Maternal Embryonic Leucine Zipper Kinase Inhibitor OTSSP167 Inhibits Osteoclast Activity and Restores Osteoblast Activity in a Murine Model of Multiple Myeloma Bone Disease

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INTRODUCTION

Multiple myeloma bone disease (MMBD) is characterized by an increased osteoclast activity and a decreased osteoblast differentiation in the multiple myeloma (MM) microenvironment, resulting in the development of lytic bone lesions. A large majority of MM patients suffer from MMBD, which not only causes morbidity but also negatively impacts patient survival. The development of novel therapies, ideally with a combined anti-resorptive and bone-anabolic effect, is of great interest because lytic lesions persist with the current standard of care, even in patients in complete remission. We have previously shown that maternal embryonic leucine zipper kinase (MELK) drives a high-risk gene network in MM and that MELK inhibition by OTSSP167 reduces tumor development in the 5TGM.1 model. In the current study, we assessed the effect of OTSSP167 treatment on bone cell activity and the development of MMBD.

1. OTSSP167 hampers osteoclast differentiation

Figure 1. (A) MELK mRNA levels increase during human (huOC) and RAW264.7 osteoclast differentiation. (B) 25 nM OTSSP167 reduces MELK protein levels in RAW264.7 cultures. (C) OTSSP167 reduces hPBM and RAW264.7 cell viability (MTT assay). (D+E) OTSSP167 dose-dependently reduces primary huOC differentiation. Similar observations were made with RAW264.7 OC cultures (not shown).

2. OTSSP167 inhibits bone matrix resorption

Figure 2. (A+B) Continuous OTSSP167 treatment dose-dependently reduces bone matrix resorption by huOC. Similar results were obtained with RAW264.7 OC cultures (not shown). (C) Continuous OTSSP167 treatment reduces CTSK and TRAP mRNA levels in huOC cultures. (D) OTSSP167 treatment initiation after the final day of OC differentiation did not affect RAW264.7 OC numbers or survival. (E+F) Initiation of OTSSP167 treatment on day 5 or 7 reduced bone matrix resorption by RAW264.7 OC after 24 hours. Similar numbers of mature OCs were present in vehicle vs. OTSSP167-treated cultures (not shown). (G) OTSSP167 treatment of established RAW264.7 OC cultures drastically reduced CTSK mRNA levels. Other OC markers were unaffected (not shown).

3. OTSSP167 stimulates osteoblast function

Figure 3. (A) MELK mRNA differentiation decrease during osteoblast differentiation. (B) 25 nM OTSSP167 reduces MELK protein levels in BMSC-TERT. (C) OTSSP167 has a limited effect on BMSC-TERT viability (MTT assay). (D-F) OTSSP167 dose-dependently increases collagen secretion and matrix mineralization. (G) OTSSP167 reduces NFATc1 and increases OSX mRNA levels in BMSC-TERT osteoblast cultures. Other markers were unaffected (not shown).

5. OTSSP167 decreases osteoclast activity and increases osteoblast activity in MM-bearing mice

Figure 5. (A) TRAP, toluidine blue and trichrome staining were performed on distal femurs from mice in the 7.5 mg/kg/2d cohort. (B) OTSSP167 restored osteoclast surface in 5TGM.1-bearing mice. (C) OTSSP167 restored osteoblast surface in 5TGM.1-bearing mice. (D) OTSSP167 partially restored osteoid surface in 5TGM.1-bearing mice. (E) OTSSP167 reduced osteoid thickness in 5TGM.1-bearing mice compared to naïve mice.

CONCLUSION – FUTURE PERSPECTIVES

This study provides a novel approach for the treatment of MMBD. OTSSP167 not only inhibits MM tumor development but also directly affects bone cell function and normalizes bone homeostasis in MM-bearing mice. The maintenance of bone anabolic activity by OTSSP167 is promising and warrants further clinical investigation. Moreover, drugs with a combined anti-MM and anti-MMBD effect, which include PIs and IMiDs, are of primary interest for a number of reasons, including reduced burden for the patient, reduced treatment costs and reduced interaction and toxicity concerns. In fact, MELK knockout mice have no discernable phenotype and display no hematopoietic toxicity. Bone parameters have not yet been reported for these mice. Also, OTSSP167 is currently in clinical trials for other malignancies and its toxicity appears low. Reducing MM patient morbidity and mortality via the combined anti-MM and anti-MMBD effect of OTSSP167 holds great clinical promise and our results warrant similar studies in other cancers with bone involvement.