Jagged-1 Improves Bone Graft Incorporation During Healing of Irradiated Critical Size Bone Defects

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DISCLOSURES: None

INTRODUCTION: Non-vascularized bone grafting (NVBG) in previously irradiated tissues is currently contraindicated due to the detrimental effects of radiotherapy on bone formation and graft incorporation ("Take"). Vascularized free tissue transfers remain the gold-standard for reconstructing bone defects in irradiated tissues; however, these arduous procedures require microsurgery and extensive tissue dissections, which enhance donor site morbidity and negatively impact patient quality of life. The purpose of this preclinical study was to therapeutically reverse the detrimental effects of radiotherapy on bone graft incorporation with implantable recombinant Jagged-1 (Jag1) in irradiated rat mandibular critical sized defects. Jag1 is a membrane-bound ligand in the Notch signaling pathway. We have previously shown that recombinant Jag1 can enhance osteoblast differentiation (Wagley 2020) and bone formation (Youngstrom 2017) when delivered on biomaterials. We hypothesized that Jag1 implanted to irradiated mandibles would demonstrate significant improvements beyond irradiated mandibles without implanted treatment. Additionally, we posited that the Jag1-mediated improvements would demonstrate metrics comparable to non-irradiated-control bone grafting levels.

METHODS: With appropriate institutional animal care and use approval, male Lewis rats received a human equivalent fractionated dose of radiotherapy (7Gy/d x 5d) to left hemi-mandibles. After recovery, a circular trephine burr (6mm) was utilized to create a critical sized defect just posterior to the third molar, and a bone graft was harvested from the right hemi-mandible of the same animal and secured with PDLLA plates and fasteners. This novel radiolucent fixation method facilitated longitudinal imaging without metal artifact. Three groups of animals were investigated: Control (no radiation but with bone defect and implant), irradiated (XRT), and irradiated + Jagged-1 (Jag1-XRT). Mandibles were imaged at 14, 40, and 60 days with *in vivo* µCT, and a 60-day healing period was allowed prior to further outcomes testing. Bone graft incorporation was judged clinically by three blinded reviewers on a scale from 0 to 4, representing the approximate percentage of robust bone formation along the circular graft-recipient site interface (where 0=0%, 1=25%, 2=50%, 3=75%, and 4=100%). Mandibles underwent biomechanical push-out testing *ex vivo*. Statistical comparisons were conducted with ANOVA ($p<0.05$).

RESULTS SECTION: Bone mineral density (BMD) demonstrated a trend for higher Control values at 14 and 40 days above XRT and Jag1-XRT, and a significant increase above the XRT and Jag1-XRT groups at 60d (**Fig 1A**). Bone volume fraction (BVF) demonstrated a higher Control value over XRT throughout the timeline, as expected (**Fig 1A&B**). Interestingly, we observed a striking initial increase in 14d Jag1-XRT BVF over XRT, followed by a loss of that significant gain at 40d, subsequently followed by a regaining of significance over XRT at the 60d mark. There were no differences between Control and Jag1-XRT BVF at any reported timepoint in the study. Bone graft incorporation demonstrated that Control had the highest score of 94% incorporation, and XRT demonstrated 35%. Jag1 treated animals demonstrated 78% incorporation (**Fig 1B**). Biomechanical testing demonstrated significant increases in Control and Jag1-XRT over XRT in yield, stiffness, and max load (**Fig 1C**). There were no significant differences between Control and Jag1 for any of the biomechanical metrics.

DISCUSSION: We observed increased NVBG incorporation, enhanced biomechanical strength, and improved radiomorphometrics, when comparing Jag1 to no-treatment XRT. The Jag1 group demonstrated a 78% bone graft incorporation rate, representing a 43% improvement above the XRT group. Significant improvements were also demonstrated for all biomechanical metrics when comparing Jag1 to no treatment. Irradiated tissue shows poor vascularization and as bone requires robust vascularization this may suggest that either Jag1 treatment can increase vascularization or drive osteoblastogenesis and bone formation in a hypoxic environment. Despite this, the BMD of the Jag1 group was inferior to the Control group at all timepoints in the study, which could indicate that the regenerated bone is immature and slower to mineralize. Based on these observations, subsequent studies may explore a sustained release delivery of Jag1, and enhanced delivery matrix to improve osteogenesis and graft incorporation.

