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Poster Sessions

651. Myeloma - Biology and Pathophysiology, Excluding Therapy: Poster III

Proteasome Inhibitors Block Myeloma-Induced Osteocyte Death *in Vitro* and *in Vivo* in Multiple Myeloma Patients

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Multiple myeloma (MM) is characterized by a severe unbalanced and uncoupling bone remodeling leading to osteolysis. We have recently shown that osteocytes are involved in MM-induced osteolysis through an increased cell death. Accordingly MM patients are characterized by a reduced number of viable osteocytes related to the presence of bone lesions. Proteasome inhibitors currently used in the treatment of MM are able to stimulate osteoblast formation but their potential effects on osteocyte death are not known and have been investigated in this study both *in vitro* and *in vivo*.

Osteocytic MLO-Y4 cells or human pre-osteocytic HOB-01 cells were co-cultured for 48 hours in the presence or absence of the human myeloma cell lines (HMCLs) JJN3 or RPMI-8226 placed in a transwell insert. A significantly reduction of ostecyte viability was observed (median percent reduction of MLO-Y4 viability: -16% and -30%, respectively). The treatment for 12–24 hours with Bortezomib (BOR) (2nM) or other proteasome inhibitors such as MG262 (10nM) or MG132 (100nM) significantly blunted MLO-Y4 and HOB-01 cell death. Similarly, Dexamethasone (DEX)-induced MLO-Y4 apoptosis, obtained at pharmacological doses (10^{-4}–10^{-5} M), was significantly reduced by the treatment with proteasome inhibitors. To translate our *in vitro* data into a clinical perspective we performed a retrospective histological evaluation on bone biopsies of a cohort of 40 newly diagnosis MM patients (24 male and 16 female, median age: 68 years) 34 of them with symptomatic MM and 6 with smoldering MM (SMM). The 58% of patients with symptomatic MM have evidence of osteolytic lesions at the X-rays survey. Bone biopsies were obtained in both symptomatic MM and SMM at diagnosis and after an average time of 12 months of treatment or observation, respectively. The

68% of patients with symptomatic MM were treated with a BOR-based regimen while 42% do not. Moreover the 58% of MM patients received DEX and the 59% Thalidomide (TAL). Zoledronic acid (ZOL) was infused monthly in the 60% of MM patients. Osteocyte viability was evaluated in a total of 500 lacunae *per* histological sections, corresponds to the total number of osteocyte lacunae in the bone biopsies. The number of viable osteocytes and the number of degenerated or apoptotic osteocytes and empty lacunae have been evaluated. In patients with SMM no significant change was observed in the number of viable osteocytes in the two histological evaluations carried out (median percent change: +1.2, *p=0.68, NS*). In symptomatic MM patients the mean percent change of the osteocyte viability was not correlated with the response rate to treatment (R^2 0.01, *p=NS*). A significant increase of the number of viable osteocytes was demonstrated in MM patients treated with BOR-based regimen as compared to those treated without BOR (% median increase of osteocyte viability: +6% vs. +1.30%, Mann-Whitney test: *p=0.017*). Patients treated with BOR alone showed the highest increase of osteocyte viability that was statistical significant in comparison with that observed either in patients treated without BOR (+11.6% vs. +1.3%, *p=0.0019*) or in those treated with BOR plus DEX (+11.6% vs. +4.4%, *p=0.01*). On the contrary, no significant difference was observed in patients treated with TAL than in those treated without TAL (*p= 0.7*, NS) as well as patients treated with ZOL compared to those untreated showed no significant difference in the number of viable osteocytes (*p=0.18, NS*). To confirm the role of the different drug treatment on the osteocyte viability we perform a multiple regression non-parametric analysis showing that BOR had a significant positive impact on osteocyte viability (*p=0.042)* whereas ZOL and TAL have not (*p>0.2,NS*) and it counterbalanced the negative effect of DEX treatment (*p=0.035*).

In conclusion our *in vitro* and *in vivo* data suggest the proteasome inhibitors block osteocyte death induced by MM cells could have a positive impact on bone integrity in MM patients.

Disclosures: No relevant conflicts of interest to declare.

Footnotes

* Asterisk with author names denotes non-ASH members.