9230 POSTER

Allogeneic stem cell transplantation induces high-titered antibody responses to cancer testis antigens in multiple myeloma patients

S. Kobold[1], Y. Cao[1], S. Tams[1], B.M. Bartels[1], C. Eberhard[1], T. Lütkens[1], C. Pabst[1], C. Bokemeyer[1], N. Kröger[1], D. Atanackovic[1]. [1]*Universitätsklinikum Hamburg-Eppendorf, Department Of Oncology Hematology Stem Cell Transplantation With The Section Pneumology, Hamburg, Germany*

Background: We set out to perform the first longitudinal analysis of antibody responses against a variety of cancer testis (CT) antigens in multiple myeloma patients undergoing allogeneic stem cell transplantation. This study was conducted to answer the question if and under which conditions humoral responses against CT antigens would occur in myeloma patients.
Methods: Antibodies directed against CT-antigens (MAGE-C2/CT10, MAGE-A1, MAGE-A3, MAGE-A4, MAGE-A8, MAGE-A11, SSX-2, SSX-4, NY-ESO-1, PRAME) were analyzed by an ELISA using full-length recombinant proteins. The specificity of those antibodies was confirmed by Western blot analysis. An epitope analysis was performed with 20 mer peptides for each of the CT-antigens. Furthermore, IgG subtype-assessment was undertaken.
Results: We screened 1100 sera of 194 patients with multiple myeloma at different time points for antibody responses against the 10 CT-antigens. The sera of 100 healthy volunteers were used as controls. Importantly, we identified a number of patients with significant titers against CT antigens, some of them consistently showing high-titered responses. Remarkably, none of the samples pre-allogeneic transplantation was positive for NY-ESO-1 nor SSX-2 antibodies. In contrast, allogeneic stem cell transplantation was able to induce high-titered and highly specific antibody responses against NY-ESO-1 or SSX-2 in three and two myeloma patients undergoing allogeneic stem cell transplantation, respectively. Interestingly, antibody responses were consistently directed against a limited number of epitopes. The range of epitopes recognized by those antibodies changed over time, but remained confined to the same group for all patients. Those antibody responses were predominantly of the complement activating subtype IgG1 and to a lower extent IgG3.
Conclusions: We show in this first comprehensive and longitudinal analysis of humoral responses against CT antigens in myeloma that antibody responses occur and often persist after allogeneic stem cell transplantation. Our IgG-subtype results suggest the ability of those antibodies to mediate complement activated cytolysis. Preleminary data indicate that this antigen-specific immune effect might improve the prognosis of these patients. We plan to further investigate such immune responses on the T cell level and we will analyse if the stimulation of the adaptive immune system is able to reverse the negative effect of CT antigen expression on the patients' prognosis.

9231 POSTER

An optimized memory B cell assay revealing CT antigen-specific B cells in myeloma patients

Y. Cao[1], M. Gordic[1], T. Luetkens[1], S. Meyer[1], K. Bartels[1], Y. Hildebrandt[1], C. Bokemeyer[1], N. Kröger[2], D. Atanackovic[1]. [1]*Med. Klinik Und Poliklinik Uni-klinikum, Oncology And Haematology, Hamburg, Germany;* [2]*Med. Klinik Und Poliklinik Uni-klinikum, Stem Cell Transplantation, Hamburg, Germany*

