Nicole Patel, BS¹, Michael Sorkin, MD¹, Charles Hwang, BS¹, John Li, MD¹, Serra Ucer, PhD¹, Sidra Kader, BS¹, Kaetlin Vasquez, MS¹, Shuli Li, MD, PhD¹, Ravi Kumar, PhD², Yuji Mishina, PhD¹, Benjamin Levi, MD¹

¹University of Michigan, Ann Arbor, MI, USA, ²Acceleron Pharma, Inc., Cambridge, MA, USA

PURPOSE: Heterotopic ossification (HO) can commonly occur after severe trauma, burn injuries, and is a debilitating consequence of the congenital disease fibrodysplasia ossificans progressive (FOP). The etiology remains poorly understood, however, it is presumed that inflammation plays a critical role with several inflammatory cell types being recruited to the site of HO development. While the role of aberrant BMP signaling is established in HO formation, there is recent indication that macrophage secreted transforming growth factor beta 1 (TGFβ1) is also involved through propagation of chondrogenesis and endochondral ossification. Here we explore the effect of Tgfβ1 inhibition utilizing a near clinical Tgfβ1 receptor ligand trap to attenuate HO formation.

METHODS: Bone marrow derived macrophages were isolated and polarized into M2 phenotype in-vitro. Secreted TGFβ was measured in conditioned medium using ELISA. A model of traumatic heterotopic ossification involving a 30% dorsal burn and Achilles tenotomy was utilized in-vivo and 6-week old male C57BL/6 mice were randomized into receiving treatment with the pre-clinical pharmaceutical grade TGFβR-Fc ligand trap (n=10) or PBS control (n=10). This was administered subcutaneously twice weekly for 3 weeks. At 3 weeks, histology samples were collected, decalcified and stained with Safranin O to assess formation of HO anlagen (n=3/group). Volume of mature formed HO was quantified using micro CT analysis and imaging reconstruction at 9 weeks (n=6/group).

RESULTS: Recruited macrophages are a known source of cytokines that influence the inflammatory microenvironment. We therefore initially assessed the secretion of Tgfβ1 in cultured macrophages. Interestingly, we observed that while M0 macrophages secrete only minimal TGFβ1, the regenerative M2 polarized macrophages, which are known to be recruited to inflammatory sites, had a 500-fold increase in Tgfβ1 secretion. Furthermore, this increase in TGFβ1 levels was completely abrogated when the TGFβR-Fc ligand trap was present in the culture media. We next aimed to assess the effect of systemically administered TGFβR-Fc on HO formation. Following treatment for 3 weeks, we observed a substantially attenuated development of HO anlagen in mice treated with TGFβR-Fc with decreased osseous deposition and marrow space formation on histologic examination. Furthermore, the volume of mature HO was significantly decreased in the treatment group.

CONCLUSIONS: Heterotopic ossification, from trauma or congenital FOP, severely limits mobility, function and quality of life of affected patients and currently, no prevention strategies exist. In this study, we demonstrate that Tgfβ1 signaling plays a critical role in HO formation and treatment with TGFβR-Fc is effective in attenuating the early stages of ectopic bone deposition resulting in decreased HO volume. This therapeutic approach reveals a novel avenue for the treatment of this condition that may translate into effective clinical applications.

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Minimally Processed Adipose-Derived Stem Cells Increase Union Rates in a Murine Model of Irradiated Mandibular Fracture Repair: Enhancing the Translational Application of Cell Based Therapeutics

Kavitha Ranganathan, MD, Jeremy V. Lynn, N/A, Kevin Urlaub, BS, Noah S. Nelson, BS, Alex Donneys, MD, Lauren Buchman, N/A, Steven R. Buchman, MD

University of Michigan, Ann Arbor, MI, USA

PURPOSE: Cell-based therapeutics represent a critically important component of regenerative medicine and tissue engineering methodologies, as these techniques inherently possess the requisite machinery for tissue growth and differentiation. More specifically, adipose-derived stem cells (ASCs) have been studied extensively throughout the past several decades due to their multipotent potential,
distinct biomarkers, and relative ease of harvest. Despite the immense potential of ASCs to enhance bone regeneration, the Food and Drug Administration (FDA) remains hesitant to approve ASC-based therapies given the consistent need for scaffolds, onerous processing techniques, and requirement of cell culture. The purpose of this study is to define the optimal method of administration of ASCs that will most readily mitigate the deleterious effect of radiation therapy (XRT) on bone healing during fracture repair while maintaining compliance with FDA guidelines; this will consequently maximize the translational applicability and clinical adoption of such therapies.

