets, haemoglobin and leukocytes, as predictor for VTE in cancer patients. **Methods:** The Cancer and Thrombosis Study (CATS) is an ongoing prospective observational study in patients with newly diagnosed cancer or progression of disease, enrolment started 10/2003. Occurrence of VTE and information on the patients' anti-cancer-treatment during follow-up are recorded. Observation ends with occurrence of VTE, death or after 2 years. At the time of enrolment, a blood sample of 3ml was taken, in order to assess the patient's complete blood count. A VTE

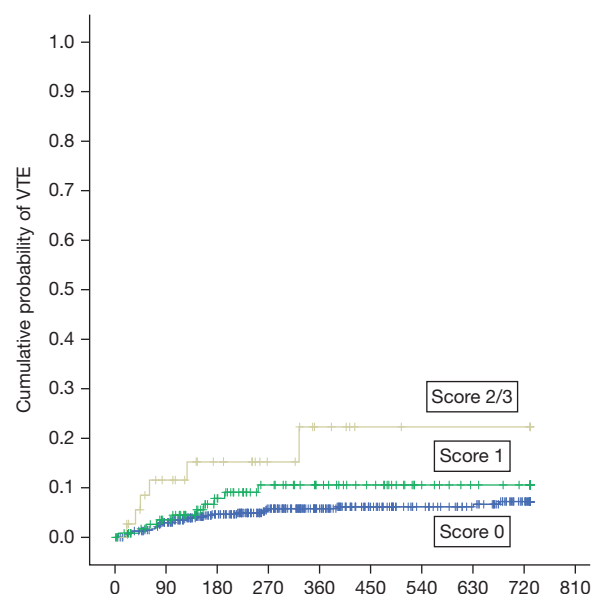


Fig. 1. Cumulative probability of VTE in patients with a score of 0, 1 and 2/3

risk score was calculated based on findings of the blood cell count. This score is a simplified version of a score first published by Khorana et al. (*Blood*. 2008 May 15;111(10):4902-7). A hemoglobin level below 100 g/L, platelet count above $350 \times 10^9/L$ and leucocyte count above $11 \times 10^9/L$ increased the score by 1, respectively. **Results:** Data on 635 patients with cancer (302 women/333 men) were available for analyses. Patients were followed for a median observation time of 366 days (interquartile range 174 – 730). 44 VTE were observed. Main tumour entities were malignancies of the breast (n=138), lung (n=112), gastrointestinal tract (n=153), pancreas (n=55), kidney (n=30), prostate (n=102) and others (n=45, rare cancer types like sarcoma or cholangiocellular carcinoma). 477 patients had a score of 0 (75%), 120 of 1 (19%), 32 of 2 (5%) and 6 patients (1%) had a score of 3. Because of the low number of patients with a score of 3 these patients were analysed together with those with a score of 2 further on. Compared to patients with a score of 0 those with a score of 1 had a hazard ratio of 1.6 [0.8 – 3.4, p=0.19] and those with a score of 2/3 had a hazard ratio of 3.8 [1.6 – 9.2, p=0.003], respectively, adjusted for age and sex. The cumulative probability of a VTE in patients with a score of 0, 1, 2/3 at 6 months was 5%, 9% and 16% respectively (Fig. 1). **Conclusions:** The BCS offers a simple and reliable method to determine which cancer patients are at highest risk of suffering a VTE. Using this score allows to identify a relative small (6%) high risk group of patients. This score could be used for risk stratification in future interventional trials.

P419

Evaluation of safety and efficacy of recombinant Factor IX in daily clinical practice: a pharmacovigilance evaluation of BeneFIX

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Introduction: Haemophilia B (HB) is a rare coagulation disorder with an incidence of about 1:25,000 in male newborns. Treatment of choice is prophylactic or on-demand substitution of coagulation factor IX (FIX) in a home-treatment setting. Today, two different types of factor concentrates are marketed in Germany: plasma-derived products and a recombinant product (nonacog alfa, BeneFIX). Nonacog alfa has proven its safety and efficacy in clinical studies including previously treated patients, previously untreated patients and during surgery. A new formulation of nonacog alfa was introduced to the market in Oct. 2007. Clinical studies have confirmed bioequivalence with the old formulation as well as good safety and efficacy. Since HB is a very rare disease and only a limited number of patients can be included in clinical trials, a non-interventional post-authorization study with a special focus on safety parameters appears to be adequate. We here report on the study design and first results of the pharmacovigilance evaluation (PE) of BeneFIX. **Methods:** To prospectively evaluate BeneFIX in the usual health care setting we started a non-interventional study including HB patients of any severity treated with ref. BeneFIX. Primary objective of the study is the long-term evaluation of BeneFIX safety profile when used outside of clinical trials. The secondary objective is the evaluation of efficacy of treatment with BeneFIX. Inclusion criteria for patients are: diagnosis of HB, treatment with ref. Ben FIX and written informed consent. The aim is to include approx. 80-100 patients within the first three years. **Results:** With regulatory and ethic committee's notification the PE started in Germany in February 2008. The study is set up and managed by the medical department of Wyeth in collaboration with a scientific advisory board. Data-collection and data-management as well as monitoring are supported by a clinical research organization. The data collection period will last for at least 3 years and is very likely to be extended. Until the end of April 2008 9 centers have been initiated, and 8 patients have been included. **Conclusions:** Non-interventional trials in the usual health care setting are adequate means to assess the safety and efficacy of a treatment in the post-authorization phase. Especially in very rare diseases such as HB the conduct of this kind of study appears to be reasonable in order to monitor a larger cohort of patients over a longer period of time. First results will be presented in Oct. 2008.

GI Onkologie

Freie Vorträge:

V420

A randomized trial in patients with gemcitabine refractory pancreatic cancer. Final results of the CONKO 003 study

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Introduction: For nearly ten years gemcitabine (G) was standard first line therapy for patients (pts) with advanced pancreatic cancer (APC). There is no consensus about second line therapy after disease progression while receiving G, but 5-FU-based regimens are considered. Randomized second line studies in APC are very rare. Our phase II study (ASCO 2002) showed activity of the OFF (Oxaliplatin/Folinic Acid (FA)/ 5-Fluorouracil (FU) [24h]) regimen in 23 pts. To examine the impact and the side effects of oxaliplatin we initiated a multicenter phase III study to compare OFF and FF in pts with G refractory APC. **Methods:** Pts with CT/ MRT confirmed PD on G in first line therapy, Karnofsky Performance Status (KPS) >60%, controlled pain, adequate hematological, renal and liver functions were eligible. Pts were stratified according to duration of first line therapy, KPS and tumor stage. We planned to randomized 165 pts to outpatient treatment with FF (5-FU 2g/m² (24h)/ FA 200 mg/m on d1, d8, d15 and d22) or OFF (FF+Oxaliplatin 85mg/m², d8, d22). Start of next cycle on day 43. Clinical follow up every week, CT/ MRI at least every 12 weeks or any sign of disease progression. **Results:** We recruited 168 pts between 02/2004 and 06/2007. Drop out rate is 4,8%. There is a full data assessment of 160 pts. OFF results in a significant longer time to progression (13 vs. 9 weeks; p = 0.012) and overall survival in secondline therapy (26 vs. 13 weeks; p = 0.014) as compared to FF. Side effects were similar, except the higher count of paraesthesia in the OFF treatment group.

V421

Randomized multicenter study of cetuximab plus FOLFOX or cetuximab plus FOLFIRI in neoadjuvant treatment of non-resectable colorectal liver metastases (CELIM-study)

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Purpose: Resectability of colorectal liver metastases (mets) can be induced by an effective chemotherapy regimen. Combinations of cetuximab with FOLFIRI or FOLFOX have been shown to increase response and resection rates. **Methods:** Patients (pts) with non-resectable liver mets were randomized to receive FOLFOX6 or FOLFIRI plus cetuximab each in this multicenter, randomized phase II study. Pts were stratified according to the reason for non-resectability (technically non-resectable vs. ≥ 5 liver mets), the use of PET scans at initial staging and EGFR status. Preoperative treatment was planned for 8 cycles. In case of persistent non-resectability, multidisciplinary evaluation was planned every four cycles. **Results:** From Dec 2004 to Mar 2008, 124 pts were screened for the study. 111 pts were randomized to receive FOLFOX-Cet. (56 pts) or FOLFIRI-Cet. (55 pts). Median age was 63 years. Out of the 111 pts, 60 pts (54%) were judged as technically non-resectable, 20 pts (18%) were staged with PET, and 81 pts (73%) were EGFR detectable. At the interim analysis in March 2008, response and resection data were available from 81 pts. Best response was 75.3% (61/81 pts, 95%CI 64.5-84.2%, combined analysis for both

arms), confirmed response 59.3% (48/81 pts, 95% CI 47.7-70.0%). KRAS status was available for 86 pts, best response rate in KRAS wild type pts was 85.4% (41/48 pts, 95%CI 72.2-93.9%), and 50% (7/14 pts) in KRAS mutant pts. Sixteen resections were performed in pts with ≥ 5 liver mets, 18 resections in technically non-resectable pts. In total, 34/81 pts were resected (42.0%, 95%CI 31.1-53.5%), twenty nine with microscopically free margins (R0). Interim data on toxicity of 98 pts demonstrated acne like rash (32%), neutropenia (20%), diarrhea (15%), allergic reaction (6%), neurologic toxicity (5%) to be the most common preoperative grade ≥ 3 toxicities in both arms. One patient had a fatal pulmonary embolism (2.9%). **Conclusion:** In the interim analysis, the combination of cetuximab with standard chemotherapy has demonstrated high activity and an encouraging rate of liver resection. Mature resection and response data per treatment arm and KRAS status will be reported at the meeting.

V422

Matrix metalloproteinase-9 (MMP-9) and vascular endothelial growth factor (VEGF) mRNA expression levels in patients with metastatic gastric cancer receiving first-line chemotherapy

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Introduction: In preclinical tumor models, VEGF induced MMP-9, resulting in the enhancement of tumor angiogenesis and the formation of metastases. This study evaluated MMP-9 and VEGF in patients (pts) with metastatic gastric cancer undergoing chemotherapy. **Methods:** Tumor samples from pts who received first-line chemotherapy for metastatic gastric cancer within a phase III trial of the AIO were prospectively collected and analyzed. VEGF and MMP-9 mRNA were isolated from paraffin embedded tissues using a new patented method based on nanotechnology. The expression was then assessed by real-time quantitative reverse transcription-polymerase chain reaction (qRT-PCR) and by immunohistochemistry (IHC). Because potentially relevant to the MMP-9 pathway, immune cell infiltrates, e.g. T-cells, NK-cells, mast cells, and macrophages, were evaluated by IHC. **Results:** Overall, 288 samples from 168 out of 220 pts enrolled were analyzed for at least one of the marker. 109 pts have been assessed for MMP9 mRNA so far; 96 pts were eligible for the analysis. In this cohort, increased MMP-9 levels were strongly associated with increased VEGF expression, resistance to chemotherapy and shorter progression-free and overall survival. At the cut-off with the highest predictive value, the rate of disease progression as best response associated with elevated MMP9 expression was 50% (vs. 22.4%; fisher's test $p=.027$) and median overall survival was 3.1 months (vs. 8.7 months, log rank $p=.0000165$). This prognostic value for survival was maintained when data were categorized according to the median ($p=.024$) or quartile ($p=.004$) distributions for MMP-9 expression. Conversely, extraordinary prolonged survival (> 24 months) correlated strongly with extremely low levels of MMP-9 and VEGF mRNA. Elevated MMP-9 levels were associated with synchronous metastases and more lymph node and peritoneal involvement. However, MMP-9 expression (mRNA) was the only independent prognostic factor in the multivariate analyses (relative hazard ratio for death 3.666, $p=.00045$). In IHC, MMP-9 was expressed in tumor cells, intratumoral immune cell infiltrates, and the extra-cellular matrix. **Conclusions:** MMP-9 mRNA expression levels correlate with increased VEGF and are linked to an aggressive clinical course in metastatic gastric cancer. These results may have significant implications for novel anti-protease/ anti-VEGF treatment strategies.

V423

Cetuximab and cisplatin/5-FU (CF) versus CF in first-line metastatic squamous cell carcinoma of the esophagus (MESCC): A randomized phase II study of the Arbeitsgemeinschaft Internistische Onkologie (AIO)

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Background: Combining the EGFR antibody cetuximab with chemotherapy has shown increased efficacy compared with chemotherapy alone in various types of cancer. This trial was conducted to assess the activity of cetuximab in combination with CF in MESCC. **Methods:** Patients (pts) with no prior treatment for metastatic disease received cetuximab (400 mg/m² at first infusion followed by weekly 250 mg/m²) in combination with CF (100 mg/m² d1 plus 5-FU 1000 mg/m² d1-5 every 29d) or CF alone. The primary endpoint was tumor response (RECIST). Patients who progressed on CF were allowed to crossover to CF+cetuximab or cetuximab alone. **Results:** 66 pts with MESCC were included (median age 61y, range 40-76y; ECOG PS 0/1: 33/33). Two pts died and 2 pts had a deterioration of their PS before therapy started and were excluded from analyses. In 62 pts (CF 30, cetuximab and CF 32), the median duration of treatment was 11 wks for CF and 16 wks for cetuximab+CF. Of 5 pts who crossed over to cetuximab after disease progression with CF, 3 showed disease control, including 2 PRs (1 on cetuximab alone; 1 on cetuximab+CF) and 1 SD (cetuximab alone). With a median follow-up of 21.5 mo, median PFS was 3.6 [1.0-6.2] vs 5.7 [3.4-8.0] mo and median overall survival 5.5 [1.9-9.1] vs 9.5 [8.4-10.6] mo for CF vs cetuximab+CF. Median survival was 9.3 mo in pts with grade 2/3 skin reactions and 7.0 mo in pts with grade 1 or no skin reactions. **Conclusions:** Cetuximab can be combined safely with standard doses of CF. Although this study was not powered to demonstrate a significant difference in survival, results suggest that the addition of cetuximab may increase the efficacy of standard CF chemotherapy in MESCC.

V424

Efficacy and safety of sorafenib in patients with alcohol-related hepatocellular carcinoma: a sub-analysis from the SHARP trial

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Introduction: The vast majority of HCC patients are diagnosed with advanced disease not suitable for curative treatment approaches. The only systemic therapy currently approved for HCC is sorafenib, a multi-kinase inhibitor. Approval of sorafenib by the FDA and EMEA for the treatment of HCC followed results from the phase III Sorafenib HCC Assessment Randomized Protocol (SHARP) trial, which demonstrated that sorafenib 400mg b.i.d. improves overall survival (OS) versus placebo in advanced HCC. The etiological factors most commonly linked to HCC are infection with either hepatitis B virus (HBV) or hepatitis C virus (HCV), and alcohol abuse. Here we report a subgroup analysis of SHARP assessing safety and efficacy of sorafenib in patients with alcohol-related advanced HCC. **Methods:** In SHARP, 602 patients with advanced, unresectable HCC were randomized to sorafenib

400mg b.i.d. or placebo. In this subanalysis, OS and time to progression (TTP) were evaluated for patients with alcohol-related HCC. Events were independently assessed. **Results:** In the intent-to-treat population, 79 patients (pts) in the sorafenib group and 80 pts in the placebo group had an alcohol abuse – history related to the underlying disease. Median OS for sorafenib vs placebo was 10.32 vs 7.99 months (HR: 0.76; 95% CI: 0.50, 1.16) and TTP was 5.52 vs 3.94 months (HR: 0.64; 95% CI: 0.40, 1.03). Drug-related, treatment-emergent adverse events were reported in 80.8% of the pts in the sorafenib group vs 53.8% of the pts in the placebo group. The most common drug-related adverse events in the sorafenib group were diarrhea (47.4%), fatigue (20.5%) and hand-foot skin reaction (20.5%); in the placebo group these adverse events occurred at rates of 10%, 12.5% and 6.3%, respectively. **Conclusions:** The small number of pts in this sub-analysis warrants careful interpretation. However, the results from pts with alcohol-related advanced HCC suggest a trend of improved OS and TTP in favor of sorafenib and are consistent with the overall results in the SHARP study population, supporting the use of sorafenib as a valid treatment option for a wide range of patients with HCC.

Indolente Lymphome

Freie Vorträge:

V425

Efficient induction of lymphoma-specific immunity with a recombinant idiotype vaccine (IDIOVAX) as remission maintenance in indolent B-NHL

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The immunoglobulin receptor of B-cell lymphomas (B-NHL) constitutes a tumor-specific antigen (idiotype, Id) and an attractive target for active immunotherapy. The immunological efficacy of s.c. immunization with Id coupled to the immunogenic carrier KLH has been assessed in phase II and III trials for indolent B-NHL patients in clinical remission. Id-specific humoral and cellular immune responses (IR) have been detected in 35-65% and 20-90% of patients, respectively, and were associated with superior outcome (Weng 2004, Inoges 2006, www.genitope.com). To ease and accelerate manufacturing of patient-individual Id vaccines, we have developed a production strategy based on expression of recombinant Fab fragments in *E. coli*. This uncoupled antigen is formulated with MF59 adjuvant and injected intradermally together with s.c. administration of GM-CSF at the same location. A phase I trial in heavily pretreated patients has shown excellent tolerability and promising immunogenicity of this vaccine formulation (Bertinetti, 2006). In a phase II trial designed to prove immunogenicity, 22 patients with indolent B-NHL (15 follicular [FL], 6 mantle cell [MCL], 1 lymphocytic lymphoma) received 6 monthly vaccinations as remission maintenance after cytoreductive therapy. Grade II toxicity (local erythema) occurred in 1 patient, and no grade III/IV toxicities were seen. 11 patients were in 1st CR, 8 in 1st PR, and 3 in 2nd CR. Despite NHL remission, patients had a functional immune defect: 12/13 (92%) anti-HbS-negative patients failed to respond to a conventional hepatitis B vaccine. Nevertheless, 13 of 15 evaluated patients (87%; 95% con-

fidence interval = 0.70-1.01) developed a humoral IR to the antigen as measured by ELISA. IFN γ ELISpot analysis demonstrated the in vivo induction of circulating, Id-reactive T cells in 8/11 patients (77%; 95% CI = 0.46-0.99). The frequency of Id-responding T cells increased from the 2nd to the 6th vaccination and could be efficiently boosted by additional maintenance immunization in 3-monthly intervals. At a median follow-up of 30 (10-85) months after start of cytoreductive therapy, 15 patients (68%; 12 FL [80%]; 3 MCL [50%]) are in progression-free survival (PFS), but too few events have as yet occurred to permit correlation of PFS with IR. This Id vaccination schedule appears to yield favorable IR rates even in comparison to the KLH-conjugated Id vaccines. Encouraging PFS and lack of toxicity warrant further clinical development.

V426

Exploratory analysis of the relevance of dendritic cells in the tumor microenvironment of follicular lymphoma

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The clinical course of follicular lymphoma (FL) may vary from spontaneous remissions in up to 15% of patients to rapid symptomatic progression. While combinations of clinical parameters such as the FL International Prognostic Index provide prognostic information, there is a clear need to develop biomarkers that predict more aggressive disease behavior and outcome. Candidate biomarkers include non-malignant immune cells, e.g. macrophages or FoxP3⁺ regulatory T cells in the FL microenvironment. The potential importance of these markers points to a prognostic role of an effective tumor defense mediated by the innate or adaptive immune system against FL. Along this line, we retrospectively analyzed subtypes of dendritic cells (DC) in formalin-fixed biopsies taken at initial diagnosis in 45 FL selected for a >8 year follow-up and 13 benign follicular hyperplasia (FH) patients to address a possible association with clinical outcome. The importance of DC content and phenotype as been shown especially for epithelial cancer, where tumor cells also modulate DC maturation and function. The average intra- and interfollicular number of immature CD1a⁺ and S100⁺ DC, mature CD83⁺ DC, CD11c⁺ myeloid and CD123⁺ plasmacytoid DC in 10 high-power fields was counted in each biopsy. Compared to FH, FL had less interfollicular immature, plasmacytoid, and possibly mature DC and less intrafollicular myeloid DC (Table). DC subsets in FL were not significantly different between FL cases with (n=14) and without (n=31) eventual histological transformation. By Kaplan-Meier analysis, no association between frequency of DC subsets and overall survival became apparent. These data fail to indicate a major role of specific DC subsets in the microenvironment for the prognosis of FL. However, the relative paucity of certain DC subsets within and in the vicinity of the malignant follicle might be associated with reduced T cell priming and hence impaired anti-tumor immunity in FL.

Table for Abstract V426

Biopsies		median number of positive cells / hpf					
		CD11c		CD1a	S100	CD83	CD123
Diagnosis	n	intra-		inter-follicular			
FH	13	111	137	4	5.5	5	44
FL	45	44	157	0.7	1.5	1.8	18
- w/o transformation	31	42	158	0.7	1.7	1.4	17
- with transformation	14	47	139	0.8	1.3	2.8	18
<i>p</i> [FH vs.FL] (Mann-Whitney test)		0.0004	n.s.	0.0015	0.001	0.08	0.02

V427

Assessment of the prognostic indices IPI and FLIPI in patients with mucosa associated lymphoid tissue (MALT) lymphoma

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Background: The prognostic value of the international prognostic index (IPI) and the follicular lymphoma international prognostic index (FLIPI) has widely been demonstrated in diffuse large B-cell lymphoma and follicular lymphoma, respectively. No attempts to assess their applicability in MALT lymphoma have been performed so far. **Patients and Methods:** A total of 143 patients with histologically verified MALT-lymphoma were analysed. Parameters of both IPI and FLIPI were assessed and correlated with relapse and time to relapse as markers of clinical course. Statistical analysis was done with SPSS 14.0. Partial correlation was assessed with the Pearson coefficient (CF) and reassessed with multiple regression analysis. Estimated time to relapse curves were calculated with the Kaplan Meier method and tested for significant differences with the Log-Rank test. **Results:** According to the IPI 96 patients (67%) were classified as low risk, 22 (15%) low-intermediate, 17 (12%) high-intermediate and 8 (6%) as high risk. FLIPI identified 99 patients (70%) at low risk, 35 (24%) at intermediate and 9 (6%) at high risk. After a median follow up time of 58 months, 123 patients are alive and 46 patients have relapsed with a median time to relapse of 38 months. IPI significantly correlated with time to relapse with the typical differentiation into a low, low-intermediate and high risk group. The FLIPI divided the patients into three risk groups, but the low and intermediate risk group showed a similar course in terms of time to relapse. Our data initially suggested autoimmune disease as well as multifocal disease to be correlated with relapse. Multiple regression analysis, however, identified only extragastric disease (found in 92 patients) as predictive factor of relapse ($p=0.001$). **Conclusion:** Our data demonstrate that both IPI and FLIPI partly are able to discriminate prognostic subgroups in patients with MALT-lymphoma.

V428

Quality of life in patients with non-hodgkins-lymphoma during maintenance therapy with the anti-CD20 antibody rituximab

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Introduction: The introduction of rituximab into the treatment of malignant lymphomas of the B-cell lineage has had a major impact on the management of these diseases. In diffuse large B-cell lymphomas (DLBCLs) and follicular lymphomas (FLs) several multicenter prospective randomized trials consistently demonstrated an improved outcome when rituximab was added to chemotherapy. In addition, prolonged exposure to rituximab as maintenance therapy has been beneficial in patients with FL and mantle cell lymphoma (MCL). For patients, the effect of any prolonged antitumor therapy on the quality of life (QoL) is a very important question. However, so far the question whether rituximab maintenance therapy may impair QoL in patients with Non-Hodgkins-lymphoma remains unanswered. **Methods:** To investigate this subject, we have performed a prospective randomized trial of rituximab maintenance therapy (8 cycles rituximab 375 mg/m² every 3 months) versus observation in patients with CD20+ B-cell Non-Hodgkins-Lymphoma in our institution. **Results:** Between July 2002 and December 2005, 106 patients (pts) were included into the trial. QoL was assessed with the standardized questionnaires EORTC-QLQ-C30 and EuroQoL-5D. After statistical analysis with the Wilcoxon signed-rank test, we found no significant differences of the QoL between the rituximab treatment group and the observation group. We conclude that rituximab maintenance therapy is safe and does not impair quality of life in this patient population.

V429

The histone deacetylase inhibitor SAHA and the proteasome inhibitor bortezomib synergistically induce apoptosis in cutaneous T-cell lymphoma

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Introduction: Histone deacetylase (HDAC) inhibitors and proteasome inhibitors are novel targeted therapies being evaluated in clinical trials for cutaneous T-cell lymphoma (CTCL). However, data in regard to tumor biology are very limited with these substances. In the present study we evaluated the effects of the HDAC inhibitor suberoylanilide hydroxamic acid (SAHA) and the proteasome inhibitor bortezomib as single agents and in combination on human CTCL cells. **Methods:** Four human CTCL cell lines (SeAx, Hut-78, MyLa and HH) were exposed to bortezomib and/ or SAHA at different concentrations over a time of 24 to 72 hours. Cell viability was quantified by the MTT assay and apoptosis was determined by flow cytometry analysis of annexin V binding populations. In addition, reactive oxygen species (ROS) were analyzed using the fluorophore H₂DCFDA. **Results:** Both agents potently inhibited cell viability and induced apoptosis in a dose- and time-dependent manner in all cell lines tested. After 48 hours of incubation, IC50 of SAHA was noted at 0.6 μM in SeAx cells, 0.9 μM in HH cells, 0.75 μM in Hut-78 and 4.4 μM in MyLa cells, respectively. For bortezomib, the IC 50 values were at 8.3 nm, 6.3 nm, 7.9 nm and 22.5 nm in SeAx, HH, Hut-78 and MyLa cells, respectively. Combined treatment with SAHA and bortezomib resulted in synergistic cytotoxic effects, as indicated by CI values <1 using the median effect method of Chou and Talalay. **Conclusion:** These results demonstrate that SAHA and bortezomib synergistically induce apoptosis in CTCL cells and provide a rationale for clinical trials of combined proteasome and histone deacetylase inhibition in the treatment of cutaneous T-cell lymphoma.

Indolente Lymphome

Poster:

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Treatment of a refractory cutaneous T-cell lymphoma with the anti-CD52-antibody Alemtuzumab

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Purpose: Cutaneous T-cell lymphomas are a heterogeneous group of diseases with different biological behaviour and outcome. Though skin-directed therapies may initially lead to high rate of responses, failure ultimately occurs in all patients and generalization of disease is frequent. **Case Report:** We report on a 24 year old male patient who suffered from lymphomatoid papulosis since October 2005. Therapy with Re-PUVA and methotrexate remained without success. In October 2006 a cutaneous anaplastic large-cell T-cell lymphoma with general lymphadenopathy was diagnosed. Due to rapid cutaneous progression and extracutaneous manifestation systemic first line therapy using the "CHOEP" protocol was initiated with 5 courses of chemotherapy, finally leading to a progressive disease. Second line therapy with 6 applications of Pentostatin (8 mg/week) failed as well. Therapy with the anti-CD52-antibody Alemtuzumab (3 x 30mg/week) was administered under usual anti-infectious prophylaxis. After achievement of a good partial remission dose was reduced to 2 x 10 mg/week. In total, 18 doses of Alemtuzumab were administered. Simultaneously, an electron-beam radiation of the affected skin areas was applied with up to 60 Gy depending on the reaction of the radiated skin area. Taking into account the young patient's age and dismal prognosis, allogeneic stem-cell transplantation was planned. In September 2007, the patient was transplanted from an HLA-matched unrelated donor after myeloablative therapy with total-body radiation (10 Gy) and Fludarabine (cumulative dose 150

mg/m²). Patient did well and after uncomplicated engraftment without GvHD the patient was discharged. Two months later he presented with a massive lymphadenopathy and severe reduction of general condition. An Epstein-Barr virus-induced posttransplant lymphoproliferative disorder (EBV-PTLD) was diagnosed. The patient died five days after admission. **Conclusions:** In refractory cutaneous T-cell lymphoma, rescue therapy with Alemtuzumab and allogeneic stem-cell transplantation might be an option for young patients. Severe infectious complications have to be considered and might be fatal. Due to the massive immunosuppression after Alemtuzumab and myoablative treatment EBV-PTLD might develop in rare cases. Therapeutic options comprise reduction of immunosuppression, donor lymphocyte infusions, cytotoxic therapy with the anti-CD-20-antibody rituximab and antiviral therapy.

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Treatment analysis in 127 consecutive follicular lymphoma (FL) patients (pts) receiving chemotherapy, radiotherapy and vaccination, with and without immunotherapy consolidation and future options with Yttrium-90 (Y90) Ibritumomab Tiuxetan (Zevalin®) radioimmunotherapy (RIT) consolidation in a single center institution

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Zevalin® is a murine anti-CD20 monoclonal antibody developed for RIT targeting CD20+ lymphoma cells. RIT consolidation is a highly promising approach, however, its mode of its best inclusion after therapy induction has to be further elucidated. Zevalin® has been released for the treatment of refractory/relapsed CD20+ follicular lymphoma (FL) after Rituximab (R)-based chemotherapies (CTxs), and because of its good feasibility and limited toxicity, is currently shifted into upfront regimens and into consolidation options after CTxs in FL. We aim to evaluate the efficacy and safety of RIT with Zevalin as consolidation in FL pts after R-CHOP-like CTxs and will compare these results with prior consecutive FL pts without RIT consolidation treatment. For the latter control group, we analysed the clinical courses of consecutive FL pts in our center within 1/2006-12/2007. Of a total of 902 lymphoma pts, seen and/or treated 2007, 201 had CLL, 235 had MM, 116 had diffuse large-cell NHL (DLCL), 127 FL, 39 MCL, 33 marginal NHL, 25 Waldenström's, 10 Burkitt-lymphoma and 116 other NHLs. FL pts, consisting of 14% of all NHL pts, had a median age at diagnosis and currently of 55 and 61 years, with male and female pts of 69 and 54, respectively. FL grade I or II was present in 111 and grade III in 12 pts. The most frequently used CTxs regimens were (R)-CHOP, (R)-VACOP-B, (R)-FC, Knospe, local radiation, autologous stem cell transplantation, and immunotherapies (vaccination and interferon), and lately, consolidation with rituximab (R). First-line (f-l) therapies, including R-CHOP-like regimens, R-FC, Knospe and radiation/vaccination, were performed in 62, 8, 10 and 19 pts, respectively, whereas 24 required no initial f-l treatment. R-consolidation was performed in 20 pts (16%), other approaches (vaccination, IFN, radiation) in 7, whereas 96 pts received no consolidation. The current remission is SD in 19, PD in 13, PR in 24, CR in 60 and initial diagnosis - currently not in need for treatment - in 7 pts. **Conclusions:** 1. FL pts consist of 14% of all NHL pts seen in our institution, 2. 65% are in need for systemic treatment, whereas in 15% local radiation or immunotherapy approaches or in even 20% no therapy (watch&wait) can be pursued. 3. Consolidation approaches with R mono were performed in 16%, whereas most FL pts may not as yet receive consolidation approaches. The comparison of 2006 and 2007 NHL and FL pts will be presented at the meeting.

P432

Successful treatment of multicentric Castleman's disease with combined immunochemotherapy in an AIDS patient with multiorgan failure

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Introduction: Multicentric castelman's disease (MCD) is a generalized lymphoproliferative disorder with poor prognosis associated with HIV and HHV-8 infection. Median survival in HIV-positive patients is 8 to 14 months. **Case:** We report on a HIV positive patient whose infection was diagnosed in 2001. In May 2006 the patient was admitted with remittent fevers up to 40°C over the last 6 months, recurrent abdominal pain and weight loss of 13kg. CT scan showed disseminated lymphadenopathy up to 5.4cm in diameter and massive hepatosplenomegaly. Under antiretroviral therapy, HIV infection was controlled but HHV-8 reactivation was present. Expansive pathogen diagnosis revealed no correlative infection although inflammatory parameters showed a 30 fold elevation of C-reactive protein (CRP). Probatory, calculated antibiotic therapy was started and evaluation of inguinal lymph node led to the diagnosis of HHV-8 associated plasmacellular MCD. Rapidly the patient's condition deteriorated and he developed multiorgan failure including cardiac and pulmonary insufficiency, acute renal failure and an acute abdomen with progressive hepatosplenomegaly necessitating mechanical ventilation, hemodialysis and vasopressor treatment. Laboratory diagnostic workup revealed 8 fold elevated lactatdehydrogenase (1979 U/l) and nearly 100 fold elevated CRP serum levels (47.89 mg/dl). These findings and clinical aggravation were interpreted as a possible transformation of MCD into a high-grade NHL. Therefore, despite existing multiorgan failure, a combined chemotherapy (cyclophosphamide, adriamycin, prednisone) including the CD20 antibody Rituximab, was initiated immediately starting with 2 courses of Rituximab followed by 3 cycles of R-CHP. **Results:** Clinical symptoms, LDH, CRP and lymphadenopathy improved significantly. In the following, treatment had to be stopped due to bowel perforation and peritonitis, necessitating surgical intervention. Despite treatment discontinuation, the patient showed rapid clinical recovery and substantial improvement of laboratory values and was discharged merely under HIV specific therapy. At the last follow-up 20 months after diagnosis of MCD, the patient presented in good general condition and no evidence of MCD relapse. **Conclusions:** Treatment of this HIV-positive MCD patient with R-CHP was effective. Therefore, we encourage physicians to administer combined immunochemotherapy in patients with MCD despite limited general condition.

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Determination of SAA1 genotype to assess the prognosis of patients with Familial Mediterranean fever

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Purpose: Familial Mediterranean fever (FMF) is an autosomal recessive disorder affecting more than 100,000 people worldwide. It is characterized by recurrent self-limiting attacks of headache, joint, chest and abdominal pain often associated with fever. The prognosis-limiting complication of FMF is the development of amyloidosis. **Case Report:** We report on a 60 year old female patient of Turkish descent, who suffered from progressive abdominal pain, headache and fatigue. An IgG kappa multiple myeloma had been diagnosed 7 years before in stage I. Clinical examinations revealed hepatosplenomegaly with portal hypertension (portal venous flow 35 cm/sec) and esophageal varices in stage II. The analysis of peripheral blood discovered pancytopenia:

leukocytes 1.900/ μ l, hemoglobin 9 g/dl, thrombocytes 74.000/ μ l. Serological testing and bone marrow cytology showed stable disease with asymptomatic myeloma with 15% plasma cells in bone marrow. Rectum and liver biopsies were performed because of organomegaly and revealed AA-Amyloidosis. Family history combined with genetic investigation proved the diagnosis of FMF with a rare mutation E230K in the MEFV (pyrin/marenostrin) gene causing the AA-Amyloidosis. The patient was homozygous for the SAA1 alpha gene. SAA protein in serum was considerably elevated: 3,1 mg/dl (0,5 mg/dl). Due to ongoing abdominal pain we started treatment with colchicine. Abdominal pain and headache rapidly improved. Clinical and laboratory control 3 months later showed response to the therapy. Pain intensity decreased from 7 to 3 by visual analogue scale and SAA-level normalized to 0,5 mg/dl. **Genetic background:** Since patients with identical mutations vary in their clinical manifestations, especially with regard to the development of amyloidosis, a role for additional genetic and/or environmental modifiers has been investigated. Of these, polymorphisms at the SAA1 (serum amyloid A1) locus, or rather the SAA1 α / α genotype, were found to influence susceptibility to amyloidosis in patients with FMF. The risk to develop amyloidosis for patients with homozygosity for SAA1 alpha genotype is 7-11 fold higher than for patients with other genotypes. Both our patient and one of her daughters exhibited the SAA1 alpha/alpha genotype rendering them at high risk for the development of amyloidosis. **Conclusions:** About 13% of patients with FMF develop amyloidosis. The determination of the SAA1 genotype might be useful in assessing the patient's prognosis. Patients with homozygosity for SAA1 α seem to require a more intensified therapy, compared to favorable genotypes. Moreover it could be necessary to start treating people with mutations in the MEFV gene and homozygosity for SAA1 α being still symptom-free closely monitored by SAA-level.

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Polyglobuly Requiring Phlebotomy: A Late Side Effect Associated with Cladribine Therapy of Hairy Cell Leukemia

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Cladribine (2-CDA) is the treatment of choice for hairy cell leukemia (HCL). Common side effects are extended myelosuppression and gastrointestinal, hepatic, and renal toxicity. Here we report polyglobuly as a potential late effect of 2-CDA. A 43 year-old male patient reported recurrent headaches 24 months after 2-CDA treatment for HCL. Laboratory analysis showed an Hb of 18.8 g/dl and Hct of 51.6%, with normal leuco- and thrombocytes. A bone marrow (BM) biopsy showed no evidence for a myeloproliferative disorder but a 40% infiltration with HCL cells. A 2nd cycle of 2-CDA was given. A 54 year-old male patient presented with plethora, palpitations, and severe headaches 48 months after having received 2-CDA treatment for HCL. His Hb was 18.6 g/dl, the Hct was 52.5%. A slightly pronounced erythropoiesis without fulfilling criteria for myeloproliferative diseases and CR of HCL were noted in the BM. In both cases, phlebotomy therapy was initiated immediately. The diagnostic work-up revealed normal erythropoietin levels and absence of JAK2 exon 12 mutations. Pulmonary and cardiac tests were normal, and there was no sonographic evidence for renal tumors. Both patients remain in 1st and 2nd CR of HCL, respectively, at 37 and 4 months after diagnosis of polyglobuly but continue to require phlebotomies to maintain an Hct <45%. We retrospectively analysed all 31 consecutive HCL patients (including the index cases) achieving CR after 2-CDA therapy at our center from 1992 and 2008. The median age was 50 (29-77) years at diagnosis, 27 patients were male. Evidence for a trend for high-normal red cell mass was indicated by a median maximum Hb of 15.7 g/dl (12.3-18.8 g/dl) and a median maximum Hct of 45.3% (36.0-52.5%). Hb >17 g/dl occurred in 6 patients, Hct of >50% in 3 patients. The peak Hct occurred at a median of 30 (3-122) months after 2-CDA therapy. Since no evidence for a concurrent hematological neoplasia or a defined etiology for secondary polyglobuly was noted in any patient, we postulate that 2-CDA treatment may induce a long-term dysregulation of erythropoiesis. Case 1 illustrates that symptomatic polyglobuly can even exist in the context of an imminent HCL relapse. Since polyglobuly is a so far unknown late effect after 2-CDA therapy for HCL, long-term follow-up is recommended for this patient group to avoid unnecessary delay of initiation of phlebotomies when indicated

and to exclude eventual development of a true myeloproliferative disorder in the long term.

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A case of generalized Malt-Lymphoma with IGM paraproteinemia and peripheral blood involvement

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Introduction: Mucosa associated lymphoid tissue (MALT) lymphomas comprise a well-defined entity of Non-Hodgkin-Lymphomas (NHL) but may also share clinical and histopathologic findings with other low-grade lymphomas thereby causing diagnostic problems. **Methods:** A 35 year old woman was diagnosed with *Helicobacter pylori* (HP) + gastritis and MALT lymphoma in July 2007. Without further staging HP eradication therapy was initiated, however, control endoscopy after 6 months showed persisting lymphoma and the patient was referred to our hospital for further evaluation and therapy in February 2008. She presented with B-Symptoms, CT-scan showed infiltration of the stomach in antrum and corpus, enlargement of epigastric lymph nodes as well as a 8 x 7 cm hypodense lesion in the liver and multiple opacities in both lungs. All of these lesions showed increased FDG-uptake by PET-scan. Histologic examination was performed on biopsies of the stomach, liver and lung and confirmed the diagnosis of MALT lymphoma revealing patchy infiltrates of small centrocyte-like monoclonal B cells with interspersed reactive and colonized follicles in the involved organs, forming typical lymphoepithelial lesions in the gastric mucosa with accompanying HP infection. Bone marrow biopsy revealed minimal infiltration with lymphoma cells. WBC was 6,71 G/l with 50% well-differentiated lymphocytes and 5% lymphoplasmacytic cells in the differential count. Flow cytometry of the peripheral blood revealed 18% CD19+/20+/slgM+/5-/10- leucemic lambda-clonal B-cells. Analysis of the serum immunoglobulins showed elevation of IgM and lambda light chain, immunofixation revealed a monoclonal paraprotein IgM lambda. Cytogenetic analysis of the peripheral blood cells revealed only normal metaphases, however, I- FISH revealed 20% cells showing 3 signals with probes for the MALT and BCL-2 locus on chromosomal region 18q21 and 3 signals with a probe for the EVI-1 locus on region 3q26 consistent with duplications or (partial) trisomies of these chromosomal regions. RT-PCR performed on tumor specimens failed to detect MALT-API2 specific RNA fusions, thereby excluding the MALT lymphoma specific t(11;18)(q21;q21). **Conclusion:** This case demonstrates the importance of complete staging procedures in MALT lymphoma, furthermore, clinical features that are more common in other low-grade lymphomas, e.g. lymphoplasmacytic lymphoma, may also be present in this entity, genetic examinations may be helpful in the evaluation of borderline cases.