Background: Immunotherapies using cancer-testis (CT) antigens as targets represent a potentially useful treatment in patients with multiple myeloma (MM) who commonly show recurrent disease following chemo-therapy. Surprisingly, until now frequency and function of CT antigen-specific B cell in cancer patients have not been analyzed. In our current study, we developed an optimized memory B cell ELISPOT assay for the quantification of CT antigen-specific B cells in MM patients.
Methods: B cells specific for tetanus toxoid (TT) influenza virus (Flu), cytomegalovirus (CMV), and CT antigen NYESO-1 were quantified in patients with MM and normal donors. Peripheral blood or bone marrow mononuclear cell (*PBMNC*/*BMMNC*) were incubated in B cell medium with different combination of Pokeweed mitogen (PWM), Staphylococcus aureus Cowan I (SAC), CpG ODN-2006, CD40 ligand (CD40L), IL-2, IL-10 and IL21 for 3 to 6 days. The stimulated cells were then used in an IgG- enzyme-linked immunospot (ELISPOT) read-out assay. Supernatant from the culture was applied for enzyme-linked immunosorbent assay (ELISA) to detect antigen specific antibodies produced upon stimulation. The proliferation and differentiation of memory B cells was monitored by the fluorometric assay.
Results: After 6 days of stimulation, CD40L, CpG ODN-2006 and IL21 were shown the strongest combination to induce TT-specific antibody-secreting B cells ($P < 0.05$). When we applied isolated B cells from stimulated PBMC to an ELISPOT assay, we yielded more specific spots and less background. Analyses by flow cytofluorometry demonstrated that after 6 days' stimulation, a group of B cells were expanded, which were $CD19^{intermediate}$ $CD27^{high}CD38^{+}IgD^{-}$ plasma cells. Applying our optimized assay, we could also determine the frequency of B cells specific for other antigens such as Flu and CMV. Most importantly, we were also able to detect for the first time NY-ESO-1 specific memory B cells in the peripheral blood of MM patients post allogeneic stem cell transplantation.
Conclusion: Here, we optimized a quantitative assay for the determination of antigen-specific B cells in different human tissues showing that the combination of CD40L, CpG ODN-2006, and IL21 is ideal for the induction of antibody-secreting B cells. Applying this methodology to patient material we demonstrated for the first time the existence of CT antigen-specific memory B cells in the peripheral blood of myeloma patients.

9232 POSTER

A phase Ib multicenter dose escalation study of carfilzomib plus lenalidomide and low-dose dexamethasone in relapsed multiple myeloma – preliminary results

R. Niesvizky[1], M. Wang[2], B. Bensinger[3], M. Vallone[4], A.A. Gutierrez[5], M. Kauffman[5]. [1]*Cornell University, Weill Cornell Medical College, New York, USA;* [2]*University of Texas, MD Anderson Cancer Center, Houston, USA;* [3]*Fred Hutchinson Cancer Research Center, Clinical Research, Seattle, USA;* [4]*Proteolix Inc., Clinical Operations, South San Francisco, USA;* [5]*Proteolix Inc., Clinical Development, South San Francisco, USA*

Introduction: Carfilzomib (CFZ) is a highly specific proteasome inhibitor with single agent activity in relapsed multiple myeloma (MM) (ASH 2008). The purpose of this study is to evaluate the safety and activity of CFZ in combination with lenalidomide (LEN) and low-dose dexamethasone (loDex).
Methods: This phase Ib trial evaluates 4 dose levels ($\geqslant$3 pts each) to define the maximum tolerated dose (MTD) of CFZ/LEN/loDex in relapsed MM pts who failed 1−3 prior therapies, including prior LEN or bortezomib (BTZ). CFZ IV 15−20 mg/m^2 (d1, 2, 8, 9, 15, 16), LEN 10−20 mg po (d1−21) and loDex 40 mg po (d1, 8, 15, 22) in 28-day cycles (C). An additional 10−15 pts will be evaluated at the highest dose level reached. Dose limiting toxicity (DLT) has been defined as grade (G) $\geqslant$3 non-hematologic; G4 neutropenia for > 7 d and/or neutropenic fever; G4 thrombocytopenia >7 d or G3-G4 thrombocytopenia in association with bleeding. Overall response (CR/sCR, VGPR/PR) is assessed by IWG criteria, with secondary assessment by modified EBMT criteria which includes MR.
Preliminary Results: 20 pts have been enrolled. 18/20 are evaluable for response and toxicity. Median prior lines of therapy was 2.5 (range 1−3). Prior therapies included DEX (18/18), BTZ (14/18), LEN (14/18), stem cell transplant (14/18), alkylators (11/18), thalidomide (7/18) and anthracyclines (6/18); 12/18 pts had received both LEN and BTZ. MTD has not yet been reached after the first 3 dose cohorts. No drug related SAEs, G3/4 treatment emergent AEs or neuropathy were reported. Two cases with transient lenalidomide-induced rash have been reported. Responses to date with a median of 4 cycles (range 1−9) are shown below. Responses were rapid and occurred within the first 28-day cycle.

