pocket of CLP patients at the time of palate repair enhances bone growth and accelerates healing, with the autologous stem cells and extracellular matrix serving as a “primary bone graft” to close the alveolar cleft.

**METHODS:** Human umbilical cords were harvested following routine delivery and WJ was isolated and purified. In vitro, WJ derived stem cells were placed in osteogenic differentiation medium for 14 days, followed by Alizarin Red S staining to evaluate mineral deposition. In vivo, we used a rat critical-size alveolar bone defect model to investigate the use of Wharton's Jelly (WJ) in formation of bone. WJ was implanted into a critical size (7 x 4 x 3 mm) alveolar bone defect model representative of cleft palate surgery in 10–11 week old male Sprague-dawley rats. The defects were monitored weekly with CT imaging of living animals to evaluate bone formation in time, followed by histology evaluation at week 24.

**RESULTS:** WJ showed significant in situ osteogenic differentiation of WJ cells as evidenced by strong Alizarin Red S staining. By contrast, no staining is observed when nWJ is maintained in control medium. In vivo, CT data showed that the defect size was critical and did not lead to the union of the bones in the control animals (n=12) for the entire duration of study. New bone growth was stimulated leading to partial-to-full closure of the defect in the animals treated with WJ (n=12). 24 weeks postoperatively, the percent increase in new bone formation in the WJ treated group (156.57 ± 26.85%) was markedly higher than that in the control group (49.97 ± 12.51%) (p<0.05). Histology data also revealed significantly greater new bone formation in WJ treated vs, control animals, confirming CT findings.

**CONCLUSION:** We show that WJ has in vitro osteogenic differentiation capacity and in vivo, enhances bone growth in animal cleft palate models indicating its potential use as a natural tissue engineering construct for regenerative clinical applications. The success of this approach would represent a paradigm shift in the treatment of CLP patients by reducing or eliminating the need for subsequent bone grafting.

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**PURPOSE:** Distraction osteogenesis (DO) is a powerful method of endogenous bone tissue engineering that has been applied to the craniofacial skeleton with great success. However, the cellular and molecular signaling that governs this process of de novo bone formation is not well understood. We aimed to establish a rigorous mandibular distraction model that is genetically dissectable, using C57/B6 mice, the most common background strain of inbred transgenic mice.

**METHODS:** Mandibular distraction devices were manufactured using computer-aided design (CAD) software and 3D printing. Animals were divided into four groups in this study: sham-operated (n = 6 per time point), fracture (n = 6 per time point), acutely lengthened (n = 8 per time point) and gradually distracted (n = 8 per time point) right hemimandibles. Gradual distraction was performed at a rate of 0.15 mm every 12 h over the course of 10 days whereas acute lengthening was performed at a single time point. The total amount of distraction (3 mm) was maintained across both groups. Specimens were harvested at mid-consolidation (POD 29) and end consolidation (POD 43) time points for μCT and histological analysis.

**RESULTS:** Detailed serial histology of gradually distracted mandibles revealed patterns of bone formation identical to those seen in our rat DO model. Two weeks into consolidation (POD 29), greater than half of the distraction gap was filled with trabecular bone. No cartilage was seen within the distraction gap, indicating that the regenerate was produced through direct intramembranous bone formation. At the end of consolidation (POD 43), trabecular bone completely bridged the distraction gap of all gradually distracted specimens. Trabecular bone along the edges of the osteotomies was remodeled to lamellar, cortical bone. Three-dimensional reconstruction of the μCT images of gradually distracted specimens clearly showed complete osseous bridging of the osteotomy gap. In marked contrast to the above data, the distraction gap of all mandibles undergoing acute lengthening was filled with fibrous tissue at end-consolidation and there was minimal evidence of new bone formation at the osteotomized bone histologically or radiologically.

**CONCLUSION:** We have developed and characterized a model of mandibular DO in C57BL/6 mice, which faithfully reproduces the ultrastructural and histological changes seen in larger animal models. Experiments utilizing this unique mouse model will offer valuable insight into the biology of
de novo bone formation and the mechanical forces guiding distraction osteogenesis, speeding the development of clinically applicable therapies.

