AAV-Based Gene Therapy for Endochondral Repair of Segmental Bone Defects

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INTRODUCTION: While most long bone fractures heal on their own, approximately 100,000 cases each year in the U.S. result in non-unions, leading to significant morbidity. Large, critical-size segmental defects are particularly challenging. One current treatment involves delivering recombinant human bone morphogenetic protein-2 (rhBMP-2) on a collagen sponge implanted into the defect. Although rhBMP-2 stimulates bone formation, its clinical effectiveness is modest, requiring high doses that trigger inflammation, produce poor-quality bone, and cause harmful side effects. Previous work from our lab has shown that gene transfer of BMP-2 can promote bone healing more effectively than rhBMP-2 protein delivery, achieving therapeutic effects at low, non-inflammatory expression levels [1]. Given that adeno-associated virus (AAV) vectors are among the least pyrogenic viral vectors available, we are developing AAV-based systems for bone regeneration. Additionally, we hypothesize that co-delivery of an immunomodulator, such as interleukin-1 receptor antagonist (IL-1Ra), could enhance bone quality by promoting endochondral ossification—a process disrupted by the immune response to high doses of rhBMP-2 [2].

METHODS: AAV6-BMP2 or AAV6-BMP2-P2A-IL1Ra at doses of 1e10 or 1e11 viral genomes were implanted into 5 mm critical-sized defects in the femurs of male rats on a collagen sponge to study their effects on bone regeneration. Each treatment was done in replicates of 7. X-rays were taken to monitor the progress of the regeneration. After 16 weeks, the rats were sacrificed, and the defects were taken for microCT and histology to determine the volume of newly synthesized bone and the ossification pathway.

RESULTS: AAV6-BMP-2 showed regeneration at the 1e10vg dose; however, histology indicated it occurred through the intramembranous ossification pathway. When increased to 1e11vg, the AAV6-BMP-2 vector produced widespread heterotopic ossification, which has not been previously documented in the rat model, suggesting a significant expression of the BMP-2 transgene. AAV6-BMP2-P2A-IL1Ra came close to bridging at 1e10vg with a small cartilaginous zone remaining. The histology confirmed regeneration took place via the endochondral ossification pathway (Figure 1). By 12-weeks post-surgery, the 1e11vg dose of AAV6-BMP2-P2A-IL1Ra appeared likely to bridge by the pending 16-week time point (Figure 2).

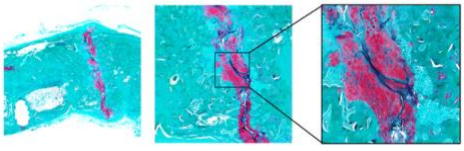

Figure 1: Safranin-O staining of femoral segmental defect treated with AAV6-BMP-IL1Ra at 1e10vg at 16-weeks post-operation.

DISCUSSION & CONCLUSIONS: The main goal is to develop a gene therapeutic able to fully bridge a 5mm defect via the endochondral ossification pathway in this rat model. Of the constructs evaluated here, the AAV6-BMP-2-P2A-IL1Ra vector showed most promise, stimulating endochondral bone formation that came close to bridging (Fig. 1) at both the 1e10vg and 1e11vg doses (Figure 2). The higher dose of AAV6-BMP-2 produced heterotopic ossification (Fig. 2) which was absent from the equivalent bicistronic vector co-expressing IL-1Ra, thus confirming the merit of expressing IL-1Ra in osseous defects [2].

SIGNIFICANCE/CLINICAL RELEVANCE: With significant endochondral bone regeneration stimulated via the vector, this therapy could form the basis for a clinical product that could heal recalcitrant non-unions in human and veterinary medicine.

REFERENCES: [1] Atasoy-Zeybek A, Coenen M, Hawse G, Logeart-Avramoglou D, Evans CH, De la Vega RE: Efficient autocrine and paracrine signaling explain the osteogenic superiority of transgenic BMP-2 over rhBMP-2. Mol Ther - Methods & Clinical Development 29: 350-363, 2023

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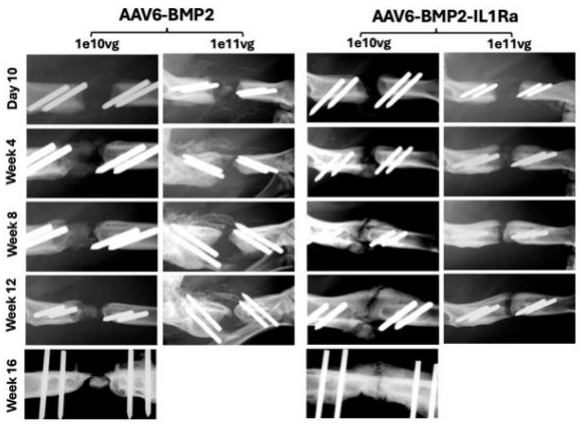

Figure 2: X-rays of femoral segmental defects treated with either AAV6-BMP2 or AAV6-BMP2-IL1Ra with 1e10 or 1e11vg of vector out to 12- or 16-weeks post-operation.