		N	VGPR	PR	MR	SD	PD
Cohort 1	CFZ 15/LEN 10	6		3	0	2	1
Cohort 2	CFZ 15/LEN 15	6	1	1	2	0	2
Cohort 3	CFZ 15/LEN 20	6	2	2	1	1	

Conclusion: CFZ/LEN/loDex in combination was well tolerated in the first 3 cohorts. The combination has achieved early encouraging responses in pts who had failed both LEN and BTZ at doses well below the single agent MTD of either LEN or CFZ. Dose escalation is ongoing. Updated data will be presented at the meeting.

9233 POSTER

The angiogenesis-related polymorphisms: roles for the risk of multiple myeloma

E.W. Faber[1], G.J. Lourenco[1], M.M. Ortega[1], P.M.R. Silva[1], C.A. De Souza[2], I. Lorand-Metze[2], C.S.P. Lima[1]. [1]*Laboratory of Cancer Genetic, Departament of Internal Medicine - Faculty of Medical Sciences - State University of Campinas, Campinas SP, Brazil;* [2]*Departament of Internal Medicine, Faculty of Medical Sciences - State University of Campinas, Campinas SP, Brazil*

Background: Angiogenesis (AG), which seems to be influenced by the *VEGF*, *GSTM1* and *GSTT1* polymorphic genes, is a crucial step in

multiple myeloma (MM) development. The wild allele of the *VEGF* C936T polymorphism and the *GSTM1* undeleted genotype were linked to a higher angiogenic phenotype compared to the respective variant genotypes in few reports, but their roles in MM is unclear. We tested herein whether the *VEGF* C936T, *GSTM1* and *GSTT1* genotypes altered the risk for MM.
Material and Methods: Genomic DNA from 117 MM patients (mean age: 55; range: 29−86; 95 Caucasians; 22 African-Americans; 55 females; 62 males) and 150 controls (mean age: 53; range: 25−60; 125 Caucasians; 25 African-Americans; 73 females; 77 males) was analysed by PCR-RFLP or multiplex-PCR.
Results: Patients' and controls' samples were in Hardy-Weinberg equilibrium for *VEGF* C936T ($\chi^2 = 0.35$, $P = 0.55$; $\chi^2 = 0.25$; $P = 0.61$). An excess of the undeleted *GSTM1* was seen in patients compared to controls (65.0% *vs* 52.0%, $P = 0.02$). Carriers of the *GSTM1* gene were under a 1.78-fold (95% CI: 1.07−2.96) increased risk for MM than others. Similar frequencies of the undeleted *GSTT1* (73.5% *vs* 79.3%, $P = 0.29$) and the undeleted *GSTM1+GSTT1* (49.6% *vs* 40.7%; $P = 0.86$) were seen in patients and controls. Individuals with the distinct genotypes of the *GSTT1* gene (OR = 0.73; 95% CI: 0.41−1.30) and the *GSTM1* and *GSTT1* combined genes (OR = 1.08; 95% CI: 0.46−2.52) were under similar risks for disease. Similar frequencies of the *VEGF* CC (76.9% *vs* 73.3%, $P = 0.51$) and the CC+CT genotypes (99.1% *vs* 97.3%, $P = 0.28$) were seen in patients and controls. Individuals with the CC (OR = 1.21, 95% CI: 0.68−2.15) and CC+CT (OR = 3.38, 95% CI: 0.36−31.55) genotypes were under similar risks for MM than others. Moreover, similar frequencies of the *GSTM1+GSTT1+VEGF CC* (41.0% *vs* 32.7%, $P = 0.86$) genotypes were found in patients and controls. Carriers of the *GSTM1+GSTT1+VEGF* CC (OR = 1.08, 95% CI: 0.45−2.61) combined genotypes were under similar risks for MM than others.
Conclusions: Our data suggests that the *GSTT1* and *VEGF* genotypes do not influence the risk for MM. However, the presence of *GSTM1* gene is associated with increased risk for disease in Brazilians. Additional studies about vascular microdensity in tumor samples from distinct genotypes individuals, as well as protein function studies, will elucidate if increased risk for disease results from stimulant effect of *GSTM1* gene into AG. Financial support: CNPq.

