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RELATIVE QUANTIFICATION OF TUMOR ASSOCIATED ANTIGENS MAGE-A1 AND MAGE-A3 IN MULTIPLE MYELOMA

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Background. Multiple myeloma (MM) is a malignant plasma cell neoplasm that often is preceded by a common pre-malignant monoclonal expansion of plasma cells called monoclonal gammopathy of undetermined significance (MGUS). MGUS is reported to be present in 1% of the adult population and to progres to MM at a rate of 1% per year. MM is an incurable tumor characterized by clonal expansion of malignant plasma cells in the bone marrow. The MAGE genes encode antigenic peptides that are presentd by HLA class I molecules and that are recognized on human tumors by T lymphocytes. They are activated in a variety of malignant neoplasma while remaining silent in normal tissues with the exception of testis and ocassionally placenta. Presence of RNA transcripts encoding members of the MAGE gene family in myeloma tumor cells and cell lines has been documented. *Aims.* The aim of this study is to evaluate the possibility of using these genes as molecular markers of the progression MGUS to multiple myeloma and the early relapse of the MM. This abstract covers our pilot and preliminary *Results. Methods.* Total of 50 samples from bone marrow were evaluated: 25 samples from myeloma patients, 8 samples of patients with early stage of MM who did not required treatment (smoldering MM 2x and stage IA 6x), 5 samples of MGUS patients, 9 samples of normal healthy donors served as control group. Total RNA was evaluated by RT-PCR and then by real-time PCR using FRET probes on the LightCycler instrument (Roche). For relative quantification we used G6PDH housekeeping gene as external standard. As positive control we used myeloma cell line U266. *Results.* None from samples of 9 healthy donors did show expression of MAGE. Only 1 of 5 (20%) samples from MGUS patient showed expression of MAGE-A1. Five (62,5%) from 8 patients with early stage of MM (IA and smoldering) showed expression of MAGE. On the contrary 11 (44%) of 25 samples from MM patients showed expression of at least one gene MAGE-A1 or MAGE-A3 or both (7 cases). *Summary/ Conclusions.* We have confirmed that expression of MAGE is not present in samples of healthy donors. There is an obvious correlation between expression of the MAGE genes and early-late stage of the disease as our preliminary evalaution confirmed the detection of low expression levels of MAGE-type mRNA in bone marrow from patients with MGUS and early stage of MM. It is possible that MAGE antigen monitoring may predict the evolution towards more advanced disease as well as this metod should be used for monitoring minimal residual disease in patients with MM. The prospective evaluation is under way. The actual results covering total of 15- 50 evaluated patients in conclusive groups will be presented. This work is supported by grant of the Ministry of Education, Czech Republic, LC06027.

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POST RELAPSE AND OVERALL SURVIVAL OF PATIENTS WITH MULTIPLE MYELOMA THAT PROGRESSED AFTER DECEMBER 1998

