MEK inhibitor treatment for osteofibrous dysplasia RASopathy

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INTRODUCTION: Osteofibrous dysplasia (OFD) is a skeletal RASopathy characterized by radiolucent, cortex-confined lesions on the periosteal surface of long bones in children as well as increased risk of persistent pseudarthrosis following pathologic fracture. Inherited and somatic *MET* gene mutations were identified in patients with OFD. All mutations impacted the juxtamembrane domain (JMD) that is required for ubiquitin-mediated degradation, leading to reduced receptor degradation and ligand-dependent gain-of-function (GOF) of the c-MET receptor tyrosine kinase. Here, we used a novel mouse model of OFD and patient-derived primary cells to complete pre-clinical studies testing targeted therapies as a potential treatment for OFD-associated skeletal disease. Finally, based on these pre-clinical results, we treated a pediatric OFD patient with the MEK inhibitor (MEKi) mirdametinib. Results from this study implicate MEK inhibition as a potential therapy for OFD.

METHODS: Primary periosteal explant cells were cultured from the long bones of 4-month-old male and female control and $Met^{\Delta JMD}$ mice harboring a deletion of the Met JMD. Primary mouse cells were cultured in MEM-alpha with 10% fetal bovine serum and antibiotic. For osteogenic differentiation, culture media was supplemented with 100ug/mL L-ascorbic acid 2-phosphate and 5mM β-glycerophosphate for 14 days, with media and osteogenic supplement refreshed every 3 days. For drug studies, cells were treated with vehicle (DMSO) or drug. Gene expression was tested by quantitative reverse-transcription PCR (qRT-PCR). Osteogenic mineralization was evaluated by Alizarin staining with quantification normalized to Crystal Violet staining. Femurs from 4-month-old mice were evaluated by micro-computed tomography (μCT) and 3-point bending. Significant differences were determined by ANOVA with multiple test correction or T-test. All animal procedures were approved by the Institutional Animal Care and Use Committee of UT Southwestern Medical Center (UTSW). An OFD patient was treated with mirdametinib (1mg twice daily, 3 weeks ON/1 week OFF) as part of a Compassionate Use Protocol (SpringWorks, Inc). Surgery and radiographic follow-up were performed as standard-of-care.

RESULTS: Consistent with ligand-dependent GOF, ERK pathway activation in $Met^{\Delta JMD}$ explant cells was significantly greater following stimulation compared to control (**Fig. 1**). Osteogenic differentiation potential of cultured $Met^{\Delta JMD}$ explant cells was significantly reduced compared to control (Fig. 2, blue). *In vivo*, the cortical bone of adult $Met^{\Delta JMD}$ femurs was significantly more porous, with reduced biomechanical integrity upon 3-point bending. In contrast to Met activation, osteogenic differentiation of explant cells from *Postn*-cre;$Met^{flox/flox}$ mice was significantly enhanced. These results implicate a bidirectional role for Met in regulating osteogenic differentiation and suggest ERK pathway inhibition may rescue osteogenic differentiation defects inherent in $Met^{\Delta JMD}$ explant cells. To test potential therapies for OFD, we treated $Met^{\Delta JMD}$ PECs with the MEKi mirdametinib, which significantly rescued osteogenic differentiation and mineralization of $Met^{\Delta JMD}$ PECs (**Fig. 2**). To translate these results to the human condition, we treated cultured pseudarthrosis-derived primary cells from five OFD patients with vehicle or mirdametinib and tested the transcriptomic response. Consistent with Western Blot results, mirdametinib treatment significantly reduced expression of MAPK-associated genes and increased expression of genes associated with skeletal development and mineralization. Finally, we tested whether MEKi therapy can improve pseudarthrosis healing in a young child with OFD. Prior to treatment, the patient required 8 surgical revision procedures over the course of 3 years for persistent pseudarthrosis (top, **Fig. 3**). Following the most recent revision surgery, the patient was prescribed mirdametinib, and fracture healing was monitored by radiograph. Fracture healing was evident 6-months on treatment and transitioned from external frame to a cast. Fracture healing continues 7-months on-treatment (bottom, **Fig. 3**). Diarrhea was noted as a grade 1 adverse even during the first month of therapy that resolved by the second month. No other adverse events have been observed.

DISCUSSION: Some patients with OFD harbor inherited or somatic *MET* gene mutations resulting in expression of a ligand-dependent gain-of-function receptor. Our results implicate defects in osteogenic differentiation in the pathogenesis of OFD, though $MET^{\Delta JMD}$ expression in other skeletal cell types may also be involved in the pathogenesis of OFD. Results from this study implicate the use of MEK inhibitors to treat OFD.

SIGNIFICANCE/CLINICAL RELEVANCE: Our results are the first to demonstrate a bidirectional role for Met signaling in regulating osteogenic differentiation, in part, through regulation of MEK/ERK pathway activation. We present clinical results from the first skeletal RASopathy patient treated with a MEK inhibitor (mirdametinib), which supports further consideration of this treatment to treat OFD and potentially other skeletal RASopathies.

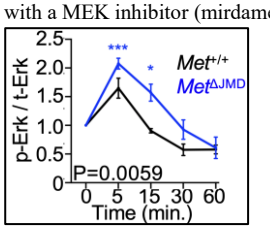

Fig1. Western blot time course quantification of ERK activation following HGF stimulation of BMSCs

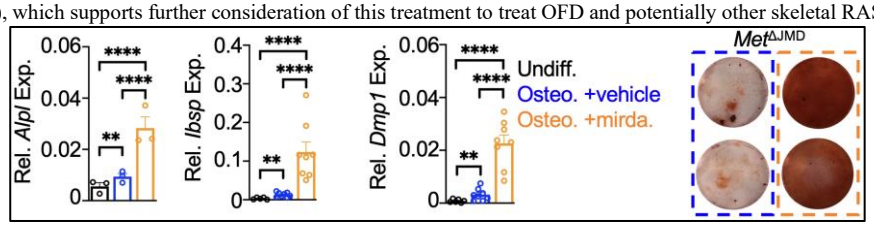

Fig2. Mek inhibition rescues osteogenic differentiation of $Met^{\Delta JMD}$ PECs

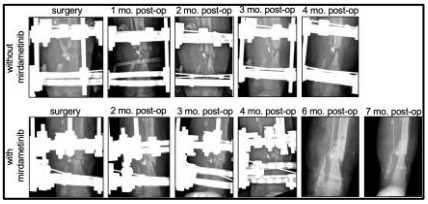

Fig3. Longitudinal post-operative radiographs of an OFD patient pseudarthrosis treated without mirdametinib and with mirdametinib.