9234 POSTER

Clinical experience with vorinostat: collated safety and tolerability data from patients with solid or hematologic malignancies

D. Siegel[1], P.N. Munster[2], M. Iwamoto[3], S. van Belle[4], M. Hussein[5], C. Belani[6], F. Robert[7], E. Galanis[8], S. Rizvi[9], E.H. Rubin[10]. *[1]Hackensack University Medical Center, John Theurer Cancer Center, Hackensack NJ, USA; [2]H. Lee Moffitt Cancer Center, Medicine / Oncologic Sciences, Tampa FL, USA; [3]Merck & Co. Inc, Clinical Pharmacology, Upper Gwynedd PA, USA; [4]University of Ghent, Internal medicine, Ghent, Belgium; [5]H. Lee Moffitt Cancer Center, Malignant Hematology Program, Tampa FL, USA; [6]Penn State Cancer Institute, Penn State College of Medicine, Hershey PA, USA; [7]University of Alabama, Comprehensive Cancer Center, Birmingham AL, USA; [8]Mayo Clinic and Mayo Foundation, Oncology, Rochester MN, USA; [9]Merck & Co. Inc, Oncology Clinical Research, Upper Gwynedd, USA; [10]The Cancer Institute of New Jersey, Department of Medicine, New Brunswick NJ, USA*

Background: Vorinostat is an orally active histone deacetylase inhibitor approved in the United States for the treatment of cutaneous manifestations of progressive, persistent or recurrent cutaneous T-cell lymphoma (CTCL), and has been widely investigated in other malignancies in a clinical trial program.
Methods: Safety and tolerability data were collated from patients who received vorinostat as monotherapy or in combination therapy for solid or hematologic malignancies in Phase I and II trials.
Results: Collated safety and tolerability data are available for 341 patients who received vorinostat monotherapy (107 CTCL, 105 other hematologic malignancies, 129 solid tumors) and 157 patients who received vorinostat combination therapy (with pemetrexed/cisplatin for advanced cancer [n = 46], bortezomib for multiple myeloma [n = 34], bexarotene for CTCL [n = 23], and erlotinib [n = 30], gemcitabine/platin [n = 21] or carboplatin/paclitaxel [n = 3] for non-small-cell lung cancer). With monotherapy, common drug-related adverse events (AEs) were fatigue (61.9%), nausea (55.7%), diarrhea (49.3%), anorexia (48.1%), and vomiting (32.8%); Grade 3/4 AEs included fatigue (12.0%) and thrombocytopenia (10.6%), and 3 drug-related deaths (ischemic stroke, tumor hemorrhage, unspecified) occurred. Thirty-eight patients (11.1%) discontinued due to drug-related AEs, 71 patients (20.8%) required dose modifications, and 1 patient (0.3%) discontinued due to Grade 2 chest pain. With combination therapy, common drug-related AEs were nausea (48.4%), diarrhea (40.8%), fatigue (34.4%), and vomiting (31.2%); the most common Grade 3/4 AE was fatigue (13.4%), and 1 drug-related death (hemoptysis) occurred. Thirty-one patients (19.7%) discontinued due to drug-related AEs and 27 patients (17.2%) required dose modifications. In 24 patients with advanced cancer, a single supratherapeutic 800 mg dose of vorinostat did not prolong the QTcF interval (monitored over 24 hours). The upper limit of the 90% confidence interval for the placebo-adjusted mean change-from-baseline of vorinostat was 30 msec, and 1 patient had a QTcF interval >450 msec (after both vorinostat and placebo administration).
Conclusions: Vorinostat was generally well tolerated, with the majority of AEs ⩽Grade 2 and no prolongation of the QTc interval observed, when administered as monotherapy or in a combination regimen in cancer patients.