Klinische Fälle Antikörper Therapie Freie Vorträge:

V436

Complete remission of severe chronic neutropenia with Alemtuzumab

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Introduction: The majority of patients with autoimmune cytopenias require only standard immunosuppression, mainly steroids, to achieve long-lasting remission. Alternative treatment such as splenectomy, or cytotoxic drugs and granulocyte colony-stimulating factor (G-CSF) is necessary in a proportion of refractory disease. monoclonal antibodies directed against B- and/or T-cells have become a new approach in treating patients with severe, refractory autoimmune cytopenias with life-threatening anaemia, thrombocytopenia, or

infections. **Methods:** We report the course of a female patient with severe autoimmune neutropenia (AIN). She was first diagnosed at the age of 11 with cyclic neutropenia. A secondary AIN could be excluded. Serum autoantibody screen was negative, especially anti-neutrophil antibodies were not found. **Results:** In the course of her disease the patient suffered from various bacterial and viral infections. Immunoglobulins, G-CSF, granulocyte macrophage colony-stimulating factor (GM-CSF) were used without improvement of the absolute neutrophil count (ANC). Immunosuppression with antilymphocyte globulin (ATG), cyclosporine A and steroids showed only a transient response. Treating the patient with Alemtuzumab (a genetically engineered human IgG1-kappa monoclonal antibody against the CD52 antigen expressed on B- and T-lymphocytes and monocytes) subcutaneously with a dosage of 30 mg three times a week resulted in a complete remission of ANC after three weeks. The therapy was well tolerated. During the treatment with Alemtuzumab there was an immediate but predictable lymphopenia and monocytopenia. ANC remained stable until reduction to a maintenance dosage of 30 mg once weekly, which led to an early relapse. A few weeks later the patient died of an acute pulmonary embolism. **Conclusions:** Alemtuzumab used as single agent therapy may provide an alternative and less toxic approach to the treatment of severe or refractory AIN. The optimal dose and schedule of Alemtuzumab as maintenance needs to be further investigated.

V437

Successful treatment of severe EBV-associated hemophagocytic syndrome in a young adolescent with rituximab and etoposide

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Introduction: Hemophagocytic syndrome (HLH) is a hyperinflammatory condition with cardinal symptoms fever, hepatosplenomegaly and cytopenias. Frequent triggers are infectious agents, mostly viruses of the herpes group. The course of untreated HLH is fatal in the majority of patients. **Case:** We describe an 18-year old patient, admitted to our intensive care unit with severe sepsis with multiorgan failure (SAPS II-Score 45, corresponding to a predicted 30-day mortality of 40%). Laboratory investigation revealed marked cytopenias, severe hypofibrinogenemia (min. 35 mg/dl), hyperbilirubinemia, a marked increase in ferritin (14070 µg/l) and a high level of sIL-2r (soluble interleukin-2-receptor, 19074 U/ml) and CRP (24,6 mg/dl). Ultrasound showed hepatosplenomegaly without any signs of malignant lymphoma. Bone marrow cytology showed hemophagocytosis, a liver biopsy revealed chronic persistent hepatitis. Diagnosis was made according to the revised diagnostic criteria for HLH (Henter et al.). Despite immediate treatment with IVIG and dexamethasone, the condition of the patient deteriorated dramatically within hours with rising temperatures, progressive edema and progressive respiratory failure. The patient required mechanical ventilation, application of vasopressors and antifibrinolytic agents. One day after admission, positive PCR results for EBV-DNA were obtained and MRSA was detected in the peripheral blood. Considering EBV-associated HLH as the primary diagnosis, combination chemotherapy with etoposide and dexamethasone was initiated. Rituximab was added in order to target EBV-infected B-cells suspected as a main trigger of the disease. Etoposide and dexamethasone were applied as outlined in the HLH 2004 protocol. Beginning at day +1 after initiation of treatment, the patients condition impressively improved and laboratory abnormalities gradually resolved. Treatment was continued according to the HLH 2004 protocol for a total of 20 weeks. In total, four courses of Rituximab weekly were applied. EBV-PCR turned negative after 20 days. 5 months after completion of therapy, the patient remains free of symptoms in a complete remission. **Conclusions:** HLH is a rare disease with a often fatal course. Prognosis of EBV-associated HLH may be improved by addition of Rituximab. Awareness of clinical symptoms and diagnostic criteria is important to enable therapeutic intervention which may be life-saving even in patients with advanced multi-organ failure.

V438

Prolonged Bone Marrow Failure following Rituximab Therapy of Follicular Lymphoma

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Rituximab (R) is a mainstay in B-NHL therapy. Apart from infusional reactions and prolonged B cell depletion, severe R side effects are relatively rare. A 65 year-old, previously healthy woman presented with anemia (Hb 9.3 mg/dl), thrombocytopenia, and cervical lymphadenopathy. A submandibular lymph node biopsy showed CD20⁺CD10⁺CD79a⁺ follicular infiltrates. Diagnosis of follicular lymphoma was supported by detection of an IgH-BCL2 gene rearrangement by FISH. CT scans revealed generalized lymphadenopathy and hepatosplenomegaly. The BM was infiltrated with 68% clonal CD20⁺CD10⁺CD79a⁺ B cells. Due to advanced (stage IVA) and high-risk disease (FLIPI 4/5), 200 mg/m² cyclophosphamide/100 mg prednisone/day (d) were given for 5 days, followed by a single dose of 375 mg/m² R. Grade 4 neutropenia developed 4 days later and precluded the planned CHOP chemotherapy. A BM biopsy on d+21 post R showed marked BM hypoplasia consistent with drug-induced changes, but no residual lymphoma. Serologic testing ruled out Parvovirus B19, HBV, HCV and HIV infections. Leukocytes recovered to >1/nl 3.5 weeks after R administration. No further cytoreduction was given due to prolonged cytopenia and documented PR. Despite this history, Bendamustin/R (200 mg) was given for eventual BM relapse at a different hospital 11 months later. Grade 4, filgrastim-refractory cytopenia with the same BM histology developed promptly and lasted for 4 weeks. The patient was transferred to our institution because of multiple opportunistic infections including pulmonary aspergillosis, E. faecium sepsis, and destructive mucor mycosis of the right maxillary sinus. Posaconazole and liposomal amphotericin were given for 5 months. 13 months after the 2nd R infusion, the patient had to undergo reconstructive surgery with a flap plasty to cover an extensive, disfiguring facial and maxillary necrosis. Since different alkylators were given at moderate doses, a causal relationship to similar episodes of rapid-onset, extended BM failure appears unlikely. R, administered immediately prior to both episodes, appears to be the causative agent in this case. Since hematopoietic precursors and the myeloid lineage do not express CD20, the mechanism for this near-fatal BM failure is unknown and suggestive of an idiosyncratic component. The BM findings are incompatible with a metamizol-type allergic agranulocytosis. Severe BM failure appears to be a rare but grave potential side-effect of rituximab.

V439

Pulmonary complications after Rituximab-containing therapy for Non-Hodgkin's Lymphoma

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Introduction: Rituximab (R) is increasingly used for the treatment of B-cell non-Hodgkin's lymphoma (B-NHL). In rare cases, however, R may be associated with lung toxicity. **Methods:** The cases of 3 pts developing interstitial pneumonitis (IP) (n=2) or pneumocystis jiroveii pneumonia (PcP) (n=1) while under therapy with R-cyclophosphamide, doxorubicine, vincristine, prednisone (CHOP) are reported. **Results: Case 1:** A 65-year old woman with a diagnosis of stage IIA diffuse large B-cell lymphoma (DLCL) was treated with R-CHOP in 2-week intervals. A complete remission (CR) was achieved after 6 courses. One week after the 8th course she became dyspneic at rest. A chest CT-scan revealed marked bilateral interstitial infiltration. She developed acute respiratory failure necessitating mechanical ventilation. No pathogens were identified by blood cultures and bronchoalveolar lavage (BAL). A diagnosis of IP was made and she was started on prednisone. Her respiratory situation steadily improved with repeat CT-scans showing marked resolution of alveolitis. She is well and in complete remission 19 months after diagnosis of DLCL. **Case 2:** A 76-year old woman received R-CHOP for stage IVA DLCL (Richter's syndrome) following a 4-year history of B-CLL. Worsening dyspnoea and a non-productive cough developed after the 6th course. Chest x-ray showed diffuse bilateral pulmonary infiltrates. Worsening oxygenation prompted bronchoscopy with BAL. No pathogens were detected but transbronchial biopsy (TBB) revealed IP. With

initiation of steroids her oxygenation improved sufficiently. The patient remains in CR 9 months after completion of chemotherapy. CT-scan does not reveal any residual pulmonary fibrosis. **Case 3:** Following the diagnosis of stage IVA DLCL a 65-year old man was treated with 2-weekly R-CHOP. After 5 courses of therapy he reported shortness of breath and dry cough. A chest radiogram showed bilateral interstitial infiltration. Pneumocystis jirovecii and Herpes Simplex Virus-DNA were demonstrated by BAL with TBB showing unspecific IP. Clinical and radiographic signs recovered following therapy with cotrimoxazole, prednisone and acyclovir. Antineoplastic therapy was terminated. After 12 months of follow-up he is well and remains in CR. **Conclusion:** IP induced by R as well as the occurrence of PcP following treatment with R are rare but potentially fatal pulmonary complications. Awareness of R-IP, rapid diagnostic proceedings and initiation of therapy are essential.

V440

Efficacy of the Terminal Complement Inhibitor Eculizumab in a Patient with Cold Agglutinin Disease (CAD)

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Background: Cold agglutinin disease (CAD) is a rare form of autoimmune hemolysis where monoclonal or polyclonal IgM autoantibodies can activate the complement system in colder areas of the body (e.g. extremities), resulting in red blood cell destruction. Typically, prednisone is ineffective and on rare occasions chemotherapy can suppress autoantibody production. Most cases are mild and self-limited, but severe cases need hospitalization and can be life-threatening. Supportive transfusions of red blood cells can be necessary. Based on CAD pathogenesis, blocking the terminal complement cascade by eculizumab could be a possible therapeutic approach. Eculizumab, a monoclonal antibody targeting complement factor C5, has shown high efficacy in patients with hemolytic paroxysmal nocturnal hemoglobinuria (PNH) through inhibiting the terminal complement system. **Aims/Methods:** To test for efficacy of eculizumab in a transfusion-dependent patient with chronic cold agglutinin disease refractory to extensive previous treatment eculizumab was dosed as follows: 600mg IV every 7±2 days x 4; 900mg 7±2 days later; and then 900 mg every 14±2 days. As eculizumab treatment puts patients at risk for meningococcal disease, a standard vaccination scheme was administered prior to treatment. Transfusion requirements as well as clinical and biochemical indicators of hemolysis were monitored. **Results:** Intravascular hemolysis, measured by the area under the curve for LDH against time, was significantly reduced from 118,858 to 79,687 U per liter (-33%; p = 0.006). Hemolysis control resulted in improvement of anemia (median 10 vs. 11.8 g/dl). Transfusion requirements decreased by 100% from 16 PRBC units during a 6 months pre-treatment period to 0. Furthermore, the patient also reported an improvement of fatigue and quality of life. There was no adverse event and the ongoing therapy with eculizumab is safe and well tolerated in this patient. **Conclusions:** This is the first report of eculizumab efficacy in CAD. As no alternative effective treatment options are available, this promising result provides a strong rationale for a clinical trial for the use of eculizumab in patients with CAD.

Klinische Fälle Hämatologie und Transplantation Freie Vorträge:

V441

Splenic artery embolization in the management of an acute autoimmune thrombocytopenia-related intracranial hemorrhage in a patient with CLL

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Purpose: Although relatively rare, cerebral hemorrhage represents a common cause of death within the scope of autoimmune thrombocytopenia. We describe here the case of a 58-year old male with CLL and refractory auto-

immune thrombocytopenia of 7.000 platelets/ μ l, who experienced an acute cerebral hemorrhage. Initial treatment with high-dose immunoglobulines and high-dose steroids followed by rituximab and chemotherapy had failed. In this situation, conventional splenectomy is a treatment option that might lead to an adequate increase of platelet counts. One year before, our patient had received expanded abdominal surgery, so conventional splenectomy was contraindicated because of an increased risk of bleeding due to an expected complicated surgical procedure. In this situation of acute cerebral hemorrhage and contraindication for conventional splenectomy, we decided to perform an embolization of the splenic arteries. **Methods:** Via a right femoral approach a catheter was advanced to the splenic artery. All excepting one of the segmental arteries of the spleen were embolized using particles (Gelfoam, Embospheres) until complete stasis was achieved. During as well as a couple of days past, intensive analgetic therapy was necessary. Pneumococcal vaccination was performed two weeks after intervention. **Results:** One day after the procedure an increase of platelets to 30.000/ μ l was observed, two days later platelets reached levels of 60.000/ μ l were noticed. Cerebral hemorrhage stopped spontaneously, making neurosurgical intervention unnecessary. Three weeks after the intervention, the patient showed normal platelet counts. CT-scan of the spleen revealed a residual organ perfusion of 20%. Through the use of prophylactic antibiotics a post-interventional infection of the spleen could be avoided. A normal platelet count has now been maintained for 6 months. **Conclusions:** Splenic embolization turns out to be an alternative to conventional splenectomy for patients with autoimmune thrombocytopenia and acute bleeding. Particularly in patients with contraindication for conventional splenectomy this approach is quickly achievable and can be life-saving.

V442

Adoptive intrathecal cellular therapy for the treatment of leukemic meningitis

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Background: Leukemia with central nervous system (CNS) relapse is correlated with a poor outcome. The failure of most chemotherapeutic agents to cross the blood brain barrier (BBB) and neurotoxicity of repeated CNS treatments are limiting factors. Although the graft vs. leukemia effect has been well documented, allogeneic stem cell transplantation is generally not recommended in patients presenting with CNS disease. Since the CNS is an immunologically privileged sanctuary, donor lymphocytes theoretically cannot cross the BBB. **Purpose:** In single patients with isolated CNS relapse of acute or chronic myeloid leukemia after allogeneic stem cell transplantation we decided to administer for the first time intrathecal (IT) donor lymphocyte infusions (DLI) in order to extend the therapeutic spectrum. **Results:** Up to eight intrathecal infusions of CD14 depleted DLI with a starting dose of 1×10^6 T cells were applied. The patients did not present either immediate or delayed side effects after the infusions. Besides leukemic blast cell counts of the CSF molecular genetic analyses for chimerism as well as for translocations were applied for monitoring in addition to MRI and to neuroclinical symptoms. Full donor chimerism of the CSF and disappearance of bcr-abl translocations were registered in one CML patient, whereas in an AML patient only a transitory disappearance of blast cells and a delay of rise of leukemia cells was recorded. The patients are still under observation (8-11 months) and have a continuing full chimerism of blood and bone marrow. **Conclusions:** Intrathecal DLI for CNS relapse of leukemia after allogeneic stem cell transplantation is a new still experimental approach. The first applications indicate no side effects e.g. cerebral or meningeal GVHD and a possible efficacy in CNS relapse of CML. This new approach merits further investigation in the setting of a clinical trial.

V443

Paraneoplastic Pemphigus in newly diagnosed Chronic Lymphatic Leukemia - a case report

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Paraneoplastic pemphigus (PNP) is a chronic disease of skin and mucosa, characterized by intraepidermal acantholytic vesicles with autoantibodies against surface antigens of keratinocytes. There is an HLA association with

HLA DR4, DRw6, and certain variations in the β -chain of HLA DQ. Neoplastic diseases associated with PNP are Chronic Lymphatic Leukemia (CLL), other Non Hodgkin Lymphomas, Castleman's disease, occult neoplasias, thymomas, solid tumours such as breast cancer and cancer of the ovary. The prognosis of PNP is poor, the course is independent of the underlying disease. Treatment consists of immunosuppression (steroids, cyclophosphamide, anti-CD20 antibody, plasma exchange, i.v. immunoglobulins etc.) Here we report on a 61 year old ill appearing man who was admitted to the department of orofacial surgery because of cervical lymphadenopathy. He reported about mucosal inflammation particularly orally, but also genital, which had started some four weeks before. Had had lost 11 kilogram in these weeks, but there was no fever nor night sweats. The diagnostic procedures revealed a CLL Binet C with multiple cytogenetic abnormalities including deletion 11 and an unmutated IgVH status. The mucositis was considered to be due to herpes simplex virus infection and treated with i.v. acyclovir followed by foscarnet without any sign of remission. The diagnosis was therefore reconsidered and PNP was diagnosed supported by a high level of anti desmoglein III antibodies. In order to suppress the paraneoplastic features treatment with high dose steroids was started, followed by rituximab, fludarabine and cyclophosphamide, leading to a discrete remission of the lymphomas without any improvement of the pemphigus. Therefore treatment was changed to 2 cycles rituximab-CHOP, again without success. The options of allogeneic stem cell transplantation or alemtuzumab were discussed. Because of the patient's poor condition the latter was chosen. A total number of 9 cycles over 6 months was given, interrupted by several infectious complications (candida mucositis, 2 times CMV reactivation, neutropenic fever, condylomata acuminata, pulmonary aspergillosis). However, the result was a complete remission of both the CLL and the PNP. Treatment of PNP is a difficult and unsatisfying task for both patient and doctor with the disease often resistant to immunosuppressive drugs. However, recently remissions have been reported by using some of the newer immunosuppressants such as cyclosporine or alemtuzumab, the latter being a humanised monoclonal antibody directed against CD52. This antibody is used in the treatment of CLL, unfortunately however associated with multiple infectious complications as also seen in our patient. To our knowledge this is the second case reported where treatment with alemtuzumab induced a complete remission in PNP and its underlying disease, CLL.

V444

Long-term mixed hematopoietic chimerism associated with clinical remission of refractory Wegener's granulomatosis

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Introduction: Animal models have shown that the induction of mixed donor chimerism after allogeneic hematopoietic stem cell transplantation (aHSCT) may lead to remission of autoimmunity which may be explained by a graft-versus-autoimmunity effect. Although only partially understood, donor hematopoietic and immune cells are able to tolerize autoreactive T and B cells. Stable mixed chimerism with a relevant proportion of recipient cells is an infrequent event in patients with malignancies receiving aHSCT but may occur in recipients without intensive chemotherapeutic pre-treatment. So far, there are very few reports on the outcome of aHSCT after minimal conditioning in patients with autoimmune diseases. **Patient:** A 23 year old male patient who was diagnosed with Wegener's granulomatosis at the age of 14 received GCSF mobilised PBSC from his HLA-matched sister after conditioning with 2 Gy total-body irradiation and 90 mg/m² fludarabine. GvHD prophylaxis was performed with CsA and mycophenolate mofetil. Before aHSCT, the patient was treated with various immunosuppressive regimens including high-dose cyclophosphamide, autologous stem cell transplantation, azathioprine, pulsed steroids, rituximab and etanercept. Progressive vasculitis lead to infiltration of the right orbita with partial blindness, chronic otitis, mastoiditis and subglottic stenosis requiring a tracheostoma. Multiple infectious complications including

suspected mycobacteriosis complicated the course prior to aHSCT. PR-3 autoantibodies started to increase prior aHSCT. **Results:** Neutrophil and platelet engraftment occurred 2 weeks after aHSCT and the further course was uneventful with no signs of GvHD. Donor chimerism in peripheral blood reached 95% at day 30 and a maximum level of 97.5% at day 105. The level of chimerism declined continuously and reached 55% at 3 years. Chimerism analysis in sorted cell populations (CD4/8, CD15, CD34 cells) showed a mixed chimerism with predominance of the donor in the myeloid compartment. Due to the pronounced mixed T-cell chimerism, donor lymphocytes were infused on 3 occasions (days 310, 347, 406). Despite this intervention chimerism analysis in sorted subsets revealed mixed chimerism in CD15+ granulocytes and CD34+ progenitors. CD4+ and CD8+ donor chimerism has remained between 15 and 25% until 3 years after aHSCT. CD4+/CD25+ Tregs were within the normal range (3.5%) at last follow-up. PR-3 autoantibodies declined to levels around the detection limit. MRI studies showed a regression of the retroorbital infiltration at last follow-up. For the first time within the last 10 years, steroids could be stopped without exacerbation of disease activity. **Conclusion:** Stable mixed chimerism after aHSCT may be sufficient to cure or at least ameliorate the course of autoimmune vasculitis. Minimal conditioning allows to significantly decrease the risks associated with the procedure.

V445

Haploidentical hematopoietic stem cell transplantation for refractory mast cell leukemia

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Introduction: Mast cell leukemia is rare and a devastating disease which is often refractory to conventional cladribine mono- and polychemotherapy treatment. Stem cell transplant has been considered a possible salvage treatment option (1;2). Here, we report the first case report of haploidentical hematopoietic stem cell transplantation for therapy refractory mast cell leukemia. **Case:** A 44 year old man presented with abdominal and cervical lymphadenopathy, hepato- and splenomegaly, B-symptoms, anemia (9g/dl) and thrombocytopenia (11/nl). Tryptase- and CD117- positive blasts were identified to infiltrate bone marrow and lymph nodes leading to the diagnosis of mast cell leukemia. Trisomy and tetrasomy 4, 8 and 19 but no KitD816 mutations were found by cytogenetic studies.

Treatment was initiated with cyclophosphamide, etoposide and cladribin based chemotherapy without evidence of clinical response. We documented progressive disease after administration of Imatinib, given in light of CD117 positivity (3). Bone marrow infiltration remained at 90% after first cycle arabinoside C and doxorubicin. Thrombocytopenia and anemia and recurrent fever were present at all stages of treatment. Because we could not identify a suitable matched related or unrelated hematopoietic stem cell donor, we conducted a haploidentical blood stem cell transplantation using a CD3/CD19 depletion strategy. The conditioning chemotherapy regimen included fludarabine, thiotepe, melphalan and OKT-3 (4). **Results:** Diarrhea and abdominal cramps during aplasia but no viral or bacterial infectious complications were observed. Granulocyte and platelet take was documented 14 and 22 days after transplantation, respectively. A complete remission of the lymphadenopathy and bone marrow infiltration, a 99.9% donor- chimerism and a near normal hematopoiesis (WBC 7.2, Hb 10.0, Platelets 72/nl) were observed two month after transplantation. **Conclusions:** This case report provides evidence that haploidentical transplantation can be a valuable treatment option for refractory mast cell leukemia.

References:

1. Valent P, et al. J Allergy Clin Immunol 2004 Jul;114(1):3-11.
2. Chen TY, et al. Bone Marrow Transplant 2003 Jul(1);11-4.
3. Akin C, et al. Exp Hematol 2003;31:686-92.
4. Bethge WA, et al. Exp Hematol 2006;34:1746-1752.

Second allogeneic stem cell transplantation with no prophylactic immunosuppression from a matched sibling donor after previous partial liver transplantation

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Introduction: Liver transplantation has been performed in selected patients with liver failure after allogeneic stem cell transplantation. We report on a patient who received two allogeneic stem cell transplants after a previous partial liver transplantation from the same sibling donor. **Methods:** A 41 yr old female patient suffered from hepatocellular carcinoma in 2000 and was treated by hemihepatectomy. She had a relapse in 2002 and her remaining liver removed and replaced by a partial liver transplant from her brother. In 2006 she was diagnosed with Philadelphia-positive acute lymphoblastic leukemia and treated by induction chemotherapy while continuing immunosuppression with ciclosporin A. She achieved complete hematological remission and bcr-abl transcript levels decreased to 0,06%. No relevant liver toxicity was found. Therefore she received a myeloablative conditioning with 12 Gy TBI and 120 mg/kg Cyclophosphamide followed by a peripheral stem cell transplant from the same brother who donated the liver graft. GvHD prophylaxis was ciclosporin A and short course methotrexate. Maximum aGvHD was Grade II (skin) which responded to steroids. Engraftment was on day +20 and the patient discharged on day +28. Chimerism analysis showed 100% donor hematopoiesis. Ciclosporin was completely stopped day +220 without signs of GvHD or liver rejection. On day +250 raising bcr-abl transcripts were found and treatment with Imatinib and donor lymphocytes initiated. Due to insufficient response, dasatinib was started and after progressive cytopenia a second allogeneic transplantation after conditioning with fludarabine and treosulfan with the same donor was performed on day +372. The patient received no prophylactic immunosuppression. Acute GvHD of the skin (Grade II) responded to a short course of ciclosporin A. Discharge was on day +24 after the second graft. CsA was stopped and maintenance with dasatinib was instituted. **Results:** No relevant problems of the transplanted liver were found throughout the whole treatment so far. Complete withdrawal of immunosuppression was tolerated without any sign of liver rejection, consistent with the hypothesis of donor organ tolerance induced by allogeneic stem cell transplantation. **Conclusion:** This case report demonstrates the feasibility of repeated allogeneic stem cell transplantation after a partial liver transplantation.

Klinische Fälle Onkologie Freie Vorträge:

Spontaneous remission of metachronous pulmonary metastasis from rectal cancer. Report of a case

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Introduction: Spontaneous remissions (SR) of cancer are rare events. Approximately 20 cases in epithelial malignancies are reported every year.¹ In colorectal cancer (CRC) SR is even more rare. Between 1900 and 2007 only 21 cases have been reported. The majority of these cases were cancers of rectal origin in male subjects. No SR of pulmonary metastasis (PM) in CRC has been reported so far. **Case Report:** A caucasian male subject was diagnosed in 10/06 with rectal cancer. Medical history revealed alcohol-induced cirrhosis of the liver (stage CHILD B), non insulin dependent diabetes mellitus and arterial hypertension. Neoadjuvant chemoradiotherapy with capecitabine and oxaliplatin up to 50,4 Gy followed by total mesorectal excision was performed. Histological examination revealed an ypT3 Nx (0/10) L0 V0 R0 G2 adenocarcinoma. Due to a prolonged postoperative course with renal failure and impairment of the hepatic function no adjuvant treatment was given. 8 month after surgery, a CEA elevation up to six times ULN was noted and a CT scan of chest and abdomen

revealed PM (11/07). The patient was screened for a clinical trial. Screening CT scans were performed six weeks later (12/07), showing partial remission of the PM. The patient was not included into the clinical trial and received no chemotherapy. Consecutively CEA levels declined to normal values. A follow up CT scan in 03/08 displayed a complete remission. During these six month of observation the patient showed neither clinical signs of infection nor CRP elevation, consistent to no significant changes in the routine laboratory values over time. FACS analysis of the immune system revealed normal values. Follow up examinations are scheduled three monthly. **Conclusion:** To our knowledge this is one of the first reports of complete SR of pulmonary metastasis in CRC. The extremely rare event of SR should be kept in mind while treating CRC as well as assessing the results of this treatment. Abdelrazeq AS. Spontaneous regression of colorectal cancer: a review of cases from 1900 to 2005. *Int J Colorectal Dis.* 2007 Jul;22(7):727-36. Epub 2006 Dec 5. Review.

Left atrial SARCOMA and adenocarcinoma of the lung presenting as ST-elevation myocardial infarction

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Introduction: Primary cardiac sarcomas are uncommon and little is known about their optimal management. Surgical resection is the mainstay of treatment, mostly with poor outcome. Adjuvant chemotherapy has been used to improve the disappointing results with surgery alone. Nevertheless, local recurrences and systemic spread of the disease remain frequent. We report on a patient with a synchronous double malignancy: a sarcoma of the left pulmonary vein with myogenic differentiation and an advanced non small cell lung cancer (NSCLC). **Case Report:** The 37-year-old man was admitted to our emergency department with a subacute ST-elevation myocardial infarction. He reported a 2-month history of atypical thoracic pain and amaurosis fugax. Whereas angiography showed normal coronaries, a left atrial mass was observed in echocardiography. A myxoma was assumed, and the patient underwent surgery. Histological examination revealed a poorly differentiated sarcoma with myogenic differentiation, with positive resection margins (R1). Since a postoperative pleural effusion and pneumonia did not resolve timely, we performed further diagnostic procedures, and an adenocarcinoma of the right upper lobe was found (stage IIIB, malignant pleural effusion). We started a combination chemotherapy with gemcitabine and docetaxel for 5 treatment cycles which resulted in a partial remission of the NSCLC. Then, erlotinib achieved a stabilisation (SD) for 9 months. With tumour progression (PD), a combination of carboplatin and vinorelbine was started and given for 2 cycles (with PD), followed by pemetrexed for 9 weeks with SD. Four months later, the patient developed CNS metastases and finally succumbed to his NSCLC 31 months after the initial diagnosis. There were no clinical signs of active sarcoma. **Conclusion:** Sarcomas of the heart are a rare cause of impaired coronary blood flow. They are associated with a poor prognosis, especially if they are not resected completely. Although there was no radical surgery for both cardiac sarcoma and NSCLC, our patient survived 31 months by means of palliative chemotherapy. This is above the expectations for both entities. We summarise the course of his disease and review the current literature.

Osteomalacia mistaken for skeletal metastases in young female immigrants

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Patients: Three young women, aged 29, 36, and 39, were referred to the Oncology Department with a diagnosis of diffuse bone metastases for search for a primary. History was reported to be unremarkable except for long lasting musculoskeletal pain. Repeated consultations and treatments were unsuccessful. **Results:** History for signs and symptoms of a primary was negative. The patients were dark coloured or black female immigrants to Germany from India, Pakistan and Somalia, respectively. They lived secluded lives, mostly

caring for their families at home and they were consistently wearing veils. The patients had five and three children, respectively, and one had three miscarriages. Physical examination was not informative. Bone scintigraphy demonstrated multifocal accentuated tracer uptake and there were multiple osteolytic lesions on X-ray interpreted as typical for diffuse bone metastases. On reevaluation they were reclassified as Looser's zones. Laboratory studies revealed high alkaline phosphatase, low calcium, low phosphate and high parathyroid hormone, consistent with secondary hyperparathyroidism. Calcidiol was decreased below reference concentrations in all 3 patients. A diagnosis of long lasting vitamin D deficiency with resultant hyperparathyroidism and severe osteomalacia was made. **Conclusions:** Osteomalacia due to vitamin D deficiency is a rare differential diagnosis in patients referred for evaluation of suspected bone metastases. In Europe, it was first described in the 70's in the United Kingdom in immigrants from Pakistan and India. Risk factors are female gender, number of pregnancies, prolonged breast feeding (loss of calcium), special diets (vegetarian, lack of milk products, phytin in pulse blocking absorption of calcium), dark skin, and inadequate exposure to sunlight due to cultural habits (reduced synthesis of vitamin D in the skin). Vitamin D concentrations tend to be low in the German population and even more so in Scandinavian countries due to less exposure to sun light compared to populations living closer to the equator. Oncologists should be aware of osteomalacia due to secondary hyperparathyroidism caused by vitamin D deficiency as differential diagnosis of diffuse bone metastases. Patients typically present with a history not immediately suggestive of metastatic cancer and they usually have risk factors for vitamin D deficiency as detailed above.

V450

The dosis makes the poison Report on a patient with malignant pheochromocytoma and the need of alloHSCT

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Introduction: Malignant pheochromocytomas are rare tumours with a 5-year-mortality rate greater than 50%. Treatment with ¹³¹Iodine metaiodobenzylguanidine (¹³¹I-MIBG) is currently the best adjuvant therapy following debulking surgery and is generally well tolerated. **Methods:** However, here we report on patient who suffered from severe haemotoxicity with prolonged leukopenia after radiotherapy with ¹³¹I-MIBG and subsequently underwent allogeneic haematopoietic stem cell transplantation (HSCT) due to myelodysplastic syndrome with unfavourable cytogenetics. To our knowledge this is the first report on such a serious, potentially life-threatening complication after ¹³¹I-MIBG therapy. **Results:** The patient was diagnosed from malignant pheochromocytoma in 1995 and underwent right-sided adrenalectomy the same year. In 2002 relapse with metastatic lesions in bone, lung and the mediastinum were diagnosed. After tumor debulking by surgery radiotherapy with ¹³¹I-MIBG was performed. Between January 2003 and November 2006 he received a cumulative ¹³¹I-MIBG dose of 63,8GBq. In November 2006 he suffered from prolonged leukopenia. Bone marrow aspiration revealed the diagnosis of myelodysplastic syndrome (RCMD) with unfavourable cytogenetic (45,XY,-7,del(12)(p11p13)[11]). Subsequently he received an HSCT from his HLA-identical sister after conditioning with treosulfan and fludarabine. Immunosuppression consisted of tacrolimus and sirolimus. On day 142 after HSCT steroid sensitive graft-versus-host disease was diagnosed after termination of immunosuppression due to mixed chimerism. Currently, one year after HSCT the patient is in remission of pheochromocytoma as well as myelodysplastic syndrome and graft-versus-host disease. **Conclusions:** In conclusion, if higher doses of ¹³¹I-MIBG are used in order to achieve better response rates, the onset of severe haemotoxicity and furthermore induction of secondary malignancies have to be taken into account. Following allogeneic HSCT immunosuppressive therapy with sirolimus might be of benefit, since it has been shown, that mTOR inhibitors have some efficacy in neuroendocrine tumours. We hope that recent development of targeted therapy interfering with signal pathways may provide a therapeutic option for patients with malignant pheochromocytoma, with the aim to improve the overall survival and to reduce toxic side effects of standard therapy.

V451

Effect of chemotherapy against high-grade B-Cell lymphoma on concomitant hepatocellular carcinoma: a case report

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Introduction: Hepatocellular carcinoma is responding poorly to systemic therapy. **Methods:** We describe the case of a 75-year old man, who was diagnosed of a diffuse large B-cell lymphoma (DLBCL) stage III B and a concomitant well differentiated hepatocellular carcinoma (HCC, stage I, segment II/III) developing in a Child class A liver cirrhosis. Due to increased perioperative risk, the decision was made to first treat the DLBCL. The patient received the standard chemo-immunotherapy protocol of rituximab, cyclophosphamide, doxorubicin, vincristin, prednisolone (R-CHOP 21). Due to prolonged neutropenia anti-lymphoma therapy had to be stopped after 5 cycles of R-CHOP. **Results:** An unconfirmed complete response of the DLBCL was achieved after 5 cycles of R-CHOP. The HCC, with an initial size of 3,5 x 2,5 cm, revealed a partial regression after chemotherapy 2,4 x 1,0 cm and could be successfully resected eight months after first diagnosis. **Conclusions:** Liver areas suspected of infiltration in advanced high grade lymphoma may harbour concomitant hepatocellular carcinoma. Therefore histology should be aimed for, especially if evidence for liver cirrhosis is present. If primary surgery of the HCC is not feasible, systemic anti-lymphoma therapy including an anthracycline may be capable to prevent progress or even induce partial remission of the concomitant HCC, to safely postpone surgery for lymphoma treatment.

Magen Poster:

P452

Documented mutation of the E-Cadherin Gene in 3 Cases of advanced gastric cancer in an Austrian family

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Purpose: Germline mutations in the E-cadherin (CDH1) gene have been found in families with hereditary diffuse gastric cancer (HDGC). So far 68 distinct CDH1 germ line mutations have been reported in HDGC. These families are characterized by a highly penetrant susceptibility to diffuse gastric cancer with an autosomal dominant pattern of inheritance. Endoscopic screening in HDGC cannot rule out diffuse gastric cancer, because macroscopically the stomach and also biopsies can be normal despite the presence of adenocarcinoma. We describe the clinical presentation of 3 cases with advanced gastric cancer, the way of confirming the suspicion of underlying HDGC and the clinical management of the other healthy family members according to the guidelines of the International Gastric Cancer Linkage Consortium (IGCLC). **Methods:** Screening for CDH1 germline mutation was done by denaturing high-performance liquid chromatography and automated DNA-sequencing. **Results:** The clinical suspicion of HDGC has been confirmed by identifying a frameshift mutation in exon 9 (1302_1303insA, 1306_1307delTT) of the E-cadherin gene. This mutation disrupts the function of the E-cadherin protein. **Conclusions:** The documented mutation in the E-cadherin gene identifies a further family with HDGC. Symptomatic HDGC is associated with a serious prognosis. The recommendation of a prophylactic gastrectomy to mutation carriers is due to a high penetrance of the mutated gene and the young age of onset of this particular tumor type.

P453

Hepatic arterial infusion chemotherapy for liver metastases from gastric cancer

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Introduction: The advantage of administering chemotherapy by arterial hepatic infusion (HAI) is the achievement of high concentrations of the given drugs in the liver. Oxaliplatin, Irinotecan and 5-fluorouracil (5-FU) are active agents for the treatment of advanced gastric cancer. Therefore a pilot study was performed to investigate the effects of these drugs administered by HAI in heavily pretreated gastric cancer patients with predominant hepatic metastases. Up to now, very limited data about HAI in gastric cancer exist in Western patients. **Methods:** Five patients with advanced gastric cancer who had received extensive systemic chemotherapy were included in the pilot study. 3/5 patients were over the age of 70 when starting therapy with HAI. All had a Karnofsky index between 60-80 and had at least received two previous systemic chemotherapy regimens including cisplatin/5-FU combinations, 3/5 had received three previous regimens. After informed consent, catheters were implanted in the hepatic artery by interventional radiological technique. Patients were given chemotherapy by HAI, Oxaliplatin 85mg/m²/2h, 5-FU 600mg/m²/2h und Folic acid 300 mg/m²/2h was the starting regimen in all patients. With development of clinically observed or radiologically proven progressive disease Oxaliplatin was switched to Irinotecan 180 mg/m² (n=1), Epirubicin (n=1) or discontinued (n=2). **Results:** 31 cycles of HAI (range 2-12) were administered in 5 patients. Administration of chemotherapy by HAI was feasible and safe, no grade 3/4 toxicity was observed. 3/5 patients developed grade I-II neurologic toxicity after Oxaliplatin therapy, which was partly reversible. The mean treatment duration was 6 cycles of HAI. One patient showed disease stabilization of liver metastases over a period of 7 months and was then switched to a different treatment because of progressing pulmonary metastases. 3/5 patients showed stabilization of quality of life. In 4/5 patients there was radiologically proven progressive disease after a mean treatment time of 11,6 weeks. **Conclusions:** In this pilot study, chemotherapy given by HAI showed anti-tumor activity in heavily pretreated gastric cancer patients. HAI showed a very favorable toxicity profile and could be safely administered even in elderly patients without negatively influencing quality of life. It might be an additional therapeutic option in patients with predominant liver metastases from gastric cancer and should be further investigated.

