effects were constipation (10 patients WHO grade 1, 8 patients WHO grade 2), polyneuropathy (14 patients WHO grade 1, 2 patients WHO grade 2) and somnolence (4 patients WHO grade 1). None of the 23 patients developed dose-limiting hematoxicity as defined by an ANC < 1,0 Gpt/L for > 7 days or an ANC < 0,5 Gpt/L for > 3 days or platelet count < 25 Gpt/L. Short neutropenia was reported in 8 patients (WHO grade 3 and 4) but no thrombocytopenia was observed. BPT with a dose between 50 and 200 mg thalidomide daily is well tolerated in patients with relapsed or refractory MM.

0241

CANTHARIDIN, A DERIVATIVE OFBLISTER BEATLES INDUCES APOPTOSIS IN MULTIPLE MYELOMA CELLS VIA INHIBITION OF IL-6-INDUCIBLE STAT3 PATHWAY: NEW AGENT FOR SIGNAL TRANSDUCTION THERAPY OF MULTIPLE MYELOMA

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Background. Multiple myeloma remains incurable despite the use of high-dose chemotherapy with hematopoietic stem cell transplantation; therefore, novel therapeutic approaches are urgently needed in clinical settings. The understanding that has recently been gained into the biology of myeloma has led to the development of biological treatments, which target the myeloma cells and its microenvironment. These agents have shown remarkable activity against refractory myeloma in early clinical trials, but prolonged drug exposure may result in the development of *de novo* drug resistance. Therefore, the identification and validation of novel targeted therapies to overcome drug resistance and improve patient outcome are necessary. *Aims.* Previous reports suggest that IL-6 promotes survival and proliferation of myeloma cells through the phosphorylation of STAT3. Thus, compounds that suppress STAT3 phosphorylation have the potential for the treatment of myeloma. Recent studies have shown that Chinese traditional medicine cantharidin (CTD), a derivative of Blister Beatles, induces apoptosis in hepatoma, colon cancer, and leukemia cells. Therefore, we assume that CTD has a potency to induce apoptosis in myeloma cells, and may lead to a novel targeted therapeutic approach. *Methods.* To address our hypothesis, myeloma cells (U266, RPMI8226), and fresh myeloma samples from patients were treated with CTD. The effects of CTD on cell growth, apoptosis, cell cycle status, and the signaling pathway were studied. *Results.* CTD inhibited cellular growth of myeloma cells as well as freshly isolated myeloma cells from 5 patients in dose (0-10 μM)- and time (0-48h)-dependent manners with IC 50 of 4.3 μM. Cultivation with 5 μM CTD did not induce cell cycle arrest, but induced apoptosis of myeloma cells and primary cells from patients, but not bone marrow cells from healthy volunteers 24h after treatment. These results suggest that CTD-induced apoptosis is cell cycle-independent manner. Treatment with CTD induced caspase-3 activity in myeloma cells, and it was completely blocked by the pre-treatment with Z-VAD. To address the molecular mechanism of CTD-induced apoptosis in myeloma cells, we next examined the effect of CTD on IL-6 signaling pathway. CTD inhibited IL-6-induced gp130 activation in a time-dependent manner. STAT3 is a transducer of the IL-6 signaling pathway; thus we examined whether CTD could inhibit the STAT pathway. CTD inhibited phosphorylation of STAT3 at tyrosine 705 residues as early as 30 min after treatment, and down-regulated the expression of anti-apoptotic Bcl-xL. It has reported that STAT3 directly binds and activates the transcription of Bcl-xL gene promoter, resulting in the induction of the expression of Bcl-xL in IL-6-treated myeloma cells. Our results suggest that the inactivation of STAT3 and the down-regulation of Bcl-xL may contribute to CTD-induced apoptosis in myeloma cells. *Conclusions.* In conclusion, we report here for the first time that CTD induces apoptosis in various myeloma cells and primary myeloma cells in cell cycle-independent manner. Down-regulation of Bcl-xL with modulation of STAT3 in IL-6-mediated signaling pathway plays an important role in CTD-induced apoptosis in myeloma cells. Therefore, CTD is one of the promising candidates for the new therapeutic agent as a signal transduction therapy of myeloma.