9235 POSTER

First-line therapy for patients (pts) with newly diagnosed multiple myeloma (MM) ineligible for stem cell transplantation (SCT): a systematic review and meta-analysis (Hemo-ONCOLGroup Study)

M.L. Rodríguez[1], J.F. Combariza[1], C.P. Casas[1], C. Alarcón[1], L. Reveiz[2], J. Buendía[2], A. Martí-Carvajal[3], A.F. Cardona[4]. *[1]Instituto Nacional de Cancerología E.S.E. (INC), Hematology and Bone Marrow Therapy Department, Bogotá, Colombia; [2]National University of Colombia, Clinical Research Institute and Health Technology Assessment Unit, Bogotá, Colombia; [3]University of Carabobo, Clinical Epidemiology and Public Health Unit Iberoamerican Cochrane Network, Valencia, Venezuela; [4]Catalan Institute of Oncology Hospital Germans Trias i Pujol, Medical Oncology, Barcelona, Spain*

Background: Treatment for newly diagnosed MM is predicated on eligibility for SCT. Pts not eligible for SCT have been treated with melphalan (M) plus prednisone (P); however, the standard of care has shifted to MP plus thalidomide (T) due to its survival benefit. Bortezomib (B) and lenalidomide have emerged as effective agents.
Methods: Randomized clinical trials (RCTs) were identified from the Cochrane Library, PUBMED, LILACS, EMBASE and Scirus. Only RCTs comparing MP vs. any other regimen were considered in the analysis.
Results: 22 RCTs were included from 2.159 potentially eligible references. *MP vs. M+dexamethasone (MD):* 3 RCTs. No differences were found between the two combinations in overall survival (OS), complete response (CR), or hematological toxicity. MD was superior in partial response (PR) (RR 1.54, 1.32−1.80; I2 = 17%) and non-hematological toxicity (RR 2.15; 1.36−3.41; I2 = 42%). *MP vs. T regimens:* 4 RCTs. Significant differences favoring T regimens were found in CR (RR 3.44; 1.86−6.39; I2 = 53%) and PR (RR 1.67; 1.28−2.17; I2 = 72%) Although the meta-analysis of 3 RCTs showed a significant difference in OS favoring T regimens (HR 0.79; 0.66−0.96; $I^2$86%), heterogeneity was high. Progression-free survival (PFS) was superior in the T group in 4 RCTs; estimated PFS at 24 mo. was 41% and 48% in pts treated with TD and MP, respectively (p = 0.02). A significant difference was found in non-hematological toxicities (RR 0.79; RR 2.14 1.80−2.55; I2 = 0%). *MP vs. B regimens:* 1 RCT. Significant differences in OS (HR 0.61; 0.42−0.89), TTP (HR 0.48; 0.41−0.56), CR (RR 8.35; 4.68−14.89) and PR (RR 1.30; 1.06−1.59) favored B according to the EBMT criteria. No significant differences were found as regards treatment-related deaths, overall toxicity or hematological toxicity. However, peripheral neuropathy was more frequent with B (RR 88.22; 5.45−1426.63). *MP vs. chemotherapy regimens without M:* 3 RCTs. No differences in CR or OS were observed between P+bendamustine (BP) and MP, but a significantly higher number of patients treated with BP achieved a CR (RR 2.55; 1.22−5.30). TTP was also significantly longer in BP-treated pts (p < 0.02). *MP vs. polychemotherapy regimens:* 13 RCTs. No significant differences in PR or OS were observed between MP and the other chemotherapy regimens. When pooling RCTs, no significant difference was noted in hematological grade 3−4 toxicity or non-hematological G3−4 toxicity.
Conclusions: MM patients ineligible for SCT should receive as a first line treatment a combination of the standard treatment (MP) plus B or T; these regimens are associated with greater toxicity. More homogeneous RCTs using a cytogenetic risk approach are required.