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Correlation of ZAP-70 and CD38 expression with IgVH mutational status in patients with B-cell chronic lymphocytic leukemia—a single center experience

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Introduction: Unmutated IgVH mutational status is correlated with early disease progression and shorter overall survival in patients with B-cell chronic lymphocytic leukemia (B-CLL). IgVH gene sequencing is a costly and time-consuming procedure. Therefore a simple, faster and thus more convenient assay would be preferable in routine diagnostics. In several studies, the expression of ZAP-70 and CD38 has been shown to be correlated with a pre-germinal type of B-CLL with an unmutated IgVH mutational status. ZAP-70 has also been described as an independent risk factor for early progression and short overall survival. **Methods:** In peripheral blood specimen of 36 patients with B-CLL we analysed ZAP-70 expression and CD38 expression and correlated it to the IgVH mutational status. Intracytoplasmic staining of ZAP-70 was measured in CD19- and CD5-positive B-CLL cells using a PE conjugated monoclonal antibody (clone G4, Santa Cruz biotechnology). CD38 was measured on CD19-positive B-cells expressing CD5, CD23 and CD43. IgVH sequences with > 2% difference in IgVH from the most similar germline gene

were considered mutated ("mutated B-CLL"), sequences with < 2% difference were considered unmutated ("unmutated B-CLL"). **Results:** ZAP-70 expression varied in a wide range from 1% to 77% (mean 19,1% in "unmutated B-CLL" and 21,5% in "mutated B-CLL"), as well as the CD38 expression from 1% to 99% (mean 41,7% in "unmutated B-CLL" and 6,8% in "mutated B-CLL"). Using ROC curve analysis we determined cut-off values and positive predictive values for IgVH mutational status according to CD38 expression we found a significant correlation of CD38 expression with unmutated IgVH (p=0,0004), while no significant correlation between ZAP-70 expression and IgVH mutational status was observed. **Conclusion:** In our experience, CD38 is a more suitable surrogate marker for mutational status prediction in B-CLL as compared with the measurement of ZAP-70 expression.

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Phase II study of Mitomycin C and capecitabine in pretreated patients with metastatic gastric cancer: interim analysis of a multicenter trial

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Background: Patients with disease progression of metastatic gastric cancer who were pretreated with platinum-based combination therapy usually have a dismal prognosis. Second line therapies may improve survival and quality of life. This multicenter phase II study investigates toxicity and efficacy of a combination of mitomycin C and capecitabine. **Methods:** Patients with pretreated locally advanced or metastatic adenocarcinoma of the stomach or gastroesophageal junction and ECOG performance status of 2 or better, measurable lesions and adequate organ functions are treated with mitomycin C 10 mg/m² day 1 and capecitabine 2,000 mg/m² (day 1 to 14; repeated day 21). CT or MRT scan of index lesions are performed every 6 weeks. **Results:** At time of abstract submission, 26 of the planned 40 patients are included in the study. Interim data was available on 22 patients. Median age was 65 years, 77% were male. The majority of patients had an ECOG performance status of <=1 (90%). Grade 3 toxicity occurred in 45% of the patients most often anemia 4%, thrombocytopenia 8%, leukopenia 8%, nausea 4%, vomiting 4%. Grade 4 toxicity was reported in one patient (elevation of alkaline phosphatase due to bone metastases). Thus far, 21 patients are evaluable for response analysis. Three patients had partial remissions and six no change adding to a tumor control rate of 43%. Twelve patients showed progressive disease. **Conclusions:** The mitomycin C and capecitabine combination regimen exhibits a favorable tolerability profile even in pretreated patients with gastric cancer. The preliminary disease control rate of 43% compares adequately with other phase-II studies for second-line therapy in gastric cancer. Accrual for the study is continuing.

P456

The expression of the epidermal growth factor receptor (EGFR) and related molecules and their impact on survival in patients with metastatic gastric cancer receiving first-line chemotherapy: results from the FLO versus FLP gastric cancer phase III trial of the AIO

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Introduction: Research on the role of EGFR in advanced gastric cancer (AGC) is warranted, with a view toward the future introduction of anti-EGFR therapy

in AGC. **Methods:** Tumor samples from a 1st-line phase III study with 5-fluorouracil/leucovorin plus oxaliplatin or cisplatin were prospectively analyzed. The expression of EGFR was assessed by immunohistochemistry (IHC) and quantitative RT-PCR. In addition, HER2/neu, Ki-67, T-Cells (IHC), and VEGF-A (quantitative RT-PCR) were evaluated. Polymorphisms of EGFR were determined using PCR based RFLP techniques and GeneScan analyses (Her1-497, -216G/T and -191C/A and intron 1 CA-repeat). **Results:** 230 samples from 149 out of 220 patients (pts) enrolled were analysed. EGFR was expressed in 47% of pts (1+, 24%; 2+, 39%; 3+, 37%). The expression was "focal" in 11% of cases. It was highest in intestinal type histology (50%) and surgically resected tissues (50%) and lowest in diffuse type histology (28%) and biopsy tissues (33%). There was no difference in EGFR expression between primary tumors and metastases (40% and 39%, respectively). HER2/neu over-expression (3+) was detected in 17% of pts. EGFR expression (any grade) correlated significantly with prolonged overall survival (median OS: 10.8 months in EGFR+ and 8.2 months in EGFR- pts; log rank $p=.026$), but not with response rate (RR) or progression-free survival (PFS). Correlation remained significant in the multivariate analysis ($p=.028$) and was independent from age, sex, ECOG, type of histology, treatment, liver and peritoneal involvement. Interestingly, within the group of EGFR+ tumors, higher EGFR expression grades correlated inversely with RR, PFS, and OS (e.g. RR, 53% in EGFR1+ patients and 19% in EGFR3+ patients; $p=.024$). EGFR mRNA levels and the analyzed EGFR genomic polymorphisms correlated neither with EGFR protein expression nor with patients' survival. **Conclusions:** Unexpectedly, EGFR-expression (any grade) was a positive prognostic factor in pts with AGC receiving first-line chemotherapy. However, once EGFR is expressed, over-expression represented a negative predictive factor.

P457

Patterns of care and trends in the treatment of patients with metastatic gastric cancer: results of a survey in Germany 2006 – 2007

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Background and Methods: Gastric cancer still represents a major health problem accounting for about 12,000 cancer deaths per year in Germany. Yet, data about the treatment reality and the impact of study results in clinical practice is rare. We have therefore conducted a clinical and epidemiological survey (TherapieMonitorä) in a representative sample of surgical and medical departments as well as oncology practices in 2006 and 2007. Herein we report on patterns of care and trends in treatment decisions regarding 1st-line chemotherapy. **Results:** A total of 1321 patients (pts) undergoing treatment decision in 2006 (n=759) and 2007 (n=562) were documented. The median age was 66 years, and 63% were male. UICC-stages at diagnosis were distributed as follows: stage I 9.5%, stage II 12.6%, stage III 19.5%, stage IV 43.2%, unknown 15.1%. 30% (2006) and 27% (2007) of the pts had tumors involving the esophago-gastric junction. A total of 555 pts receiving 1st-line chemotherapy are evaluable. Median age of these pts was 65 years, (15% older than 75 years). Sites of metastases (%; 2006/2007): peritoneum 46/42, liver 47/51, abdominal lymph nodes 54/54. Regarding 1st-line therapy, 5-fluorouracil was frequently used as backbone in combination regimens. Further drugs were (%; 2006/2007): cisplatin 60/51, oxaliplatin 10/21, docetaxel 13/18, irinotecan 12/7, capecitabine 4/14. Cisplatin was used more frequently in pts with Karnofsky Performance Status (KPS) $\geq 80\%$ vs $< 80\%$ (73% vs 35%; $p < 0.0001$) but less frequently in elderly pts (≥ 65 vs < 65 years) (47% vs 64%; $p < 0.0001$). Older pts were also less likely to receive 3-drug combination regimens (11% vs 27%; $p < 0.0001$). 2nd-line therapy was given to 189 pts. Treatment was preferably given to pts with KPS $\geq 80\%$. Frequently used drugs were irinotecan 37% and docetaxel 19%. **Conclusion:** Among German pts with advanced gastric cancer, younger pts (< 65 years) and those with KPS $\geq 80\%$ are more likely to receive cisplatin and 3-drug combinations as 1st-line treatment. The use of oxaliplatin, capecitabine, and docetaxel increased during the observation period. These data suggest that it might be justified to test different treatment strategies in clinical trials for elderly and/or unfit pts with advanced gastric cancer.

P458

Neoadjuvant chemotherapy in patients with resectable esophagogastric adenocarcinoma using docetaxel-based triplet chemotherapy: a two center based study of 35 patients

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Background: Docetaxel-based triplet chemotherapies proved highly effective in patients (pts) with metastatic gastric cancer. Therefore, the evaluation of these combinations in the neoadjuvant setting has considerable appeal. **Methods:** A consecutive series of 35 pts with localized or limited metastatic esophagogastric adenocarcinoma who underwent neoadjuvant treatment with docetaxel in combination with cisplatin and 5-fluorouracil (DCF regimen) or 5-fluorouracil, leucovorin, and oxaliplatin (FLOT regimen) were evaluated. Limited metastatic disease was defined as potentially resectable primary with paraaortal lymph node involvement only, or resectable metastases involving one additional organ and ECOG < 2 . Pts received 8-9 weeks of induction chemotherapy and resection was attempted 3-4 weeks after chemotherapy was completed. Data were collected prospectively. Clinical staging was performed by endoscopic ultrasound and hydro-CT. Centralized evaluation of pathological responses was performed by an experienced pathologist. **Results:** Two institutions participated. Thirty-five pts were treated (median age 61; range 31-77). 51% of pts had tumors of the esophagogastric junction. Preoperative clinical TNM-stages were: T0/T1 0%, T2 14%, T3 77%, T4 8%; N+ 100%. Twenty-six (74%) pts had localized and 9 (26%) had limited metastatic disease. Three pts received DCF and 32 received FLOT. Median number of preoperative cycles administered was 4 and 54% of pts received postoperative chemotherapy. A partial or complete clinical response was achieved in 28 (80%) pts. Seven (20%) pts had stable disease and disease progression during neoadjuvant treatment was not observed. The pathological complete remission and R0 resection rates were 18% and 94% in the entire population and 20% and 100% in pts with localized disease. Postoperative pathological stages were: T0/T1 29%, T2 60%, T3 9%, T4 3%; N+ 56%. Postoperative mortality and morbidity were 5.8% and 51%, respectively. The toxicity of the chemotherapy regimens did not differ from that known from previous trials using DCF or FLOT in the palliative setting. **Conclusion:** Neoadjuvant chemotherapy with DCF or FLOT resulted in high pathological complete remission and R0 resection rates. Postoperative mortality and morbidity were comparable to those reported in previous trials. Patients with limited metastatic disease seemed to benefit from the multimodal treatment strategy. The regimens clearly warrant further development in this setting within prospective clinical trials.

P459

Alternating 5-FU, FA, Cisplatin with 4-Epirubicin, Docetaxel as first line therapy in patients with advanced gastric cancer

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Introduction: Gastric cancer is one of the most common malignancies. On the basis of the known anti cancer activity of Cisplatin and 5-FU several additional drugs have been evaluated in recent studies. Combination of more than 2 cytostatic agents usually induces severe toxicity in these patients. Therefore we performed a mono-center Phase II study using alternating administration of effective drugs on a monthly basis. **Methods:** Patients with histologic proven advanced (inoperabel, locally advanced or metastatic) gastric adenocarcinoma were enrolled. ECOG Status 0-2. The treatment regime consisted of 5-FU 2000 mg/sqm and FA 500mg/sqm d 1,8,15,22; CISPLATIN 35mg/sqm d 1,2,15,16; 4-EPIRUBICIN (E)60mg/sqm and DOCETAXEL (D) 60mg/sqm d 29, 43. Q 56 d. A total of 4 cycles were administered in patients with PR and CR and 3 cycles in SD, respectively. **Results:** A total of 45 patients were eligible. (m:f = 34:11) The median age was 62 y. Overall response rate was 47% (21/45), PR = 33% (15/45), SD = 31%, PD = 22%. Progression free survival

was 6,1 months and median overall survival was 10,2 months. PFS for patients with PR+CR was 9,9 months. Toxicity mainly affected haematopoesis with leucopenia WHO Grade 3 - 4 with 73% and thrombopenia Grade 3 with 16% observed in the treatment section upon E and D. **Conclusions:** Monthly alternating treatment as demonstrated in this study induces tumour response comparable to other studies reported recently. The overall toxicity was rather low. To handle the significant haematotoxicity we recommend the use of haematopoietic growth factors after D + E. This outpatient regimen seems to be a good basis to be added with modern monoclonal antibodies.

P460

TROP2 as prognostic marker in gastric cancer

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Introduction: Gastric carcinoma is one of the most common neoplasias and most frequent causes of cancer related deaths worldwide. Although surgical resection remains the only means of cure, the recurrence rate is unacceptably high even after R0 resection and (neo)adjuvant chemotherapy. Therefore, there is an urgent need for identification of predictive and/or prognostic biomarkers to select high-risk patients who might benefit from additional tailored therapies. Expression of TROP2 was demonstrated to be associated with tumor aggressiveness and poor prognosis in patients with various epithelial cancers. The aim of this study was to investigate TROP2 expression in gastric cancer and its correlation with clinicopathological features and disease outcome. **Methods:** Expression of TROP2 was investigated by immunohistochemistry of paraffin-embedded tumor specimens from 104 patients who underwent resection for gastric cancer at the Department of Surgery, Innsbruck Medical University. Overall survival (OS) and disease-free survival (DFS) was calculated using Kaplan-Meier estimates. Parameters found to be of prognostic significance in univariate analysis were verified in a multivariate Cox regression model. **Results:** TROP2 was found to be overexpressed in 58 (56%) of the tumor samples. According to Lauren classification significantly higher expression of TROP2 could be detected in intestinal-type gastric cancer than in diffuse-type carcinoma ($p=0.03$). In intestinal-type gastric cancer, TROP2 overexpression was significantly correlated with shorter DFS ($p=0.03$). Among the total patient group, TROP2 overexpression was a prognostic marker for poor disease-free ($p<0.01$) and overall ($p=0.03$) survival in lymph node positive patients but not in those without locoregional lymph node metastasis. Multivariate Cox regression analysis revealed TROP2 overexpression to be an independent prognostic marker for poor DFS in the subgroup of patients with intestinal-type gastric cancer irrespective of lymph node involvement. **Conclusion:** Our results demonstrate that TROP2 is an independent prognostic marker for disease recurrence in intestinal type gastric cancer. Due to its wide distribution TROP2 may become an attractive therapeutic target in a subgroup of patients with gastric cancer at particularly high risk for relapse.

MammaCa

Freie Vorträge:

V461

Combination therapy with Ixabepilone plus Capecitabine is effective in ER/PR/HER2-negative breast cancer resistant to Anthracyclines and Taxanes

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Introduction: A large proportion of basal-like breast cancers, a disease subgroup with poor prognosis, lack expression of estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2). Patients with ER/PR/HER2-negative disease have fewer treatment options than those with receptor-positive tumors. Ixabepilone, a novel epothilone that was developed to have less susceptibility to tumor-resistance mechanisms, showed activity in phase 2 studies of anthracycline- and taxane-pretreated metastatic breast cancer (MBC), including ER/PR/HER2-negative disease. **Methods:** In a phase 3 trial of 752 patients with anthracycline/taxane-resistant MBC, ixabepilone, 40 mg/m² IV over 3h q3wk, in combination with capecitabine, 2000 mg/m² PO d1-d14 q3wk, was compared with capecitabine alone, 2500 mg/m² on the same schedule. Progression-free survival (PFS) and objective response rate (ORR) were prospectively analyzed for the ER/PR/HER2-negative patient subgroup (25%) and compared to the group as a whole. **Results:** The combination of ixabepilone with capecitabine prolonged PFS and improved ORR compared with capecitabine monotherapy in patients with ER/PR/HER2-negative MBC (PFS hazard ratio, 0.75; $P=.0003$; ORR 27% for combination therapy and 9% for monotherapy), as well as in the total population. Most frequent Grade 3/4 treatment-related adverse events for the total population in the combination arm were: sensory neuropathy (21%), fatigue (9%), and neutropenia (68%). **Discussion:** Ixabepilone plus capecitabine was superior to capecitabine alone in anthracycline/taxane-resistant advanced breast cancer and a similar result was evident in patients with ER/PR/HER2-negative tumors. The combination of ixabepilone and capecitabine may offer a specific advantage in this subset of breast cancer including ER/PR/HER-2-negative tumours.

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Soluble E-cadherin and its prognostic impact on response to neoadjuvant anthracycline-based chemotherapy in breast cancer patients

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Introduction: To date, no reliable markers are available to predict response to or to assess prognosis after neoadjuvant chemotherapy (NCT) in patients with breast cancer. Previous studies demonstrated that different levels of soluble E-cadherin (sE-cadherin), a product of proteolytic cleavage of cell surface E-cadherin, are associated with metastatic disease and poor prognosis in various tumor types. Therefore, it was hypothesized that serum sE-cadherin levels measured before NCT may correlate with pathological response to NCT. **Methods:** In a retrospective analysis, sE-cadherin levels were measured in sera of 108 female patients with histologically proven breast cancer before initiation of NCT by using a commercially available quantitative sandwich enzyme immunoassay technique. Patients received a median number of 4 (range 3-6) cycles of anthracycline-based chemotherapy. The median patient age was 50 (range 21-71) years. Tumor size was measured clinically in one dimension and translated into the TNM-system before start of chemotherapy. Histopathological response in surgically removed specimens was evaluated

using the Sinn regression score. Her2neu and EGFR status was determined from preoperative tumor biopsies using standard immunohistochemical methods. In univariate and multivariate analyses the correlations between levels of sE-cadherin and pathological response to NCT were calculated. **Results:** The histopathological regression scores correlated significantly with age ($p=0,010$), histopathological grading ($p=0,043$), hormone receptor status ($p=0,017$), type of chemotherapy used (anthracycline-taxane based vs anthracycline-cyclophosphamide based; $p=0,001$) and sE-cadherin levels ($p=0,003$). No correlation was seen between histopathological regression scores and Her2-neu status or EGFR-status. **Conclusion:** sE-cadherin may be a marker predicting response to NCT for patients with breast cancer and therefore these findings warrant the evaluation of sE-cadherin in a prospective trial.

V463

Asynchronously growing and synchronized human MCF-7 breast cancer cells differentially respond to the CDK inhibitor roscovitine

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Introduction: Roscovitine (ROSC), a selective blocker of cyclin-dependent kinases (CDKs) efficiently inhibits proliferation of exponentially growing human MCF-7 breast cancer cells by induction of cell cycle arrest and p53-mediated apoptosis. ROSC blocks MCF-7 cells in G₂-phase in a time- and concentration-dependent manner. On the other hand, ROSC exerts much weaker anti-proliferative effect on normal human diploid fibroblasts. Therefore, in this contribution we raised the question whether and if yes, to which extent the anti-proliferative effect of ROSC does depend on the cell cycle status of cancer cells exposed to the drug. **Methods:** MCF-7 breast cancer cells were synchronized in G₁ phase of the cell cycle by serum deprivation for 24 h or in S-phase by exposure to hydroxyurea for 4 h prior to the treatment with ROSC in medium supplemented with 10 % FCS. Cells were stained with propidium iodide. Sensitivity of human MCF-7 breast cancer cells and normal MRC-5 fibroblasts to ROSC was determined by proliferation assay using a microtiter plate CellTiter-Glo luminescent cell viability assay (Promega Corporation). This is a method for determining the number of viable cells in a culture based on quantification of the ATP levels. **Results:** After serum re-feeding G₁-synchronized cells started to re-enter the active cell cycle after 12h. Exposure of G₁-synchronized cells to ROSC prolonged the cell cycle arrest accompanied by a decrease of S-phase cells after 24h. A similar but weaker trend occurred after ROSC administration to cells released from G₁ arrest for 4h prior to the onset of treatment. ROSC diminished the frequency of S-phase cells. Exposure of MCF-7 cells released from G₁ arrest to ROSC for 24h resulted in an increase of G₁ cell population by 20%. Exposure of MCF-7 cells released from S-phase block to ROSC increased the ratio of S-phase cells. **Conclusions:** The effect of ROSC on cells being synchronized in G₁ by serum deprivation or in S-phase by hydroxyurea strongly differs from its effect on asynchronously and exponentially growing human MCF-7 breast cancer cells. Diminished effect of ROSC on G₁ arrested MCF-7 cells resembled that exerted on normal human fibroblasts. The data clearly evidence that the cell cycle status prior to the onset of treatment with the CDK inhibitor ROSC strongly determines the outcome of the therapy and substantiate our former observations that inhibition of CDKs has low effect in healthy cells.

V464

Molecular profiling and predictive value of circulating tumor cells in patients with metastatic breast cancer: an option for monitoring response to breast cancer related therapies

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Purpose: The purpose of the study was the molecular analysis of circulating tumor cells (CTC) in blood of metastatic breast cancer patients and to determine the ability of this method to predict the response to breast cancer related therapies. **Experimental Design:** 10 ml blood was obtained from 42 metastatic breast cancer patients before and during therapy. All patients had measurable disease and were either patients with relapse of breast cancer diagnosed years before and to start chemotherapy or patients with documented progressive breast cancer who were to begin a new endocrine, chemo-, trastuzumab- or experimental therapy. CTC were analyzed for EpCAM, MUC-1 and HER2 transcripts with the *AdnaTest BreastCancer* (AdnaGen AG). Expression of the estrogen (ER) and progesterone (PR) receptor was assessed in an additional RT-PCR. The analysis of PCR products was performed by capillary electrophoresis on the Agilent Bioanalyzer 2100. Blood of healthy controls were used to confirm the specificity of the test. **Results:** The overall detection rate for CTC was 52% (thereof 86% EpCAM; 86% MUC-1; 32% HER-2; 35% ER; 12% PR). CTC were ER, PR and Her2 negative in 45% (ER), 78% (PR) and 60% (Her2) of patients with receptor-positive tumors. Interestingly, 29% of patients with HER2-negative tumors had HER2-positive CTC. A complete therapy monitoring could be performed in 32/42 patients. The test predicted therapy response in 78% of all cases. Persistence of CTC significantly correlated with shorter overall survival ($p=0.005$). **Conclusions:** The presence of CTC in metastatic breast cancer is a predictor of therapy response. Molecular profiling of CTC may offer more differentiated prognostic information with respect to higher accuracy of risk assessment for recurrence and towards predictive judgement of a response to therapeutic regimens.

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Study of transforming potential and drug sensitivity of ErbB2 variants identifies lapatinib-resistant kinase domain mutations

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Overexpression of ErbB2 kinase is a frequent event in breast carcinogenesis. The dual ErbB1/ErbB2 tyrosine kinase inhibitor lapatinib recently was approved for the treatment of advanced ErbB2-positive breast cancer. Recently variants of ErbB2 were reported in breast, gastric, colorectal and lung cancer. Importantly polymorphism at aa I654 to V654 was reported to be associated with higher risk of breast cancer. However, the effect of this polymorphism on kinase activity and drug sensitivity was not known. In this study, both V654 and I654 showed similar kinase activity and transformed BaF3 cells to IL-3 independence. Moreover, sensitivity of both variants were similar towards gefitinib, erlotinib, AEE788 and lapatinib. However, ErbB2 variants were more sensitive to dual inhibitors AEE788 and lapatinib. Conversely, the ErbB1 L858R, which is frequently found in ErbB1 mutated NSCLC was very sensitive to gefitinib, erlotinib and AEE788, but displayed higher IC50 values to lapatinib. Development of secondary drug resistance due to kinase domain mutations in BCR-ABL (CML) and ErbB1 (NSCLC) was previously reported. We hypothesized that mutations in ErbB2 kinase domain might confer lapatinib resistance. To test this, we used an in vitro screen to identify mutations that cause lapatinib resistance. BaF3 cell line transformed by wt ErbB2 was used for this purpose. Analysis of resistant clones revealed two lapatinib resistant mutations L755S and T862A. Interestingly, the L755S exchange was

reported in breast cancer patients. Thus, lapatinib treatment in patients harboring L755S, in contrast to ErbB2 wt or the polymorphism I654V may not be effective. However, L755S is relatively sensitive to alternative ErbB2 inhibitors such as AEE788, with an IC50 of 0,5 µM. Thus, alternative ErbB2 inhibitors may be superior to lapatinib in breast cancer patients with the L755S mutation. We then tested a panel of ErbB2 kinase domain mutations that were reported in breast, colorectal and lung cancer. All mutants transformed BaF3 cells and displayed differential sensitivity to specific drugs. ErbB1 T790M causes gefitinib resistance in NSCLC. We therefore cloned the analogous mutation in ErbB2 (T798M) and tested for drug sensitivity. T798M was fully resistant to all drugs tested with IC50 values above 2 µM. Thus, ErbB2 T798M probably will emerge in the future as more breast cancer patients are treated with ErbB2 inhibitors, similar to the Bcr-Abl T315I mutation in CML. We believe that specific activity and resistance profiles will be helpful in selecting patients with specific ErbB2 variants for treatment with a particular drug not only in the setting of ErbB2-positive breast cancer, but also in selected cases of gastric, colorectal and lung cancer. Identification of drug resistant mutations also is a prerequisite for the development of new inhibitors that may overcome secondary resistance.

V466

A novel method to capture and characterize occult bone marrow metastases in patients with breast cancer

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Introduction: The presence of disseminated tumor cells (DTC) in the bone marrow (BM) of breast cancer patients is an independent prognostic factor. We have recently shown that the majority of these DTC have the putative stem cell phenotype (CD44+/24-). The clinical use and biological characterization of DTC has been hindered by lack of efficient capture and analysis methods. **Methods:** We have recently developed parylene membrane filter microdevice for size-based capture of DTC circulating in peripheral blood (PB) with recovery rates of spiked tumor cells > than 90%. Since the frequency of DTC has repeatedly been reported to be much higher in BM aspirates in comparison to PB, it is essential to characterize the performance of the parylene membrane filter in DTC detection from BM aspirates. Due to presence of larger cells and higher viscosity and cell density of BM aspirates, such analysis could be more challenging than analysis of blood. **Results:** In this pilot project, we have successfully studied BM aspirates from 8 patients with hematological diseases utilizing a model system where 10 - 100000 cultured breast cancer cells (MCF7) were spiked in BM aspirates. At least one DTC was detected when 10 cells were spiked into 5ml of BM. Up to 6 ml of BM successfully passed through a filter (6x6mm square area) with 8µm pores. The captured cells were subjected to multiple markers analysis by immunofluorescence (cytokeratin, MIB1, CD44, CD24), with direct evaluation on the membrane. Cytokeratin-positive DTC ranging from 1 to 12 cells were isolated with laser microdissection and pressure catapulting (LMPC) and subjected to whole genome amplification followed by comparative genomic hybridization (CGH) analysis, which reflected the amplification on chromosomes 17q and 20q, characteristics of the MCF7 cell line. **Conclusion:** The ability to efficiently capture DTC on a very small filtering area and then analyze these cells on the same platform will substantially facilitate further detailed analysis of early and late events in metastasis. Further, effective, multiplexed examination of the molecular and cellular characteristics of DTC should lead to the identification of new therapeutic targets, as well as efficient, cost effective methods for assessing prognosis and therapeutic efficacy, thus permitting the design of rational strategies to target and treat breast cancer.

MammaCa

Poster:

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Quality of life: learning to cope with the changes in body image and treatment effects of breast cancer therapy – The role of rehabilitation

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Purpose: Various treatment modalities for breast cancer, such as surgery, chemotherapy, radiotherapy and anti-hormone therapy, may lead to major alterations in subjective and objective assessments of body image, functional deficits, sleep disturbances, sweating and impairment of mood, activity and vigilance. Disturbances of body image range from mild to severe traumatisation. For the complex task to reconstitute traumatised patients, rehabilitation with a multidisciplinary team consisting of specially trained physicians, physiotherapists, lymphtherapists, psychologists, dieticians as well as creativity therapists, ergotherapists and social workers can offer comprehensive help. The aim of the present study was to investigate how body image and acceptance will change in patients who had undergone various surgical procedures, chemotherapy, radiotherapy and hormone therapy for breast cancer. Especially variables such as their subjective feeling and motivation for rehabilitation were targets of the study. Furthermore, we tried to shed light on the role of preceding treatments as well as the emotional status of the patient for improvement of the previously described items. **Methods:** We investigated 200 consecutive patients who underwent rehabilitation in an inpatient setting. Their status was investigated with the following inventories: Acceptance of body image questionnaire (testing satisfaction with the results of surgery), Paremo (motivation for rehabilitation, readiness to change, ability to seek help and social support, impairment of everyday life activities, hopelessness, initiative and knowledge) and BSKE – assessing current well-being by 6 categories of adjectives (positive affect, fear, hostility, inactivity, vigilance and introvertedness). These inventories were applied at the beginning and at the end of the rehabilitation period. **Results:** demonstrate a significant improvement regarding the acceptance of the body image as well as an increase of vigilance and activity and a decrease of hostility. Patients with a positive mood upon admission improved more in accepting their body image than those with negative feelings; whereas type of preceding treatment had no effect on improvement. **Conclusions:** Rehabilitation - favourably in an indoor setting - has proven effective as a major tool for improving the quality of life for patients with breast cancer. Interestingly, acceptance of body image was mainly dependent on positive feelings at the beginning of rehabilitation.

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Analysis of risk factors associated with early development of brain metastases in patients with metastatic breast cancer

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Introduction: Following the introduction of trastuzumab, a number of groups observed an increased incidence of brain metastases (BM) in patients (pts) with Her2-positive breast cancer (BC). Beside prolonged survival, the inability of antibodies to pass the blood-brain-barrier is held responsible for this development most often. Furthermore, based on preclinical data, it was suggested that Her2-positive BC had an increased affinity to brain tissue. We investigated, which risk factors were significantly associated with early development of BM. **Methods:** 197 pts with BM from breast cancer were identified at two Austrian centres and included. All had whole brain radiotherapy (WBRT) of 30 Gy with or without boost irradiation or surgical resection. Data concerning epidemiology, histology, and outcome is available. A Cox regression model was used as multivariate analysis to identify risk factors (age at diagnosis, grading, ER/PgR-status, Her2-

status, tumour stage at diagnosis, lung metastases, liver metastases, trastuzumab before diagnosis of BM) predicting shorter time to development of BM. **Results:** Median age was 55 years (y), range 28 – 81 y, and time to development of BM was 29 months (m), range 2 – 254 m, 95% CI 22.97-35.03. Overall survival following WBRT was 7 m, range 1 – 83 m, 95% CI 5.08-8.92. The following factors predicted early development of BM: endocrine receptor negative disease ($p=0.026$), Her2-positive disease ($p=0.003$), stage IV at first presentation ($p<0.001$), and presence of lung metastases ($p=0.043$). **Conclusions:** Different groups have already described risk factors associated with increased incidence of BM: ER/PgR-negative, Her2-positive disease, high tumour grade, and lung metastases. Also, a higher risk in patients on trastuzumab treatment was reported. Here, we describe a population at risk for early development of BM. Our data show that Her2-positive BC is significantly associated with early development of BM, while no protective influence of trastuzumab treatment was observed. These results underline preclinical data suggesting an increased affinity of Her2-positive tumours to the brain. In conclusion, we strongly suggest trials evaluating the combination of trastuzumab with agents able to pass the blood-brain-barrier in this population.

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Clinical activity of Ixabepilone, a novel Etoposide analog, across the breast cancer disease continuum

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Ixabepilone is a semi-synthetic analog of etoposide which has a low susceptibility to multiple mechanisms of resistance. Ixabepilone has been evaluated both as a single agent and as combination therapy in more than 1200 patients across the continuum of breast cancer – as primary systemic therapy in patients with locally-advanced and metastatic breast cancer

- first line in taxane-naïve and taxane-resistant patients
- second and third line in taxane-resistant or -naïve patients
- third or fourth line in multi-resistant patients.

Ixabepilone is effective in patients as a neoadjuvant therapy, as well as in later stages of disease progression, including in heavily pretreated patients. Ixabepilone has efficacy as a single agent (administered as 40 mg/m² or 6 mg/m²/day on days 1-5 every 3 weeks) and in combination with capecitabine (40 mg/m² ixabepilone every 3 weeks plus 2000 mg/m²/day capecitabine on days 1-14). Ixabepilone has a manageable safety profile.

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Gemcitabine plus vinorelbine versus gemcitabine plus cisplatin versus gemcitabine plus capecitabine: A randomized trial in pretreated metastatic breast cancer

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Methods and Patients: This randomised phase II trial investigated pretreated MBC patients who were attributed to one of three gemcitabine-based regimens

applied at 3-week intervals. Patients received either Gem/Vin (gemcitabine 1000 mg/m² days 1+8 plus vinorelbine 25 mg/m² days 1+8) or Gem/Cis (gemcitabine 1000mg/m² days 1+8 plus cisplatin 30 mg/m² days 1+8) or Gem/Cap (gemcitabine 1000 mg/m² days 1+8 plus capecitabine 650 mg/m² bid orally days 1+14). ORR was evaluated as a primary end-point, while TTP, OS, and safety were analysed as secondary endpoints. **Results:** 134 eligible MBC patients were randomized and received either Gem/Vin (n=41), Gem/Cis (n=44), or Gem/Cap (n=49). Baseline characteristics regarding median age (58 vs 59 vs 61 years), and estrogen receptor positivity of the primary tumor (46% vs 49% vs 38%) were well balanced between Gem/Vin, Gem/Cis, and Gem/Cap treatment arms. All patients had previously received anthracyclines for adjuvant or palliative therapy. A median of 3 cycles was performed. According to an ITT analysis, overall response rates were in the Gem/Vin arm 32.5% (95% CI, 18.6-49.1%), in the Gem/Cis arm 47.7% (95% CI, 32.5-63.3%), and in the Gem/Cap arm 30.6% (95% CI, 18.3-45.4%). Progression-free survival for Gem/Vin, Gem/Cis, and Gem/Cap was 5.8 months (95% CI, 3.9-8.2), 7.3 months (95% CI, 5.8-10.4), and 8.2 months (4.3-9.1), respectively, while overall survival was 17.8 months (12.5-37.2), 13.2 months (11.1-24.8), and 20.2 months (17.0-34.3), respectively. When overall grade 3-4 toxicities were compared, no significant difference between arms was detected (chi-square test). **Conclusion:** Three gemcitabine-based chemotherapy regimens, independent of anthracyclines and taxanes, were shown to be effective treatment options for pretreated in MBC patients.

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Vinorelbine (oral and intravenous) plus Trastuzumab for 1st-Line Treatment of HER2-Overexpressing Metastatic Breast Cancer (MBC). A Trial of the German AIO Breast Cancer Group

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Background: High activity of intravenous vinorelbine (VNR) plus trastuzumab (T) has been demonstrated in previous clinical trials performed in HER2-overexpressing MBC patients (pts). The present phase II study evaluates a new regimen investigating the combination of oral and intravenous vinorelbine plus trastuzumab. **Methods:** This phase II trial investigated HER2-overexpressing MBC pts (IHC 3+ or FISH+) who received VNR at an intravenous dose of 25mg/m² on day 1 followed by oral VNR applied at a dose of 60mg/m² on days 8 and 15 of a 3-week cycle. Trastuzumab was given at 3-week intervals with a starting dose of 8mg/kg on day 1 of the first cycle, and a maintenance dose of 6mg/kg in all subsequent cycles. Overall response rate was evaluated as a primary end-point, while progression-free survival (PFS), overall survival (OS), and safety were analysed as secondary endpoints. **Results:** 42 pts were recruited. Median age was 60 years (range 34 – 75 years), and median baseline Karnofsky performance status was 100% (range 70-100%). 67% of pts were hormone receptor positive. A HER2 IHC score of 3+ was determined in 95% of pts, while 5% of pts had an IHC score of 2+, but were FISH positive. 27 pts (64%) had received previous (neo)-adjuvant therapy (21 anthracycline-based, 8 taxane-based). Visceral metastasis was evident in 69% of pts. A median of 8 cycles (range 1-18) were applied. Hematological toxicity was generally low with grade 3-4 neutropenia of 19%. Complete remission was observed in 8 pts (19%), partial remission in 18 pts (43%), and stable disease in 8 pts (19%) for an ORR of 62%. Median remission duration was 7.5 months, median PFS was 7.9 months. The median of OS has not been reached so far. **Conclusion:** The combination of oral plus intravenous vinorelbine and trastuzumab has shown high antitumor efficacy and excellent tolerability in 1st-line treatment of HER2 overexpressing MBC.

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Tumor Markers for Early Detection of Recurrent Metastatic Breast Cancer

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Background: With the aim of early detection of metastatic disease (=detection in asymptomatic patients) breast cancer patients are monitored in a prospective non randomized trial in intervals of 6 weeks by using kinetics of CEA and CA 15-3. **Methods:** Among the 494 patients participating in our trial we analysed the clinical data of those who developed distant metastases (n=61). A reproducible increase of CEA (Abbott, AxSYM) or CA 15-3 (Roche, Elecsys) $\geq 100\%$, corresponding to a predefined specificity of 98%, was the indicator for metastatic disease. **Results:** 61 patients developed distant metastases. 35 patients (57%, of these 80% ER+ and/or PR+, 38% HER2 positive primary tumors) showed the previously defined increase of CEA or CA 15-3 at the time of first metastases (true-positive, TP). In 24 patients (39%) CA 15-3 alone, in 8 patients (13%) CEA alone and in 3 patients (5%) both markers increased. 26 patients (43%, of these 69% ER+ and/or PR+, 12.5% HER2 positive primary tumors) did not show an increase in tumor marker levels at the time of first metastases (false-negative, FN). At further progression, a delayed increase of markers was determined in 92% of the FN patients. The median disease-free interval until detection of distant metastases did not differ between TP (44.1 months) and FN patients (42.3 months) (p=0.8). All marker-positive patients were asymptomatic at the time of recurrence. ER/PR and HER2 expression did not differ significantly between TP and FN patients (p=0.4 and p=0.1, respectively), but triple negativity (ER-, PR- and HER2-) was more frequent in FN patients (p=0.03). All patients with liver-only metastases were TP (n=11, p=0.0002), 8 (73%) of them suffered from ligometastatic disease. All patients with brain-only metastases were FN (n=3, p=0.1). **Conclusions:** 57% of patients with distant metastases were TP, including all patients with liver-only metastases. Of these, 8 (73%) suffered from oligometastases. If early detection of asymptomatic and oligometastatic disease will allow early interventions and thereby improves treatment outcome needs to be shown in the future.

P473

Systems-directed targeted therapy in chemorefractory metastatic breast and ovarian cancer

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Introduction: Targeting tumor systems biology in patients with advanced systemically pre-treated carcinoma with a combined multi-targeted (anti-inflammatory, angiostatic and immunomodulatory) treatment strategy seems to be promising. **Methods:** In a prospective multi-center phase II trial patients with measurable, histologically proven, unresectable, progressive carcinoma and systemically pre-treatment received continuous oral pioglitazone (Actos) 60mg daily, day 1+, etoricoxib (Arcoxia) 60 mg daily, day 1+ as anti-inflammatory treatment, and 1g/m² capecitabine (Xeloda) bid for 14 days, every 3 weeks as angiostatic therapy (starting day 1+) until tumor progression. Tumor response was assessed every 6 weeks using WHO criteria. Primary endpoint was progression-free survival (PFS). **Results:** Five patients suffering from metastatic ovarian cancer were enrolled onto study protocol for 3rd to 8th line therapy, eight patients for 2nd to 5th line therapy in metastatic breast cancer. PFS following the respective preceding pulsed chemotherapy was mean 3.4 months (range 0.9 to 6.8) in patients with ovarian cancer and 6.1 months (range 1.2 to 17) in patients with metastatic breast cancer. The corresponding PFS on study medication nearly doubled for patients with ovarian cancer, mean 6.6 months (range 1.4 to 14.1 months) including one patient with a partial remission, and was mean 4.0 months (range 1.3 to 6.3 months) for patients with breast cancer including three patients with a $> 100\%$ prolongation of PFS on study medication. Capecitabine was shortly interrupted and then continued with 1g absolute bid due to a hand-foot-syndrome WHO grade 2 in 4 patients.

No $>$ grade 2 WHO toxicities have been observed. The tumor-associated C-reactive protein levels (mean 78.1, range 3.6 to 335) in patients with ovarian cancer decreased to normal after 4 weeks on study medication in four patients and rose continuously during final tumor progression. **Conclusions:** Systems-directed biomodulatory therapy targeting complex aggregated action effects of stroma components (for instance inflammation, angiogenesis), tumor cells, and cells of the involved organ may prolong response in patients with metastatic, multiple pre-treated ovarian or breast cancer as compared to response to the preceding reductionist therapy approach. Biomodulation of systems biological processes facilitates comparatively high efficacy at moderate toxicity.