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Background. The prognosis of multiple myeloma (MM) patients progressing after autologous BMT (ABMT) was documented to be poor, ranging between 14-18 months in various reports. Since December 1998, a variety of novel methods were introduced for the salvage therapy of MM, namely Thalidomide, reduced intensity allogeneic stem cell transplantation (RISCT) and later on Bortezomibe and Lenalidomide. *Aims.* To evaluate the impact of the introduction of novel methods in progressing MM. *Methods.* We report the outcome of a non-selected group of MM patients that progressed from ABMT after December 1998 and were treated in our center. The treatment strategy for this group of patient was based on the nature and the risk score of the relapse, according to the following mile stones: 1. Treatment only at clinical indication; 2. Thalidomide with or without steroids: A. As first line of salvage therapy. B. At relapse from RISCT in a combination with donor lymphocyte infusion (DLI); 3. RISCT or ABMT: A. for consolidation of response in patients resistant to thalidomide (after an induction of response with platinum containing regimen) and /or with high risk responding relapse. B. At escape from thalidomide effect; 4. Bortezomib and Lenailidomide: A. in patients resistant to or escaping from Thalidomide effect, with an attempt to consolidate response by high dose therapy with allogeneic or autologous stem cell support. B. At progression from RISCT that did not respond to DLI and Thalidomide. *Results.* 84 patients (pt's) that their disease progressed after ABMT between December 1998 and May 2004 were enrolled. All patients were treated with Thalidomide as first salvage therapy at a clinical indication, followed by the various options according to the scheme. At a later stage, 32 patients underwent RISCT (22 from related and 10 from unrelated donors) and 13 patients had an ABMT. 16 patients were treated with Bortezomibe and 8 patients received Lenalidomide, for further progression. The median interval from detection of progression to initiation of therapy was 5.5 months. Response rate to thalidomide + steroids was 59% with a median duration of response (for responders not transplanted immediately at response) being 15 months (the longest exceeding 5.5 years). Transplant related mortality in RISCT was 22%. The 3 years overall survival (OS) for all the patients that underwent RISCT is 42%, and for those transplanted at response 61%. The median OS rate from progression, of the entire group of 84 patients, is 39 months. The median OS from first ABMT of this group is 84 m. *Summary*. The introduction, since 1998, of novel tools for the treatment of progressing MM, significantly prolongs the post relapse and the overall survival of patients with MM that undergo ABMT as a part of the initial therapy.

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OPG/ RANKL SYSTEM IN MULTIPLE MYELOMA

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Background. According to the contemporary 'convergent' hypothesis the major osteoresorbtive and antiresorbtive factors converge to the system osteoprotegerin (OPG)/receptor activator of nuclear factor kB-ligand (RANKL) and influencing its delicate balance they affect osteoclast proliferation, activation and apoptosis. Clinical results concerning the importance of the system in myeloma bone disease (MBD) are controversial. *Aims.* To analyse the serum levels of OPG and RANKL in patients with multiple myeloma (MM) and their correlations with clinical stage (Durie et Salmon), degree of MBD (according to the Merlini scale) and basic parameters of disease activity. *Methods.* We studied 66 newly diagnosed patients with MM, 29 male, 37 female, median age±SD: 61,8±8,6; range: 45-81years. In I / II / III clinical stage were 13,7% / 33,3% / 53,0% patients, renal failure (RF) was found in 40,9%, MBD in 84,8%, hypercalciemia in 31,8%. Serum levels of OPG and RANKL (ELISA kits Biomedica, Vienna) were compared to a control group of healthy individuals (n=30). Statistics were performed by SPSS for Windows v. 11.0. *Results.* OPG levels were higher in myeloma patients: 5,36±0,46 pmol/l vs 3,77±0,33 pmol/L (p<0,001) but OPG/creatinin ratio (thus eliminating the influence of RF) does not differ between the groups. The lowest OPG levels were measured in patients with MBD grade 2+3: 4,26±0,36. A positive correlation between β2-microglobulin and OPG was found- p<0,001; r= +0,375. We found strong negative correlations of OPG/creatinin with clinical stage (p<0,001; r= -0,616), MBD (p<0,001; r = -0,521) and bone marrow plasmocytic infiltration (p<0,001; r= -0,530). Levels of RANKL were higher in MM compared to controls: 0,458±0,046 pmol/L vs 0,203±0,031 pmol/l (p<0,001).

Table 1. Rankl and Rankl/OPG tatio-clinical correlations.

Parameter	*Rankl*		*Rankl/Opg*	
	p	*r*	*p*	*r*
Clinical Stage	<0.001	0.524	<0.001	0.690
Myeloma Bone Disease	<0.001	0.524	<0.001	0.690
Bone marrow plasmocytosis	0.004	0.346	0.011	0.39
β2-microglobulin*	<0.001	0.577	<0.001	0.543
LDH	0.001	0.397	0.015	0.299
CRP	NS	-	0.024	0.277

**Patients without RF.*