SIGNIFICANCE/CLINICAL RELEVANCE: This study provides preclinical evidence in support of developing Jag1 as a therapeutic to improve non-vascularized bone graft incorporation in irradiated bone defects. Potential translation of this promising therapeutic offers a notable expansion of the surgical armamentarium to reconstruct these challenging bone defects for cancer patients exposed to radiotherapy.

REFERENCES: 1. Wagley et al. *Stem Cells* 38.10 (2020): 1332-1347. 2. Youngstrom, et al. *NPJ Regenerative medicine* 2.1 (2017): 32.

IMAGES AND TABLES:

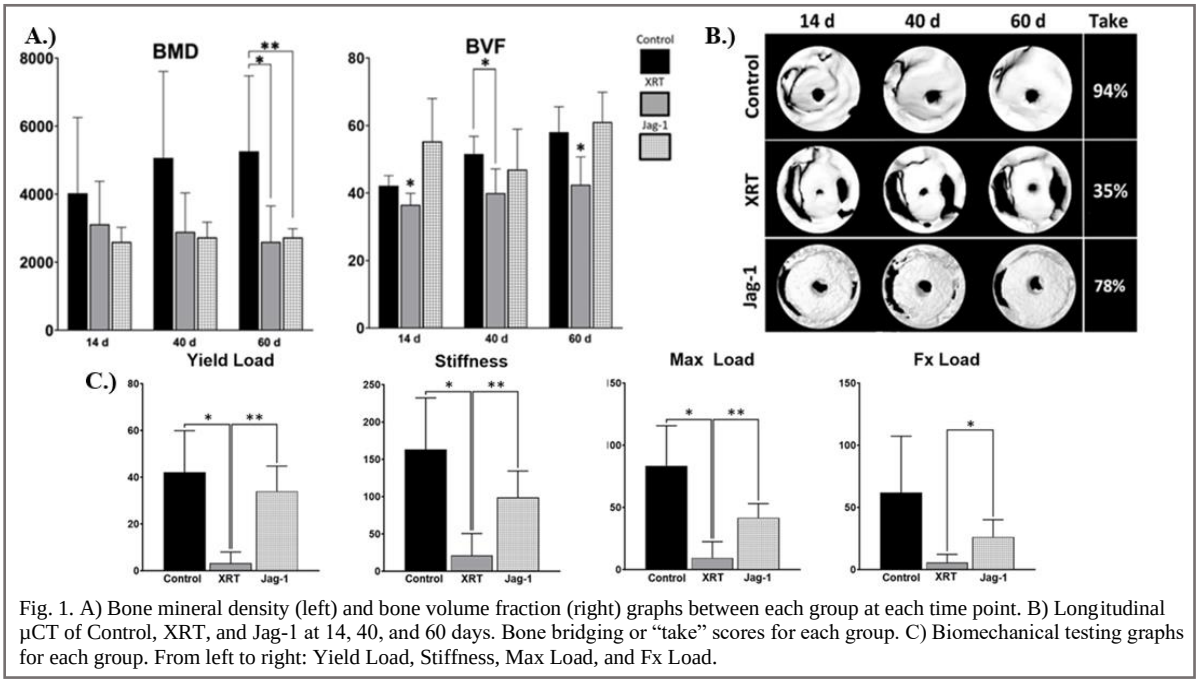

Fig. 1. A) Bone mineral density (left) and bone volume fraction (right) graphs between each group at each time point. B) Longitudinal µCT of Control, XRT, and Jag-1 at 14, 40, and 60 days. Bone bridging or "take" scores for each group. C) Biomechanical testing graphs for each group. From left to right: Yield Load, Stiffness, Max Load, and Fx Load.