P474

Improved survival of patients with metastatic breast cancer in routine care. Results of a retrospective study in a community based oncology group practice 1995-2005

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Introduction: Metastatic breast cancer (MBC) is an incurable disease, only 3% of patients live disease free beyond 5 years after diagnosis. Median survival in prospective trials is approximately 24 months. Because less than 2% of patients above the age of 60 participate in clinical trials, data about the treatment reality in routine care are not known. We therefore undertook this analysis to evaluate the actual treatment reality for unselected patients with MBC in routine care. **Methods:** All patients with MBC who were treated in our community based group practice between 1995 and 2005 were analysed retrospectively concerning prognostic factors, treatment and survival. Data were collected from patient files, transferred into a data base and evaluated statistically using the SPSS programme. **Results:** 403 consecutive patients were evaluated with a median age of 60 (32-93) at diagnosis of metastatic disease. Antihormonal therapy consisted of tamoxifen plus a LHRH-agonist in 31% of premenopausal women and aromatase inhibitor therapy in 87% of all patients undergoing antihormonal treatment. 83% of all patients received chemotherapy with the median number of lines being 3 (1-15). An anthracycline based chemotherapy was given to 49%, a taxane was used in 55%, vinorelbine in 42%, capecitabine in 36%, gemcitabine in 28%, and a platinum compound in 9% of these patients. 94% of patients with bone metastasis received a bisphosphonate and 63% of HER-2-neu-positive patients were treated with trastuzumab. Median survival since the start of palliative therapy was 30 months. Statistical analysis revealed as major prognostic factors nodal status, ER-PR-status, site and number of metastasis and DFI (time from initial diagnosis to the diagnosis of metastatic disease) while age at time of diagnosis has not shown any influence on OS. Patients could be treated almost entirely as outpatients and treatment related toxicity and hospitalisation was low. 39% of patients could die at home. **Conclusions:** Evaluation of the treatment reality of MBC in routine care reveals a prolonged median survival of 30 months which is probably due to the sequential use of the most effective treatment modalities.

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An all-oral chemotherapy in locally advanced and metastatic breast cancer

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Introduction: Capecitabine (C) and vinorelbine (V) are among the most active cytotoxic agents in pre-treated breast cancer. The new oral formulation for V allows an all-oral combination-chemotherapy. Such regimen would be advantageous for the patients as well as for the hospital staff, as the frequency of hospital visits and the number of venous punctuations can be reduced. The purpose of this study was to evaluate retrospectively the efficacy and feasibility of C plus oral V in patients with locally advanced inoperable or metastatic breast cancer. **Methods:** Thirty consecutive patients (median age 48 years, range 32-70) were entered as eligible for C/V treatment. Capecitabine was administered at a dose of 1000 mg/m² BID on days 1 to 14 in combination

with 60 mg/m² of vinorelbine on days 1 and 8; this schedule was repeated in three-week cycles. Patients with Her2-positive tumors received additional Trastuzumab (8 mg/kg loading dose then 6 mg/kg every three weeks). Treatment continued until progression, unacceptable toxicity or patient refusal to continue. **Results:** As of April 2008, all patients are evaluable for response and toxicity. One patient (3%) had complete response, 12 (40%) had partial responses and 9 (30%) experienced disease stabilization for more than 6 months. This results in an overall response rate (ORR) of 43% and a clinical benefit rate of 73% (CBR=CR+PR+SD>6 months). One patient had stable disease of less than 6 months (3%) and 7 patients (23%) had progressive disease despite treatment. Median time to progression (TTP) was 8 months (95% CI 6.56-9.44). Overall survival (OS) was not reached after a median time of observation (TOO) of 14 months (range 2-48). Toxicity was recorded for 326 cycles. Main toxicities consisted of anemia, neutropenia, nausea and gastrointestinal effects. The only grade 3/4 toxicities observed were neutropenia (5 patients, 17%) and hand-foot-syndrome (2 patients 7%). **Conclusions:** The all-oral combination of capecitabine/vinorelbine at this schedule appears to be an effective and well-tolerated regimen in the treatment of advanced breast cancer and offers a promising alternative to single agent capecitabine and vinorelbine or intravenous poly-chemotherapy.

P476

A prospective observation of fulvestrant in postmenopausal patients: the Austrian Fulvestrant Registry

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Background: Endocrine therapy is the preferred treatment in oestrogen- and/or progesterone-receptor (ER/PR) positive breast cancer. Fulvestrant (F) is a pure ER antagonist with similar efficacy as aromatase inhibitors in pre-treated patients (pts). We present results from 58 Austrian centres that contributed pts to the Austrian Fulvestrant Registry. **Methods:** F was administered at the registered dose and schedule. For baseline evaluation, CT-scans of chest and abdomen, mammography, gynaecologic examination, and bone scan were mandatory. Tumour re-assessment was performed every three months using WHO criteria. Time to disease progression (TTP) was defined as primary study endpoint; secondary endpoints were clinical benefit rate (CBR; CR+PR+SD ≥ 6 months), and toxicity. TTP was estimated using the Kaplan-Meier product limit method. A multivariate analysis (Cox regression model) was performed to identify factors significantly associated with TTP. **Results:** Three-hundred-fifty consecutive pts, median age 66 years (y), range 35 – 92 y, were included. 36% had prior adjuvant chemotherapy (CT), and 64% adjuvant endocrine treatment; 42% had prior CT for MBC. 49% had visceral involvement; 13.4% Her2-positive disease; 45% were > 65 y. 92 pts (26.3%) received F as 1st-line treatment, 170 pts (48.6%) as 2nd-line, 67 pts (19.1%) as 3rd-line, and 21 pts (6%) as 4th-line respectively. Median TTP was 7 months, range 2 – 34 (95% CI 6.09-7.9). 2.3% experienced CR, 12.3% PR, and 46% SD ≥ 6 months, resulting in a CBR of 60.6%. 6.9% had SD < 6, and 32.6% had PD. In the Cox regression model, 1st-line treatment and non-visceral metastases only were significantly associated with longer TTP; other variables (including age, time to recurrence ≤ 1 y, prior CT, Her2-status, PR-status) had no influence. 1.4% experienced grade 3 joint pain, and 0.3% grade 3 hot flashes. Other toxicities (grade 1 and 2 only) consisted of nausea, headache, fatigue, weight gain, and depression. 16 pts developed mild local reactions. **Conclusions:** Our results confirm previous data that F is an effective and well tolerated treatment option. Of note is the efficacy also in elderly pts. TTP and response rates are superior to other trials, probably due to the large number of pts receiving F as 1st- or 2nd-line treatment. Activity of F is apparently independent of Her2- and PR-status.

P477

Interim safety analysis of a randomized phase III study evaluating pegylated liposomal doxorubicin (PLD) versus capecitabine as first line chemotherapy for metastatic breast cancer (MBC): The PELICAN study

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Introduction: MBC is still an incurable disease. Effective single agent chemotherapy is increasingly used in the frontline setting to lower toxicity and maintain patient's quality of life (QoL). The phase III PELICAN trial was designed to evaluate efficacy and safety of first-line PLD (50 mg/m², q28 days) vs. capecitabine (1250 mg/m² BID x 14 days, q21 days) as approved dose for both agents. **Methods:** Pts with MBC were eligible. They received either PLD 50 mg/m² iv every 28 days or capecitabine 1250 mg/m² orally twice daily for 14 days followed by a 7-day rest period (qd21) as a first line treatment. Toxicity was evaluated continuously, efficacy and quality of life every 3 months; cycles were repeated until disease progression or unacceptable toxicity. **Results:** The PELICAN trial is actively enrolling pts. 159 pts (PLD n= 79; capecitabine n=80) were analyzed for safety. Median age was 61 years (range, 35-80. Pts received a median of 4 cycles (1-20). Both drugs were associated with comparable rates of grade 1-3 hand foot syndrome (HFS) (PLD, 61%; Capecitabine, 62%); Capecitabine was associated with more diarrhea (36% v 17%) and more grade 3 or 4 thromboembolic events (8.4% v 1.5%). All other grade 3 or 4 toxicities affected less than 5% of pts in both arms. **Conclusions:** These preliminary results showed no unanticipated toxicity. The toxicity observed was manageable and did not lead to treatment discontinuation. Thus, the IDMC recommended the continuation of patient accrual to the pre-set target of 346.

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5-Fluorouracil continuous infusion in heavily pretreated patients with metastatic breast cancer

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Introduction: Despite of a survival benefit achieved in the past decades due to newer therapeutic options, optimal therapy for patients with metastatic breast cancer (MBC) is still a matter of debate. Taxanes, anthracyclines, capecitabine, and vinorelbine are mainstays of the sequential treatment in the metastatic setting. The treatment remains difficult as it needs to be individually tailored to the patient taking into account various factors such as sites of metastases, pretreatment, toxicity and co-morbidity. Frequently palliative treatment is hampered by liver metastasis with hepatic failure (contra-indication for anthracyclines, taxanes and vinorelbine) or treatment resistance after multiple pre-treatments. In these situations 5-fluorouracil (5-FU) offers a treatment option with low toxicity. **Methods:** We retrospectively analyzed all MBC patients who were treated with low-dose 5-FU continuous infusion. Continuous 5-FU was administered at a daily dose of 150-300 mg/m² continuously in a 7-day non-electronic infusion pump through a permanent central venous line. **Results:** Between 1993 and 2007, 43 patients were treated with continuous 5-FU. Median age at continuous 5-FU was 60 years; median time between first evidence of metastasis and continuous 5-FU was 32 months (range 0-229 months). Median numbers of metastatic sites was 3 (range 1-6). Most patients were heavily pretreated with a median of 3 previous palliative chemotherapy regimens (range 0-11). 41 patients were evaluable for objective response: five had partial response (12%), 6 had stable disease lasting at least 6 months (15%) leading to a clinical benefit rate (CR + PR + SD>6months) of 27%. Median time to progression of patients with clinical benefit was 10 months (range 3-22). The overall survival of all patients from time of continuous 5-FU was 8 months (range 1-75); from time of first metastases 42 months (range 9-281). Toxicity in general was low. Grade 3 toxicity as follows:

2 patients hand-foot-syndrome, 1 patient asthenia, 1 patient stomatitis and 1 patient diarrhea. **Conclusions:** Continuous 5-FU offers a palliative treatment option with low toxicity for heavily pretreated patients. It can safely be initiated even in case of beginning liver failure due to metastases and results in a satisfying clinical benefit rate of 26% taking into account the number of pre-treatments.

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Remaining issues in metastatic breast cancer (BC) patients with long lasting response to trastuzumab: a case series

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Introduction: Trastuzumab (T) has improved the prognosis of patients with Her2-positive metastatic BC dramatically. Despite a multitude of trials, several questions remain: Can T be interrupted when a remission has been achieved? Is a maintenance therapy of T as monotherapy beneficial? Should T be continued when progressive disease is restricted to the CNS? **Methods:** In the absence of clinical trials providing definite answers, we present three cases adding to the rationale of our practice guideline. **Results:** Patient A was diagnosed with metastatic BC in 1999. Hormonal treatment (tx) yielded disease stabilization lasting a few months. The disease progressed early during subsequent therapies with EC, paclitaxel, capecitabine, and gemcitabine. In March 2002 T/vinorelbine was initiated and resulted in complete remission of cutaneous and soft tissue metastases. According to patient's preference, tx was stopped after only 10 weeks. Another 8 weeks later, disease progressed with new metastases in various organs. On reintroduction of T, the tumor responded again and remained stable for 22 months until May 2004. Patient B presented with bone metastases 13 years after initial surgery for BC. Different hormonal therapies controlled the disease for 5 years. On occurrence of liver metastases T/paclitaxel was initiated. Due to neurotoxicity, paclitaxel was stopped. Maintenance tx with T was continued. 25 months after initiation of T, cerebral metastases were detected. Whole brain irradiation (WBI) resulted in a partial remission, T was continued ever since. Currently, the patient is progression-free 16 months later. Patient C was diagnosed with node-positive disease in 1998. Following surgical tx, adjuvant EC/CMF was applied. In 2000, palliative chemotherapy with paclitaxel and doxorubicin was applied for liver metastases. After short-lived response, the disease progressed during capecitabine and finally gemcitabine tx. T/vinorelbine was started in May 2003, vinorelbine was discontinued after 10 applications for toxicity reasons. Ever since, the patient is in continuous remission on T given at 6 mg/kg q3w for almost 5 years. **Conclusions:** After achieving a remission on T-based immunochemotherapy, we continue with T as maintenance and advise against complete stopping of the treatment. When disease-progression during this treatment is limited to the brain, we do not alter the regimen as long as cerebral metastases are controlled by WBI.

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Neoadjuvant immuno-chemotherapy with non-pegylated liposomal doxorubicin, docetaxel and trastuzumab for stage II and III HER-2 overexpressing breast cancer

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Introduction: Combinations of anthracyclines and taxanes are the most active cytotoxic treatment regimens in the neoadjuvant therapy of breast cancer. Adding neoadjuvant trastuzumab improves outcome for patients with human epidermal growth factor receptor 2 (HER-2) overexpressing breast cancer. However, both trastuzumab and anthracyclines are associated with cardiotoxicity. We report on a single-center experience with a neoadjuvant immuno-chemotherapy regimen of non-pegylated liposomal doxorubicin (Myocet®), docetaxel and trastuzumab in patients with HER-2 overexpressing breast cancer. **Patients and Methods:** Between October 2005 and October 2007, 20 patients with non-inflammatory, HER-2 overexpressing breast cancer were

treated with neoadjuvant immuno-chemotherapy consisting of concomitant administration of Myocet® 60 mg/m², docetaxel 75 mg/m² and trastuzumab 8 mg/kg, followed by 6 mg/kg every three weeks for 6 cycles. All patients received peg-filgrastim prophylaxis 24 hours after chemotherapy. The mean age of the patients was 48.5 years (range 35-66 years). The mean clinical tumour size at baseline assessment was 4.5 cm (range 2.2 – 7cm). Thirteen patients (65%) had stage II and 7 patients (35%) had stage III disease according to the TNM-AJCC staging system. **Results:** In 6 of 20 (30%) patients treated with 6 cycles of neoadjuvant immuno-chemotherapy, pathologic complete response (pCR) was achieved. Clinical downsizing of the tumour diameters was observed in 18 (90%) patients. Breast conserving surgery (BCS) was achieved in 15 (75%) patients. Decrease of left ventricular ejection fraction below the normal range was documented in one patient after the 5th cycle of immuno-chemotherapy. Febrile neutropenia was observed in 1 of a total of 116 immuno-chemotherapy cycles administered. Non-haematological WHO grade III and grade IV toxicities: grade III alopecia was observed in 20 patients. **Conclusion:** Neoadjuvant treatment with Myocet®, docetaxel and trastuzumab is well tolerated and leads to a high pCR rate in patients with HER-2 overexpressing breast cancer. Replacing conventional anthracyclines by liposomal doxorubicin could reduce cardiotoxicity in neoadjuvant treatment regimens containing anthracyclines, taxanes and trastuzumab.

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Outcome of autologous and allogeneic hematopoietic stem cell transplantation for multiple myeloma with and without deletion 13q

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Introduction: Deletion 13q (DEL13) is associated with an unfavourable outcome in multiple myeloma patients (pt). Tandem autologous hematopoietic stem cell transplantation (HSCT) is standard of care in younger multiple myeloma pt, but only allogeneic HSCT is potentially curative in these pt. The latter therapeutic approach is limited by a high transplantation related mortality. Lower intensity conditioning regimens might be an option for DEL13 positive high risk pt. Aim of the present retrospective analysis was to compare the outcome of multiple myeloma patient with and without DEL13 following autologous or allogeneic HSCT. **Methods:** The files of the BMT Registry at the Martin-Luther-University were screened for records of pt who received an autologous or allogeneic HSCT for multiple myeloma from 10/1997 to 04/2006. Basic demographic data, disease status, treatment data and results of the cytogenetic analysis were noted. Pt received either tandem autologous HSCT with high dose melphalan or a reduced intensity conditioning regimen with 2Gy total body irradiation (day 0) and fludarabine (30 mg/m²/d, days -4 to -2) followed by an allogeneic HSCT. **Results:** 34 consecutive patients who received HSCT for multiple myeloma were identified. Allogeneic HSCT was performed in 7/11 pt with and in 4/23 pt without the DEL13. The remaining pt were treated with tandem autologous HSCT. Comparison of basic demographic data and disease status revealed no significant difference neither between pt with or without DEL13 nor between pt with DEL13 and autologous respectively allogeneic HSCT. The overall survival (OS) of the autologous HSCT group was 21,13 month (m) for pt with DEL13 and 56,18 m without DEL13 respectively (p = 0,012). Pt with DEL13 had a much better OS following allogeneic HSCT (29,42 month) than after autologous HSCT (21,13 month; p= 0,046). The log rank test revealed no difference between pt with DEL13 and allogeneic HSCT and pt without DEL13 and autologous HSCT in the OS (p= 0,099). **Conclusion:** The more aggressive therapeutic approach of allogeneic HSCT seems to mitigate the unfavourable outcome in multiple myeloma pt with DEL 13 compared to standard tandem autologous HSCT. These findings should be confirmed in a larger prospective study.

Growth and metastasis of MAT B III cell-derived adenocarcinomas are inhibited by stromal endothelin B receptor-deficiency

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Introduction: Endothelins (ETs) and their receptors are overexpressed in many cancers, usually correlated with increased invasiveness and poor prognosis. While previous studies have mostly focussed on the biological characteristics of the tumor cells, there is now growing evidence that interactions with benign cells in the stromal compartment play a critical role for cancer progression. The function of the stromal ET B receptor (ET_BR) in this context is still unclear. **Results:** Here we demonstrate that MAT B III rat mammary adenocarcinoma cells, which overexpress ET-1 and ET_AR, but are negative for ET_BR, show decreased proliferation rate and local tumor growth in homozygous ET_BR-deficient spotting lethal rats (sl/sl), a model of constitutive ET_BR deficiency. Metastasis to the lungs is also strongly reduced as compared to non-ET_BR-deficient heterozygous and wild type rats. There is no difference between the genetic subgroups regarding neoangiogenesis, VEGF serum concentration and rate of apoptosis. However, the lack of functional ET_BR in the host-derived stromal compartment is associated with diminished infiltration of tumor-associated macrophages and reduced production of TNF-alpha, both known as powerful promoters of tumor progression. As a proof of principle, reconstitution of ET_BR function by transgenic expression of an intact ET_BR counteracts these effects. In conclusion, tumor growth and metastasis are critically dependent on signalling via ET_BR in cells of the tumor microenvironment, especially the tumor-associated macrophages

Identification of a novel breast cancer specific antigen expressed on cell surface

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Introduction: Breast cancer is the most common cancer among women in the western world. Despite improved screening and advances in anti-tumor therapy, the overall mortality remains at about 50%. As antibody based passive immunotherapy has been successfully applied for many years, we set out to identify novel tumor specific antigens for use in active and passive immunotherapy. **Methods:** A cDNA library was generated from breast carcinoma tissues and cloned into a yeast display system. The library was screened according to the RAYS method and reactive antigen presenting yeast clones were sorted out by high speed cell sorting. 330 sera of breast cancer patients and 120 sera of healthy donors were analysed for specific antibody binding to the selected antigens. Subsequently a phage display Fab-library was screened for specific antibodies against reactive antigens. The selected Fab-antibodies were evaluated their ability to directly stain tumor cell lines by flow cytometry and immunohistochemistry. **Results:** The characterisation of the sorted cDNA-clones highlighted 10 potentially interesting targets according to the literature. These clones underwent serological analysis. One clone reacted strongly with 10% of the sera from tumor patients but did not show any reactivity with control sera. The antibody binding was confirmed by antibody titration analysis. In order to detect this antigen in tumor tissue, we selected specific Fab-antibodies out of a phage display library. Selected Fab-antibodies were checked for binding to this peptide by ELISA. Further antibody analysis showed that the selected Fab fragments competed with antibodies from the sera of tumor patients for binding to the selected peptide. Furthermore, these Fab-antibodies specifically targeted breast cancer cell lines MCF7 and SKBR7 as evidenced by FACS analysis and immunohistochemistry. **Conclusion:** We

identified a novel breast cancer specific antigen and selected specifically binding Fab-antibodies for this target. Competition assays with human antibodies from the sera of tumor patients indicated high antigen affinity of the selected Fab-antibodies. Staining of breast cancer celllines with the Fab-antibodies highly suggested the cell surface expression of the discovered antigen in tumor. Further analysis with tissue micro-array technology as well as mass-spectrometry will be needed to definitely prove its existence in breast cancer and evaluate its real potential as immunotherapeutic target.

MDS / MPS

MDS

Freie Vorträge:

Detailed Morphologic findings in 2773 patients with Myelodysplastic syndromes

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Introduction: The diagnosis of Myelodysplastic syndromes (MDS) mainly is based on cytomorphic findings in blood and marrow. The first detailed description of MDS provided by the FAB group in 1982 still serves as a methodological tool for diagnostic procedures. Besides the peripheral and medullary blast count, the presence of ring sideroblasts and Auer rods, as well as the absolute monocyte count are essential for the classification of MDS. Since morphologic findings that can only be seen in MDS, the assessment of many different dysplastic features have to be performed in order to assign the patients to the WHO types. **Methods:** In order to better describe detailed morphologic features in MDS, we turned to the Düsseldorf registry that stores detailed morphologic features of 2773 patients that have been diagnosed at our laboratory. The following items have been assessed in all patients: Erythropoiesis: Megaloblastoid changes, multinuclearity, nuclear changes, nuclear bridges, atypical mitoses, cytoplasmatic changes, defects of maturation. Granulopoiesis: left shift of granulopoiesis, Auer-rods, hypogranulation, Pseudo-Pelger-Cells, hypersegmentation of neutrophils, MPO deficiency. Megakaryopoiesis: mononuclear cells, Micromegakaryocytes, Hypersegmentation, multiple segmented nuclei, defects of maturation. **Results:** The most frequent signs of Dyserythropoiesis were defects of maturation (57%), Megaloblastic changes (56%), nuclear changes (40%) and multinuclearity (35%). The most frequent signs of Dysgranulopoiesis were left shift of granulopoiesis (54%), Pseudo-Pelger-cells (39%) and Hypogranulation (37%). The most frequent signs of Dysmegakaryopoiesis were defects of maturation (55%), mononuclear cells (35%), Hypersegmentation, multiple segmented nuclei (32%) and Micromegakaryocytes (29%). There were no substantial differences in the degree of Dyserythropoiesis between all WHO types. The degree of Dysmegakaryopoiesis and Dysgranulopoiesis was not different between RCMD, RCMD-RS, RAEB I, and RAEB II. Special morphologic features could only be found within the MDS with del (5q) type. The degree of Dyserythropoiesis was not different between the WHO types. The types with multilineage dysplasia without elevated blasts (RCMD and RCMD-RS) were detectable by focussing on Hypogranulation, Pseudo-Pelger cells, Micromegakaryocytes and mononuclear cells. Using these 4 parameters, 85% of all RCMD and RCDM-RS could be clearly separated from the unilineage dysplastic types (RA and RARS). The remaining 15% could only be identified as multilineage dysplasia by focussing on less frequent features or by means of special staining like POX (MPO-deficiency). **Conclusions:** Detailed assessment of dysplasia is necessary in the diagnostic work up of MDS patients. Focussing on 4 major morphologic features together with the peripheral and medullary blast count allow the correct allocation to WHO subtypes in the vast majority of MDS patients. The correct identification of unilineage RA and their discrimination from secondary anemias is not possible in a subset of patients without considering cytogenetic findings, clonality analyses as well as the course of the disease and repeated marrow punctures.

5 – azacytidine prevents imminent relapse defined by decreasing CD34+ subset donor chimerism in patients with high-risk myelodysplastic syndromes (MDS) or acute myeloid leukemia (AML) after allogeneic peripheral blood stem cell transplantation

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Introduction: Besides graft versus host disease (GVHD), disease relapse is one of the major challenges in the care of patients with acute myeloid leukemia (AML) or myelodysplastic syndrome (MDS) undergoing allogeneic peripheral blood stem cell transplantation (PBSCT). However, we and others have shown that relapse can be predicted in case of CD34-expression on the malignant clone by a sensitive chimerism analysis in sorted CD34+ peripheral blood cells. If the percentage of donor cells in this compartment drops below 80%, leukaemia relapse is inevitable within 6-8 weeks in the absence of clinical interventions like cessation of immunosuppressive drugs or the administration of donor lymphocyte infusions (DLI). However, both approaches often result in clinically significant GVHD. Therefore, new strategies are warranted in order to treat imminent relapse in MDS/AML patients. **Methods:** We report results of an ongoing phase II clinical trial evaluating the efficacy of 5-azacytidine (5-aza) to prevent hematological relapse in patients with CD34+ AML or MDS and a decreasing CD34-donor chimerism after allogeneic PBSCT. Therefore, a total of 18 patients with CD34+ MDS or AML were prospectively screened on day 56, 84, 112, 140, 184, 365 and at later time points after PBSCT for a decreasing chimerism of donor CD34+ cells in the peripheral blood. In case of imminent relapse, defined as a drop of donor CD34+ cells below 80%, 5-aza was given at a dose of 75mg/m²/d s.c. day 1-7. A total of 4 cycles every 28 days were allowed in the absence of hematological relapse or toxicity. **Results:** A median of 274 days after PBSCT, eight out of 18 patients screened entered the treatment phase of the study with a median of 25% (range 0-53%) CD34+ donor cells in the peripheral blood (PB). However, a complete overall PB donor chimerism and less than 5% marrow blasts were documented in all patients before 5-aza treatment. Reversible neutropenia and thrombocytopenia grade 3/4 occurred in 60% of the patients. Only three patients still had immunosuppression prior 5-aza, which in two of them was slowly tapered during the period of 5-aza administration. With a median follow-up of 150 days after starting 5-aza six patients are eligible for response evaluation. Of these, CD34-chimerism was reverted to complete donor type (>90%) in 5 patients. Only 1 patient showed a further decrease of donor CD34+ cells and relapsed 4 weeks after having completed 4 cycles of 5-aza. Two patients (one without immunosuppression) developed limited cGVHD during 5-aza treatment. No hematological relapse occurred in the responders and in the screening cohort without decreasing CD34+ chimerism. **Conclusions:** Preemptive treatment with 5-aza triggered by minimal residual defined by decreasing donor CD34+ subset chimerism seems to be a potent strategy to prevent imminent relapse of myeloid malignancies after allogeneic PBSCT.

Molecular Mechanism of JAK2V617F oncogenic activation: The JAK2-V617F mutant utilizes the SH2 domain for constitutive kinase activity in the presence of unstimulated heterodimeric cytokine receptor

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The JAK2-V617F mutation has been reported in the majority of MPDs including PV, ET, and IMF. This mutation leads to the constitutive activation of the JAK2 tyrosine kinase activity and overexpression of JAK2-V617F renders hematopoietic cell lines growth factor-independent. However, the molecular

mechanism leading to constitutive activation of JAK2V617F is largely unclear and the requirement of homodimeric or heterodimeric cytokine receptors needs to be determined. Since the SH2 domain is adjacent to the pseudokinase domain containing the V617F mutation, we hypothesized a possible role of the SH2 domain for the constitutive phosphorylation of JAK2-V617F. To investigate the functionality of the SH2 domain, we mutated the conserved arginine 426 within the SH2 domain to a lysine. Ba/F3 cells expressing JAK2-V617F grew IL3-independent and showed constitutive activation of JAK2, STAT5, and ERK1/2. In contrast, introduction of the SH2 mutation in JAK2-V617F abrogated both transformation as well as constitutive activation of downstream signaling pathways. Membrane localization studies showed equal membrane distribution of SH2-mutated and unmutated JAK2-V617F in both parental Ba/F3 and EpoR-Ba/F3 cells. These data indicate that the SH2 domain mutation does not affect subcellular distribution of JAK2. It has been reported that JAK2 binding to homodimeric type I cytokine receptor may facilitate JAK2-V617F-mediated transformation. We therefore studied the role of the SH2 domain in EpoR-transduced Ba/F3 cells. Interestingly, coexpression of EpoR with SH2 domain mutant JAK2-V617F led to Epo-independent growth and showed constitutive activation of JAK2, STAT5 and ERK1/2 similar to SH2-unmutated JAK2-V617F. These results suggest that a functional SH2 domain is not necessary for JAK2-V617F induced transformation in the presence of overexpressed EpoR. In contrast, reconstitution of JAK2 mutants in a JAK2-negative cell line with IL-3R coexpression revealed reduced activation of JAK2 when the SH2 domain was mutated. These findings indicate that the SH2 domain plays a crucial role for oncogenic JAK2 signaling in cells expressing endogenous heterodimeric cytokine receptors such as IL-3R. Notably, co-IP experiments revealed that the SH2 domain was able to bind JAK2 itself indicating a possible role in the dimerisation and transphosphorylation of JAK2. Consequently, reduced transphosphorylation was only seen in IL-3R- and not in EpoR-expressing cells again emphasizing a critical role for the SH2 domain in heterodimeric but not homodimeric receptor signaling. Finally, we checked the role of the SH2 domain in a BM transplantation model. Surprisingly, we found that an intact SH2 domain in JAK2-V617F was required for the induction of a PV-like disease. Overall, our results point to an important role of the SH2 domain for the constitutive activation of JAK2-V617F in cells expressing heterodimeric cytokine receptors. This mechanism seems to be crucial for the induction of a PV-like murine disease. This is the first report demonstrating a functional important role for the SH2 domain in JAK kinases.

A Limited Number of 5-Azacytidine Cycles can be Effective Treatment in MDS

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Introduction: Hypomethylating agents, such as 5-azacytidine (5-AZA) and 5-aza-2'-deoxycytidine (decitabine), have recently been approved for the treatment of myelodysplastic syndromes (MDS). Several randomized trials have shown favorable results concerning response rate, survival, transformation to acute leukemia and quality of life. In these trials, treatment was administered continuously until progression. **Methods:** In the retrospective study presented here, we evaluated the outcome of patients with higher risk MDS or secondary acute myeloid leukemia (sAML) treated with a limited number of 5-AZA cycles. **Results:** A total of 32 patients received 5-AZA alone (n = 30) or in combination with valproic acid and all-trans retinoic acid (n = 2). 5-AZA was administered subcutaneously at a fixed dose of 75 mg/m²/day for 7 days and repeated every 28 days. 5-AZA was given for a median of 4 courses. Treatment was continued for two more cycles as consolidation in patients who had achieved complete remission (CR), marrow CR or stable disease with hematologic improvement. The overall response rate was 50% according to the modified International Working Group criteria. Complete remissions were achieved in 15.6% and stable disease in 34.4% of patients. Peripheral blood counts normalized in 6.3% of patients while hematologic improvement was achieved in 25%. The median time to AML in responding patients was 45 weeks while AML occurred after a median of 14 weeks in non-responding patients (P = .038). The median survival of all patients was 60 weeks; the median survival of responders was 74 weeks compared with 26 weeks in non-responders (P = .047). **Conclusions:** In this retrospective analysis, 5-AZA was associated with a survival advantage in responding patients with higher risk MDS or

sAML. These favorable results demonstrate that patients may benefit even from a limited number of courses of 5-AZA.

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At which interval should prognostic scores for preleukemic duration and survival in Myelodysplastic Syndromes be re-evaluated?

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Introduction: In MDS standard prognostic scores for preleukemic duration and survival are calculated at diagnosis only. As Malcovati et al. showed in the development of the WPSS, patient's characteristics constituting scores, change considerably over time. Their proposal, the WPSS, is intended to be dynamically re-evaluated, but as it is partly based on important but rather subjective aspects (transfusion dependency), the question, if established scoring systems (e.g. the IPSS) would equally qualify for dynamical re-evaluation is still of interest. We examined the predictive values over time, starting with well known scores. **Methods:** The study is based on data of a series of 243 primary MDS patients treated with supportive care only. Median survival was 31 mo and the median follow up 34 mo. The median age was 72 years. 124 patients (med. OS 29,5 mo) were male and 119 (OS 31 mo) female. According to the FAB classification the patient numbers and median survival months were: RA 74, 68m; RARS 27, 65m; RAEB 55, 14m; CMML 62, 25m; RAEBT 25, 9m. For these patients the following scores were computed: IPSS, PI-Score, Lille, Duesseldorf, Sanz, Bournemouth, Lausanne-Bournemouth, c-IPSS. Preleukemic duration as well as survival time were split in two periods with different cutpoints (6, 9, 12, 18, 24, 30, 36, 42 months) and effect parameters, based on Cox-proportional-hazards-models were estimated. **Results:** Regarding survival time all scores showed the highest predictive value when prediction was limited to the first 9 months; only the Duesseldorf-score had a higher coefficient at 6 months (b=1.89). The nine-months-coefficients were as follows: IPSS (b=.95), c-IPSS (b=.73), PI-Score (b=.73), Lille-score (b=1.50), Duesseldorf-score (b=1.60), Sanz-score (b=1.22), Bournemouth-score (b=1.21), Lausanne-Bournemouth-score (b=1.65); all of them significant with p<.01. The estimates for the one year period were a little, but not substantially lower. The estimates for c-IPSS and PI-score exhibited little change over time. Concerning preleukemic duration results are similar. All scores showed good predictive values for the first year. The cytogenetically based scores (PI-Score, c-IPSS) substantially maintain their predictive power over more than three years. **Conclusions:** The examined scoring systems, especially those containing clinical characteristics would gain predictive power if dynamically re-evaluated. Re-evaluation on a yearly basis appears to be a reasonable starting point. Cytogenetically based scores inherently are in less need for re-evaluation.

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GATA and BCLx1 downregulation in erythropoiesis during in vitro lineage specific differentiation of MDS hematopoietic progenitor cells is not induced by activated Notch pathway

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Introduction: Notch signals have recently been shown to inhibit erythroid and megakaryocytic differentiation of hematopoietic progenitor cells. In myelodysplastic syndrome (MDS) its role in dyserythropoiesis has not been fully elucidated. Therefore we have analyzed whether dysregulation of Notch pathway elements might be associated with impaired GATA1 and BCLx1 expression and ineffective erythropoiesis being a hallmark of MDS hematopoiesis. **Methods:** We have generated an in-vitro model of MDS lineage-specific hematopoietic differentiation by culturing CD34+ bone marrow cells from healthy donors

(n=7) and MDS patients (low risk: RA/n=6, RARS/n=3; high risk: RAEB/n=4, RAEB-T/n=2) with EPO and TPO. Cell harvest was at days 0, 4, 7 and 11. Expression of GATA1, BCLx1, DLK1, Notch1, HES1 and HERP2 was measured by real time RT-PCR (qPCR). Methylation analysis of CpGs flanking cis-regulatory elements (including N-box and GATA box) of the GATA1 erythroid promotor was analyzed by pyrosequencing of bisulfite treated genomic DNA at any specific time point. **Results:** In normal erythropoietic cells, RNA expression of GATA1 and of BCLx1 was steadily upregulated, particularly during late erythropoiesis. In contrast, during MDS erythropoiesis a loss of typical late upregulation of GATA1 and BCLx1 was observed. Notch ligand DLK1 showed increased expression during erythropoiesis particularly in high risk MDS as compared to normal controls. Furthermore, expression of HES1 was increasing during the course of normal erythropoietic and megakaryopoietic differentiation but not in lineage specific cells from MDS patients. **Conclusion:** Our data show that the transcription factor GATA1 which is critical for erythropoietic differentiation as well as the anti-apoptotic molecule BCLx1 are markedly downregulated during MDS erythropoiesis. This may contribute to the ineffective erythropoiesis seen in this disease. However, an upregulation of the Notch pathway leading to increased expression of the GATA1 repressor HES1 could not be detected.

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Life expectancy in low and intermediate risk essential thrombocythemia (ET) – Results of the Soillet Study

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Introduction: Prognostic data on the life expectancy of patients with low- and intermediate essential thrombocythemia (ET) are generally impaired by relative short follow-up intervals and altering diagnostic criteria. Furthermore, deaths from other causes than the underlying disorder are usually not considered. **Methods:** This multicenter observational study included a total of 277 patients (112 men and 165 female, median age 55 yrs) with a clinical and histological established diagnosis of ET according to the WHO criteria. Patients were followed up for 2,698 person-years with a median interval of 9.5 yrs. Low- and intermediate risk status at diagnosis was determined by absence of previous ET-related complications like severe thromboembolic or severe hemorrhagic events and/or a platelet count of less than 1,500 x 10⁹/l. Effects of mortality from other age-related causes were eliminated by calculation of relative survival rates which compare the individual survival with an age and sex-matched general population. To measure the impact of disease and prognostic stratification, life expectancies and the proportion of expected life loss were determined. **Results:** Within the first five years of follow-up no loss of age-adjusted relative survival rates was observed, 10 and 15-years relative survival were 89% and 81%. Accordingly, an overall life expectancy of more than 24 yrs. is calculated with a disease specific rate of only 12%. Interestingly, in female patients a higher impact of disease was observed, older age-groups (> 60 yrs.) showed no higher mortality. Contrasting this finding, younger patients (< 40 yrs.) had a slight increase in disease-specific mortality (10 yrs: 85%, 15 yrs: 77%). In only 36 patients ET-related thromboembolic or hemorrhagic complications were observed during the follow-up. These cases revealed a significant higher mortality with a disease-specific loss of life expectancy of more than 30% and 10- and 15-year relative survival rates of 80% and 76%, respectively. No patient showed a transformation into acute leukemia of severe myelofibrosis. **Conclusions:** According to our data, overall survival in low- and intermediate risk ET is not significantly different from the expected mortality in the general population. Higher age at diagnosis (> 60 yrs) is not as important as reported in former studies. Since occurrence of ET-related complications was confirmed as the major prognostic factor, prevention of these events has to be the most important therapeutic objective.

Coalescence of the German-Austrian and IMRAW Cytogenetic MDS databases: Modification of patient risk groups

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Introduction: Based on a patient cohort comprising 816 MDS patients from the IMRAW, the IPSS defines the standard for risk assessment in MDS. Recently, the GACMSG published cytogenetic data including 2124 MDS patients. Coalescence of these two large databases offered the opportunity to analyze the cytogenetic data jointly and to propose a modified cytogenetic risk stratification system by correlating previously undefined recurrent abnormalities with survival, as well as characterizing the IPSS intermediate risk group in more detail. **Methods:** 1971 patients with karyotype and survival data originating from the IMRAW and the GACMSG cohorts were included in this study. The collectives comprised patients with primary MDS treated with supportive care exclusively. By reviewing the ISCN karyotypes, the patients were grouped into cytogenetic categories defined by median survival (MS). The categories comprised karyotypes with the respective abnormality alone or in combination with one additional anomaly. Karyotypes with 3 or more than 3 abnormalities were considered separate categories. **Results:** We found 15 cytogenetic categories comprising 10 or more patients. These categories were combined into 4 prognostic groups according to the MS: MS>3 years; MS 1.5-3 years; MS 1-1.5 years and 4 MS<1 year. Further stratification of these categories led to a system with 4 distinct risk strata (n pts.): good (1374), int-1 (160), int-2 (99), and poor (166). 172 patients (9%) could not be classified according to this system. Survival analysis of these 4 groups showed distinct MS (p<0.0001): good: 50 months; int-1: 24 months; int-2: 15 months; poor: 6 months. When comparing this new classification with the original system defined by the IPSS, 66 formerly intermediate risk patients shifted into the good risk group and 114 poor risk patients into the intermediate risk group. **Conclusions:** Combined examination of the two databases introduces 7 new cytogenetic categories with distinct survival times as compared to the IPSS. With respect to future refined integrative scoring in MDS we present an approach that distinguishes groups of intermediate risk and a heterogeneous group of as yet unclassified rare cases harboring uncertain prognoses. In the latter cases, risk assessment should be based on other prognostic parameters rather than assigning an intermediate risk to this group. This new cytogenetic risk stratification system needs to be validated and tested using multivariate approaches.