0242

CITRULLINE CONCENTRATION AFTER HIGH-DOSE MELPHALAN IN AUTOLOGOUS HSCT RECIPIENTS

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Background. Mucosal damage to the intestines induced by intensive myeloablative conditioning for an allogeneic HSCT can be determined by the concentration of citrulline which is a functional marker of small intestinal enterocytes. However, there are no data available about the kinetics of citrulline levels after high-dose melphalan used to prepare for an autologous HSCT. Aims We were interested to know whether and when the citrulline concentrations declined after starting myeloablative therapy. Methods We selected 29 patients who underwent an autologous HSCT following conditioning with HDM 100 mg/m^2 HSCT day -3 & -2. We collected plasma samples from each patient via a central venous catheter at 9.00 hour on the first day of conditioning therapy and 3 times per week (Monday,Wednesday, Friday) thereafter until discharge. The samples were stored frozen until citrulline concentrations could be determined by HPLC.[1] Oral mucositis was registered using a Daily Mucositis Score. Results The baseline mean citrulline concentration was 28 mM which is lower than the 35 mM that is found normally. The mean citrulline concentrations declined rapidly thereafter reaching a nadir of 6.7 μmol/L 11 days after starting HDM which is HSCT day +7. Citrulline concentrations then only increased gradually and were still significantly low at 12 mM when patients were discharged. The most severe oral mucositis coincided with the nadir of citrulline. Conclusion Citrulline appears a valuable marker of small intestinal mucosal barrier injury induced by HDM to prepare for an autologous HSCT.

Reference

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0243

PLASMA CELL PROPIDIUM IODIDE (PC-PI/CD138) AND ANNEXIN V (PC-AI/CD138) INDICES IN MULTIPLE MYELOMA AND MONOCLONAL GAMMOPATHY OF UNDETERMINED SIGNIFICANCE EARLY PREDICTORS OF TRANSFORMATION?

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Background. Propidium iodide and annexin V indices have a close relation to prognosis in multiple myeloma (MM) and have been proved to be significant and independent prognostic factors in its evaluation. The aim of this study is to evaluate these indices also in monoclonal gammopathy of undetermined significance (MGUS) in correlation with MM, especially with stage I (D-S) to determine their importance as early predictors of transformation from benign into symptomatic phase of the disease. *Methods.* Analysed group consists of 257 patients (70 MGUS and 187 MM patients -21 st. I, 78 st. II and 88 st. III), all at the time of diagnosis, before therapy. 20 of MGUS patients were measured also in regular 6-12 month intervals to evaluate the course of MGUS. Proliferative activity of plasma cells was measured using propidium iodide index (PC-PI /CD138), rate of apoptosis using annexin V index (PC-AI/CD138), followed by method of flow-cytometry (DNA- Prep Reagents Kit, Coulter, Software Multicycle fy. Phoenix). For statistical estimation Student´s t-test, ANOVA and non-parametric Mann-Whitney test were used. *Results.* Within the evaluation of propidium iodide and annexin V in MGUS and MM there was a significant difference between the values of both indices ' in MGUS the values of proliferation were lower (M: 1,9%) than in MM (M: 2,6%, p<0,0001) and the values of apoptosis were higher (M: 7,45% vs 4,6%, p<0,0001). If we depicted only stage I MM there was also statistically significant difference in proliferation when comparing with MGUS (M: 1,9% vs 2,3%, p=0,016). In apoptosis we found higher values in MGUS (M: 7,45% vs 6,2%), however not statistically significant (p=0,121). In next step we tried to analyse differences in PC-PI and PC-AI within the stages of MM. The corresponding medians of PC-PI were for stage I, II and III (D-S) values 2,3%, 2,6% and 2,75%, none of them being significant (p=0,539). For apoptosis the