A Translational Strategy Targeting Type I BMP Receptors to Prevent Heterotopic Ossification

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PURPOSE: Trauma-induced heterotopic ossification (tHO) is the aberrant growth of ectopic bone in soft tissue, which develops in patients following severe musculoskeletal trauma. Much of HO literature focuses on a related pathology known as fibrodysplasia ossificans progressiva (FOP), which is caused by a hyperactivating mutation in the type I bone morphogenetic protein receptor (T1-BMPR) ACVR1 (ACVR1 R206H). Consequently, emphasis has been placed on developing inhibitors with improved specificity for ACVR1. However, patients who develop tHO do not harbor known ACVR1 mutations, and it is unclear whether emphasis on ACVR1-specific inhibition is beneficial for the treatment of tHO. Here investigate whether any single T1-BMPR is required for tHO, or whether these receptors perform overlapping roles during tHO development. We further evaluate the efficacy of the BMP ligand trap, Alk3-Fc, as a broad-spectrum inhibitor of T1-BMPR receptors in the treatment and prevention tHO.

METHODS: Wild-type, tamoxifen-inducible Acvr1 knockout (Acvr1 tmKO: Ub.creERT/Acvr1 flox/flox), tamoxifen-inducible Bmpr1a knockout (Bmpr1a tmKO: Ub.creERT/Bmpr1aflox/flox), and Bmpr1b knockout (Bmpr1b−/−) mice received hindlimb Achille’s tendon transection and dorsal 30% total body surface area partial-thickness burn. A second cohort of wild-type mice received daily i.p. injections of Alk3-fc (2 mg/kg) or saline for 6-weeks post-injury or during weeks 0–2 (inflammation), weeks 2–4 (chondrogenesis), or weeks 4–6 (ossification) after injury. Mice receiving abbreviated treatment with Alk3-fc received intraperitoneal saline injections for the remainder of their 6-week treatment period. HO was quantified by microCT 9-weeks post-injury, and cartilage formation was assessed via pentachrome staining.

RESULTS: Genetic loss of Acvr1 (p>0.05), Bmpr1a (p>0.05), or Bmpr1b (p>0.05) alone was unable to significantly or substantially reduce tHO after injury. However, genetic loss of both Acvr1 and Bmpr1a (Acvr1;Bmpr1a tmKO: Ub.creERT/Acvr1 flox/flox;Bmpr1aflox/flox) led to a substantial and significant reduction in tHO volume (16.7-fold decrease vs. wild-type; p<0.05). Based on these findings we studied the effect of Alk3-Fc, a BMP ligand trap which inhibits BMP receptor signaling capable of broadly T1-BMPR inhibition at different points during tHO development. Mice treated daily with Alk3-Fc for 6-weeks post-injury demonstrated a significant and substantial reduction in tHO volume (2.9-fold; p<0.05). Mice treated daily with Alk3-Fc from 0–2 weeks demonstrated similar reductions in tHO volume (2.3-fold; p<0.05). This was confirmed histologically, with diminished cartilage content in 6-week and 0–2 week treated groups when compared with controls.

CONCLUSIONS: Here we demonstrate that although BMP signaling is required for tHO, no single T1-BMPR (ACVR1/ALK2, BMPR1a/ALK3, or BMPR1b/ALK6) alone is necessary for this disease. This suggests that, unlike with genetic forms of HO such as FOP, these receptors have functional redundancy in the setting of tHO. Based on these data we developed an approach based on broad-spectrum T1-BMPR blockade using the ligand trap Alk3-Fc to prevent tHO formation. Our findings suggest that Alk3-Fc treatment during the earliest period after injury is sufficient to block tHO and provides a translational approach to targeting this disease process.

Fabrication of the First Full-Scale Human Auricular Chondrocyte Derived Ear Scaffold for Clinical Application

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