Different Treatment Strategies in their Influence on The Prognosis Of Patients With Myelodysplastic Syndromes (MDS)

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Introduction: So far, there are no survival data on MDS patients who received treatment apart from best supportive care (BSC) outside studies, with the exception of allogeneic stem cell transplantation. As phase III trials are largely lacking, matched-pair analyses can be a tool to compare treatment groups with BSC groups. **Methods:** In order to obtain data on the influence of treatment on survival we performed matched-pair analyses using the MDS registry Duesseldorf (n=3067). We focused on Thalidomide (n= 55), Valproic acid (n=76), low-dose Ara-C (n=65), ATG (n=17), allogeneic stem cell transplantation (ASCT, n=39) and induction chemotherapy (n=172). We identified pairs corresponding in age (+/- 5 yrs.), gender, cell counts, WHO type and IPSS. **Results:** In a first step, we showed the prognosis of patients with BSC did not differ regarding the year of diagnosis (1975-2005) to ascertain there is no confounding influence of the time of diagnosis. Patients treated with Thalidomide at any time during the course of the disease differed in terms of the probability of survival (33 vs. 25 mo., p=0.0291). In none of the subgroups, except for

patients with an IPSS of 2 and 3 (31 vs. 8 mo., p=0.007), a benefit could be demonstrated. In patients treated with Valproic acid, only the subgroup of RAEB I and II patients showed a higher median survival time (46 vs. 22 mo., p=0.019). Patients treated with ATG had a survival benefit (158 vs. 61 mo., p=0.04) and especially RCMD patients that received ATG as their first therapy (158 vs. 61 mo., p=0.0293). In a next step, low or intermediate I risk patients, according to the IPSS, who either received ATG, Thalidomide or Valproic acid were compared with their matches. Patients of the treatment group showed a beneficial survival of 112 versus 75 months (p=0.008). Regarding low-dose Ara-C, there was no significant advantage in the overall analysis. Induction chemotherapy was not beneficial for patients over 60 years of age (21 vs. 16 mo., p=0.7946), regardless of subgroups. Patients who underwent ASCT had a survival benefit (49 vs. 14 mo., p=0.04), especially patients with an IPSS of 2 or 3 (65 vs. 8 mo., p=0.0017). **Conclusions:** Our data show that specific treatment of MDS need not result in an overall improvement of prognosis. Only within subgroups moderate effects on the outcome could be shown. Within the low and intermediate I risk group, treatment with either ATG, Valproic acid or Thalidomide potentially leads to a better survival. This indicates that risk-adapted therapy is mandatory to improve prognosis.

Cytogenetic monitoring by sequential FISH analyses of circulating CD34+ cells in MDS

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Purpose: Most chromosomal anomalies in MDS detected by classical cytogenetics of bone marrow (bm) metaphases are provable by fluorescence in situ hybridisation (FISH), too, and can be detected in circulating CD34+ cells from peripheral blood (pb). **Methods:** Chromosomal aberrations initially diagnosed by classical cytogenetics using bm metaphases were followed by sequential FISH analyses of circulating CD34+ cells after immunomagnetic cell sorting. We compared FISH analyses of enriched circulating CD34+ cells, non-enriched pb, enriched CD34+ bm cells and non-enriched bm cells with classical cytogenetic analyses of bm metaphases. Twenty pts receiving 5-azacytidine (5-aza) and 3 pts treated with lenalidomide (len) were followed during therapy. **Results:** For every abnormal karyotype a suitable FISH-probe was found. In all cases a sufficient quantity of circulating CD34+ cells could be enriched for FISH analyses (85-420x10³ CD34+ cells/20ml pb). Microscopically the sorted cells are morphologically homogeneous populations. The results of FISH analyses of circulating CD34+ cells are more representative and comparable to the clone size measured by classical cytogenetics of bm metaphases than FISH analyses of non-enriched pb or bm cells. Out of 20 pts treated with 5-aza, 16 (2 f, 14 m; 1 RA, 3 RCMD, 1 RAEB-1, 4 RAEB-2, 7 AML) received at least 4 cycles and were analysed: Median age 66.6 years (49-81), median number of 5-aza cycles 5.6 (4-9), median observing time 36.2 weeks (19-60). The karyotypes were: 1 normal, 10 with 1-2 anomalies, 5 with complex aberrant karyotype. Out of 16 pts 12 (75%) responded to therapy (according to modified IWG criteria): 5 CR, 1 PR, 1 marrow-PR, 1 HI, 2 with at least partial cytogenetic remission (cyPR), 1 cyPR/HI, 1 marrow-PR/cyPR. Five further pts stayed in SD. We defined cyPR as a reduction of >50% of aberrant interphase nuclei and a complete cytogenetic remission (cyCR) as a reduction of below 10%. In 9/15 pts (60%) with chromosomal anomalies cyPR was possible, 5 of 9 even reached cyCR. Interestingly, cyPR preceded HI by 4-8 weeks. By the same method we observed 3 pts (2 m, 1 f) with 5q-syndrome treated with len. All pts reached CR, cyPR/CR and HI correlated with each other. **Conclusions:** Analysing circulating CD34+ cells is a practicable, representative and less invasive method for cytogenetic monitoring in MDS pts and even pts with low-risk MDS can be monitored frequently. Obviously, there is a predictive value of cyPR under 5-aza.

Sequential cytogenetic monitoring of response to lenalidomide in progenitor cells of MDS patients with isolated deletion del(5q) (Sequenzielles zytogenetisches Monitoring des Ansprechens auf Lenalidomid in Vorläuferzellen von Patienten mit MDS und isolierter Deletion del(5q))

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Purpose: Myelodysplastic Syndromes (MDS) are a heterogeneous group of acquired diseases originating from an early pluripotent hematologic progenitor cell causing an ineffective hematopoiesis. Recently, novel effective therapy options have emerged with a new generation of drugs possessing immunomodulatory and anti-angiogenic properties. Lenalidomide belongs to this class of compounds. The response rate including complete cytogenetic remission is highest among patients with an interstitial del(5q). A main question is whether lenalidomide can eradicate the aberrant clone also at the stem cell level. The latter has not been addressed so far. **Methods:** Two patients with an isolated del(5q) and the diagnosis of red blood cell (RBC) transfusion dependent MDS (5q-syndrome, RAEB, patients A, R) as well as one RAEB (patient H) in CR but with an imminent relapse as defined by an increase of recipient derived and del(5q) positive CD34 cells after allogeneic stem cell transplantation (allo SCT) received lenalidomide (5 mg/d or 10 mg/d). The therapy monitoring included blood and bone marrow cytology, conventional cytogenetics, SKY, and FISH. FISH was also done on FACS isolated CD34+/CD45+ cells. **Results:** Patients A and R achieved transfusion independence after 2 and 6 months, respectively. In both patients a complete cytogenetic remission defined as eradication of the del(5q) clone was obtained also by FISH at the CD34+ stem cell level. One patient is still in remission also in the CD34+ cells 10 months after stop of lenalidomide. In the other patient (R), however, an unrelated clone (del(18q)) in the absence of del(5q) developed. In the patient (H) after allo SCT lenalidomide stopped the further increase of the recipient del(5q) stem cells for about one year. Then karyotype evolution (der(6)t(1;6)) was noticed and the patient displayed a hematological relapse. **Conclusion:** These data suggest that lenalidomide can eradicate the del(5q) clone also at the stem cell level. However, the emergence of clonal progression and acquisition of an unrelated clone, respectively, on the basis of genomic instability and selection pressure could be caused by lenalidomide. Therefore, closely cytogenetic monitoring of patients under lenalidomide seems to be important.

Prospective multidimensional geriatric assessment in 210 patients \geq 60 years with MDS/AML

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Background: MDS and AML are increasingly viewed as a biological continuum particularly in older pts. Treatment options in these pts range from best supportive care (BSC) to intensive chemotherapy (IC), with low-dose chemotherapy or novel non-intensive agents (e.g. demethylating agents) as alternatives. Due to frequent age-related physical and/or mental impairments, intensive treatment is not always feasible. As the basis for treatment decision-making is not well defined, Geriatric Assessment (GA) is expected in the future to offer rational support in this process. Validation of instruments for age-specific functional and quality of life (QOL)-assessment in addition to disease-specific risk factor definition thus is urgently needed. **Methods:** Since Jan. 2004, we have prospectively evaluated the prognostic impact of GA on overall survival (OS) in 210 pts \geq 60yrs. with AML (n=159) or MDS (n=53) according to WHO, receiving either BSC or BSC plus low-dose decitabine (DAC) or IC. GA, including eight instruments evaluating QOL, frailty and activities of daily living, depression, mental functioning, mobility, comorbidities

and performance status (PS), has been applied to 210 pts in three participating centers. In addition, disease- and patient-specific laboratory parameters were obtained. **Results:** Median age of pts was 71 yrs (range: 60-87 yrs). The primary treatment allocation was: BSC: n=58 (median age: 74 yrs); BSC+DAC: n=74 (74 yrs); IC: n=80 (68 yrs). 63% of IC pts received a matched related/unrelated stem cell transplantation. Application of age-specific tests on psycho-social and physical well-being were readily feasible, as was multi-center organisation. Multivariate statistical analyses will evaluate the possible association of the initial multidimensional GA with treatment allocation, age, hematological, cytogenetic, laboratory parameters and treatment outcome. Also, at defined time points follow-up assessments are performed to ask whether treatment influences GA and individual QOL values. Follow-up will be closed by August 2008. **Conclusion:** With a duration of 30 min. per examination, the multidimensional GA showed very good acceptance amongst study participants. Multivariate analysis demonstrating the most relevant geriatric parameters for overall survival and quality of life under three different treatment allocations will be presented. *This work is supported by the Deutsche José Carreras Leukämie-Stiftung; Grant: DJCLS F 06/04*

Thrombocytes, Megakaryocytes and bleeding in myelodysplastic syndromes (MDS)

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Introduction: Patients with Myelodysplastic syndromes (MDS) present with different types of cytopenia. Although low platelet counts have been identified to be associated with a poor prognosis, the exact incidence of thrombocytopenia and its relationship with the clinical course of the disease is not well known. In this study, we evaluated the influence of platelet count at the time of diagnosis, morphology of Megakaryopoiesis, signs of bleeding and platelet transfusion dependency in the course of disease in MDS. **Methods:** We turned to the Düsseldorf MDS Registry and analyzed 2900 patients, who have been diagnosed centrally at our lab between 1982 and 2007. Dysmegakaryopoiesis was diagnosed if at least ten out of 25 megakaryocytes were mononuclear or micromegakaryocytes or if they had multiple widely separated nuclei. **Results:** At the time of diagnosis, 60% of the patients had platelets $>100,000/\mu\text{l}$. Platelets between 20,000 and 50,000/ μl were found in 11% and platelets $<20,000$ in 7% of the patients. Platelet anisometry was found in 35% and "giant platelets" in 18%. In the bone marrow, signs of dysmaturity of the megakaryocytes were found in 41%. Hypocellularity of Megakaryopoiesis was present in 25%. Signs of bleeding, most frequently petechia, at the time of diagnosis were evident in 19% and a dependence of platelet transfusion during course of the disease occurred in 22% of the patients. Patients with advanced MDS according to WHO present more often with signs of bleeding, lower platelet counts and required more often platelet transfusions while course of disease. Patients with either $<50,000$ platelets, presence of platelet anisometry, hypocellularity or maturation defects of megakaryocytes presented more often with signs of bleeding ($p<0.0005$). Patients with platelets $<20,000/\mu\text{l}$ had a median survival of 7 months as compared to 41 months in patients with normal platelets. The risk of progression into AML increased with low platelets at diagnosis. The cause of death was in 89% disease related, infections and the progression in AML being the most frequent causes with 32% and 30%, respectively, followed by dead associated with bleeding in 14%. **Conclusions:** 1. Dysmegakaryopoiesis, hypocellularity of Megakaryopoiesis as well as low platelet count are associated with clinical signs of bleeding. 2. In patients with either more advanced MDS stages according to the WHO or high IPSS status platelet counts are lower and need for platelet transfusion is higher.

MDS / MPS

Poster:

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Differential expression of the tumor suppressor candidate Calreticulin (CALR) in CD34+ cells of MDS patients is not regulated by promoter methylation

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Purpose: The multifunctional Ca^{2+} binding protein Calreticulin (CALR) plays an integral role in the regulation of Ca^{2+} homeostasis and chaperone activity. Furthermore a potential tumor suppressive function of CALR by its mediation of apoptosis dependent phagocytosis has been shown. Recent studies using whole genome copy number and gene expression analysis revealed a loss of heterozygosity and transcriptional downregulation of CALR in CD34+ bone marrow (BM) cells from patients with Myelodysplastic Syndrome (MDS). Therefore, we have elucidated the influence of CALR promoter methylation on differential gene expression. **Methods:** Isolation of RNA and genomic DNA (gDNA) from CD34+ BM cells of de novo MDS patients (n=13) and healthy individuals (n=4) was performed using standard TRIZOL technique. DNA was subsequently bisulfite converted using the Qiagen Epi-Tect Bisulfite Kit. Methylation of the CALR promoter region was quantitatively measured using the PyroMark ID pyrosequencing system. Reverse transcription of RNA was carried out with the Qiagen QuantiTect Reverse Transcription Kit and CALR expression levels were determined by quantitative Taqman PCR on a Rotor-Gene 6000 system. **Results:** Real time PCR analysis of CALR expression validated the differential downregulation observed by micro array studies in MDS patients as compared to healthy donors. However, no increased levels of promoter methylation could be detected by pyrosequencing in MDS cells. Hence, no cohesion between CALR downregulation and its promoter methylation profile could be demonstrated. **Conclusions:** In this study we investigated promoter methylation as a possible reason for a strong downregulation of the multifunctional protein CALR in CD34+ BM cells of MDS patients as compared to healthy donors. Our results demonstrate that promoter methylation profile of the CALR gene does not affect its differential regulation in MDS. Therefore we are currently performing further analyses on interacting transcription factors and epigenetic effects such as miRNA silencing on CALR gene expression.

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Guillain-Barré syndrome associated with CMML

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Introduction: Chronic myelomonocytic leukaemia (CMML) is a clonal disorder of haematopoietic stem cells. Peripheral neuropathy is uncommon in haematological malignancies. We present a patient with Guillain-Barré syndrome (GBS) associated with CMML, which to our knowledge has only been described once in the literature. **Patient:** An 82-year-old man was admitted because of muscle weakness. Extended leukocytosis without any signs of an infection caused the presentation to the haematologist (Tab., 1st adm.). The constellation of findings in bone marrow biopsy, aspirate and flow cytometry confirmed the diagnosis of CMML. Because of the patient's good general condition and stable WBC, monthly laboratory tests were arranged (Tab., 2nd adm.). One month later the WBC had risen to 76.5 G/L, so he was readmitted to treat his CMML in acceleration (Tab., 3rd adm.). Hydroxyurea was started. The following week WBC was regressive and the patient remained clinically stable. 10 days later he was readmitted because of muscle weakness and serious general condition (Tab., 4th adm.). Hydroxyurea was stopped. Neurologic examination revealed bilateral symmetric muscle weakness that started distally, spreading proximally over a period of a few days. No dysphagia or dyspnoea was present. The results of the MRI (cervical spine) and CCT showed no pathologies. His weakness worsened until by day 5 the patient was no longer able to walk. The protein concentration in the cerebrospinal fluid was elevated.

The WBC was progressive and therapy with thioguanin 40mg was started (Tab., 5th adm.). Electromyographic testing revealed peripheral neuropathy. Guillain-Barré syndrome associated with CMML was diagnosed. High dose immunoglobulin therapy resulted in improvement. The leukocyte counts were within normal range (Tab., 6th adm.). A week later, the WBC started to rise again and another episode of GBS occurred. This time there was severe respiratory failure in addition to the general muscle weakness, and the patient died on the same day (Tab., 7th adm.). **Conclusion:** Both CMML and GBS are uncommon diseases making a chance association unlikely. This case illustrates a potential complication in the setting of chronic myelomonocytic leukaemia.

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Dysplastic features detected by multiparameter flow cytometry in patients with myelodysplastic syndromes

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Introduction: Multiparameter flow cytometry (MFC) is capable of identifying dysplastic features in different cell lineages in patients with myelodysplastic syndromes (MDS). We aimed at correlating dysplastic features as detected by MFC and previously published to occur in MDS with findings in cytomorphology (CM) and cytogenetics (CG). **Methods:** We analyzed 307 bone marrow samples from patients with suspected (n=130) or proven (n=177) MDS by MFC, CM, and CG in parallel. **Results:** In cases clearly rated MDS or no MDS by CM there was a high degree of concordance with MFC: both methods MDS, 75.8%; both methods no MDS, 17.9%; CM MDS, MFC no MDS, 2.2%, CM no MDS, FC MDS, 4.0%, $p < 0.001$. Blast counts as determined by CM and MFC, respectively, ranged from 0% to 21% (median, 3.5%) and from 0% to 23% (median, 3%; $r = 0.271$, $p < 0.0001$). Differences between CM and MFC were found in cases with monoblasts as well as due to limited quality of samples. As compared to cases with no indication of MDS by CM (=non-MDS) cases with MDS according to CM were significantly associated with a reduced side-scatter signal in granulocytes (ratio granulocytes:lymphocytes 6.53 ± 1.27 vs. 7.44 ± 1.17 , $p < 0.0001$) as well as a higher number of dysplastic features in granulocytes (1.98 ± 1.09 vs. 1.00 ± 1.31 , $p < 0.0001$), monocytes (0.81 ± 0.84 vs. 0.35 ± 0.63 , $p < 0.0001$), and erythroid cells (0.33 ± 0.47 vs. 0.20 ± 0.40 , $p = 0.061$). Particularly, an aberrant expression of CD56 in monocytes occurred more frequently in 33 cases with CMML as compared to non-MDS cases (84.8% vs. 15.7%, $p < 0.0001$). In cases with possible MDS according to CM the differences to non-MDS cases were less pronounced (reduced side-scatter signal in granulocytes 7.77 ± 1.47 vs. 7.44 ± 1.17 , n.s., dysplastic features in granulocytes 1.90 ± 1.21 vs. 1.00 ± 1.31 , $p = 0.003$, monocytes 0.50 ± 0.63 vs. 0.35 ± 0.63 , n.s., and erythroid cells 0.30 ± 0.47 vs. 0.20 ± 0.40 , n.s.). In cases with aberrant cytogenetics (n=80, excluding those with Y as sole aberration) dysplastic features by MFC occurred more frequently as compared to cases with normal karyotypes (reduced side-scatter signal in granulocytes 6.35 ± 1.18 vs. 7.09 ± 1.38 , $p < 0.0001$, dysplastic features in granulocytes 2.05 ± 0.95 vs. 1.68 ± 1.31 , $p = 0.021$, monocytes 0.80 ± 0.89 vs. 0.71 ± 0.81 , n.s., and erythroid cells 0.38 ± 0.49 vs. 0.31 ± 0.48 , n.s.). A total of more than two dysplastic features in blasts, granulocytes, and monocytes and/or a blast count $> 5\%$ occurred in 68.4%, 63.3%, and 35.3% of cases with MDS, possible MDS, and non-MDS according to CM ($p = 0.0005$). Interestingly, in some cases aberrant antigen expression has been observed in cell lineages not rated dysplastic by CM. **Conclusions:** This evaluation suggests that MFC may be used to identify dysplastic features in patients with suspected MDS. Sensitivity and specificity varies between MDS subtypes and should be clearly defined in future studies.

P500

Altered immunophenotype of peripheral blood B lymphocytes in myelodysplastic syndromes as detected by routine flow cytometry

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Introduction: Myelodysplastic syndromes (MDS) are clonal hemopoietic stem-cell disorders characterized by ineffective and dysplastic hemopoiesis. Data on the involvement of lymphatic cells into the clonal alteration are scarce. On the other hand, immune system deregulation may play a pathogenetic role in MDS. The goal of our study was to find alterations in B- and T-cell subsets as well as phenotype in peripheral blood (pb) of MDS patients applying a routinely used flowcytometric approach. **Methods:** Pb samples of patients with diagnosed MDS (n=32) as well as control subjects without MDS were investigated by four-color flow cytometry. The following markers were investigated: CD3, CD4, CD8, CCR7, CD45RA (naïve, central and effector memory T cell subsets), CD2, CD5, CD28 (T phenotype); CD19, CD5, IgD, CD27 (B subsets), CD21, CD22, CD81, CD79b, CD268, CD45R-B220 (B phenotype). For each marker percentage of positive cells and mean fluorescence intensity (MFI) were measured in a light scatter defined lymphocyte gate. **Results:** Twenty patients with low risk (RA, RCMD, RARS) and 12 with high risk MDS (RAEB-1/2, MDSu) with a median age 69 years as well as 17 controls with non-hematologic medical disorders or healthy blood donors (median age 60) were included into the study. Compared to controls the MFI of the B-cell markers CD21 (30 vs. 16, p=0.004), CD22 (306 vs. 133, p=0.036) and CD268 (852 vs. 502, p=0.004) was significantly decreased in MDS patients. This also was observed for the percentage of CD22⁺ (98.9 vs. 93.5%, p=0.00056) and CD268⁺ (99.3 vs. 97.2%, p=0.001) B cells. In high risk MDS lowest MFI for those markers were measured: 13 for CD21 (p=0.002), 91 for CD22 (p=0.005) and 368 for CD268 (p=0.002). Also in high risk patients, MFI for CD2 (p=0.013), CD5 (p=0.014), and CD45R (p=0.024) and percentage of CD45R⁺ B (p=0.026) cells was significantly reduced in high risk patients. We found no significant differences in the distribution of T- and B-cell subsets. **Conclusion:** Our data show an alteration of phenotype in the B cell compartment in MDS patients which warrants further investigation. The diminished expression of markers modulating B cell receptor signaling (CD21, CD22) or supporting activation and survival of B cells (the BAFF receptor CD268) may point to an altered immune response in MDS or possibly be caused by MDS associated clonal changes in B cells.

P501

Identification of a novel mode of kinase inhibitor resistance: An F604S exchange in FIP1L1-PDGFRα modulates FIP1L1-PDGFRα protein stability in a SRC-dependent manner

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FIP1L1-PDGFRα is a constitutively activated protein kinase which was reported in chronic eosinophilic leukemia (CEL) and in cases of hypereosinophilic syndrome and mastocytosis with eosinophilia. Imatinib is clinically active against FIP1L1-PDGFRα positive disease. However, clinical resistance to imatinib has been observed in FIP1L1-PDGFRα positive leukemia and was shown to occur due to a secondary mutation (T674I) in the PDGFRα kinase domain. Using a screening strategy to identify imatinib resistant mutations, we generated numerous imatinib resistant cell clones. Analysis of the PDGFRα kinase domain in these cell clones revealed a broad spectrum of resistance mutations including the clinically reported exchange T674I. Interestingly, one of the abundant mutations was a Phe to Ser exchange at position 604 (F604S), which occurred alone or in combination with other exchanges. Surprisingly, FIP1L1-PDGFRα/F604S in contrast to D842H and F604+D842H did not increase the biochemical or cellular IC50 value to imatinib when compared to wild-type (wt). However, F604S and F604S+D842H transformed Ba/F3, NIH3T3 and mouse bone marrow more efficiently compared to wt and D842H, respectively. Also, F604S and

F604S+D842H showed strong activation of Stat5, ERK and Akt compared to wt and D842H. Immunoprecipitation and immunoblotting indicated increased amounts of FIP1L1-PDGFRα protein in F604S versus wt cells. Moreover, SRC coimmunoprecipitated with FIP1L1-PDGFRα in wt, but not F604S cells. We hypothesized that F604S might interfere with FIP1L1-PDGFRα protein stability, and that SRC might be involved in this process. GST pull down experiments using SRC-SH2 domain showed lesser binding of FIP1L1-PDGFRα/F604S compared to wt. Similarly, using a GST-PDGFRα fragment, more SRC was precipitated with wt compared to F604S. Importantly both, the SRC inhibitor PD166326 and SRC siRNA mimicked the F604S phenotype and resulted in stabilization of the wt protein. Also, co-expression of SRC in 293T cells augmented degradation of wt, but not F604S FIP1L1-PDGFRα, indicating that SRC is a negative regulator of FIP1L1-PDGFRα protein stability. Similar results were obtained with an exchange in near proximity to F604. However, kinase-defective SRC had no effect on FIP1L1-PDGFRα stability, indicating that kinase activity of SRC is necessary for its effect on FIP1L1-PDGFRα stability. Moreover, kinase defective FIP1L1-PDGFRα (G610R) was not degraded indicating that kinase activity of FIP1L1-PDGFRα is necessary for its own degradation. Taken together, imatinib resistance screening in FIP1L1-PDGFRα identified a novel class of resistance mutations, that do not act by impeding drug binding to the target, but rather increase target protein levels by interfering with its SRC mediated degradation.

P502

Levels of Beta 2 Microglobulin have a prognostic relevance for patients with Myelodysplastic syndrome with regard to survival and the risk of transformation into acute myelogenous leukaemia

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Purpose: The international prognostic scoring system (IPSS) is currently the “gold standard” for risk assessment in patients with myelodysplastic syndromes (MDS). Still, additional parameters would be helpful to improve the prognostic power of the IPSS. A promising candidate is the Beta 2 Microglobulin (B2M) which is a cellular surface subunit of the human leukocyte antigen-class I. **Methods:** We retrospectively evaluated the prognostic relevance of B2M plasma concentration in 109 patients with MDS (9 RA, 1 RARS, 35 RCMD, 14 RSCMD, 5 (5q-), 12 RAEB I, 19 RAEB II, 10 CMML I, 4 CMML II) from the Duesseldorf registry. Patients with serum creatinine levels > 1.5 mg/dl, receiving single new investigational agents, intensive therapy or blood stem cell transplantation were not included in this study. There were 54 females and 65 males with a median age of 68 years (range 17 – 86 years). The classification of the patients based on the WHO, FAB and on the IPSS classification as well as cell counts, LDH, medullary blast count, karyotype, age and gender were recorded. **Results:** With a median time of follow up of 24 months (range 1 - 99 months) 47 patients are alive, 62 patients died within the observation period (57%) after a median time of 21 months (range 14 to 92 months). Twenty nine (27 %) patients developed overt acute myelogenous leukaemia (AML) after 12 months (range 1 to 60 months) following the diagnosis of MDS. According to a multivariate analysis the time of survival and the risk of transformation into AML was independently related to B2M level ≥ 2 mg/dl (p = 0.006). In the entire group of patients, patients with B2M level ≥ 2 mg/dl showed a significantly shorter overall survival time with a median of 23 months in comparison to 61 months for the 44 patients with B2M serum concentrations below 2 mg/dl. Looking at the risk of developing AML a proportion 46 % and 72 % of patients with B2M ≥ 2 mg/dl suffered from this transformation after 2 and 5 years whereas in the group of patients with B2M levels below 2 mg/dl the respective proportion of patients was 5 % and 19 %. When the analysis was performed for the patients allocated according to the IPSS groups the B2M level was only of prognostic power for patients belonging to the intermediate type 2 and high risk but not for those ones of the low and intermediate type 1 IPSS risk groups. **Conclusion:** In conclusion measurement of B2M serum concentration in patients with MDS should be envisaged at the time of initial diagnosis as it might be helpful in the decision what kind of treatment can be offered to the patient.

P503

Treatment responses to cladribine and dasatinib in rapidly progressing mastocytosis

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Background: Systemic mastocytosis (SM) is a myeloid neoplasm involving mast cells (MC) and their progenitors. In a majority of cases, neoplastic cells display the D816V-mutated variant of *KIT*. Cladribine (2CdA) and dasatinib are two antineoplastic drugs that counteract the *in vitro* growth of neoplastic MC bearing *KIT* D816V. However, only a few reports on the *in vivo* effects of these drugs in patients with SM are available. **Patient and Methods:** We report on a patient with highly aggressive SM who was treated with 2CdA and dasatinib. *In vitro* pre-testing revealed a response of neoplastic MC to both compounds with reasonable IC₅₀ values. **Results:** The patient was subsequently treated with 6 cycles of 2CdA (0.13 mg kg⁻¹ i.v. daily on 5 consecutive days). Despite a short-lived major clinical response and a decrease in serum tryptase, the patient progressed into mast cell leukemia (MCL) after the sixth cycle of 2CdA. The patient then received two further courses of 2CdA followed by treatment with dasatinib (100 mg p.o. daily). However, no major response was obtained and the patient died from disease-progression after 2 months. **Conclusions:** In patients with rapidly progressing ASM or MCL, neither 2CdA nor dasatinib may produce a long lasting response *in vivo*, despite encouraging *in vitro* results obtained in this and in previous studies. Therefore, for these patients, alternative treatment strategies have to be developed.

P504

Lenalidomide in Patients with isolated del 5q and Transfusion dependent Anemia

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Introduction: Recent data have shown that treatment of MDS patients with del5q with Lenalidomide (Len) results in very high rates of transfusion independency and is also capable to induce cytogenetic responses in these patients. Here we present updated results for 6 patients with MDS and del5q treated at our institution. **Methods:** Since October 2006 we treated 6 patients with an isolated del 5q with Len. The median age was 84 (59-86) years with a median time since diagnosis of 7.5 months. All patients were female with a median Karnofsky index of 65% (30-100) and dependent on transfusions with packed red blood cells (median: 4 PRBC 1 month before treatment). According to the WHO-classification 3 patients had a 5q-Syndrome, 1 patient had RAEB-2, one RARS and one patient had an AML with del 5q (50% blasts in the BM). The prognostic score according to IPSS was low in 3 patients, int-1 in 1, int-2 in 1 and high risk in 1 patient. All patients received an initial dose of 10mg Len orally for 21 days every 4 weeks. Dose reduction was necessary in one patient due to nausea (G II). Dose interruption was necessary for one week in one patient due to G IV neutropenia (ANC 50/μl). Patients received a median of 6 (2-15) cycles of Len. FISH analyses on del 5q were performed from peripheral blood every 4 weeks during the last 4 months to evaluate this method as a potential monitoring tool. **Conclusions:** 4 out of 6 (67%) patients became transfusion independent ("responders") after a median of 6 (4-7) weeks. Two patients had a complete cytogenetic remission and are transfusion independent since 28 and 40 weeks, respectively. The other two responders were transfusion free for 73 and 35 weeks. The patient with AML showed no response. One patient could not receive Len as planned due to severe rash and dyspnea during cycle 1. The median response duration for all 4 responding patients so far is 37 weeks. Grade III/IV hematologic toxicity occurs frequently but 3 out of 6 patients had a preexisting grade III/IV cytopenia. In all patients hematologic toxicity resolved or improved in case of response.

P505

De Quervain's thyroiditis as a rare cause of fever in a patient with MDS and severe neutropenia

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Introduction: Neutropenia is a common complication of malignant hematologic diseases and predisposes to bacterial or fungal infections associated with high morbidity and mortality. Fever is the most common presentation of infection and is usually empirically treated to prevent a dismal course. We report on a female patient with myelodysplastic syndrome (MDS) and severe neutropenia who presented with fever (40°C) and was subsequently found to have autoimmune thyroiditis. **Patient:** A 50-year-old female patient with unclassifiable MDS and granulocytopenia (600/ml) presented to our hospital with high grade fever (40°C) and a sore throat that had begun 4 days earlier. The physical exam as well as a chest x-ray and an abdominal ultrasound were within normal limits as was a CT-scan of the lungs. Endocarditis was ruled out by transesophageal echocardiography. Multiple blood cultures were negative. Ceftazidime 3 x 2g was started immediately and later switched to meropenem 3 x 1g according to our institutional protocol for neutropenic patients with no resolution of the fever. Since the patient continued to complain about her sore throat a thyroid ultrasound was performed showing poor echogenicity of an organ of normal size. TSH was low (0,03mU/l) whereas free T4 was elevated (38,1pmol/l) consistent with hyperthyroidism. High titer anti-thyroglobulin antibodies were present (812 mU/ml), whereas MAK and TRAK were negative. De Quervain's thyroiditis was diagnosed and oral prednisolone 30 mg/d was started with prompt resolution of fever and pain. Steroids were tapered during the following 3 months and the patient is currently doing well waiting for an allogeneic stem cell transplantation. **Discussion:** Although retrospectively the clinical presentation of this patient was typical for de Quervain's thyroiditis, we failed to make a prompt diagnosis, since our major concern was a potentially severe infection. De Quervain's thyroiditis is a rare disease that usually occurs in adults < 50 years. Women are more often affected than men. A sore throat without typical infectious findings suggestive of pharyngitis should warrant further work up including TSH, T4 and a thyroid ultrasound. **Conclusion:** This case shows that although usually helpful in every day practice institutional standards for treatment and workup of common complications tempt physicians to overlook subtle presentations of unsuspected eventually rare diseases.

Melanom Poster:

P506

Palliative care in progressive Merkel cell carcinoma (MCC) an interdisciplinary challenge

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Introduction: Palliative care is a form of medical care that concentrates on reducing the severity of disease symptoms, rather than providing a cure. The goal is to prevent and relieve suffering and to improve quality of life for people facing serious illness. Palliative wound care should be centered on symptom management and is a viable option for patients whose chronic wounds do not respond to standard interventions. MCC is a relatively rare neuroendocrine carcinoma of the skin. It arises in the head and neck regions. Its aggressive behavior predisposes patients to local-regional recurrence and distant metastases even under aggressive therapeutic regimens. Here we report a case of palliative wound treatment in progressive MCC. **Case Report:** A 85 year old man with the diagnosis of MCC stage I underwent excision of the tumor. Histological analysis revealed tumour-free resection margins. After covering the wound irradiation was performed. Due to relapse systemic chemotherapy was performed but could not be completed due to side effects. Standard local irra-

diation could not stop the progression in tumor mass, rapidly affecting almost the entire head region including the pharynx. Therefore, the patient was admitted to our palliative care unit with the leading symptom of fetid necrotizing tumor, dysphagia and pain. In an interdisciplinary setting palliative wound treatment was performed with metronidazole gel, fuchsin solution and absorbent dressing containing activated charcol. In addition a systemic antibiotic therapy with metronidazole and an aroma therapy were administered, controlling the leading symptom of malodor. Additional psychological support helped in addressing the disgust felt by the patient himself. Furthermore treatment resulted in complete relief of pain and dysphagia. **Discussion:** MCC is often resistant to surgery, radiation and chemotherapy regimens. The outcome for patients with unfavourable disease remains poor and 25-50 % of patients die as a direct result of MCC. Standardized treatment regimens have not been established, and difficulties arise finding appropriate treatment for the elderly. In our case a novel palliative wound strategy could achieve successful control of malodor, as the leading symptom dramatically impairing patient's quality of life. Furthermore this case underlines the importance of early initiation multimodal therapeutic approach including palliative care with palliative wound management to prevent such drastic clinical cases.

P507

Efficacy of transarterial chemoembolization (TACE) of liver metastases from uveal melanoma

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Introduction: The liver is the predominant site of metastases in the majority of patients with uveal melanoma. Response rates to systemic chemotherapy are low and median survival is very short with 6-9 months. Locoregional treatment like transarterial chemoembolisation (TACE) is of interest especially in patients in whom the liver is the only site of disease or disease progression after systemic chemotherapy. **Methods:** 26 patients with documented disease progression in the liver treated with fotemustine- or cisplatin-based TACE between 2003 and 2008 at our institution were analysed. TACE of liver metastases was performed by interventional placement of a catheter into the hepatic artery followed by the administration of spherex (900mg) admixed either with fotemustin (100mg/m²) or with cisplatin (50mg). A median of 2 TACE (range 1 to 8) was applied. Median time between diagnosis of metastases and TACE was 8 months (range 1 to 24). All but one patient had prior systemic therapy. **Results:** Grade III toxicity consisted of nausea (n=1), resorption fever (n=3), splenic infarction (n=1), thrombocytopenia (n=1), and gastric ulcer (n=1). No grade IV toxicity or catheter associated complications were observed. 14 of 26 patients had stable disease for at least 2 months, three a partial, and one a complete remission, resulting in an objective response rate of 15% and a disease control rate of 69%. Median time to progression (TTP) was 3 months (95% CI: 2-4 months), median survival (OS) 5 months (95% CI: 4-6 months), and the 1-year survival rate 15%. Both TTP and OS were significantly longer in case of pre-treatment lactate dehydrogenase (LDH) below the twofold upper limit of normal (n=11): median TTP 5 versus 2 months (p<0.001) and median OS 11 versus 5 months (p=0.008). **Conclusions:** TACE was effective and well tolerated in patients with liver metastases from uveal melanoma and should further be evaluated as first line therapy in prospective clinical trials.

P508

Stealth Adeno-associated virus vectors identified by codon randomization of immunogenic epitopes

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Introduction: efficiency of therapeutic gene transfer by adeno-associated virus of serotype 2 (AAV2) vectors is hampered in patients with pre-existing immunity against the natural virus. Genetic engineering by rational design or directed evolution has been employed in the last 3 years to generate capsids

that escape antibody neutralization and has led to identify several amino acid residues of the capsid proteins that can be mutated in order to decrease antibody recognition. We aimed to exploit the comprehensive knowledge gathered so far by generating novel capsid variants that carried multiple mutations of amino acids whose substitution yielded antibody evading phenotypes in previously published studies conducted in our and other groups (Perabo et al., 2006; Maheshri et al, 2006; Lochrie et al., 2006). **Methods:** capsid libraries were generated by codon randomization of several of these immunogenic residues and screened to isolate mutants that most efficiently infected human cells despite the presence of anti-AAV2 neutralizing antibodies. Besides testing novel combinations of concomitant mutations at these sites, this approach allowed for the first time an exhaustive scanning of combinations of all 20 natural amino acids at each position, maximizing stealth properties and minimizing loss of packaging ability, particle stability and transduction efficacy. **Results:** This procedure has allowed us to identify several novel capsid mutants that remain highly infectious even when incubated with serum concentrations that completely neutralize wild type AAV2. These vectors can be used for therapeutic gene transfer to patients with pre-existing immunity, or for repeated treatment after antibodies are generated upon first application.

Microenvironment

Vortrag:

V509

The microenvironment in Chronic Lymphocytic Leukemia: new approaches to dissect and target the social network of CLL cells

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Purpose: There is growing evidence suggesting that interactions between Chronic Lymphocytic Leukemia (CLL) cells and accessory stromal cells in the microenvironment within secondary lymphatic tissues and the marrow make pivotal contributions to disease progression, drug resistance, and minimal residual disease. Therefore, we anticipate a paradigm shift from targeting primarily the malignant cells towards combinations of cytotoxic agents with compounds that target the tumor microenvironment. However, identification of key pathways that regulate the cross talk between CLL cells and accessory cells is a prerequisite for targeting the microenvironment. **Methods:** We previously reported that chemokine receptors expressed at high levels on CLL cells (CXCR4, CXCR5), and integrins, such as VLA4, are essential for interactions between CLL cells and the stroma. In this study, we define which genes become induced by co-culture of CLL cells with different stromal cells using gene expression profiling with Affymetrix arrays. **Results:** Co-culture with nurselike cells (NLC) induced a homogeneous gene expression response in CLL cells with high-level expression of B cell maturation antigen (BCMA) and two chemoattractants (CCL3, CCL4). CCL3/CCL4 expression correlated with ZAP-70 expression, and supernatants from CLL-NLC co-cultures revealed high CCL3/CCL4 protein levels. B cell receptor triggering also induced a rapid and robust induction of CCL3 and CCL4 expression. CLL patients displayed higher CCL3 and CCL4 plasma levels than healthy donors, and these levels correlated with markers of disease activity. Through these chemokines CLL cells can recruit accessory cells, particularly T cells, and thereby create a microenvironment that favors their growth and survival. In another set of experiments, we examined the importance of phosphatidylinositol 3-kinases (PI3-K) for migration and survival of CLL B cells in stroma co-cultures. Using a panel of isoform-specific PI 3-K inhibitors (PI-103, PIK-90, IC87114, TGX-115, ZK-75), we observed inhibition of chemotaxis by the multi-targeted compounds PI-103 (51.4±0.2%) and PIK-90 (57.5±8.9%), whereas p110beta and delta inhibition had no effect. Because adhesion to stroma mediates protection from spontaneous and drug-induced apoptosis, we tested PI3-K inhibitors alone and in combination with fludarabine in CLL-stroma co-cultures. Pre-treatment of CLL cells with PI3K inhibitors resulted in a significant decrease in viability of CLL cells co-cultured with and without NLC. PI3-K isoform specific inhibitors enhance the

cytotoxicity of fludarabine and partially reversed the protective effect of stromal cells on fludarabine-induced apoptosis. **Conclusions:** Collectively, these studies provide new insight into the molecular cross-talk between CLL cells and the microenvironment, and identify the PI3-K signaling pathways as a mediator of pro-survival and drug-resistance signals, that can be targeted by novel, isoform-specific kinase inhibitors.