results were similar - for stage I, II and III values 6,2%, 4,9% and 4,3%, p=0,196. Finally we compared the values of PC-PI and PC-AI within the course of 20 MGUS patients - there was no statistical significance between the values, either. *Conclusion.* Our measurements support the hypothesis of PC-PI and PC-AI being independent prognostic factors and also the indicators of early transformation of MGUS into MM. Within the course of MGUS there exists no significant change in either of the indices, however, when transforming into symptomatic multiple myeloma, there is a significant increase in PC-PI together with decrease of PC-AI. The above results also confirm the major importance of proliferation in the process of transformation into MM - there is significant difference even between MGUS and stage I MM, on the other hand, decrease in apoptosis is probably not the fundamental part of this process. Measurement of proliferation and apoptosis contributes to the assessment of MM prognosis, and plays also a prominent role in the evaluation of the course of MGUS, especially as an early predictor of transformation into multiple myeloma.

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0244

EPIDEMIOLOGY OF ANEMIA IN 720 PATIENTS WITH MULTIPLE MYELOMA: RESULTS FROM EUROPEAN ANAEMIA SURVEY

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Background. Although anemia is a common complication of multiple myeloma (MM) patients (pts), information on the evolution of anemia during follow up, relation with age and performance status, risk factors for anemia and treatment practices was not available. *Aims.* Identify the incidence, prevalence and evolution of anemia during an up to 6 months follow up period, analyse possible correlations between anemia and clinical characteristics, identify risk factors for evolution of anemia (in pts with myeloma and lymphoma) and study patterns of anemia treatment in European myeloma pts. *Methods.* 720 patients with multiple myeloma (male 52% and female 48%) were enrolled into a prospective, epidemiologic survey, ECAS (European Cancer Anemia Survey), which included an additional 1640 pts with lymphoma (L) and a total of 15,370 pts with cancer at any stage of their disease. Survey data were collected for up to 6 data points or 6 months of scheduled visits (Ludwig, EJC 2004; 40 (15): 2293-2307). *Results.* Median age in MM pts was 65.7 years (range 31-94), with 28% of patients presenting with age <60 years (yrs), 32% with age 60-69 yrs, and 40% with age ≥70 yrs. 28% of the 720 pts with MM were newly diagnosed; 55% had persistent/recurrent disease and 17% were in remission. In terms of cancer treatment, 50% were receiving chemotherapy (CT), 46% were not receiving any cancer treatment, with the remainder receiving radiotherapy or concomitant CT and radiotherapy. At enrollment, 69% of patients were anemic (Hb <12g/dL), 30% had Hb < 10 g/dL and 39% Hb of 10 to 12 g/dL. 85% were anemic at some time during the survey. 78% of those <60 yrs, 85% of those 60-69 yrs and 90% of those 70+ were ever anemic. 44% had a WHO score of 2-4. The incidence of anemia in MM who were not anemic at enrolment and who started CT during ECAS was 75%. Incidence of anemia increased with increasing age (60% in pts < 60 yrs, 88% in those 60-69 yrs and 100% in those 70+). Adverse WHO score correlated with low Hb (r=-0.346). Despite the 59% of those who became anemic having a nadir Hb < 10 g/dL, 41% received no anemia treatment, 2% received iron, 22% transfusion and 35% received epoetin. Logistic regression analysis of MM/L pts revealed 4 variables significantly predicting anemia development: Initial Hb (adjusted odds ratio (AOR) 4.2), persistent/recurrent disease (AOR 1.5), female gender (AOR 2.8), and treatment with platinum-based CT (AOR 5.5) were found to independently predict anemia (p<0.001). *Conclusions.* Frequency of anemia in MM pts remains substantial and important: prevalence of anemia (ever anemic) was high (85%) in MM pts, increased with age and correlated with poor PS. Follow up during the 6 month post-enrollment period indicated that 75% of initially non-anemic pts developed anemia after starting CT. Anemia treatment was given to 41% of ever anemic MM pts, although 59% had at least once Hb levels <10g/dL. With the identification of important risk factors, anemia management in MM pts could be improved.