METHODS: Forty-four male Lewis rats were randomly divided into four groups: control, XRT, ASC, and minimally processed ASC (MP-ASC). Excluding the control group, all rats received a fractionated dose of 35Gy of radiation, and all groups underwent subsequent mandibular osteotomy. The ASC group was treated with cultured ASCs, while the MP-ASC group received non-cultured ASCs. More specifically, for both groups, ASCs were harvested from the inguinal fat pads of isogenic Lewis rats. Cultured ASCs were processed, plated, and achieved confluence within 10–12 days. These cells were subsequently implanted into the osteotomy site at passage two. In the MP-ASC treated group, ASCs were harvested, centrifuged, and immediately implanted into the osteotomy site without the need for cell culture. After animals were sacrificed on post-operative day 40, gross pathology and MicroCT analysis were utilized to determine union rates and the quality of the bony regenerate within the osteotomy site.

RESULTS: The implantation of MP-ASCs significantly increased union rates compared to XRT alone based on MicroCT results and pathology (60% vs. 15%). Although MP-ASC administration resulted in slightly decreased union rates compared to cultured ASCs (60% vs. 100%), the quality of bone regenerated was similar between the groups based on bone mineral density (687.25±92.02 vs. 619.64±42.5; p=0.17) and bone volume fraction (0.755±0.097 vs. 0.721±0.057; p=0.75). The implantation of cultured ASCs resulted in similar union rates as non-radiated mandible fracture sites (100% vs. 100%)

CONCLUSIONS: Mesenchymal stem cells (MSCs) are adult stem cells with immense potential to enhance bone healing and regeneration following injury. This study identifies the ability of MP-ASCs, a minimally processed type of MSCs, to enhance bone regeneration in the absence of cell culture. With these results in mind, additional studies are required to further maximize the osteogenic potential of ASCs while also maintaining adherence to FDA regulations that mandate the minimal processing of tissues prior to implantation.

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Changes in Mouse Skeletal Progenitor Cells in a Model of Osteoporosis

Tom Andrew, MBChB, MSc¹, Danielle Struck, BS¹, Michael Lopez, BS¹, Tatiana Boyko, MD¹, Michael Longaker, MD¹, Charles Chan, BS PhD¹, George Yang, MD PhD¹,²

¹Stanford University, Palo Alto, CA, USA, ²Palo Alto VA Health Care System, Palo Alto, CA, USA

PURPOSE: Osteoporosis is a disease characterized by low bone mass and structural deterioration of bone tissue. People with osteoporosis are more prone to fracture due to the decrease in bone mass with 40% of post-menopausal women developing an osteoporotic fracture. Of those suffering a fracture, 20% will die and only 30% will fully recover indicating that there is also a deficit in fracture healing. Our group recently described mouse skeletal progenitor cells. One sub-population, the mouse skeletal stem cell (mSSC) has been shown to be capable of forming all components of the skeleton. Another sub-population, the bone cartilage skeletal progenitor (BCSP) has been found to be important in fracture healing. We hypothesized that the changes in skeletal anatomy following estrogen depletion will be reflected by alterations in skeletal progenitor cell populations.

METHODS: C57/BL6 mice underwent either sham operation or oophorectomy. Bone mineral density (BMD) was assessed by microCT. For fracture healing, mice underwent femur fracture 10 weeks after oophorectomy. Skeletal progenitor cells were harvested from unjured bone or fracture callus as previously described and sorted by FACS. For colony forming units (CFUs), sorted cells were placed in growth medium for two weeks prior to colony counting.