Morbus Hodgkin

Freie Vorträge:

V510

HIV-associated Hodgkin's disease (HIV-HD): Interim analysis of a prospective multicenter trial

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Introduction: Hodgkin's disease (HD) is one of the most common non-AIDS defining malignancies. Recent data indicate an improved outcome of pts with HIV-HD treated since the introduction of highly active antiretroviral therapy (HAART). This trial was initiated to investigate a risk adapted treatment strategy in pts with HIV-HD in accordance with standard treatment procedures established for HIV-negative pts with HD. **Methods:** Pts with HIV-infection and histologically proven HD are included in the ongoing study. Pts are planned to receive 2x ABVD + 30 Gy involved field (IF) radiation for early stage (ES) favourable HD, 4x BEACOPP baseline + 30 Gy IF for ES unfavourable HD (extranodal involvement, large mediastinal mass, ≥ 3 lymph node areas involved), and 6-8 x BEACOPP baseline for advanced stage HD. BEACOPP should be replaced by ABVD in pts with far advanced HIV-infection. HAART is given to all pts in parallel to chemotherapy (CT). **Results:** Since March 2004, 59 pts (57 males, 2 females) with a median age of 44 yrs (range 29 – 64) were included in (n=54) or treated according to the ongoing trial (n=5). 35 of 52 pts had mixed cellularity subtype. 5/52 pts (10%) were diagnosed with stage I, 11 (21%) had stage II, 18 (35%) stage III and 18 (35%) stage IV disease. B-symptoms were present in 32 of 50 cases (64%). HAART was given prior to HD in 39 of 49 cases (80%) and 15/49 pts (31%) had a prior AIDS defining illness. The median CD4 counts at HD diagnosis was 210/ μ l with a median viral load of <50/ml (range 0 – 454.000). Pts received/are receiving ABVD (n=24) or BEACOPP baseline (n=33) as initial CT with no data available yet in 2 pts. Grade 3/4 toxicity was reported in 27 of 40 pts (68%) and 14 pts developed a documented infection. Response data are available in 36 pts [CR 25 (69%), PR 8 (22%), SD 1 (3%), PD 2 (6%)]. So far, 3 pts have relapsed. After a median follow up of 10.5 months 6 pts have died, all of them diagnosed with stage IVB. Causes of death were neutropenic sepsis during the 1st and the 7th course of BEACOPP (n=2), progressive HD (n=1), progressive HIV-infection (n=1) and both, progressive HD and HIV-infection (n=2). **Conclusions:** In pts with HIV-HD risk-adapted CT and concomitant HAART is safe and effective. However, pts are at increased risk for neutropenic and opportunistic infections. These data suggest that the prognosis of HIV-HD may approach results achieved in the HIV-negative population with HD.

V511

Lenalidomide in chemotherapy – refractory or multiple relapsed Hodgkin lymphoma patients

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Introduction: Lenalidomide is an immunomodulatory drug with multiple properties including inhibition of TNF α and VEGF (vascular endothelial growth factor), modulation of T-cell activity and impact on the microenvironment. These characteristics suggest that lenalidomide may play a role in the treatment of Hodgkin Lymphoma (HL), since Hodgkin cells are highly dependent on their microenvironment. **Methods:** To investigate the activity in HL, lenalidomide was provided in a named patient program. Patients were eligible if they had active disease and no curative therapeutic options. Lenalidomide was given at 25 mg daily for 21 days, followed by one week rest. Response was assessed according to Cheson criteria. **Results:** So far, eight are available for safety and efficacy after two cycles of Lenalidomide. Seven were refractory to prior chemotherapy, and one had an early relapse (8 weeks) after allogeneic transplantation. Six patients were male, the age ranged from 20–64 years. The median number of prior treatments was 4 (range 3–7), all patients had received radiotherapy and autologous stem cell transplantation, and two had received an allogeneic transplant. Disease stage was stage II in two patients, stage III in one patient, and stage IV in five patients. Six patients suffered from B-symptoms. Three patients achieved a partial remission and three patients had stable disease as determined by CT. B-Symptoms resolved in all patients. No severe hematological or non-hematological toxicity was documented. Treatment is ongoing in all patients. **Conclusions:** These data indicate that lenalidomide is active in heavily pretreated patients with progressive, refractory HL. It is too early to judge on the duration of the responses. Since therapeutic options in these patients are very limited and their prognosis is very poor, Lenalidomide warrants further evaluation in this setting. The lack of side effects suggests that higher doses might be tolerated in HL patients.

V512

Erythropoietin (EPO) in patients with Hodgkin Lymphoma: an analysis of the GHSG HD15-EPO trial

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Anaemia is frequent among cancer patients and impacts organ function and quality of life of anemic patients. In addition, it has been shown to be a negative prognostic factor in several malignancies including Hodgkin Lymphoma (HL). At the time the HD15 study for patients with advanced-stage HL was initiated, there were some data suggesting that EPO might also be associated with improved overall survival in cancer patients. Thus, apart from the major chemotherapy-related question of this study, ie 6 or 8 cycles of BEACOPP, patients in HD15 were randomized between Epoetin- α and placebo. Study drug was given at 40 000 i.e. weekly with a haemoglobin (Hb) target level of 12–13 g/dl. Primary endpoint was fatigue directly and 6 months after the end of chemotherapy (CT) measured with the EORTC QLQ-C30 questionnaire (0–100 scale). With respect to the current safety discussion we also analysed efficacy and safety endpoints being aware that the trial is not sufficiently powered for time-to-event analyses. Between 1/2003 and 12/2006 a total of 1 379 patients were randomized for the EPO question of whom 688 were randomized before July 05 and included in this analysis. Patient characteristics were generally balanced between the placebo and the EPO arm. The CR/Cru rate was 90,6%, with a median follow-up of 28 months a total of 58 progressions/relapses (8,4%) and 33 deaths (4,8%) were observed without difference between study arms. There was no difference in terms of freedom from treatment failure (95%-confidence interval (CI) of hazard ratio (HR) 0.6–2.3) and overall survival (95% CI of HR 0.6–1.4). 51 serious adverse events (7,4%) and

35 thrombosis (5.1%, prospective documentation started 6/04) were observed with no difference between arms. However, this needs all cases for a final conclusion. The median number of red blood cell units given significantly favored patients receiving Epoetin- α (2 vs 4; test for trend $p < 0.001$). The latest interim analysis for fatigue gave an average value of 61 ± 25.8 (mean \pm SD) directly after and a reduced fatigue of 33 ± 25.3 six months after CT without reaching the sequential stopping rule. **Conclusion:** In this large prospectively randomized, placebo controlled double-blind trial performed so far using Epoetin- α in patients with advanced-stage HL, we found no difference in terms of fatigue, response, relapse or side effects between those patients receiving Epoetin- α or placebo. There was a significantly reduced number of RBC units given in the EPO group. The final analysis needs to confirm these findings and will be presented.

V513

Quality of life (QoL) in long-term survivors of Hodgkin Lymphoma (HL) treated within the German Hodgkin Study Group (GHSg) in the Czech Republic: A cross-sectional study of patients treated 1995 to 2003

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Introduction: We investigated the current QoL in long-term survivors of HL treated within the GHSg between 1995 and 2003 in the Czech Republic. The aim was to describe patients (pts) QoL after cure and returning to normal life during the years (yrs) after end of treatment in a cross-sectional design. **Methods:** EORTC QLQ C-30, MFI-20, subjective retrospective evaluation of treatment and a life situation questionnaire (LSQ) were used for the assessment of the pts' situation after end of treatment. An authorized Czech version of the questionnaires was sent to 172 pts who were followed-up for four and more yrs. **Results:** 142 (82.5%) pts replied. Median age at time of assessment was 33 yrs, median follow-up 66 months. Regarding the QoL functional and fatigue scales, pts report a mixed pattern of responses but indicate quite severe limitations in their perceived QoL during later years of follow-up. Emotional functioning and global QoL recovers fully only in 50% of pts 4-12 years after end of treatment and about 25% report constant severe strain. General fatigue remains high with 45% of pts reporting "high fatigue" levels but only 15-30% of pts report high levels of reduced motivation and activity. In physical functioning 70% recover fully and only 6% report very low functioning. In general, women report lower QoL and higher symptom scores over time than men. **Conclusions:** QoL data from the reintegration process of pts into normal life during the years of follow-up reveal substantial strain and limitations of QoL, particularly in subsets of patients and in specific areas of QoL. Longitudinal QoL assessment within the GHSg trials is ongoing also for Czech pts and will add the QoL course of recovery to the current cross-sectional data. Results regarding also socio-demographic variables will be included in the final presentation. **Supported by Grant MZ CR IGA NR 8033 – 6/2004**

V514

FDG-PET for assessment of early therapy response after 4 cycles of chemotherapy in advanced stage Hodgkin Lymphoma

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Introduction: As positron emission tomography (PET) seems to be a powerful predictive tool in therapy control of Hodgkin lymphoma (HL) we analysed the prognostic value of PET after 4 cycles of different BEACOPP variants. **Methods:** Patients were treated in or according to the protocol of the prospective HD15 multicenter trial of the German Hodgkin Study Group (GHSg), which evaluates the impact of different BEACOPP variants and the prognostic value of PET after chemotherapy in advanced-stage HL patients. Between January 2004 and March 2007, 50 patients with newly diagnosed, histology-proven HL in clinical stages IIB with large mediastinal mass or extranodal disease, III and IV were enrolled and received 8 cycles (standard arm A) or 6 cycles (arm B) BEACOPP_{escalated} compared with 8 cycles time-condensed BEACOPP_{baseline} (arm C). 35 patients received 4 cycles BEACOPP_{escalated} (pooled arm A+B), 15 patients had 4 cycles BEACOPP_{baseline} (arm C) before performance of the intermediate PET. Additional RT was restricted to PET positive patients after the end of completed chemotherapy. **Results:** 14 of 50 patients had a positive PET, 36 patients had a negative PET scan in residual tissue after 4 cycles chemotherapy. 13/14 patients with a positive PET and 16/36 with a negative PET had a large mediastinal tumor at diagnosis. At a median observation time of 25 months, 2/14 patients with a positive PET had progressed or relapsed (PPV=14%), while there was no progression or relapse in PET negative patients. One PET negative patient died in the last cycle chemotherapy due to acute toxicity of treatment (bleomycin induced pneumonitis) and was counted as failure resulting in a NPV in PET-negative patients of 97%. **Conclusion:** Our results indicate a high negative predictive value of PET after 4 Cycles of BEACOPP variants suggesting a possible reduction of therapy in these patients. **Supported by Grant MZ CR IGA NR 8033-6/2004**

V515

Apoptosis of Hodgkin lymphoma cells in response to the anti-CD30 antibody 5F11 and bortezomib is increased by selective metalloproteinase inhibition

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Introduction: CD30-overexpressing Hodgkin lymphoma cells are targeted with the fully human monoclonal antibody 5F11 but efficacy is limited due to NF- κ B-dependent apoptosis resistance of some malignant cells. Our previous data indicate that combination of the NF- κ B inhibitor bortezomib (Velcade) and 5F11 shows cytotoxic synergy in killing Hodgkin lymphoma cells both in vitro and in vivo (Böll et al., 2005, Blood, 106, 1839-42). Surprisingly, synergy was only observed when NF- κ B inhibitor was delivered in sequence, more than 30 min after antibody treatment. Here, we analyzed the underlying mechanism of this finding. **Methods and Results:** We showed that bortezomib stimulated CD30 shedding. This caused a decrease of CD30 expression on the cell surface and the adverse release of a soluble targeting competitor. TNF- α converting enzyme (TACE) was identified as releasing enzyme

using TACE-deficient cell lines generated from an embryonically lethal ADAM17^{-/-} mouse. Inhibition of CD30 shedding by non-toxic concentrations of a TACE-selective hydroxamate inhibitor results in the blocking of the bortezomib-stimulated CD30 release. As demonstrated in vitro by annexin-staining and XTT-viability assays, this inhibitor not only improved the efficacy of 5F11 and subsequent bortezomib combination but also rescues therapy failure after inverse drug delivery. **Conclusion:** General inhibition of metalloproteinases caused severe side effects in previous clinical studies. These data suggest a therapeutic value of selective metalloproteinase inhibition in targeted immunotherapy against targets susceptible to metalloproteinase cleavage.

Morbus Hodgkin

Poster:

P516

Massive intravascular hemolysis and limb pain in a patient with Hodgkin's disease and septic shock

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Introduction: Intravascular hemolysis is generally caused by red cell enzymopathies, thrombotic-thrombocytopenic purpura, malaria or mechanic heart valves. Here we present the case of a 38 year-old male Caucasian patient who had been diagnosed with lymph node, pericardial and pleural involvement of relapsed Hodgkin's disease. He was started on a salvage protocol incorporating rituximab, etoposide, adriamycin, cyclophosphamide, procarbazine, and prednisone. **Case Report:** Ten days after discharge following administration of the fourth treatment cycle, he presented to the emergency service feeling generally unwell with a history of fever, chills and back pain for two days. On physical examination severe pallor, tachycardia and tachypnea were noticed. Abnormal laboratory values were WBC, $0.1 \times 10^9/l$; haemoglobin, 4.4 g/dl; PLT, $19 \times 10^9/l$; bilirubin, 6.6 mg/dl; lactic dehydrogenase, 299 U/l; CK, 454 U/l, and procalcitonin, 42.0 $\mu g/l$. Examination of the peripheral blood smear was negative for fragmentation and direct antiglobulin test showed no reactivity. Abdominal ultrasound and chest X-ray were normal. Blood cultures were obtained and treatment initiated with transfusion of packed red cells, intravenous broad-spectrum antibiotics, and granulocyte colony-stimulating factor with the patient being admitted to the intermediate-care unit. Analgesics were started for severe pain in the limbs without evident of perfusion disturbance. The patient developed septic shock two hours later necessitating administration of vasopressors and mechanical ventilation. At that time, hemoglobin was 4.3 g/dl; lactic dehydrogenase, 6940; CK, 9036 U/l; potassium, 7.3 mmol/l; and phosphorous, 3.95 mmol/l. Initiation of hemofiltration for severe metabolic acidosis and oliguria was prevented by central venous line clotting. Despite massive transfusion there was a further decrease in hemoglobin and hematocrit. Metabolic as well as hemodynamic situation were refractory to treatment, so the patient died eight hours from admission. Clostridium perfringens bacteremia was diagnosed by blood cultures two days later. **Conclusions:** We report on one of the very rare cases of fatal C perfringens sepsis associated with hematologic malignancies. The dramatic nature and refractoriness of intravascular haemolysis have repeatedly been described in the literature. Often C perfringens sepsis is due to postpartum or gangrenous abdominal infections, trauma or diabetes mellitus. In this special case, the patient presented late what along with profound neutropenia and the underlying malignancy may have contributed to the overwhelming course. However, devastatingly short survival times of only a few hours from admission to death have remained unchanged since the very first reports more than 50 years ago.

P517

Chemotherapy Alone Versus Chemotherapy Plus Radiotherapy For Early Stage Hodgkin Lymphoma: A Systematic Review With Meta-analysis

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Introduction: The optimal choice of treatment in patients with early-stage Hodgkin lymphoma (HL) is still being discussed. A recently conducted randomised controlled trial (RCT) reported no significant difference in 5-year overall survival (OS) in patients treated with either 4 to 6 cycles of chemotherapy (CT) alone or 2 cycles of CT plus radiotherapy (RT) (Meyer et al. 2005). We performed a systematic review evaluating chemotherapy plus radiotherapy (CT+RT) compared to CT alone with respect to OS, freedom from disease progression (FFP) and complete response rate (CR) in patients with early-stage HL. **Methods:** The Cochrane Library, MEDLINE, EMBASE and conference proceedings were searched for RCTs from January 1975 to December 2007. Both abstracts and full texts were accepted. Hazard ratios (HRs) were used for survival data and relative risks (RRs) for binary data. The analysis was done with RevMan 5. **Results:** After screening 2193 references six RCTs with 1521 patients with early-stage HL (CS IA, IIA, IB and IIB) were included. 1484 patients from 5 trials were analysed for OS, 428 patients from two trials were analysed for FFP and 653 patients from 4 trials were analysed for CR. Patients treated with CT+RT had a non significant improvement of overall survival (HR=0.52, 95% CI 0.26 to 1.06) in the random effects analysis compared to CT alone. There was strong heterogeneity in the analysis. Examining only completely identical chemotherapy, not only with respect to the regimen but also to the number of cycles, overall survival was better in patients receiving CT+RT compared to CT alone (4 trials, HR = 0.40, 95% CI 0.24 to 0.66), with minimal heterogeneity. Both the CR (4 trials, RR = 1.08, 95% CI 1.02 to 1.14) and FFP (2 trials, HR = 0.49, 95% CI 0.28 to 0.88) were better in the group receiving CT +RT compared to CT alone; outcomes not reported by Meyer 2005. **Conclusion:** Adding radiotherapy to a chemotherapy regimen improves overall survival in patients with early stage Hodgkin disease.

P518

Hodgkin lymphoma in Tyrol – a population-based study

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Introduction: We aimed to analyze the epidemiology, clinical characteristics and outcome of patients with Hodgkin lymphoma (HL) diagnosed in Tyrol. **Methods:** All patients with newly diagnosed HL between 1993 and 2005 were included in this study. **Results:** Among the 158 cases included, nodular lymphocytic predominant HL (nodular paragranuloma, NLPHL) was identified in ten cases (6%) whereas the majority of patients had a classical Hodgkin lymphoma (cHL). Age ($p < 0.01$), sex ($p = 0.03$), risk-groups according to the German Hodgkin Study Group (GHSG) stratification ($p < 0.01$), and bone marrow infiltration ($p < 0.01$) were of prognostic significance considering overall survival (OS) whereas histological subtype and bulky disease were not. Patients without EBV-association had a significantly better OS ($p < 0.01$), but not disease-specific survival. The five- and ten-year OS rates for the total group were 89% and 85%, respectively. Notably, in patients with advanced stage HL ($n = 49$) combined modality treatment resulted in significantly better OS than chemotherapy alone ($p = 0.01$). Three patients developed a second hematological malignancy and one patient developed breast cancer. However, five patients (3%) had a malignant hematological disorder before occurrence of HL. Concerning treatment-related toxicity, bleomycin-associated lung toxicity

was observed in six (4%) patients and five (3%) developed lethal treatment-related infectious complications. **Conclusion:** Our results provide evidence that the incidence rate of HL in Tyrol is comparable to other Western countries. Modern risk-adapted treatment results in excellent long-term prognosis but may be complicated by serious non-hematological side effects, in particular infections and bleomycin-induced lung toxicity. Furthermore, 3% of HL patients had an antecedent malignant hematological disease before occurrence of HL.

Myelom

Freie Vorträge:

V519

The novel Hsp90 inhibitor NVP-AUY922 induces cell cycle arrest and apoptosis in myeloma and overcomes protection by bone marrow stroma

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Introduction: Recent findings have broadened our understanding of the mechanisms involved in myeloma cell proliferation and survival. Heat shock protein 90 (Hsp90) has been identified as a key regulatory protein in multiple myeloma (MM). By stabilization of kinases of essential signaling pathways, Hsp90 contributes to MM growth and survival. We tested the highly specific Hsp90 inhibitor NVP-AUY922 for its effects on MM proliferation, apoptosis and cell cycle distribution and its impact on signaling pathways. **Methods:** Multiple myeloma cell lines OPM-2, U-266, MM1.S and freshly isolated MM cells from myeloma patients were incubated with the Hsp90 inhibitor NVP-AUY922. Proliferation, cell cycle distribution and apoptosis were investigated. The effect of NVP-AUY922 on MM cell survival in cocultures with bone marrow stromal cells was tested. Several Hsp90 key client proteins like Akt and p38 were investigated by western blot. **Results:** We could show a time- and dose-dependent inhibition of MM proliferation with NVP-AUY922, IC₅₀ values at 72 hours were 18 nM, 57 nM and 73 nM for MM1.S, OPM-2 and U-266, respectively. The inhibitor potently induced apoptosis and cell cycle arrest in G₂. The protective effect of human bone marrow stromal cells on myeloma cell survival was overcome by NVP-AUY922. The analysis of phospho-p38 and Akt by western blot showed a marked decrease after 4 and 24 hours treatment with the Hsp90 inhibitor. **Conclusions:** The data demonstrate the anti-myeloma effects of the novel Hsp90 inhibitor NVP-AUY922. The compound is a highly potent inhibitor of proliferation and induces apoptosis and G₂ cell cycle arrest in myeloma cell lines. NVP-AUY922 treatment can overcome the protective effect of coculture with human bone marrow stromal cells. As a mechanism of action, we found a downregulation of key Hsp90 client proteins like phospho-p38 and Akt by NVP-AUY922. These data build the framework for NVP-AUY922 as a novel anti-myeloma agent to be evaluated in clinical trials.

V520

Serum concentrations of DKK-1 decrease in patients with multiple myeloma responding to therapies with bortezomib, thalidomide, lenalidomide or autologous stem cell transplantation

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Introduction: Osteolytic bone destruction is a major clinical problem in multiple myeloma and is due to an uncoupling of the normal bone remodeling. Secretion of Dickkopf (DKK)-1 by myeloma cells is a major factor which causes inhibition of osteoblast precursors. We could recently show a correlation between the DKK-1 serum concentration and the extent of lytic bone disease. In the present study, the effect of different treatment regimens for multiple

myeloma on DKK-1 serum levels was evaluated and correlated with the response to treatment. **Methods:** Concentrations of DKK-1 were quantified in 133 myeloma patients before initiation of treatment and after 3 months by ELISA, using a monoclonal anti-DKK-1 antibody (R&D systems). The following therapies were administered: bortezomib (n=29), thalidomide (n=18), lenalidomide (n=13), adriamycin & dexamethasone (n=26), high-dose melphalan (n=32) and induction plus high-dose chemotherapy followed by autologous stem cell transplantation (ASCT) (n=15). 33 MGUS patients served as controls. **Results:** At baseline, myeloma patients had increased serum concentrations of DKK-1 as compared to controls (mean 3403 pg/mL vs. 1993 pg/mL). A significant decrease of serum DKK-1 was seen after chemotherapy in the following groups: administration of bortezomib (4059 pg/mL vs. 1862 pg/mL, *P* = 0.016), adriamycin & dexamethasone (1668 pg/mL vs. 1241 pg/mL, *P* = 0.016), lenalidomide (11837 pg/mL vs. 4374 pg/mL, *P* = 0.039), and induction plus high-dose chemotherapy followed by ASCT (2446 pg/mL vs. 1082 pg/mL, *P* = 0.001). Treatment with thalidomide led to a nonsignificant decrease in DKK-1 (1705 pg/mL vs. 1269 pg/mL, *P* = 0.081). However, within all groups, a significant decrease of DKK-1 was only seen in responders (i.e. patients achieving a complete or partial remission), but not in nonresponders. High dose melphalan alone did not decrease DKK-1 levels, which is most likely due to the previous reduction of DKK-1 concentration after induction treatment. **Conclusion:** We could show for the first time that serum DKK-1 levels decrease in myeloma patients responding to treatment, irrespective of the regimen chosen, but not in nonresponders. This suggests that DKK-1 is mainly produced by myeloma cells, and successful treatment of myeloma reduces DKK-1 serum levels.

V521

Elevated circulating proteasome levels at diagnosis are associated with poor outcome in multiple myeloma

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Background: The proteasome is a proteolytic complex for intracellular degradation of ubiquitinated proteins which are involved in cell cycle regulation and apoptosis. A constitutively increased proteasome activity has been found in myeloma cells. Recent studies have shown that inhibition of the ubiquitin-proteasome system can be successfully used as a targeted therapy in multiple myeloma (MM). Recent data suggest a significant correlation between circulating proteasome levels (CPL) and outcome in patients with MM. Therefore, we investigated CPL in 110 patients in order to assess the role of CPL in MM.

Experimental Design: CPL were measured in serum samples from healthy controls (N=10) as well as from patients with monoclonal gammopathies of undetermined significance (N=27), indolent MM (N=15) and symptomatic MM (N=68) using enzyme-linked immunosorbent assay (ELISA) techniques detecting circulating 20S proteasome components. All serum samples were collected at the University Hospital in Ulm at time of diagnosis. **Results:** The median CPL were 123.5 ng/mL (range, 95-185 ng/mL) in healthy controls, 180 ng/mL (range, 100-485 ng/mL) in patients with MGUS (N=27) or indolent MM (N=15), and 227.5 ng/mL (range, 100-985 ng/mL) in patients with symptomatic MM (N=70). The CPL of patients with symptomatic MM were significantly elevated compared with healthy donors (*p*=0.0017) and to persons with asymptomatic gammopathies (*p*=0.046). While CPL were also significantly higher in the MGUS/indolent MM cohort as compared to controls (*p*=0.03), CPL in MGUS and indolent MM were comparable. Using ROC analysis in the symptomatic MM cohort patients with CPL ≥150ng/mL (N=50) had a significantly shorter progression-free survival (PFS) time than patients (N=18) with CPL <150 ng/mL levels (median PFS: 40 versus 57 months, log rank test *p*=0.026). Of note, all six patients who died in the observation interval had CPL ≥150ng/mL. **Conclusions:** Here we demonstrate that increased CPL at diagnosis correlates with poor outcome in symptomatic MM patients. Evaluation of the prognostic significance of CPL in a larger cohort of uniformly treated patients with symptomatic MM is currently under way. This data will be presented at the meeting.

Heat shock proteins Hsp72 and Hsp73 as novel therapeutic targets in multiple myeloma

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Introduction: Heat shock proteins (Hsp) can assemble multi-chaperone complexes that stabilize protein conformation. We could recently show that Hsp90 alpha and beta support survival of myeloma myeloma (MM) cells by maintaining a positive feedback loop consisting of Hsp90 and major signaling pathways like IL-6R/STAT3, Ras/MAPK, and PI3K/Akt pathways. However, the role of other Hsp, like the Hsp70 family, for protein stability and therefore for the malignant growth of MM cells, remains unclear. **Methods:** Western analyses were performed to determine Hsp70 expression in different myeloma cell lines. 60 bone marrow biopsies obtained from myeloma patients were immunohistochemically stained to analyze expression of Hsp70 *in situ*. SiRNA expression vectors based on the pSUPER backbone were transfected into well-defined myeloma cell line models to selectively knock down either Hsp72 or Hsp73. **Results:** Here, we show that Hsp72/Hsp73 are frequently overexpressed in MM cell lines *in vitro* and in half of the analyzed MM biopsies *in situ*, but not in MGUS or in normal plasma cells. SiRNA-mediated knockdown of either Hsp72 or Hsp73 led to induction of apoptosis in MM cells. Interestingly, the MM cell line AMO-1, which shows a weak Hsp72 expression and lacks activation of the PI3K/Akt pathway, was largely resistant to Hsp70 inhibition, but not to Hsp90 inhibition. Furthermore, we observed upregulation of Hsp70 after treatment with a novel Hsp90 inhibitor, and therefore tested the effects of concomitant Hsp90 and Hsp70 inhibition. **Conclusion:** Our first data indicate that Hsp70 in addition to Hsp90 contributes to the pathogenesis of MM. Unfortunately, a detailed analysis of Hsp70 function is currently limited due to the lack of specific inhibitors. Thus, further development of specific Hsp70 inhibitors is warranted both for a better scientific understanding of Hsp70 function and for new potential treatment modalities.

Dexamethasone induction therapy, mobilization and high-dose melphalan chemotherapy followed by autologous stem cell transplantation in patients with systemic AL Amyloidosis: An interim analysis of an unicenter prospective phase II trial

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Introduction: Amyloid light chain (AL) amyloidosis is a fatal monoclonal plasma cell disorder which leads to depositions of insoluble fibrils in different organs. Treatment with conventional chemotherapy results in low numbers of complete remission (CR). High-dose melphalan (HDM) with autologous haematopoietic stem cell transplantation (SCT) induces the highest rates of CR (about 40%) which is a prerequisite for longterm survival. An attempt to further increase CR rate might be the administration of induction therapy. **Methods:** In 2003, a prospective phase II trial has been initiated in our institution evaluating the toxicity and efficacy of dexamethasone (dexa) pretreatment. Until November 2007 27 patients (pts) with AL amyloidosis (median age 55 years; median number of involved organs 2) were included and treated with sequential therapy of 1-3 courses of pulsed dexa, mobilization chemotherapy with cyclophosphamide / adriamycin / dexa followed by HDM and auto-SCT. Exclusion criteria were age > 70 years, symptomatic heart involvement > NYHA stage II and WHO performance status > 2. **Results:** The median follow-up is 22 months (1-55) after study inclusion. In the first 5 pts 3 toxic deaths occurred during dexa. After amendment of antibiotic prophylaxis no further treatment-related deaths were observed. Further 3 pts have not been transplanted due to AL progression. Other serious adverse events

were arterial thromboses in 2 pts with nephrotic syndrome. Five pts stopped dexa due to toxicity before completing the planned 3 cycles. Haematological response was induced in 11 / 21 (52%) evaluable pts (CR n=4) following induction treatment. After SCT CR was obtained in 11 / 21 (52%) and partial remission in 9 / 21 (43%). This led to organ response (OR) in 11 / 20 (55%) evaluable pts so far. 18 out of 27 pts are alive. No pt. died of transplant-related mortality (TRM). Overall, 6 pts died due to AL progression. Median overall survival (OS) after SCT (not reached vs. 24 months, p=0.03) was significantly improved in pts achieving CR compared to pts without CR (figure 1). No relapse occurred in pts with CR. **Conclusions:** Induction therapy prior HDM is feasible and effective, but has remarkable toxicity. CR rates can be increased compared to historical controls. A low TRM can be achieved in a single institution with large experience in AL amyloidosis. HDM is in our view still the treatment of choice for pts who are eligible for this treatment.

A novel functional SNP in PSMA6 is associated with multiple myeloma and poor outcome independent of circulating proteasome serum levels

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Introduction: The proteasome system plays a crucial role in several malignant diseases, especially in multiple myeloma (MM). Recently, the serum 20S circulating proteasome level (CPL) was shown to be an independent prognostic factor in MM. A single nucleotide polymorphism (SNP) -8C>G in *PSMA6*, one of seven α -subunit genes of the 20S proteasome, was currently demonstrated to be associated with myocardial infarction. Additionally, it has been shown that *PSMA6* expression is genotype-dependently altered e.g. in human B cells, whereas the G allele is associated with a 1.8 fold higher expression. Demonstrating the extensive role of the proteasome system in MM we investigated the role of the novel SNP in our cohort of 116 patients with MM. **Methods:** DNA-samples of 116 patients with MM, all treated at the University Hospital Essen, and 125 healthy controls were genotyped for *PSMA6* -8C>G. CPL of 70 patients were studied by an anti-20S proteasome enzyme-linked immunoabsorbant assay (ELISA). *PSMA6* -8C>G genotypes were correlated with patients' survival and CPL. **Results:** Patients' genotype distribution (69 CC, 44 CG, 3 GG) and genotype distribution of healthy controls (90 CC, 31 CG, 4 GG) were consistent with Hardy-Weinberg equilibrium. Genotypes were significantly associated with MM in a dominant genetic model (CC vs. CG+GG), with an odds ratio of 1.75 (95% confidence interval (CI): 1.02-3.00, p=0.043). Kaplan-Meier curves revealed a significant association of *PSMA6* -8C>G with 5-year survival (p=0.014). Median survival time was 43 months for the GG genotype and 50 months for the CG genotype. It was not reached within follow-up by the CC genotype (CC 5-year survival rate 61.2%). Following hazard ratios (HR) were calculated: CC vs. CG: 2.007, 95%CI 1.11-3.63, p=0.022; CC vs. GG: 2.515, 95%CI 0.58-10.86, p=0.217 and in the dominant genetic model CC vs. CG+GG: 2.038, 95%CI 1.14-3.65, p=0.017. In multivariate analysis the GG/GC genotypes were independent prognostic factors (HR 2.1, p=0.014). To prove if the detected effect of individual *PSMA6* genotypes was dependent or independent from CPL, ELISA experiments were performed. There was no detectable difference in CPL between the genotypes. Mean CPL was 255 ng/mL for CC homozygous and 205 ng/mL for G allele carriers (p=0.718). **Conclusions:** These results suggest the *PSMA6* -8C>A polymorphism as a survival prognosticator as well as indicator of a high risk group within patients with MM. *PSMA6* genotypes were not associated with CPL. Therefore, the SNP is independent of this known prognostic factor and could lead to additional prognostic information for MM patients.

Myelom

Poster:

P525

Hypercalcemia as initial manifestation of multiple myeloma

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Introduction: Multiple myeloma is a disease predominantly affecting patients in their 6th and 7th decade of life. Typical clinical symptoms include recalcitrant back pain, higher disposition for infections and signs of anemia. Laboratory findings show typically an accelerated erythrocyte sedimentation rate (ESR) as well as a paraproteinemia in the serum electrophoresis. We report on a 36 year old female patient, who was diagnosed a plasma cell tumor within the diagnostic of hypercalcemia. **Case Report:** A so far healthy 36 year old female presented to the emergency ward with nausea and vomiting. She also complained about intermittent back pain for the last few weeks. Previous diseases were negated. Findings of the physical examination were, aside from mild signs of dehydration, regular. Neither the psychiatric examination showed any irregularities. Laboratory findings were a mild anemia, a distinct hypercalcemia, as well as an elevated creatinine level (table 1). Total protein and serum electrophoresis (fig.1) were without pathological findings.

Table 1. Chosen laboratory findings at admission

parameter	reference range	finding
leukocytes	4.000-10.000 / μ l	7.250
hemoglobin	12.0-18.0 g/dl	10.8
thrombocytes	150-400 *1000/ μ l	159
potassium	3,6-5,2 mmol/l	3,2
calcium	4,4-5,5 mval/l	9,6
creatinine	0,7-1,5 mg/dl	3,2
total protein	6,6-8,7 g/dl	71
ESR	< 20 mm/h	50

The x-ray of the skull showed multiple osteolysis (fig.2). Immune electrophoresis detected a bence jones protein (κ -light chain). Bone marrow biopsy gave the result of a 70% infiltration by a plasma cell tumor. After recompensation of the exsiccosis, lowering of the serum level of calcium by the use of intravenous infusions and administration of furosemide as well as *bisphosphonates*, a high dosis chemotherapy was induced, followed by autogene stem cell transplantation. Therapy is ongoing. **Summary:** The most important differential diagnosis of hypercalcemia are shown in table 2.

Fig. 1. TP53 mutation profile in CLL

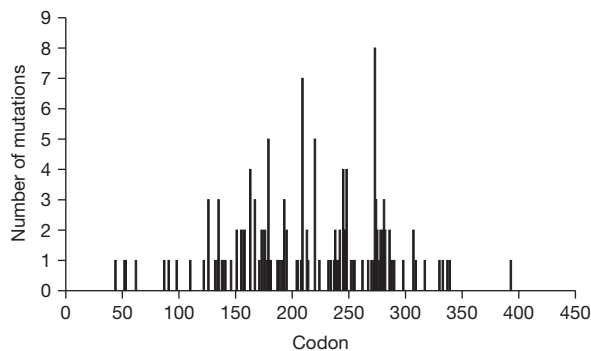


Figure 2: X-ray of the skull

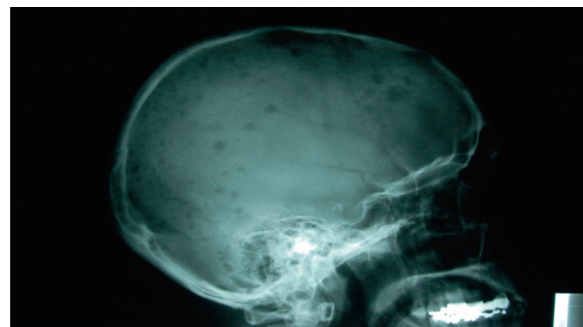


Table 2. Differential diagnosis of hypercalcemia

Malignant tumors (mainly bronchial carcinoma, breast cancer, thyroid- and renal cell carcinoma, multiple myeloma)
Paraneoplastic syndrome by parathyroid related protein
Hyperparathyroidism
Immobilization
Vitamin D-intoxication
Thiazide diuretics
Sarkoidosis

At detection of hypercalcemia there should always be performed an immune electrophoresis to exclude the multiple myeloma as possible cause.

P526

Lenalidomide (Revlimid®) maintenance therapy after high-dose melphalan and subsequent autologous stem cell transplantation

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With the introduction of the immunomodulatory effective therapeutics thalidomide and lenalidomide (Revlimid®) for multiple myeloma, the response of the disease significantly improved. In contrast to the thalidomide the structural analogon lenalidomide has a lower rate of neurotoxic and thrombotic events for at least the same clinical efficacy in this indication. In primary therapy lenalidomide is usually given in combination with dexamethasone. For a final assessment the data on maintenance therapy with this substance after subsequent remission induction are still insufficient. We report on our experience with lenalidomide in maintenance therapy in four patients (2 women, 2 men) aged 44-61 years. The first patient (light-chain myeloma, Kappa) was given a primary therapy consisting of 3 cycles VAD. Bone marrow biopsy showed a lack of response. The patient now received 3 cycles Thal / Dexa. Due to pancytopenia it was not possible to gain a sufficient quantity of CD34 positive cells for autologous stem cell transplantation. Another bone marrow biopsy resulted in the additional diagnosis of therapy-related myelodysplastic syndrome, which is why therapy with lenalidomide (5-15mg/d/21d) was initiated. In the second patient (multiple myeloma, IgG / Kappa), the initial treatment consisted of 4 cycles VAD, a low stem cell yield allowed only one high dose therapy (melphalan 200) followed by an autologous stem cell infusion. Due to constant myeloma-infiltration maintenance therapy with lenalidomide 10mg/d/21d was started. The third patient (multiple myeloma, IgG / Kappa, trisomy 11) initially received 3 cycles Thal / Dexa, autologous stem cell transplantation after prior high dose therapy with melphalan was performed according the Tandem-protocol. This resulted in bone marrow remission, but progress of osteolytic lesions led to first re-induction treatment with 6 cycles lenalidomide 25mg/d/21d, followed by a maintenance therapy with

10mg/d/21d. The fourth patient (light-chain myeloma, Kappa) received 4 cycles Thal / Dexamethasone, followed by two high dose high dose treatments with melphalan 200mg/m² and subsequent autologous stem cell rescue. With insufficient clinical and hematological response maintenance therapy was shifted from thalidomide 100mg/d to lenalidomide 10mg/d/21d. In these four cases maintenance therapy with lenalidomide resulted a sustained stabilization of disease, in the case of the patient with additional MDS even a hematological and histological remission could be achieved. In our poster, we present the clinical course of these four patients receiving maintenance therapy with lenalidomide.

