**Maternal Embryonic Leucine zipper Kinase (MELK) inhibition has direct effects on bone cells and prevents the development of osteolytic bone disease in multiple myeloma**

Keywords: multiple myeloma, bone disease, MELK, OTSSP167

Objectives:

Osteolytic bone disease is a hallmark of multiple myeloma (MM) and remains a major cause of morbidity and mortality. New therapeutic approaches for the treatment of MM bone disease are of great interest. In this study, we investigated the role of MELK, a kinase involved in cell cycle progression, in MM cell, osteoclast and osteoblast biology. In addition, we determined the effect of MELK inhibition with OTSSP167, a potent and selective MELK inhibitor, on the development of MM bone disease *in vivo*.

Methods:

A publically available gene expression profiling study (GSE755) was used to assess relative MELK mRNA expression in MM cells of patients with and without bone lesions. mRNA expression of various genes was assesses by real-time PCR (SYBR green) and analyzed using the dCt method while protein expression was determined by Western blot. For loss-of-function experiments, MELK was knocked down with a lentiviral shRNA construct or cells were treated with OTSSP167 (Biorbyt) dissolved in DMSO. Proliferation, cell cycle progression and apoptosis were determined by MTT, PI staining and AnnV/PI staining respectively. RAW264.7 and primary human osteoclasts differentiation was induced with RANKL and assessed by TRAP staining of cultures. Osteoclast resorption activity was studied in Osteo Assay plates (Corning) and determined by a Von Kossa staining. Actin ring formation in mature osteoclasts was assessed by a phalloidin staining. Alkaline phosphatase activity was measured in BMSC TERT+ osteoblast cultures. For the *in vivo* experiments, 8 weeks old female C57/KaLwRij mice were injected with 5x105 5TGM.1GFP+ cells and sacrificed after they showed signs of paraplegia, i.e. around 12 weeks. MM-bearing mice were treated with 15mg/kg/d OTSSP167 or vehicle solution (0.5% methylcellulose) by oral gavage. After sacrifice, bone architecture parameters were determined by CT (Skyscan1172). All reported results are mean+/-standard error.

Results:

MELK mRNA expression is increased in MM cells of patients with bone lesions compared to MM cells of patients without lesions (250+/-16 vs. 190+/-13, p<0.05) and knockdown or inhibition of MELK lead to strongly decreased DKK-1 mRNA expression by KMS12 BM, MM.1S and OPM2 MM cell lines (up to -99%, p<0.001). RAW264.7 treatment with OTSS167 at concentration higher than 10nM resulted in cell cycle inhibition or apoptosis and a dose-dependent decrease in RAW264.7 (N.Oc/FOV: 53.1+/-5 (DMSO), 13.3+/-2 (10nM) and 0 (100nM), p<0.001) and primary human (N.Oc: 142.9+/-40 (DMSO), 34.3+/-10 (10nM) and 2.1+/-1 (25nM), p<0.05) osteoclast differentiation. Interestingly, 1nM OTSSP167 did not affect RAW264.7 osteoclast differentiation but did result in decreased bone resorption (Von Kossa- area: 267857+/-57371 vs. 82308+/-24846, p<0.05) and a corresponding decrease in TRAP mRNA expression (0.08+/-0.007 vs 0.05+/-0.008, p<0.05). We observed no effect of MELK inhibition on actin ring formation. Of note, expression of MM stimulatory factors OPN (0.65+/-0.04 vs. 0.30+/-0.05, p<0.01), ANXA2 (0.19+/-0.005 vs. 0.11+/-0.01, p<0.01) and IGF1 (0.013+/-0.0003 vs. 0.004+/-0.0003, p<0.001) by osteoclasts was decreased after OTSSP167 treatment, suggesting that MELK inhibition inhibits the positive feedback loop that exists between osteoclasts and MM cells. Experiments on osteoblasts are ongoing and initial results indicate a dose-dependent decrease of AP activity by osteoblasts. Finally, treatment with 15mg/kg/d OTSSP167 prevented the development of bone disease in the murine 5TGM.1 model, reflected by a restoration of Tb BV/TV (3.3+/-0.3 vs. 5.0+/-0.4, p<0.05), Tb.Sp (296.4+/-8.4 vs. 269.5+/-2.3, p<0.05) and Tb.N (0.0009+/-0.00007 vs. 0.0015+/-0.00009, p<0.01) to levels observed in naïve mice.

Summary and conclusion:

We report an inhibitory effect of OTSSP167 on the development of MM bone disease. Our results indicate that OTSSP167 exerts this effect via the direct modulation of osteoclast, osteoblast and MM cell function. Additional analyses to expand on our findings are currently ongoing, including determination of downstream effects of MELK knockdown or inhibition, study of the role of MELK in osteoblasts and in MM cell-bone cell co-cultures and immunohistomorphometry analyses on bone sections.