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POSTER

**Allogeneic stem cell transplantation induces high-titered antibody responses to cancer testis antigens in multiple myeloma patients**S. Kobold<sup>1</sup>, Y. Cao<sup>1</sup>, S. Tams<sup>1</sup>, B.M. Bartels<sup>1</sup>, C. Eberhard<sup>1</sup>, T. Lütken<sup>1</sup>, C. Pabst<sup>1</sup>, C. Bokemeyer<sup>1</sup>, N. Kröger<sup>1</sup>, D. Atanackovic<sup>1</sup>.<sup>1</sup>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. Preliminary 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.

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**An optimized memory B cell assay revealing CT antigen-specific B cells in myeloma patients**Y. Cao<sup>1</sup>, M. Gordic<sup>1</sup>, T. Luetkens<sup>1</sup>, S. Meyer<sup>1</sup>, K. Bartels<sup>1</sup>, Y. Hildebrandt<sup>1</sup>, C. Bokemeyer<sup>1</sup>, N. Kröger<sup>2</sup>, D. Atanackovic<sup>1</sup>. <sup>1</sup>Med. Klinik Und Poliklinik Uni-klinikum, Oncology And Haematology, Hamburg, Germany; <sup>2</sup>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 chemotherapy. 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 (PBMC/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 cytometry demonstrated that after 6 days' stimulation, a group of B cells were expanded, which were CD19<sup>intermediate</sup> CD27<sup>high</sup> CD38<sup>+</sup> IgD<sup>+</sup> 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.

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**A phase Ib multicenter dose escalation study of carfilzomib plus lenalidomide and low-dose dexamethasone in relapsed multiple myeloma – preliminary results**R. Niesvizky<sup>1</sup>, M. Wang<sup>2</sup>, B. Bensinger<sup>3</sup>, M. Vallone<sup>4</sup>, A.A. Gutierrez<sup>5</sup>, M. Kauffman<sup>5</sup>. <sup>1</sup>Cornell University, Weill Cornell Medical College, New York, USA; <sup>2</sup>University of Texas, MD Anderson Cancer Center, Houston, USA; <sup>3</sup>Fred Hutchinson Cancer Research Center, Clinical Research, Seattle, USA; <sup>4</sup>Proteolix Inc., Clinical Operations, South San Francisco, USA; <sup>5</sup>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 ( $\geq 3$  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<sup>2</sup> (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)  $\geq 3$  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.

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**The angiogenesis-related polymorphisms: roles for the risk of multiple myeloma**E.W. Faber<sup>1</sup>, G.J. Lourenco<sup>1</sup>, M.M. Ortega<sup>1</sup>, P.M.R. Silva<sup>1</sup>, C.A. De Souza<sup>2</sup>, I. Lorand-Metze<sup>2</sup>, C.S.P. Lima<sup>1</sup>. <sup>1</sup>Laboratory of Cancer Genetic, Department of Internal Medicine - Faculty of Medical Sciences - State University of Campinas, Campinas SP, Brazil; <sup>2</sup>Department 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