P527

Cytotoxic effects and synergy analyses between multikinase inhibitor sorafenib (S) and the proteasome inhibitor bortezomib (B) in vitro: induction of apoptosis as a potentially attractive therapeutic target in multiple myeloma (MM) cells

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B is used in MM, but despite its potency, drug resistance may occur. Therapeutics may include S, a multikinase inhibitor, which has shown synergism with B in various cancer cell lines (C.Yu et al. Mol Cancer Ther 2006). The pathways being induced by S and B therefore also support their investigation in MM. S and B were tested on L363 (n=9) and U266 (n=9), cultured in RPMI1640, 10% FCS and 0.2% penicillin/streptomycin. B was used with 1, 10 and 100nM, and S with 1, 10 and 100 μM, added on day (d) 0, and compared to controls. Cell death via trypan blue and propidium iodide (PI), cell number and CD138 by flow-cytometry were determined on d 0, 3 and 6 as described (M. Zlei et al. Exp Hematol 2007). L363 as compared to U266 was growing more rapidly and showed pronounced S toxicity: median PI positivity in L363 was 7% on d0, did not increase on d3 in controls nor with 1μM S, but increased significantly to 78% with 10μM and 96% with 100μM S. On d6, this was even more impressive with median cytotoxicity in controls of 37%, whereas with 1, 10 and 100μM S, this was 33%, 84% and 92%, respectively. L363 increased from d0 to d3 a median of 9- and on d6 24-fold in controls, with 1μM S this was 5- and 24-fold, with 10μM 1.7- and 1.8-fold, and least with 100μM S both 1.4-fold, respectively. Viable L363 cells largely decreased with 10 and 100μM S, on d3 35- and 143-fold and on d6 60- and 165-fold, respectively. Correlating with these data, 138 expression was downregulated from 90% on d0 to 15% with 10μM S and 0.1% with 100μM S on d3, and to 4.5% and 0.3% on d6, respectively. The effect of S on U266 cells was similarly potent, albeit cell death with 10 and 100μM S on d3 was 2.6- and 1.2-fold decreased as compared to L363: U266 cells substantial decreased as compared to controls: this was 2.7-fold on d3, decreasing to 1.3 and 0.5 with 10 and 100μM S, and on d6 4.2 for controls, decreasing to 1.3 and 1.6, respectively. This correlated with cell viability and CD138 downmodulation. B showed a time- and dose-dependent response also. We conclude that S and B show impressive cytotoxic effects. As single agents, S was most potent with 10 and 100μM, and B with 10 and 100nM, which was true early (d3) and later (d6) in culture. Much lower concentrations should suffice for their synergism, which is currently tested as well as the protective effect of co-cultural stroma and/or cytokine support. Both agents are also analyzed in our in vivo IL2^{-/-} mouse model.

P528

Risk and clinical implications of renal impairment (RI) in multiple myeloma (MM) patients (pts): future indication for selecting most appropriate anti-myeloma therapies?

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RI has been shown to be one major risk factor in various diseases and the influence of milder RI in cancer pts is less well defined, especially not in MM. In this multicenter study, we determined RI and MM-specific prognostic factors to ascertain their difference on progression free survival (PFS) and overall survival (OS). We determined RI in 198 MM pts, receiving standard (std; n=103) or high-dose chemotherapy (n=95). Besides disease specific parameters, serum creatinine (crea) and estimated glomerular filtration rate (eGFR by Modification of Diet in Renal Disease [MDRD] and Cockcroft-Gault [CG]) were determined. MM pts reflected a typical risk profile, since their median age was 61 years (y), median BMI was normal and Satariano-index (SI) was low with 1 (range: 0-6). Despite 94% of pts had advanced disease (stage II/III by Durie&Salmon), only 31 (16%) had stage B. Their serum crea appeared normal with 0.9mg/dl, whereby the eGFR_{MDRD} revealed RI much better, which was decreased with 87ml/min/1.73m². Moderate and mild RI (eGFR_{MDRD}<60 and <90) was prominently detected in MM, in 29% and 58%, respectively. In univariate analysis, significant HRs for OS with decreasing eGFR_{MDRD} values from 1.3 with eGFR <90 to 2.9 with eGFR <30, and similarly for CG from 1.2 to 2.7, respectively, were determined, albeit HRs for PFS (HR 1.7-1.8 for eGFR_{MDRD} <90 to <30) did not increase with RI for PFS as much as for OS. In multivariate analysis for OS, relevant variables were eGFR<30, <50 and age>59y. In addition, age >59y, decreased Karnofsky index (<90%), stage B, β2-microglobulin >3.5mg/dl and std-therapy were all associated with decreased eGFR and crea (p<0.0001, Wilcoxon-test), but for the latter less pronounced. The SI did not significantly influence eGFR or PFS/OS. In conclusion, RI in MM pts is frequent, can best be detected via eGFR and is an important prognostic factor for diminished PFS/OS. Therefore, grouping pts according to eGFR allows to determine specific risk groups. Since OS is influenced by comorbidities (CI) - albeit the SI is less valuable in MM - we are currently comparing various CI scores, including a self-defined MM score. In addition, we are evaluating endothelial progenitor cells in MM bone marrow specimens, defining whether these are also influenced by RI. Further analyses should evaluate the prognostic significance of mild, moderate and severe RI via eGFR and new treatment options, which will aid to avoid RI in MM.

P529

Phenotypic characterization of primary myeloma (MM) cells. Multiparametric flow cytometry (FACS) analysis of bone marrow (BM) plasma cells (PC) from MM, MGUS and healthy donor (HD) specimens and in MM cell lines (MMCLs: L363, U266 and RPMI)

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Multiparametric FACS analysis is used to assess the expression of different receptors or antigens on mPCs. We analyzed BM aspirates of 48 MM pts, 7 MGUS pts, 3 HD and 3 MMCLs. Our objective was to improve mPC characterisation, therefore we analyzed CD38, CD138, CD45, CD56, CD126 (IL-6-R), CD221 (IGF-1-R), CD28, CD19, kappa, lambda and B-cell maturation antigen (BCMA) by 4-colour FACS. We gated both CD38-high and CD38-intermediate (int) cells and divided each into 4 quadrants A-D, according to

their CD45 + CD138 expression (A: CD45+/CD138+; B: 45-/138+; C: 45-/138-; D: 45+/138-). Mean fluorescent intensity (MFI) was determined for the quadrants A-D. Mean BM-mPCs as determined via histology, cytology and FACS were 35%, 16% and 4%, respectively. BCMA via surface- and intracellular-staining was high in all MMCLs, but much decreased or negative in mPCs of MM pts. To determine the differences between the CD38 int and high gates, both were compared in MGUS and MM pts (n=55), thereby detecting substantial differences for MFI of CD45 (gates A-C), CD138 (C+D), CD56D, CD126 (A-D), CD221 (A-D), CD19 (A-D); CD28 (A-C). Comparison between MM, MGUS and HD showed significant differences between MM vs. MGUS for CD221 int (A,C+D), CD56 int (B+C), CD28 int (B+C), CD126 int (C+D), CD19 int (C+D), and between MM vs. HD for CD38 high (%), CD38 high A, CD138 high (C+D), CD45 int (A+B), CD45 high (A-D), CD19 int B, CD19 high (B+D), CD28 high (A,B+D), CD56 high (B+C), CD126 high (B+C), CD221 high B. This suggests that for defined differences between MM and MGUS, the CD38 int gate, and for MM and HD, the CD38 high gate can profitably be used. We also determined, whether specific markers correlate with higher (>40%) BM infiltration: a positive correlation was found for CD38 int (%), CD56 int C, CD126 int (C+D), CD28 int C and CD221int D, which suggests that these gates are specifically valuable for determination of mPCs with increasing BM infiltration rates. We conclude, that 1. mean CD38 int and high cells are markedly increased in MM, elevated in MGUS and low in HD, 2. gating on CD38 int and high cells allows to more accurately evaluate mPCs, 3. for MM-MGUS differences, the CD38 int gate and for MM-HD, the CD38 high gate is most useful and 4. defined gates of increased mean MFI as described correlate with increased BM PCs. This study demonstrates important biological correlates on antigen expression profiles of mPCs.

P530

The active metabolite of the DHODH inhibitor leflunomide, A771726, inhibits multiple myeloma cell growth and proliferation at achievable plasma concentrations

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Background: Myeloma (MM) is still an incurable disease. Myeloma cells become resistant to cytotoxic drugs and patients die of disease progression. Therefore, new cytotoxic drugs are urgently needed. Inhibition of DHODH in order to inhibit cell growth of myeloma cells has never been studied before. Leflunomide is a well known inhibitor of DHODH with immunosuppressive and virostatic characteristics, and is clinically well tolerated. **Methods:** The active metabolite of leflunomide, A771726, was characterized by several assays. Induction of apoptosis was shown by annexin-V-FITC staining. Basal and cytokine stimulated cell growth rates of myeloma cells were measured by the WST-1 assay. Myeloma cell proliferation was determined by the BrdU assay. Alterations of the cell cycle were determined by flow cytometry after staining with propidium iodide. Modulation of intracellular signalling was shown by western blotting. **Results:** Clinically achievable concentrations of about 10µM to 300µM were used in this study. A771726 induced apoptosis in multiple myeloma cell lines and primary cells, as shown by the annexin V assay (H929: 73%, OPM-2: 70%, RPMI-8226: 49%, U266: 26%, primary cells: 72%). A771726 totally abrogated myeloma cell growth at 100µ-200µM. Furthermore, increased cell growth induced by conditioned medium obtained from bone marrow stromal cells was inhibited by A771726. The BrdU assay revealed that inhibition of cell growth was due to inhibition of myeloma cell proliferation (H929: -92%, OPM-2: -94%, RPMI-8226: -65%, U266: 86%). Cell cycle analysis showed that incubation of myeloma cells with A771726 induced a G1 cell cycle arrest, and this was due to time dependent down regulation of the proteins cdc25A, cyclin D2, cyclin E, pRb, and the increased expression of p21 in myeloma cells. Incubation of myeloma cells with A771726 resulted in downregulation of phosphorylated 4E-BP-1, P70S6k and BclxL and induction of Bax and Bim expression. Furthermore, A771726 shows synergistic effects when combined with melphalan, doxorubicin, dexamethasone, treosulfan and bortezomib. **Conclusions:** The DHODH inhibitor A771726 inhibits MM cell proliferation and induces apoptosis. This is accompanied by a strong inhibition cyclin D2, cyclin E, pRb, cdc25A, bcl xL and 4E-BP-1 and P70S6k phosphorylation as well as activation of p21. Since

clinically achievable concentrations of A771726 inhibit myeloma growth and proliferation, this study provides the rationale for the clinical evaluation of leflunomide in the treatment of multiple myeloma.

P531

Incorporation of the bone marker carboxy-terminal telopeptide of type-I collagen (ICTP) improves prognostic information of the International Staging System (ISS) in newly diagnosed symptomatic multiple myeloma.

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Introduction: The identification of novel prognostic factors is crucial for the current understanding of disease biology as well as for characterization of risk groups and improvement of treatment in multiple myeloma (MM). The heterogeneity in outcome can be explained by factors intrinsic to the malignant plasma cell as well as by host factors. In addition to the parameters included in the Durie and Salmon staging system, several clinical and laboratory markers, such as β2-microglobulin (β2M), C-reactive protein (CRP), plasma cell proliferative activity or cytogenetics were adopted to define high-risk patients in MM. Recently the International Staging System (ISS), including β2M and albumin, was introduced for patients with symptomatic MM. Since bone disease is a hallmark of MM, we investigated the prognostic impact of the bone resorption marker ICTP in a multivariate analysis with established prognostic markers. **Methods:** The bone resorption marker ICTP was measured by a radioimmunoassay. Other factors included in the multivariate analysis for overall survival were ISS, β2M, albumin, deletion of chromosome 13 and high-dose-therapy (HDT) in 100 patients with newly diagnosed symptomatic MM. **Results:** β2M alone, albumin alone, ISS, HDT, del(13q14) and ICTP (cut-off: normal value) were significant prognostic factors for overall survival in the univariate analysis. In a multivariate analysis, ICTP and HDT were the most powerful prognostic factors (log rank $P < 0.001$ for both, hazard-ratio: 9-fold increase and 6-fold risk reduction, respectively). ICTP clearly separated two subgroups with a good and a worse prognosis within each of the three ISS stages (ISS I: $P = 0.027$, ISS II: $P = 0.022$, ISS III: $P = 0.013$). Incorporation of ICTP in a combined ICTP-ISS score significantly ($P < 0.001$) separated four risk groups with a 5-year overall survival rate of 95%, 65%, 46% and 32%, respectively. The combined ICTP-ISS score identifies a very low-risk group (ICTP < normal value, β2M < 3.5 mg/l, albumin ≥ 3.5 g/dl) with long-term overall survival and without benefit from HDT. **Conclusions:** These data demonstrate that the inclusion of the collagen-I degradation product ICTP, as a biomarker of bone resorption, adds to the prognostic value of ISS and, in newly diagnosed symptomatic MM.

P532

Polymorphisms of the transforming growth factor beta 1 (TGFB1) gene define a subgroup of patients with late onset of disease and poor outcome in multiple myeloma

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Introduction: Common genetic variants in immune and inflammatory genes can affect the risk of developing multiple myeloma. The aim of this study was to determine the effect of polymorphic variants in the interleukin 10 (IL10), transforming growth factor beta (TGFB1) and tumor necrosis factor alpha (TNFα) genes on the predisposition to multiple myeloma and the effect of these polymorphisms on survival after treatment with chemotherapy. **Methods:** Genotype data of 7 single-nucleotide polymorphisms and clinical follow-up

information were available for 239 consecutive patients with multiple myeloma who presented at our outpatient department. All patients were treated with chemotherapy, including high-dose chemotherapy and peripheral blood stem cell transplantation in 191 patients. In particular, there were 155 males and 84 females with a median age of 55 years (range, 30 - 84). The median follow-up period for the entire cohort was 75 months (range, 3 - 190 months). **Results:** The TGFBI low producer genotype *10Leu/Leu* (n=78) was associated with a 3.5 years older age at start of treatment (57.5 versus 54 years, p=0.005), a higher level of beta2-microglobulin (3.4 versus 2.5 mg/l, p=0.01), a higher frequency of ISS-scores II and III versus I (OR=0.50, p=0.01) and an inferior median survival rate of 71.8 versus 91.6 months (p=0.02) as compared to other genotypes, respectively. In a multivariate analysis, the TGFBI low producer genotype *10Leu/Leu* was identified as an independent factor for survival (p=0.03), indicating that the poor prognosis of these patients is not due to the older age at the start of treatment. All other gene polymorphisms analyzed (*IL10 -1082A>G*, *IL10 -819C>T*, *IL10 -592C>A*, *TGFBI Arg25Pro*, *TNFα -308G>A*, *TNFα -238G>A*) showed no statistically significant effect on overall survival. **Conclusions:** Our findings suggest that patients carrying the TGFBI low producer polymorphism show a later onset of disease and need for chemotherapy treatment as compared to other genotypes. In addition, our findings indicate that TGFBI genetic variants influence prognosis in patients with multiple myeloma who received chemotherapy and suggest that genetically determined cytokine production affects the clinical course of the disease possibly through regulation of immune surveillance.

P533

Functional analysis of signaling and survival pathways in primary myeloma cells reveals novel subgroups and points to Akt as the most relevant therapeutic target

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Introduction: The IL-6R/STAT3, the MEK/MAPK, and the PI3K/Akt pathways have all been proposed to contribute to the pathogenesis of multiple myeloma (MM). However, because a systematic functional analysis of these pathways and of the consequences of pathway blockade in primary MM samples is lacking, further insights are needed to appraise their significance for therapeutic intervention. **Methods:** Freshly isolated primary MM samples (n=42) were cultured with and without presence of bone marrow stromal cells (BMSCs) and exposed to Sant-7 (abrogating function of the IL-6R/STAT3 pathway), the MEK inhibitor PD98059 or the Akt inhibitor Akti-1/2. Pathway activity was monitored by intracellular antibody staining of phosphorylated pathway components and flow cytometric detection. Apoptosis induction was measured by annexin V-FITC/PI staining. Where available, the phospho-Akt status was also determined in cognate bone marrow biopsies. **Results:** Whereas coculture with BMSCs led to stronger and almost invariable activation of the IL-6R/STAT3 and MEK/MAPK pathways, phosphorylation of Akt was not dependent on the culture conditions and was virtually undetectable in about half of primary MM samples analysed. Susceptibility against the Akt inhibitor correlated well with the phosphorylation status, i.e. P-Akt positive samples were very efficiently killed even in co-culture with BMSCs, whereas P-Akt negative cells were always much less affected. Conversely, treatment with Sant-7 and – in the large majority of cases – with PD98059 had little or no effect on the viability of co-cultured MM cells, even though both pathways were efficiently downregulated by these inhibitors. **Conclusions:** These results show that of the three pathways investigated, Akt is of paramount potential therapeutic significance and the P-Akt status may be a good predictor for sensitivity. Conversely, blockade of STAT3 and MEK/MAPK signalling may be of little or no therapeutic use. Combination experiments (synergism/antagonism determination according to Chou & Talalay) for Akti-1/2 and drugs (potentially) relevant for myeloma therapy (melphalan, doxorubicin, bortezomib, 17-AAG, SAHA) are currently being conducted and the results will be presented.

P534

Detailed analysis of six patients (pts) with concomitant chronic lymphocytic leukemia (CLL) and multiple myeloma (MM): a rare condition or more common phenomenon?

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According to the literature, the concomitant occurrence of B-CLL and MM in one pt is perceived as a rare phenomenon. With regard of the clonal relationship of CLL and MM, two hypotheses are postulated: either that each neoplasia derives independently (biclinal) or that both evolve from a single clone (monoclonal). Three different clinical sequences of the CLL/MM occurrence have been reported, that 1. both are diagnosed simultaneously (most frequent), 2. B-CLL precedes MM or 3. MM precedes B-CLL (1 case described worldwide). Interestingly, with concomitant B-CLL and MM, the former remains mostly stable and indolent, whereas MM is more aggressive. Here, we report of 6 pts with concomitant CLL and MM, diagnosed and treated at our center between 2004-2008. We determined the clinical course of both CLL and MM in all pts, aiming to decipher the clonal origin with multiparametric flow cytometry (FACS), interphase FISH and Affymetrix single nucleotide polymorphism (SNP) mapping assays. All our pts were males with advanced age (median: 72 years). According to the time point of diagnosis, three groups could be defined: in three pts, CLL preceded MM and all pts had different genomic aberrations in B-CLL and MM cells (revealed by FISH- on bone marrow smears or SNP-analyses), indicating a biclinal origin. A simultaneous diagnosis of CLL and MM was stated in two pts, showing the same genomic aberration in both cell types and strongly implying a monoclonal origin. In one case, MM was diagnosed prior to CLL without information about genetic abnormalities. All except one pt had MM stage III, while CLL remained stable and indolent in 5/6 pts (3/6 RAI 0, 2/6 RAI II, 1/6 Rai IV). Due to a more aggressive MM course, the treatment was in 5/6 focussed on MM rather than CLL. Up to now, two pts died due to distinct MM progression and the median overall survival from initial MM diagnosis was 24 months. The prevalence in our cohort of >400 MM pts - and concomitant CLL and MM - was 0.014%. We conclude that the concomitant occurrence of B-CLL and MM is a rare condition. However, it may nowadays be observed more often due to the longer disease duration, better treatment and extended survival of pts with CLL and MM. Genomic analyses suggested biclonality in 3/6 patient and monoclonality in 2/6. The enforced awareness of concomitant CLL and MM should allow identifying larger pt groups with both CLL/MM and more defined treatment options in the near future.

P535

Does scientific progress influence daily practice in multiple myeloma?

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Aim: To analyse therapeutic behaviour in multiple myeloma (MM) in Germany in 2006 with respect to previously identified prognostic markers and the use of new effective drugs. **Methods:** 500 patients (pts) with MM were analysed retrospectively. All pts presented during 1.Q 2006. Data were obtained from 66 centres constituting a representative statistical average of the medical care system (university hospitals, community hospitals, office-based haematologists). An external monitoring system assessed plausibility and correctness of the information provided. **Results:** Mean age at first diagnosis was 67 years. 60% of the pts had stage III disease (Salmon & Durie), 20% had stage II and another 20% stage I disease. Overall, 20% of the pts suffered from impaired renal function. No information about the deletion status of chromosome 13 was available in 75% of

pts. B2M was determined in 58% of all cases. First line treatment was initiated at community hospitals in 53% of pts, in 23% at university hospitals and in 24% by office-based haematologists, respectively. This distribution remained virtually unchanged throughout following lines of therapy. 60% of the pts with stage I disease received antitumour therapy, whereas 95% of pts in stage II – III were treated for MM. First line treatment was administered within a clinical trial in 16% of all cases. The MP regimen was used in 49% of pts., 30% of pts underwent irradiation of bone lesions either in addition to chemotherapy or as single modality. Autologous transplantation (autoTx) was implemented into first line procedures in 33% of pts. Of note, 45% of pts treated at university hospitals were not scheduled for autoTx in first line. The use of Bortezomib (Vel) or Thalidomide (Thal) depended on the line of treatment: First line Vel 3% , Thal 3%; second line Vel 25%, Thal 11%; third line Vel 30%; Thal 13%. **Summary:** Discrepancies to international recommendations become evident. The high number of pts treated in stage I demands further exploration. Cytogenetic / FISH analyses seem to be rather uncommon, while B2M determination is performed in only a weak majority of pts. The low number of pts treated in clinical trials may reflect inhibitory effects of current regulatory issues, but could also be due to strict inclusion / exclusion criteria. So, trials for the elderly and comorbid pts are needed. AutoTx is part of first line treatment only in a minority despite its superiority over conventional chemotherapy in pts up to 70 years of age. Finally, a notable number of pts is treated with one of the newer innovative drugs. In particular, Vel seems to be established in second as well as in third line therapies.

P536

Multiple myeloma: cIg-fish for molecular cytogenetic analysis

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Introduction: During the last decade, studies using conventional and molecular cytogenetics revealed a high incidence (almost 100%) of specific chromosomal anomalies in the malignant plasma cells (PCs) of multiple myeloma (MM), the second most common hematologic malignancy in adults. Several of these genetic changes have proven to be important independent prognostic parameters for disease course and survival. As conventional cytogenetics in MM is frequently hampered by the low proliferative activity of the clonal plasma cells (PCs), the introduction of specific molecular cytogenetic methods like cIg-FISH is of great importance in the diagnosis and follow-up of MM. **Methods:** For molecular cytogenetic analysis, mononuclear cells of MM-patients were isolated from bone marrow samples by density gradient centrifugation and subsequently immobilized onto cytospin slides. Immunofluorescent staining of cytoplasmic light chains (cIg-FISH) was used for plasma cell identification (Fonseca et al. *Blood* 100 (4), 2002). Probes for the detection of various aberrations including monosomy13/del(13)(q14), del(17)(p13), t(11;14)(q13;q32) and t(4;14)(p16;q32) represented our standard set for initial analysis. **Results:** The standard diagnostic panel revealed abnormalities in about 75% of patients. The most frequent aberrations were IgH-rearrangements (40%), -13/del(13q) (32%), and del(17)(p13) with loss of p53 (12%). Supernumerary signals indicating hyperdiploidy were seen in 20% of cases. In 32% of specimens, cIg-FISH demonstrated the concomitant presence of more than one abnormality, most commonly -13/del(13q) and IgH-rearrangements (50%). **Conclusions:** The molecular cytogenetic analysis by means of cIg-FISH is one of the two internationally recommended diagnostic methods in MM. Employment of cIg-FISH markedly improves the sensitivity of interphase FISH in cytogenetic analysis of MM at diagnosis and during the course of disease. This method facilitates specific analysis of plasma cells even in samples with very low PC count. Identification of clonal PCs by simultaneous use of cytoplasmic light chain immunostaining and cytomorphology thus provides an important diagnostic tool for the detection of prognostically relevant

genetic aberrations in MM. It can be expected that extension of the current standard panel by additional probes, some of which are commercially available, will permit the detection of diagnostically important clonal abnormalities in virtually all MM-patients.

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Feasibility of Bendamustine 180 mg/m² (d1+d2) followed by stem cell support (d4) for therapy of refractory multiple myeloma

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Introduction: Despite the development of new therapeutic agents, multiple myeloma remains an incurable disease. Therefore therapeutic options after treatment failure with conventional therapy or new drugs are needed. Bendamustine is a bifunctional alkylating agent that is not cross-resistant to other DNA-interacting substances including anthracyclines and oxazaphosphorines. It has been shown to have single-agent activity in lymphoma, myeloma, and some solid tumors. **Methods:** From September 2005 to April 2008 we treated eight patients with progressive disease not eligible for high-dose melphalan and refractory to immunomodulatory agents as well as bortezomib with high-dose bendamustine. The median patients age was 61 years (range 50-68 years). All patients were heavily pretreated (median number of previous therapies: 5.5, range 4-8) and underwent at least one course of high-dose melphalan followed by autologous stem cell transplantation. Bendamustine 180 mg/m² per day was given on day 1 and 2, followed by stem cell support on day 4. **Results:** High-dose bendamustine followed by stem cell support was well tolerated. Neutropenia was seen in five of eight patients (median number of days 12.6, range 1-30). Major complications included fever of unknown origin (FUO), nausea, and mucositis. Ileus was observed in one patient and resolved with conservative treatment. None of the patients showed life-threatening infections. The estimated overall survival is 152 days (range 45-393). **Conclusion:** High-dose bendamustine (180mg/m² d1+d2) followed by stem cell support (d4) is a feasible treatment for heavily pretreated patients including patients pretreated with the newer therapeutic agents (e.g. thalidomide, lenalidomide, bortezomib) and not eligible for or refractory to high-dose melphalan; even in progressive disease.

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Immune modulatory drugs in POEMS syndrome

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Introduction: POEMS syndrome is a rare multisystem disorder characterized by polyneuropathy, organomegaly, endocrinopathy, paraproteinaemia and skin changes. Proangiogenic and proinflammatory cytokines seem to play an important role in the pathogenesis of the POEMS syndrome. Prognosis of the disease is poor, median survivals of 33-165 months^{1,2} have been reported. The classical treatment options include mainly alkylating agent based regimes, high-dose chemotherapy with PBSCT, corticosteroids and radiation for patients with osteosclerotic lesions. To the best of our knowledge, therapy with immune modulatory drugs are described in three cases, twice with thalidomide^{3,4}, once with lenalidomide⁵. We report the case of a 52-year old man with known diagnosis of POEMS syndrome since two and a half years. The patient was treated previously with corticosteroids, cyclophosphamide and azathioprine. During treatment, a progression of the disease was observed. The bone marrow examination showed

Table for Abstract 537

Nausea	Mucositis	Neutropenia	Neutropenic fever	Blood Transfusion	Platelet Transfusion	Ileus
2/8	3/8	5/8	4/8	3/8	2/8	1/8

a patchy infiltration with atypical plasma cells. At initial presentation, severe peripheral polyneuropathy, fatigue and hyperpigmentation were the leading symptoms. After two cycles of therapy with lenalidomide 25mg per day for 21 days of a 28-day cycle and dexamethasone 40 mg once-weekly coordination, strength and fatigue showed a significant improvement. After four cycles, the patient could climb stairs without help and was able to do longer walks. The analysis six month after starting the therapy showed a normalisation of the free lambda lightchains which were eightfold elevated before therapy. As side effect we noticed a transient mild elevation of the liver enzymes and constipation. No cytopenia was observed. **Conclusions:** Lenalidomide plus low dose dexamethasone seems to be a new effective and well tolerated treatment option for patients with POEMS syndrome. The optimal duration of therapy remains to be evaluated in further studies.

References

1. Nakanishi T, Sobue I, Toyokura Y, et al. The Crow-Fukase syndrome: a study of 102 cases in Japan. *Neurology* 1984; 34:712-20
2. Dispenzieri A, Kyle RA, Lacy MQ, et al. POEMS syndrom: definition and long-term outcome. *Blood* 2003; 101: 2496-506
3. Sinisalo M, Hietaharju A, Sauranen J, Wirta O. Thalidomide in POEMS syndrome: case report. *Am J Hematol* 2004; 76:66-8
4. Kim SY, Lee SA, Ryoo HM, Lee KH, Hyun MS, Bae SH. Thalidomide for POEMS syndrome. *Ann Hematol* 2006; 85:545-6
5. Dispenzieri A, Klein CJ, Mauermann ML. Letter to the editor: Lenalidomide Therapy in a Patient with POEMS Syndrome. *Blood* 2007; 110:1075-76

P539

Primary cutaneous involvement in a patient with multiple myeloma

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Primary skin involvement is exceedingly rare in patients with multiple myeloma (MM), while sometimes observed in the setting of advanced disease. We report the case of a 62 years old female with multiple myeloma IgG lambda stage IIIA who initially presented with cutaneous lesions at the right elbow in addition to a monteggia fracture of the right and pathological subcapital humerus fractures of both sides. The skin showed a purple discoloration. First surgery of these three osteolytic bone fractures was done. The histology of the skin biopsy who was taken of the right elbow showed the infiltration of plasmacells. After induction combination chemotherapy (VAD) a palliative radiation was delivered with 39,6 Gy (SD 1,8 Gy) to involved sites. Because of infection complications we refrained from giving a second cycle of VAD but pulsed dexamethason. The disease was progressive at the extramedullary site with a red indurated exanthem of the right arm outside the radiation field. (photo) The histology of a biopsy of these lesions confirmed the exanthem as a massive progression of the skin involvement reflecting a large tumor mass. With two cycles of pulsed cyclophosphamide the skin involvement quickly regressed, but the patient died from a septic infection. Despite this unusual clinical presentation, radiosensitivity and response to chemotherapy of this disease were comparable with what may be expected in MM.

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Impact of molecular assessment of disease activity in the peripheral blood of patients with multiple myeloma undergoing high-dose therapy

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Purpose: Clonotypic myeloma cells circulating in the peripheral blood (PB) of patients with multiple myeloma (MM) may be an early indicator of relapse or may even play a role for disease progression. Therefore, we measured the

amount of clonespecific cells in the PB of patients with MM using real-time quantitative (RQ) IgH-PCR. **Methods:** During routine diagnostics 953 samples were obtained of 47 patients, who received first-line therapy for symptomatic MM. Treatment consisted of conventional induction therapy and high-dose therapy (HDT) with autologous blood stem cell transplantation followed by maintenance treatment with interferon or thalidomide or RIC allogeneic transplantation. RQ-IgH-PCR was performed with a sensitivity of 10^{-5} and the proportion of clonotypic cells was assessed as IgH/2 beta-actin ratio in percent. **Results:** A median of 10 (range: 5-24) PB samples per patient were analysed. Clonotypic cells could be detected in 42 % of samples (n=36) at the time before HDT, in 23% of samples (n=31) obtained three months after HDT and in 26% of samples obtained 3-6 months after the beginning of maintenance therapy. There was no correlation of MRD levels with clinical parameters. After a median follow-up of 61 months 28 patients (62%) had suffered from relapse while 17 patients (38%) were in remission. Sequential monitoring of clonotypic cells in PB showed that in 23 of 28 patients (82%) with progressive disease at the time of analysis, an increase of IgH/2β-actin ratio of at least one log step could be observed. This rise in circulating clonotypic cells occurred 4 months earlier (range: 1- 24) before the relapsed was diagnosed on the basis of the usual diagnostic procedures. Interestingly, clinical relapse could be predicted by IgH-PCR in all patients receiving thalidomide maintenance therapy (n= 9) whereas clonotypic cells could be detected in only 56% of patients with INF treatment before relapse(n=16, p = 0.01). Assuming that clonotypic cells precede relapse, it is tempting to speculate, that INF treatment can attenuate the rise of clonotypic cells only in some patients, whereas thalidomide therapy is able to keep down the number of clonotypic cells in most patients for at least some time and thus can prolong the duration of remission after HDT. **Conclusions:** Assessment of disease in PB using RQ-IgH-PCR can detect disease progression earlier than conventional diagnostic parameter and thus may help to guide therapeutic interventions in the future.

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Higher vascular endothelial growth factor (VEGF) and endothelial progenitors (EPCs) in multiple myeloma (MM) patients (pts) as a reflection of their governing role in pathological angiogenesis: comparison of VEGF and EPC levels between healthy donors (HD), MGUS and MM pts and correlation analysis with MM activity

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In MM pathogenesis, angiogenesis and growth factors (GFs) have a governing role. VEGF, secreted by malignant bone marrow (BM) plasma cells (PCs) and stroma, acts as one crucial factor in MM and is one important mediator of tumor angiogenesis. VEGF has been suggested as an adverse prognostic factor, being elevated in advanced and plasmablastic MM. Our objective was to study endothelial cells and other subtypes via multiparametric flow cytometry, including hemangioblasts (defined: VEGF+/CD34+), EPCs (defined: VEGF+/CD133+/CD34+) and others (e.g. CD34, CD45, CD38) in BM, peripheral blood (PB) and leukapheresis (LP) specimens, comparing these subsets and between HD, MGUS and MM pts. We analyzed BM from 8 HD, 5 MGUS and 50 MM pts. From MM pts, also 8 PB and 14 LP specimens were available. MM pts' age, BM-infiltration and serum creatinine were 62 (range: 35-84 years), 24 (0-96%) and 1.2 (0.5-8.6mg/dl), respectively. HD showed mean VEGF levels of 0.32%, which were similar in MGUS (0.18%), and 4-fold increased in MM. EPCs were elevated in MM with 0.08%, and much lower in HD (0.02%) or MGUS (0.01). VEGF and EPCs in MM subsets (BM, PB, LP) did not significantly differ (VEGF: BM: 1.39%, PB: 1.11%, LP: 1.24%; EPCs: each 0.08). In terms of other markers, CD45-/CD38+ and hemangioblasts were increased, CD38 and CD45+/CD38+ were comparable, and CD34+ and CD45+ cells were decreased in MM compared to HD BM specimens. The direct comparison of these markers in MM subsets (BM, PB, LP) showed similar values for CD34, CD45, CD38, CD45+/CD38+, CD45-/CD38+ and hemangioblasts. Of interest, EPCs and hemangioblasts were elevated in MM pts with renal impairment (RI=eGFR<60) than in those with eGFR>60. These results confirm that EPCs, hemangioblasts and VEGF+ cells are higher in MM than HD and MGUS. Lower CD34 and CD45 numbers

in MM suggest this as a result of the disease and possibly also due to anti-MM therapy. We postulate that elevated EPCs and hemangioblasts in MM may reflect disease activity and may be useful as MM biomarkers. The quantification of EPCs and hemangioblasts in MM may also be informative to monitor the efficacy of anti-angiogenic treatment. Further analyses will evaluate the prognostic significance of EPCs, hemangioblasts and other markers in MM, in pts with RI and MM, and correlate these with disease outcome. This study demonstrates important correlates on antigen expression profiles of mPCs in MM compared to HD and MGUS pts.

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V1810, a novel NFκB inhibitor, induces apoptosis and cell cycle arrest in multiple myeloma cells by downregulation of cyclin D1/2

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Purpose: Multiple myeloma (MM) is a fatal malignancy characterised by the accumulation of antibody producing plasma cells in the bone marrow. Evidence is increasing that aberrant NFκB activation plays a major role in the pathophysiology of MM and may be a promising target for new anti-myeloma therapies. In this study, we assessed the *in vitro* anti-myeloma activity of V1810, a novel NFκB inhibitor. **Methods:** MM cell lines (OPM-2, U266, NCI-929 and RPMI-8226), primary myeloma cells obtained from patients and PBMCs from healthy donors were incubated with V1810. Cell viability was measured using the WST-1 assay and apoptosis was determined by flow cytometry after staining with annexin V-FITC and propidium iodide. Cell cycle analysis was performed by flow cytometry after PI staining. NFκB activity was quantified by the NoShift transcription factor assay. Cell lysates were subjected to Western Blotting to assess modulation of intracellular signaling pathways. **Results:** V1810 potently induces cell death in all four myeloma cell lines assessed (with IC₅₀ ranging from 5μM to 10μM) as well as in primary myeloma cells. In contrast, viability of PBMCs is not affected even at very high concentration (up to 100 μM). Cell death induced by V1810 clearly shows biological features of apoptosis such as DNA fragmentation and caspase 3 cleavage. In three of four cell lines, induction of apoptosis is accompanied by cell cycle arrest (OPM2 and U266: G1-Arrest, RPMI-8226: G2-Arrest). Western blots revealed downregulation of cyclin D1 (U266) or cyclin D2 (OPM2, NCI, RPMI) respectively, but not cyclin D3. In contrast, neither protein levels of cyclin dependent kinases 2/6 nor levels of Cdk-inhibitory proteins (p16, p18, p21, p27) showed significant modulation by V1810. Consistent with downregulation of cyclin D1/2, retinoblastoma protein was found to be hypophosphorylated. Considering that cyclin D1 and D2 are known to be NFκB target genes, this is in line with our finding that V1810 inhibits base-line NFκB activity in myeloma cells (36% relative reduction). Importantly, V1810 also inhibits NFκB activation induced by cytotoxic drugs. **Conclusions:** V1810 induces apoptosis and cell cycle arrest in myeloma cells by inhibition of NFκB, downregulation of cyclin D1/D2 and hypophosphorylation of retinoblastoma protein. Further research on the *in vivo* efficacy of V1810 alone and in combination with other substances is warranted.

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The Y-box binding protein YB-1 is associated with progressive disease and mediates survival and drug resistance in multiple myeloma

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Introduction: Current knowledge about molecular mechanisms underlying disease progression and drug resistance in multiple myeloma (MM) is still

limited. The Y box binding protein YB-1 is a member of the cold shock domain protein superfamily and involved in the regulation of transcription and translation of genes. It has also been implicated in proliferation control and drug resistance of tumor cells. We therefore analyzed the potential pathogenetic role of YB-1 in MM. **Methods:** YB-1 expression was analyzed in bone marrow biopsies of MM patients by immunohistochemistry and in MM cell lines by Western blotting. To determine the role of YB-1 for survival and proliferation, siRNA technology was exploited to transiently knock down the expression of YB-1 in MM cell lines. In addition, MM cell lines were transfected with a pcDNA6-based YB-1 expression vector to transiently overexpress YB-1. **Results:** Immunohistochemical analyses revealed that neither normal bone marrow (BM) plasma cells (PCs), premalignant PCs of patients with monoclonal gammopathy of unknown significance (MGUS) nor MM cells with a mature morphology showed expression of YB-1 *in situ*. In contrast, YB-1 was strongly expressed *in situ* in normal PC precursor blasts as well as in a MM subset. Additionally, YB-1 was expressed *in vitro* in all of the evaluated MM cell lines. Phosphorylated YB-1 was detected in the nuclei of MM cells *in vitro* and *in situ*, indicating that YB-1 is activated and might act as a transcription factor. The YB-1 expressing MM cells were characterized by an immature morphology and a highly proliferative phenotype as defined by Ki 67 expression. We observed that siRNA-mediated knockdown of YB-1 decreased proliferation and induced apoptosis in MM cells even in the presence of BM stromal cells. Furthermore, we found that overexpression of YB-1 mediated resistance towards doxorubicin-induced apoptosis in MM cells. **Conclusions:** Taken together, our results show that YB-1 is expressed in immature and anaplastic MM cells, suggesting that it contributes to disease progression, survival and drug resistance and might therefore provide an attractive therapeutic target for the treatment of an aggressively growing MM subset.