0245

A PHASE I/II STUDY OF ARSENIC TRIOXIDE, BORTEZOMIB, AND ASCORBIC ACID IN RELAPSED OR REFRACTORY MULTIPLE MYELOMA

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Background. Arsenic trioxide (ATO), a trivalent arsenite salt, is believed to exert its cytotoxic effect by causing DNA fragmentation characteristic of apoptosis. Clinical studies have shown that ATO has antitumor activity as a single agent in patients with relapsed or refractory multiple myeloma (MM). Bortezomib (B) is a proteasome inhibitor that is currently approved for the treatment of relapsed or refractory MM. Preclinical studies have shown that combining ATO and B results in synergistic antitumor activity against human MM cells in tissue culture and xenograft animal models. Furthermore, the addition of ascorbic acid (AA) can sensitize human MM cells to the cytotoxic effects of ATO. These observations suggest that the combination of ATO/B/AA may be an effective treatment regimen for patients with MM. *Aims.* The primary aim of this study was to determine the safety and tolerability of the ATO/B/AA regimen in patients with relapsed or refractory MM. The secondary aims were to determine overall response rate, time to response, time to progression, progression-free survival, and overall survival in these patients. *Methods.* Patients with relapsed or refractory MM were enrolled in this Phase I/II dose-escalation trial in 6 cohorts. Patients were given ATO (0.125 or 0.250 mg/kg), B (0.7, 1.0, or 1.3 mg/m^2), and a fixed dose of AA (1000 mg) IV on days 1, 4, 8, and 11 of a 21-day cycle for a maximum of 8 cycles. *Results.* At the time of this interim analysis, 22 patients (median age, 63 years) have been enrolled, and accrual has been completed on all cohorts. This group had failed a median of 4 (range, 3-9) prior therapies. One occurrence of grade 4 thrombocytopenia was observed. One occurrence of asymptomatic arrhythmia led to patient withdrawal. All other adverse events were grade 1 or 2. For the 21 patients evaluable for efficacy, objective responses were observed in 9 patients (43%), including 2 complete (CR; 10%), 2 partial (PR; 10%), and 5 minor (MR; 24%) responses. Only 1 (1 MR) of 6 patients receiving the lowest dose of B (0.7 mg/m^2) showed a response, whereas 4 (1 CR and 3 MR) of 6 patients receiving the middle dose of B (1.0 mg/m^2) responded, and 4 (1 CR, 2 PR, and 1 MR) of 9 patients receiving the highest dose of B (1.3 mg/m^2) responded. *Conclusions.* The ATO/B/AA regimen was well tolerated by the majority of patients and produced objective responses in 43% of the patients in this heavily pretreated group. Eight of 15 patients receiving the higher doses of B had clinical responses to this regimen. The results of this Phase I/II study warrant further clinical evaluation of the ATO/B/AA combination regimen for the treatment of patients with relapsed or refractory MM.

0246

PREVALENCE OF RAS GENE MUTATIONS IN THE CONTEXT OF A MOLECULAR CLASSIFICATION OF MULTIPLE MYELOMA

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Background. Earlier studies have reported that activating mutations involving *RA* genes, in particular *NRAS* and *KRAS*, occur frequently in multiple myeloma (MM). The reported prevalence of mutated tumors varies from 10 to 40% at presentation, rising to 70% at relapse, suggesting a role of this lesion in tumor progression. Notably, the occurrence of such mutation in MGUS and indolent tumors is very low. Mutations of *KRAS*, but not *NRAS*, have been found to be associated with higher bone marrow burden and shorter survival. *Aims.* In the present study we investigated the prevalence and type of *RAS* mutations in MM in the context of a proposed molecular stratification, named as *TC classification*, based on the presence of IGH translocation and dysregulation of cyclin D genes in MM. *Methods.* The presence of *NRAS* and *KRAS* gene mutations was investigated in a panel of 82 MM at diagnosis, 13 patients with extramedullary myeloma or plasma cell leukemia, 9 patients with