Pre-clinical Testing Of Treatments For Congenital Pseudarthrosis Of The Tibia

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Disclosures:
A. Schindeler: 5; N8 Medical. J. El-Hoss: None. N. Deo: None. K. Mikulec: None. L. Peacock: None. D.G. Little: 5; AMGEN, Novartis Pharma AG, Celgene, N8 Medical.

Introduction: Congenital pseudarthrosis of the tibia (CPT) is a severe orthopedic condition with a poor prognosis. A majority of the children who develop CPT possess the autosomal dominant genetic disorder, Neurofibromatosis type I (NF1). Bone dysplasias are a diagnostic feature of NF1 and the natural history of NF1/CPT involves a congenital tibial dysplasia (a distinctive anterolateral bowing of the tibia) that is prone to fracture. Subsequent bone healing is recalcitrant and even with modern surgical interventions amputation is not an uncommon outcome. Analysis of human samples and murine models of the disease have identified a number of features that contribute to the pathology. These include deficient new bone formation, excessive osteoclast-driven bone resorption, and invasion of mesenchymal/fibrous tissue into the pseudarthrosis site that prevent subsequent repair.

In 2006 it was reported that tibial pseudarthrosis was linked to a number of cases to double inactivation of NF1 locally within the fracture site (1). Consequently, we developed a murine model of local NF1 double inactivation and this pre-clinical model demonstrates all of the features of a human pseudarthrosis including poor a bone healing response, and excessive fibrous tissue that supports the formation of abundant osteoclasts (2). In this report we describe the testing of a number of interventions for the treatment of CPT including rhBMP-2 (rhBMP-2/ACS, Medtronic), a combination of rhBMP-2 and the bisphosphonate zoledronic acid (ZA) the MEK inhibitor PD0325901 and the combination of rhBMP-2 and PD0325901.

Methods: Fractures were induced in the Nf1flox/flox mouse line and double inactivation of the Nf1 locus was induced via transduction with a Cre-expressing adenovirus, as previously published. This model results in a high non-union rate. Interventions included 10µg rhBMP-2 introduced locally at the fracture site via a collagen sponge, 5 doses of 0.02mg/kg ZA delivered systemically starting from 3 days post-operatively, and daily dosing with 10mg/kg PD0325901 in a jelly cube. Group size was n=15. Animals were harvested for analysis at 3 weeks. Experiments were performed with the approval of the institutional animal ethics committee.

Outcome measures included radiographic union rate (Faxitron XR), as assessed by a blinded observer; callus bone volume (BV) and other microarchitecture parameters assessed by microCT (Skyscan 1174); descriptive histology and histomorphometry; and in mechanical testing (Instron). Mechanical testing was restricted to specimens that had achieved radiographic union.

Results: In the first study examining MEKi treatment, animals dosed with vehicle, PD0325901, rhBMP-2, or PD0325901 + rhBMP-2 combination showed union rates of 0%, 8%, 69%*, or 80%* respectively at 21 days post-fracture, (*P<0.05 vs. control). Mice with the rhBMP-2 + PD0325901 combination treatment displayed a 6-fold greater callus volume than the vehicle controls and 2-fold greater than rhBMP-2 alone. While MEK inhibition with rhBMP-2 led to increases in bone formation and union, the proportion of fibrous tissue in the callus was not significantly reduced.

In the second study examining BMP/bisphosphonate treatment, animals dosed with rhBMP-2 + ZA combination treatment showed a high rate of bone union (93%) compared to vehicle (7%, p<0.01) and ZA alone (86%, N.S.) at 21 days post-fracture. ZA treatment alone led to a significant increase in BV compared to the vehicle group (p<0.05), but did not lead to increased union. Treatment with rhBMP-2 produced a 3-fold increase in BV compared to vehicle (p<0.01). The combination of rhBMP-2 + ZA led to even further increases in BV compared to all groups (p<0.01). Treatment with rhBMP-2 + ZA also led to a decrease in fibrous tissue present in the callus (measured as a proportion of the total callus area) compared to both vehicle and rhBMP-2 groups and less unresorbed cartilaginous tissue than vehicle, ZA and rhBMP-2 alone after 21 days of healing.

Discussion: Treatment of NF1+- fractures with systemic MEK inhibitor PD0325901 did not rescue the bone healing deficit. In contrast, rhBMP-2 treatment led to improvements in bone anabolism, but not complete rescue. However, rhBMP-2 treatment did not overcome issues associated with fibrosis and while PD0325901 co-treatment enhanced bone volume it also did not reduce fibrosis. These data suggest that the MEK pathway contributes to the poor bone anabolism seen in NF1 but is not the only pathway responsible for invasion and proliferation of fibrotic tissue.

Previous experiments have shown synergy between rhBMP-2 and bisphosphonates in a more simplistic model of Nf1+- bone healing (3). Notably however this model does not feature fibrosis or the same excessive osteoclastogenesis that is associated with a human CPT. Nevertheless, the combination of rhBMP-2 and ZA led to significant improvements in the repair of Nf1+- fractures suggesting that an approach that does not specifically target the deficient pathways in NF1 can still be effective in promoting repair. The non-union rate in rhBMP-2 treated animals was halved with the addition of ZA (14% vs 7% non-union) however this did not reach statistical significance. Notably however, the rhBMP-2 and ZA combination led to a significant
reduction in fibrosis, unlike rhBMP-2 alone which potentiated fibrosis in the MEKi model.

**Significance:** These data demonstrate the utility of this pre-clinical model for screening approaches for treating NF1/CPT. A combination of pathway-targeted and generic anabolic/anti-catabolic strategies were examined. Notably, the rhBMP-2/ZA intervention gave the most favorable outcomes in terms of histology and union rate. These data support the advancement of clinical trials to assess the efficacy of this combination therapy, which has reported anecdotal benefits in this context (4). This pre-clinical model remains as a practical and rapid screening tool for new pharmaceutical interventions for the treatment of NF1/CPT.

**Acknowledgments:** This work was supported by funding from the National Health and Medical Research Council of Australia.

**References:**