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RHAMM-R3 peptide vaccination for patients with acute myeloid leukemia (AML), myelodysplastic syndrome (MDS), multiple myeloma (MM) and chronic lymphocytic leukemia (CLL): 300 versus 1000 mcg RHAMM-R3 peptide

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Introduction: RHAMM is an immunogenic antigen that is strongly expressed in several hematological malignancies and induces humoral and cellular immune responses. We initiated a phase I/II RHAMM-R3 peptide vaccination for patients with AML, MDS, MM and CLL overexpressing RHAMM. In this clinical study, patients were included with RHAMM expression but with a limited tumor load or a minimal residual disease. **Methods:** 26 patients were enrolled. The first 12 patients were vaccinated with 300 mcg and further patients with 1000 mcg R3 peptide. Only mild drug-related adverse events were observed such as erythema and induration of the skin. Immunological analysis were performed using ELISpot assays for Interferon gamma and Granzyme B, tetramer staining and chromium release assays. Moreover, regulatory T cells were quantified during vaccination. **Results:** In the first cohort, we detected specific immune responses in 70% of patients. In most patients, we found an increase of effector T cells in flow cytometry in accordance with an increase of R3-specific CD8+ T cells in ELISpot assays. In chromium release assays, a specific lysis of RHAMM-positive leukemic blasts was shown. Moreover, we measured IL-2 and IL-10 levels in sera before and after vaccination. While IL-10 levels remained at a rather low level, we detected an increase of IL-2 in most patients who showed also clinical responses. Patients with positive clinical results showed a decrease of RHAMM expression after vaccination in RT-PCR. We detected positive clinical effects in several patients with myeloid disorders showing a reduction of blasts in the bone marrow. One MDS patient did not need any longer erythrocyte transfusions. Two patients with MM showed a reduction of free light chain serum levels. In the second cohort vaccinated with 1000μg peptide, we could also found a high frequency of T cell responses. However, patients received higher doses of peptide showed no further increase of T cell responses in contrast to patients vaccinated with 300μg R3 peptide. In several patients with positive clinical effects we detected a decrease of regulatory T cells. Taken together, RHAMM-R3

peptide vaccination induced both immunological and clinical responses. **Conclusions:** Therefore, RHAMM constitutes a promising structure for further targeted immunotherapies in patients with hematological malignancies. However, higher doses of peptide do not improve the frequency and intensity of immune responses.

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Autologous Transplantation after Salvage-therapy with Lenalidomide and Dexamethasone in patients with Multiple Myeloma

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Introduction: Since more than 20 years autologous stem cell transplantation (ASCT) is established in the treatment of newly diagnosed patients (pts) with Multiple Myeloma (MM) and considered as standard of care in younger patients. Nevertheless ASCT is currently challenged by the introduction of novel agents like bortezomib, thalidomide and lenalidomide. In case of relapsed or refractory MM pts results of randomized trials evaluating ASCT after a salvage therapy are lacking. Here we describe the combination of a salvage-ASCT in combination with the introduction of lenalidomide. **Methods:** From August 2007 to April 2008 21 pts with relapsed or refractory MM were selected for the treatment with induction therapy of 3 cycles lenalidomide/dexamethasone (len/dex) followed by high dose chemotherapy and autologous transplantation at our center. At the 10th of May 11 pts were transplanted, the remaining 10 are in the part of induction therapy. Len/dex was performed with 25mg day 1-21 and dexamethasone 20mg day 1-4, 9-12, 17-20, the cycle was repeated at day 29. The dosage of lenalidomide was adjusted to the kidney function. After 3 cycles len/dex, high dose melphalan 100mg/m² day -3 to -2 was given, followed by stem cell transplantation at day 0. **Results:** Of the 11 pts being transplanted 7 achieved a very good partial remission, 2 a partial remission and 1 a minimal response after ASCT. 1 pt will be evaluated for remission within the next weeks. There was no progression during the treatment and no abnormal termination of therapy due to severe side effects. **Conclusions:** Salvage therapy with len/dex followed by high dose melphalan and autologous transplantation is a remarkable option for patients with relapsed or refractory MM with high remission rates. Therefore we planned the ReLApsE-Study (Randomized, open, multicenter phase III study with Len/dex versus len/dex followed by autologous transplantation and lenalidomide maintenance therapy for patients with relapsed MM) which will start at the end of 2008.

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Efficacy of bortezomib under routine conditions. Results of a prospective, non-interventional study

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Introduction: This observational study was aimed at assessing the efficacy and safety of bortezomib under the conditions of routine use in patients with relapsed multiple myeloma (MM). **Methods:** Patients aged ≥ 18 years with relapsed/refractory MM were included into this prospective non-interventional study. All diagnostic and therapeutic decisions were at the physicians' discretion. The observation period amounted to a maximum of 24 weeks. **Results:** A total number of 334 patients were evaluable. Another 32 patients were considered for safety analysis only due to incomplete documentation. The median age of the patients was 69 years [37-87 y]. The majority (73.7%) had an ECOG status of 0 or 1. The majority of patients (63.8 %) were in Durie-

Salmon stage 3. The median number of previous therapies was 2 [1-10]. The most common previously administered therapy was the MP regimen (39.5%), followed by VAD (33.3%). 25.2% of the patients were treated with high-dose chemotherapy. 16% of patients had been treated with bortezomib before. Most common concomitant diseases were hypertension (27% of pts), renal failure (23%), diabetes (13%) and coronary heart disease (8%). **Efficacy:** Response rate (CR + PR) was 50.6%, including 6.9% CR, 43.7% PR.; 11.1% showed MR, 14.7% SD and 9% PD. Response rates were similar in pts ≤ 70 and > 70 y of age (CR + PR in 52% and 50%, resp.). Responses occurred rapidly, and 77% of pts with CR/PR/MR achieved their best response during the first four treatment cycles. 43/169 (25%) PR and CRs occurred after cycle 4 and 10/169 (6%) even in cycle 8. **Safety:** Adverse events occurred in 270 patients. 61 out of these 270 patients (22.6%) experienced serious adverse events. Most frequent grade 3 or 4 toxicities were thrombocytopenia (5.7%), leukocytopenia (0.9%), infections (1.2%) and anemia (0.9%). Sensory neuropathy was documented in 5.6% (grade 3+4 0.7%). 21 patients died during the study, only in one patient (cerebral hemorrhage) a possible relationship to bortezomib therapy was reported. **Conclusions:** Bortezomib is an efficient treatment in ≥ 2 nd-line therapy of MM under routine conditions with similar response rates in pts below and above 70 years of age. Responses were usually observed early. Nevertheless 25% of best responses occurred after cycle 4. Safety data documented are in line with the current SmPC of Velcade. Findings of this study confirm the results of large randomized clinical trials under routine conditions.

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Iron overload in patients with multiple myeloma

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Introduction: In patients with multiple myeloma, iron overload has been implicated as a risk factor for severe infection after autologous stem cell transplantation. So far, there are no data concerning iron overload in myeloma patients in Germany, and data available worldwide are extremely scarce. Since iron overload can easily be treated using approved drugs, it becomes important to get data about the percentage of myeloma patients with iron overload and furthermore about the severity and clinical relevance of this condition. **Methods:** The concentration of serum ferritin, transferrin, and iron were quantified in 93 multiple myeloma patients. Bone marrow iron stores were evaluated by light microscopy and categorized as adequate, decreased or increased based on the amount of stainable iron. In addition, biochemical markers for myeloma activity and prognosis, number of transfusions given and severe infections within one year were documented. Statistical analyses were performed using the SPSS software. **Results:** The median serum ferritin was 157 $\mu\text{g/L}$ (range 17-5360 $\mu\text{g/L}$). In 39.8% of the patients, serum ferritin levels were above the upper normal limits (i.e. $> 200\mu\text{g/L}$ in women and $> 300\mu\text{g/L}$ in men). In 20.4% of the patients, serum ferritin values were twice the upper normal limits. Median transferrin was 2.4 g/L (range 1.3-139 g/L), and median serum iron concentration was 78 $\mu\text{g/dL}$ (10.7-214 $\mu\text{g/dL}$). Correlation with severe infections and other biochemical markers will be presented. **Conclusion:** Our study shows that iron overload is common in myeloma patients. Since iron overload can easily be treated using approved drugs, our data build the framework for iron chelation therapy in selected subgroups of patients with multiple myeloma.

Bortezomib retreatment in relapsed multiple myeloma - results from a retrospective multicenter survey in Germany and Switzerland

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Introduction: Bortezomib (Velcade) has demonstrated highest single agent response rates in anti-myeloma therapy. There are no data from preclinical studies to suggest that resistance develops from repeated treatment with bortezomib. It was therefore of interest to investigate, whether repeated bortezomib treatment is safe, feasible and efficient. **Methods:** We have retrospectively collected data from multiple myeloma patients (pts) who had previously responded to bortezomib (previous bortezomib treatment), presented with relapsed disease and who received bortezomib for a second time (retreatment). **Methods:** Treatment and retreatment during the course of this multicenter non-interventional survey (26866138MMY4014) had been on discretion of the treating physician according to prescribing information. **Results:** Data from a total of 36 centers and 91 pts were obtained: these pts had all received bortezomib and were eligible for safety analyses. So far, data from 49 pts have been analysed. Pts had a mean age of 66 (range 43-85) years and had been treated with a mean of 4 prior therapies before receiving bortezomib for the first time. Mean cycle number for previous bortezomib therapy and retreatment was 5.0 and 4.4, respectively. The majority of pts (85.7%) received doses of 1.3 mg/m² body surface area. Concomitant dexamethasone was given in 38.8% of pts with previous bortezomib treatment, and in 61.2% with retreatment. 6 pts (12.2%) received various anti-myeloma therapies between bortezomib treatment and retreatment. Efficacy data are summarized in the table below, revealing encouraging response rates for bortezomib retreatment.

Subgroup analysis according to the duration of treatment free interval (TFI) after previous bortezomib therapy demonstrated a higher response rate when preceding TFI was > 6 months. For 33 (50.8%) pts a total of 107 adverse drug reactions (ADRs) were documented, the most frequent being thrombocytopenia and peripheral neuropathy. For 6 (9.2%) pts a total of 11 SADR were documented. In 2 (3.1%) pts, these SADR were life-threatening or disabling. At the time of analysis, 21 pts had died (32.8%). **Conclusions:** This retrospective survey suggests that the safety profile of bortezomib retreatment is in line with the current SmPC of Velcade and that high remission rates can be achieved. A treatment free interval > 6 months after previous bortezomib treatment increases the likelihood of obtaining CR or PR.

	Prev. bortezomib treatment	Retreatment
Overall response rate	100% (per protocol)	63.3%
Time to response (median)	3.2 months	3.0 months
Time to Progression (median)	10.9 months	6.7 months
Duration of response (median)	6.3 months	4.5 months
Treatment free interval (med.)	6.6 months	4.1 months
	<=6 months after prev. bortezomib	> 6 months after prev. bortezomib
CR/nCR after retreatment	9.1%	18.5%
PR after retreatment	40.9%	55.6%

Appraisal of integrin linked kinase as therapeutic target in myeloma

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Introduction: The application of novel drugs has begun to translate into a significant improvement in survival of patients with multiple myeloma (MM), but the problems of relapse and resistance persist. Therefore, further exploration and validation of additional molecular targets, as well as further development of treatments are warranted. Integrin linked kinase (ILK) is a ubiquitously expressed protein whose adaptor and/or kinase functions may relay adhesion-mediated signals to growth and survival pathways. **Methods:** We have used molecular (siRNA knockdown, Western blotting, cDNA analyses) and immunohistochemical approaches to characterise ILK expression in MM cell lines and primary samples, as well as the consequences of ILK depletion in MM cell lines. This was compared to the effects of a small molecule ILK inhibitor (QLT0267). **Results:** ILK is expressed in all cell lines and in the majority of primary MM samples, yet staining of MM biopsies revealed that only a small number displayed strong signals, indicating that overexpression of ILK may not be a common requirement for MM propagation. ILK knockdown in four transfectable MM cell lines did not impair survival or proliferation, and did not affect the activation status of the Ras/MAPK, the IL-6R/STAT3 or the Akt pathway. Conversely, application of the pharmacological ILK inhibitor led to apoptosis in all MM cell lines tested (EC50 3-10 µM) and in the majority of primary MM samples, whereas non-tumour cells were hardly affected. Treatment with QLT0267 led to strong decreases in phospho-STAT3 and Akt levels, and to strong increases in phospho-ERK1/2. Caspase inhibition blocked apoptosis and voided the effects on survival pathway activity, suggesting that they were a consequence rather than a cause of caspase activation. The fast kinetics of apoptosis induction indicated that a death receptor-mediated pathway might be involved in the anti-myeloma activity of the ILK inhibitor. Accordingly, caspase-8 cleavage and synergistic apoptotic activity with Fas-ligand were observed, and we are currently investigating if clustering of death receptors in lipid rafts is induced. **Conclusion:** In summary, while ILK is unlikely to be a good therapeutic target in MM, the selective anti-myeloma properties of QLT0267 suggest that the drug may prove useful for clinical applications.

PKC412 shows strong anti-myeloma effects in in-vitro studies

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Background: The protein kinase C (PKC) inhibitor PKC412 (*N*-benzylstaurosporine) is a derivative of the naturally occurring alkaloid staurosporine and has been shown to inhibit the conventional isoforms of PKC (alpha, beta1, beta2 and gamma). PKC412 has been shown to have an antitumor effect on non-small cell lung cancer and acute leukemia with FLT3 mutations, but little is known about its effect on multiple myeloma up to date. **Methods:** Since PKC is also an inhibitor of a tyrosin kinase which is associated with VEGF, and inhibits the release of Interleukin-6 and TNF alpha, and that of growth factor dependent C-FOS, we postulated that PKC412 might have also strong anti-myeloma features. Here we evaluated the anti-myeloma effect of PKC412 in the multiple myeloma cell lines INA-6, OPM-2 and RPMI 8226 by measuring its effect on their proliferation rate, the apoptosis rate and the Interleukin-6 mRNA expression. **Results:** PKC412 showed strong anti-myeloma effects in all three celllines. 50nM of PKC412 was enough to drop the proliferation rate in all three cell lines under 10% compared to untreated cells (p<0.01). The apoptosis rate increased in INA cell line up to 2.5 times and in RPMI cell line up to 3 times (p<0.05), whereas only a moderate increase was observed in the OPM2 cell line with 500nM of PKC412. As expected, the IL-6 mRNA expression decreased also after PKC412 treatment in all three cell lines more than 50%. The addition of Bevacizumab to PKC412 in RPMI and OPM-2 cell lines did not increase the apoptosis rate significantly. **Conclusions:** PKC412

shows strong anti-myeloma effects and might be effective also in the treatment of patients with multiple myeloma. These in-vitro studies might encourage to initiate clinical trials with PKC412 in patients with multiple myeloma.

P551

Analysis of Multiple Myeloma treatment schedules from 1999-2007 at 'Donauspital' Vienna

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Introduction: Since the introduction of the Alexanian-protocol in 1962 basic treatment principles for multiple myeloma remained unchanged for more than 30 years. Autologous stem-cell transplantation (ASCT) and the tandem-transplant concept have revolutionized treatment for younger patients (1996, 2003, respectively). The advent of the new drugs Thalidomide (1999), Bortezomib (2003) and Lenalidomide (2005) has vastly expanded the armamentarium for all patient groups including those unfit for transplantation, potentially improving overall survival. Here we report data from 1999 to 2007 which show the acceptance and growing usage of new treatment modalities in the routine setting of a community-based Viennese Hospital. **Methods:** All patients with multiple myeloma presenting at our institution are documented in our electronic database ODS (Onkologisches Datensystem) on an ongoing basis since 1999. Statistical analyses were performed using R software package. Kaplan Meier method was used to analyze overall survival. **Results:** Data for 132 patients from 02/1999 to 10/2007 were available for analysis with a median observation time of 361 days. The median age at time of admission was 66 (38-89) years. Information on myeloma subtypes was available for 98 patients: 65% had IgG, 22% IgA, 2% IgM, 2% non secretory myeloma, 8% isolated plasmocytoma. 32 patients underwent ASCT with VAD as the most common induction regimen. Melphalan-Prednisone based schedules were predominantly used as first line treatment in non-transplant patients. The novel agents were quickly introduced in our treatment algorithms (1st Thalidomide in May 2000, 1st Bortezomib in April 2004, 1st Lenalidomide in July 2006). Since the first usage of Thalidomide 54 patients have received one of the novel drugs. Survival analysis could be performed on 132 patients. The median overall survival since admission in our population was 73,3 months (95% CI 52,0 – not reached). **Conclusions:** New treatment paradigms of multiple myeloma like ASCT and the novel drugs were quickly introduced into our routine treatment algorithm. This early adoption might contribute to the favorable survival data for our patient population. Kumar et al. were able to show a clear survival benefit for patients diagnosed between 2001 and 2006 compared to previous decades (1971-2000). Our survival data nicely compare to the superior outcomes for these patients in the “novel drug era”.

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Epigallocatechin-3-gallate from Green Tea Antagonizes Bortezomib but has anti-myeloma activity when used alone

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Introduction: The tumoursuppressive, antiinflammatory, and antioxidant activity of the green tea polyphenol epigallocatechin-3-gallate (EGCG) is currently being investigated in a number of clinical studies. In multiple myeloma (MM), EGCG induces cell growth arrest and apoptotic cell death via binding to the 67-kDa laminin receptor-1 (Shammas et al., Blood 108, 2006), the molecular mechanism of EGCG action, however, is not fully understood. We have investigated the effects of EGCG on malignant plasma cells to delineate potential mechanisms of its anti-myeloma activity. **Methods:** Human myeloma cell lines including the IL-6 dependent INA-6 cell line were assessed for their sensitivity to EGCG in a colorimetric (MTS) based assay and by trypanblue exclusion. The number of apoptotic and dead cells was determined by flow cytometry upon staining with annexin-V-FLUOS/7-amino-actinomycin D. Western blot analysis

was performed on whole cell lysates from INA-6 cells pretreated with EGCG for two hours before IL-6 was added. **Results:** EGCG inhibited *in vitro* growth of human myeloma cell lines in a time and dose-dependent manner. IC₅₀ concentrations at a 72 hour culture time were between 12.5 µM and 50 µM. In long-term culture assays, however, concentrations of 10 µM or even less resulted in reduced cell growth and death. Bone marrow stromal cells or overexpression of Mcl-1 and Bcl-xL could not protect from EGCG induced cytotoxicity. Pretreatment of INA-6 cells with EGCG resulted in a dose-dependent inhibition of IL-6 induced STAT3 tyrosine phosphorylation. In accordance with the essential role of STAT3 for INA-6 cell survival, EGCG induced apoptosis. In cell lines not dependent on exogenous IL-6, EGCG induced growth inhibition was abolished by pretreating the cells with catalase, an enzyme which reduces reactive oxygen species (ROS). Surprisingly, growth inhibition by bortezomib was antagonized by EGCG at low concentrations (1-10 µM). **Conclusions:** EGCG exerts growth inhibitory activity on myeloma cells through at least two mechanisms: inhibition of IL-6/STAT3 signalling and induction of oxidative stress. Notably, at pharmacologically achievable concentrations, EGCG antagonized bortezomib activity. Thus, the intake of natural polyphenols (high consumption of green tea or taking green tea extracts) may be critical during therapy with bortezomib. Our work provides the rationale for further studies to evaluate the effect of EGCG not only in B-CLL, but also in plasma cell tumors.

P553

Stimulatory potential of MAGE antigens for the generation of T-cells for active Immunotherapy in Multiple Myeloma

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Induction: Multiple myeloma (MM) is a hematological malignancy characterized by clonal expansion of terminally differentiated malignant plasma cells and is incurable by conventional therapy. In allogeneic hematopoietic stem cell transplantation (allo-Tx), however, T-cells originating from the donor have been shown to be directed against antigens expressed by MM cells, possibly leading to prolonged graft vs myeloma effect. A tumor antigen frequently expressed by MM cells is MAGE-A3, a Cancer/Testis antigen found in various tumors but silent in normal tissue. Therefore, it is a suitable target for specific immunotherapy. Spontaneous immune response against MAGE antigens are rarely observed and mostly limited to patients with unusual favourable clinical course. In MM, MAGE-specific cytotoxic T-cells (CTL) after protein vaccination are preferentially detectable following allo-Tx. Aim of the project is to induce MAGE-specific cytotoxic T-cells for adoptive transfer following allo-Tx in vitro. Due to the very low frequency of precursor CTL in the blood of healthy individuals (< 10e-7 of CD8 cells) we intend to improve MAGE antigen uptake and presentation by dendritic cells (DCs). **Methods:** We compared the antigen loading of DCs with recombinant MAGE-A3 protein or a MAGE-A3 expressing myeloma-cell line (U266). Myeloma-cells were previously γ-irradiated or treated with a proteasome inhibitor (Bortezomib) before uptake by DCs. Opsonization of U266 cells with CD138 and immune complex of rMAGE-A3 with anti-MAGE antibody are thought to enhance crosspresentation. Phagocytosis of U266 by DCs and apoptosis staining were measured by flow cytometry, rMAGE-A3 up-take was detected by Scan[^]R. **Results:** Uptake of U266 cells by DCs was superior after pretreatment with Bortezomib after 24 hours than after γ-irradiation (30Gy) regardless of opsonization with anti-CD138 antibody. An increased apoptotic population reflected the uptake of Bortezomib exposed cells (75,20±5,62 %) compared to irradiated cells (24,33±2,28 %) by DCs. Incorporation of the tumor cells dependent on the maturation of DCs was analyzed. The CD83 expression in two cytokine cocktail supplemented media were compared over 48 hours. Both types resulted in phenotypic mature DC. However, PGE2 proved superior, as poly(I:C) suppressed phagocytosis. Blocking Fcγ receptor I and III (CD16/64) resulted in decreased MAGE protein uptake. We now further want to analyze the uptake and the stimulation capacity of the opsonated MAGE antigens. **Conclusion:** Optimized phagocytosis of myeloma cells and rMAGE-A3 immune complex uptake by DC demonstrate suitable strategies to crosspresent MAGE antigens. A long-term goal would be to treat allo-transplanted MAGE expressing multiple myeloma patients preemptively with “MAGE-specific” donor lymphocyte infusion, hopefully resulting in less graft vs host disease and efficient anti-myeloma immunity.

Newly diagnosed multiple myeloma patients treated with an induction chemotherapy consisting of Bortezomib, Doxorubicin and Dexamethasone followed by autologous stem cell transplantation

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Background: Treatment for patients with symptomatic multiple myeloma eligible for autologous stem cell transplantation (ASCT) consists of an induction chemotherapy followed by high dose melphalane. Incorporation of new agents like the proteasome inhibitor bortezomib increases response rates in induction therapy significantly and may also improve the results after autologous transplantation. **Methods:** Twenty-seven patients (male: 12, female: 15) with newly diagnosed multiple myeloma received 4 cycles of bortezomib (PS-341: 1,3mg/m² on days 1, 4, 8 and 11) in combination with doxorubicin (9mg/m² on days 1-4) and dexamethasone (40mg on days 1-4, 8-11 and 15-18 during cycle 1 and days 1-4 during the following cycles). Stem cell mobilisation was performed successfully in all patients after PAD. All patients but three qualified for ASCT by achieving at least SD. One patient discontinued therapy before ASCT, another patient is waiting for ASCT. Until now, 22/27 patients underwent ASCT after conditioning with melphalane 200 mg/m². The pre-transplantation remission status (EBMT criteria) of these patients was: 2 CR, 8 nCR, 10 PR and 2 SD. Evaluation of treatment response was performed 3 months after ASCT. Maintenance therapy after transplantation with thalidomide was determined for all patients who achieved only SD or PR before ASCT. **Results:** Twenty-one patients could be evaluated. One patient did not reach the first evaluation point yet. According to EMBT criteria, 3 patients achieved CR, 12 nCR and 3 PR. One patient died of pneumonia and two patients showed progressive disease. The median follow-up after ASCT for these 21 patients is 13 months (range: 7-25). **Conclusion:** These preliminary data show that improved response rates after PAD induction may contribute to increased CR/nCR rates after single ASCT.

Neuro-Onkologie

Vortrag:

V555

Quality of life in Hodgkin Lymphoma survivors: cognitive functions of patients treated in the trials HD10-12 from the German Hodgkin Study Group (GHSG)

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Purpose: As Hodgkin Lymphoma (HL) has become one of the best curable cancers in adults long term consequences of therapy are more and more in the focus of current research. Especially health related quality of life (QoL) needs thorough investigation for its high relevance, complexity and limited knowledge up to now. QoL encompasses different aspects like general QoL, fatigue, emotional, physical, role, social, sexual and cognitive functions. We report results on QoL in the HD10-12 trials of the GHSG with special emphasis on cognitive functions (CF) and discuss methodological approaches. **Methods:** Patients of the GHSG trials HD10-12 completed the QLQ-C30, the MFI20 and some additional items at the time of diagnosis, after chemotherapy, after radiotherapy and at follow-up examinations. We describe the courses of the QLQ-C30 scales with means and 95%-confidence intervals for each measurement point and in relation to the respective norm values. In accordance with well established criteria, we used a cut off point of 10 points below the expected value to define self reported cognitive deficits (CD). The long term influence of the intensity of the chemo- and radiotherapy and socio-demographic factors like age and sex was analysed for patients with data at baseline and at 3 years follow-up. **Results:** In the sample of 3608 patients all scales showed abnormal values from the beginning, a deterioration to the end of chemotherapy and

continuous recovery from the end of radiotherapy on. Most scales improved clearly beyond their pre-treatment values but only physical functions approached normal values finally. The CF scale showed a remarkable flat long term course and, opposed to the other scales, reached no improvement beyond the pre-treatment level. In 639 patients with baseline and 3 year results, 275 met the criteria of relevant CD at baseline. Of these, 65,8% still felt handicapped after 3 years and 34,2% were in the range of normal values. 364 patients reported no CD at baseline and 74,5 % of them remained unaffected after 3 years (25,5% had developed CD) So far we detected no influence of therapy intensity on CF. Socio-demographic factors as employment, sex and age were related to CD. **Conclusion:** In this large prospective study most QoL domains are remarkably improved after recovery from HL. In contrast CD occur frequently and show virtually no improvement. Surprisingly, we found no influence of therapy intensity on CF but a relation to baseline values of the scale. Further analyses are necessary and underway to define influencing factors, to identify subgroups of patients being at particular high risk for CD, to assess the impact on patients daily life and to complete QoL data by objective CF tests. In the face of the complex interactions between the different aspects of QoL, socio-demographic factors and other patient characteristics these results will be used to develop appropriate models and intervention strategies.

Nicht maligne Hämatologie

Freie Vorträge:

V556

High relevance of hypochromic reticulocytes and ferritin index in the diagnosis of chemotherapy-associated anemia before therapy with erythropoiesis stimulating factors

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Introduction: Before therapy with erythropoiesis stimulating factors (ESF) the corresponding guidelines call for an analysis of haematopoiesis and iron metabolism, to exclude other treatable causes of anemia. Recently Thomas et al. (Clin Chem 2002;48:1066-76) combined the reticulocyte hemoglobin (Hb) content (CHr) with the C-reactive protein (CRP)-dependent ferritin (Fer) index (FI: soluble transferrin receptor/log Fer ratio) in a diagnostic plot (DP). We conducted a prospective phase II trial to analyze the diagnostic value of CHr, FI and the DP in anemic chemotherapy treated cancer patients (pts). **Methods:** Informed consenting pts with an indication for ESF therapy as per EORTC guidelines and a ferritin level > 20ng/ml were screened with the DP in a central laboratory. DP classifies pts in 1 of 4 quadrants (Q1 – Q4). Those with FI values above threshold (FI > 3.2 if CRP < 5mg/l, or FI > 2.0 if CRP ≥ 5 mg/l) were assigned to Q2 (CHr > 28 pg) or Q3 (CHr ≤ 28 pg), and those with sub threshold FI values to Q1 (CHr > 28 pg) or Q4 (CHr ≤ 28 pg). Pts in Q1 received Epoetin beta (Epo, NeoRecormon®) 30.000E/we sc., those in Q4 additionally Fe-saccharat 200mg/we iv up to 1g. Pts in Q2 + 3 were treated with oral or iv iron only. **Results:** 11 centers recruited 303 pts (median age 65 y, 59% female) from 10/04 to 10/06; 207 (68%) fell in Q1. Only 17% (50 pts = 27 Q3 + 23 Q4) had CHr ≤ 28 pg; 21% (63 pts = 46 Q2 + 27 Q3) had an increased FI, indicating relative iron deficiency. There was no correlation between assignment to Q1-4 and age, gender, body-mass-index, type or stage of tumor, measurable metastasis, bone-marrow infiltration, performance status, endogenous epo level, and haematocrit. However, Hb was lower and CRP was higher in Q4, RBC was higher in Q2+3, leucocytes and thrombocytes were higher in Q3+4. Transferrin saturation was higher in Q1. Response to treatment was analyzed concerning Hb-increase and rate of transfusions (table). **Conclusions:** Over all response rate was comparable to other studies. However the diagnostic plot identifies about a quarter of pts, which were classified in Q2/3 due to elevated FI and had a good response receiving iron only. 8% pts with hypochromic reticulocytes and a sub threshold FI (Q4) had the best Hb-response with epo + iv iron. Supported by Roche-Pharma AG, Grenzach-Wyhlen, Germany.

Table: Response to treatment

Response to treatment	Q1 Epo only	Q2 Iron only	Q3 Iron only	Q4 Epo + iv. Iron	All
Hb g/dl N / %	207/68%	46/15%	27/9%	23/8%	303/100%
Mean \pm SD dl	9.9 \pm 1	9.6 \pm 2	9.2 \pm 1.1	9.2 \pm 1	9.8 \pm 1.2
4. we	10.7 \pm 1.3	10.4 \pm 1.3	10.9 \pm 1.1	10.6 \pm 1.4	10.7 \pm 1.3
(Hb 8.we-d1 mean \pm SD	1.3 \pm 1.5	1.6 \pm 2.4	1.5 \pm 1.5	2.3 \pm 2	1.4 \pm 1.7
Median g/dl	1.1	1.5	2	2.2	1.3
Quartile g/dl	0.3-2.1	-0.1-2.4	0.1-2.6	0.3-4.1	0.3-2.4
Pts without transfusion					
Within 12 weeks	59%	81%	84%	67%	64%
\geq 5. week	78%	97%	95%	81%	82%

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Eculizumab for the Treatment of PNH: The Essen Experience

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Background: In PNH, the lack of the GPI-anchored terminal complement inhibitor CD59 on erythrocytes renders these cells susceptible to continuous complement mediated hemolysis. In previous studies, eculizumab, a terminal complement inhibitor, significantly reduced intravascular hemolysis characterized by significant reduction of LDH levels, significantly reduced transfusion requirements as well as thromboembolic events. **Aims/Methods:** PNH patients (n=15, median therapy duration=28 months, range=1-38) were treated with eculizumab as follows: 4 x 600mg IV every 7 \pm 2 days; 900mg 7 \pm 2 days later; and then 900mg every 14 \pm 2 days. Hemolysis and serum iron parameters as well as transfusion requirements were analyzed over time of treatment. Here the results of eculizumab treatment of PNH patients from a single center are reported. **Results:** Eculizumab effectively inhibited intravascular hemolysis in all PNH patients in our center characterized by an 82% decrease of LDH levels (mean \pm SD: 1923 \pm 744 to 327 \pm 155 U/l (normal range: 100-247); p=0.007) and reduced transfusion requirements. Persistent elevation of reticulocytes as well as the reduction of haptoglobin and hemopexin were observed in most patients, that suggest continuing extravascular hemolysis. Interestingly, we observed an increase of ferritin levels in treated patients (+239%; median 80 to 300 μ g/l (normal range: 20-290); p=0.001). This was more pronounced in patients still requiring some transfusions. One patient was started on oral iron depletion therapy. No thromboembolic or serious adverse events were observed in eculizumab-treated patients. Two PNH patients were diagnosed with PNH-associated hematological disease (MDS, myelofibrosis). **Conclusions:** Eculizumab is safe and well tolerated in our Essen cohort of PNH patients. Iron parameters in PNH-patients treated with eculizumab should be monitored to determine if iron supplementation should altered or iron depletion therapy considered. While some extravascular hemolysis may persist, intravascular hemolysis is effectively controlled with eculizumab and is associated with a concomitant improvement in anemia and quality of life.

V558

Safety of outpatient long term red blood cell (RBC) transfusions with leukocyte depleted RBC units

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Introduction: Long-term transfusion support is an important part in overall management of many hematological and oncology disorder. Studies addressing adverse events like iron overload, allosensitization or transmission of infectious

disease have been performed in cohorts of long-term transfusion dependent patients. However, many of these studies do not longer reflect current practices since they have been performed before adoption of a universal leukoreduction and before implementation of measures for further reduction of transfusion transmitted infections. Therefore we performed an observational single center study to reassess safety and efficacy of transfusion therapy in adult outpatients, many of which received with long term transfusion therapy. **Methods:** Analysis of patient (pt) characteristics and transfused units of red blood cell (RBC) and platelet units. Ferritin-levels, antibodies and CMV-/HBV-/HCV-/HIV –status were checked every 6 months. Therapy efficacy was evaluated by blood count and vital parameters before and after transfusion as well as by questions about clinical symptoms addressed to the patients (pts). **Results:** characteristics of the examined 247 pts.: 147 male, 100 female; median age: 65 years (17-95 years); underlying diseases: hematopoietic diseases (n= 164), solid tumors (n= 55), iron deficiency anemia (n=14), renal anemia (n= 8) and other (n=10). 48 pts had undergone stem cell transplantation (SCT), 23 pts autologous SCT, 25 pts allogenic SCT and 3 pts both. Median duration of transfusion therapy: 256 days (1-4954 days). In 82 pts chronic transfusion continued for at least 1 year. Median number of transfused RBC units: 6 (0-213). At first outpatient transfusion 118 pts were CMV-IgG positive, 74 pts Anti-HBs positive, 29 pts Anti-HBc-IgG positive, 4 pts HBV-PCR positive, 2 pts HCV-positive and 1 pt HIV-positive; 7 pts (4 without transfusion history, 6 with hematologic diseases) had erythrocyte allo-antibodies. During transfusion therapy CMV-seroconversion was observed in 4 pts (1.6%) after a median of 48 not CMV-tested blood products. One patient CMV- and HCV- seroconverted after allo-PBSCT. In 6 pts (2.4%) with hematologic disorders erythrocyte allo-antibodies were newly diagnosed (2x Anti-Kp^a, Anti-Lu^a, Anti-Wr^a, 2x Anti-C, Anti-D, 2x Anti-E). 6 transfusion reactions were observed, none was severe. In the 82 pts with RBC transfusions \geq 1 year a median ferritin increase of 666 ng/ml was observed. There was no hint for a decrease of clinical efficiency of RBC transfusions during observation interval. **Conclusions:** Our data show that outpatient transfusion therapy is safe and efficient. Alloimmunization rate was lower compared to earlier reports before universal leukoreduction. We recommend a routine HBV-/HCV-/HIV-testing before first transfusion. Ferritin should be checked routinely at least every 6 months.

Comparison of iso-haemolysin and –agglutinin testing of platelet donors in the context of the prevention of haemolytic transfusion reactions

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Introduction: Testing for iso-haemolysins (IH) in platelet donors is mandatory in Switzerland. However, the few case reports of haemolytic reactions after minor incompatible platelet transfusion are related to high iso-agglutinin (IA) titres (>1:50 or >1:100). Data about a correlation between IH and IA titres are lacking. **Methods:** Testing for IA titres and in parallel IH with 2 different methods (IH1, IH2) by tube technique were performed in consecutive platelet donors. IH1: serum dilution 1:4 with addition of AB plasma as complement source (single titre analysis); IH2: classical titration without complement source. Specificity (Spec), sensitivity (Sens), positive (PPV) and negative (NPV) predictive values of IH1 (cut off >1:4) and IH2 (cut off >1:2) in detecting or excluding IA titres >64 and >128 are calculated. **Results:** Our results show 71/188 donations (37.8%) with IA titres >64 and 34/188 (18.1%) with IA titres >128. The correlations with the specified cut offs of IH1 and IH2 are summarized in the table:

(%)	IH1/>64	IH1/>128	IH2/>64	IH2/>128
Sens	45.1	55.9	29.6	41.2
Spec	89.7	83.8	90.6	88.3
PPV	72.7	43.2	65.6	43.7
NPV	72.9	89.6	67.9	87.2

Conclusions: Despite 1/3 false positive results (low PPV), IH1 and IH2 detect only 1/2 of the donations, using the accordingly defined cut off IA titres (low Sens). Our preliminary data suggest that IH testing may be of limited significance in the discrimination of high titre IA donations. But its value in platelet donation screening will be further assessed after completion of the data collection, including analyses for additional IH cut offs, different donor ABO blood groups and donor follow up.

V560

Implementation of transfusion guidelines for Red blood cells (RBC) in elective hip and knee replacement: a prospective, multicenter, before-and-after study in 10 Swiss hospitals

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Introduction: Limited data and no general guidelines on transfusion practice in elective orthopedic surgery are available in Switzerland. Therefore we launched a Swiss study group and initiated a study, analyzing our pre-intervention transfusion practice in elective hip and knee replacement and the effects following the introduction of a straightforward guideline on RBC transfusion. We report the data of the first, observational phase. **Methods:** Prospective, multicenter before-and-after study comparing the use of RBC in adult elective hip or knee replacement before and after the implementation of a transfusion guideline in 10 Swiss hospitals. During the first 6 months (08.2007-02.2008) we monitored RBC use and patient outcomes. In a follow-

ing transition period a RBC transfusion guideline, jointly developed by the participating hospitals, was introduced and implemented. Currently we continue monitoring the data of the post-intervention period (03.-10.2008). **Results:** Preliminary data of 1168 patients are available (45.5% male, 43% knee and 57% hip replacements, median ASA physical status 2). 19.9% of the patients received a total of 595 RBC units (including 81 autologous), corresponding to a mean of 0.51 RBC units per patient. These rates varied between the hospitals (4.4%-42.4%; 0.09-1.12 RBC units per patient). Transfused patients received a median of 2.53 units/patient. In-hospital mortality and cumulative complication rate (cardiovascular, bleeding, infections) after a median hospitalization of 9 days were 0.4% and 9.3%, respectively; and after follow up (639 patients, median 48 days) 0.5% and 12.4% respectively. **Conclusions:** This first Swiss multicenter study in elective hip and knee replacement revealed a considerable variation of transfusion practice between the participating hospitals. Although we observed a lesser degree than reported for other countries, there may be a further harmonization by the introduction of a straightforward practice guideline. Our preliminary data don't show any correlation between transfusion practice and patient outcome.

V561

Thromboembolic complications after splenectomy: A rare but serious complication

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Introduction: Splenectomy is being performed for a variety of conditions, such as trauma, hemorrhage and malignant or hematologic disease. Laparoscopic splenectomy has replaced open surgery in most cases and is associated with less blood loss and quicker recuperation of the patient. Infections with encapsulated bacteria as well as bleeding are common postoperative complications. However, within a period of 6 months we observed severe (one lethal) venous thromboembolic complications (VTE) in 3 patients. Therefore we initiated this retrospective study. **Patients and Methods:** All medical records of patients who underwent splenectomy at Magdeburg University Hospital between 1994 and 2005 were carefully reviewed. **Results:** A total of 432 patients underwent splenectomy during this time period. Patients had either hematologic diseases (n = 82), visceral cancer (n = 164), traumatic injuries (n = 95), hemorrhage (n = 18), pancreatitis (n = 25) or other conditions (n = 48). There were 396 open and 25 laparoscopic surgeries, 11 procedures had to be converted intraoperatively. Venous thromboembolisms occurred in 3 patients (0.7%), all of whom had an underlying hematologic disease, namely refractory autoimmune haemolytic anemia (AIHA), immunothrombocytopenia (ITP) and marginal zone lymphoma (MZL). One patient with AIHA developed portal and mesenteric vein thrombosis after laparoscopic surgery and eventually died of multiorgan failure. The patient with ITP had pulmonary embolism (PE) as well as portal vein thrombosis that resolved promptly on oral anticoagulation following laparoscopic splenectomy. Another patient had symptomatic PE as late as 1 month after open surgery. Among patients with hematologic diseases 3.6% developed VTE following splenectomy. **Discussion:** VTE occurring after splenectomy has received little attention so far, although an increased incidence between 2-38% has been described in the literature. Our results suggest that the presence of hematologic disease makes this complication much more likely, since no VTE occurred in the other, much larger patient cohort. The occurrence after laparoscopic surgery in 2 patients suggests that this procedure does not decrease the risk for VTE. However, the number of VTE in this study is too low to compare both surgical methods. Since no recommendations exist preventive measures rely on personal experience. Presently at our institution all patients receive low molecular weight heparin until day +30 after splenectomy and imaging studies are rapidly employed if patients develop symptoms such as fever, abdominal pain or shortness of breath.