Synergistic Gains in Vertebral Bone Mass When Sclerostin Antibody is Combined with Bisphosphonate in Brtl/+ Mouse Model of Osteogenesis Imperfecta

Diana Olvera 1,2, Rachel L. Stolzenfeld 1, Joan C. Marini 1, Michelle S. Caird 1, Kenneth M. Kozloff 1,2

1 Orthopaedic Research Laboratories, Department of Orthopedic Surgery; 2 Dept. of Biomedical Engineering, University of Michigan, Ann Arbor, MI, USA

Disclosures: The authors have nothing to disclose.

Introduction: Treatments for pediatric osteogenesis imperfecta (OI) have focused on improving bone density to promote functional strength and consequently reduce bone fragility. Though antiresorptive agents like bisphosphonates (BP) are currently the most common intervention for the treatment of OI, anabolic drugs like sclerostin antibody (SclAb) have shown efficacy at increasing bone mass and strength in animal models [1]. The short-term effects of the concurrent use of pamidronate (PAM) and SclAb have shown that in rapidly growing Brtl/+ mice, the agents induce additive gains in bone mass by independently increasing trabecular number and thickness [2]. To develop a potential treatment plan to maximize combination therapy for pediatric OI, we evaluated the long-term effects of cyclic combination therapy, where one cycle consisted of a single PAM injection followed by a two week SclAb treatment. We hypothesize that accumulating cycles of combination therapy may induce a synergistic effect on improving bone mass by first preventing resorption of primary trabecular bone through PAM, creating added substrate for new bone formation to occur via SclAb.

Materials and Methods: Animal Studies: To model the chronic effects of PAM and SclAb (Scl-Ab VI, provided by Amgen, Thousand Oaks, CA & UCB, Brussels, Belgium) in OI, Brtl/+ mice, with a G349C mutation on col1a1 and WT controls received two cycles of combination therapy. At 21 days, male mice began cycle one with a single intravenous injection of PAM at either 0.3mg/kg or 0.625mg/kg or saline control. After a three-day latency period, mice were randomly assigned to SclAb treatment or saline groups and injected subcutaneously twice a week for two weeks, at 25mg/kg. Cycle two was repeated starting on day 38 and mice were euthanized at day 55. In the attempt to minimize systemic bone turnover, PAM doses were chosen as a fraction of those shown to induce trabecular retention in growing mice [3]. SclAb was chosen at a dose that consistently induced an anabolic effect in our prior studies [4]. During necropsy, L5 vertebrae were harvested for further analysis. MicroCT: L5 vertebrae were scanned using micro-computed tomography (eXplore Locus SP, GE Healthcare Pre-Clinical Imaging, London, ON, Canada) at 18 micron voxel size. A ROI was placed mid-distance between cranial and caudal endplates to separately evaluate cortical and trabecular bone. Cortical and trabecular bone were segmented using a 2000 Hounsfield units threshold and local threshold, respectively. Standard bone architecture parameters were analyzed using commercially available software (MicroView Advanced Bone Analysis Application, GE Healthcare Pre-Clinical Imaging, London, ON, Canada). Statistical comparisons among the different treatments were made using two-way ANOVA and p < 0.05 was considered significant.

Results: MicroCT analysis of L5 trabecular bone (Fig. 1A) showed that treatment with PAM alone induced an average preservation of trabecular number of 10% for Brtl/+ and 3% for WT vs. untreated controls. SclAb alone also led to an average trabecular number preservation of 14% for Brtl/+ and 10% for WT. While PAM had no effect on trabecular thickness, SclAb consistently increased trabecular thickness across all doses (Brtl/+ 36%; WT 57%). Together, these effects led to a synergistic effect on overall bone volume fraction where the combined effects of monotherapy from PAM (Brtl/+ 11%; WT 3%) and SclAb (Brtl/+ 34%; WT 64%) showed lower gains in bone volume fraction than combination therapy at 0.3 mg/kg (Brtl/+ 59%; WT 72%) and 0.625 mg/kg (Brtl/+ 73%; WT 81%) compared to untreated control. While PAM had no effect on lumbar vertebral cortical thickness, SclAb alone induced average cortical thickening gains of 40% in Brtl/+ and 35% in WT. Functionally, PAM alone induced a dose dependent increase in stiffness at 0.3 mg/kg (Brtl/+ 66%; WT 19%) and 0.625 mg/kg (Brtl/+ 78%; WT 52%) vs. untreated control, but had no influence on maximum load (Fig. 1B). SclAb, when combined with PAM, consistently increased stiffness by 91% for Brtl/+ and 101% for WT. Unlike PAM, SclAb monotherapy increased maximum load by 33% for Brtl/+ and 71% for WT. However, when combined with a higher dosage of PAM, a synergistic effect was observed in both Brtl/+ (98%) and WT (90%).

Discussion: The present results demonstrate that antiresorptive BP and anabolic SclAb exhibit a synergistic effect on vertebral bone mass and ultimate load when administered chronically in cycles. These results suggest that modest doses of PAM, which would otherwise not induce a sustained therapeutic effect, may be sufficient to preserve trabecular architecture enough to permit the anabolic action of SclAb on bone that would otherwise be remedied. In addition these results extend prior studies that have explored single-drug therapy [5,6], but also demonstrate the beneficial synergistic effect that is achieved with multiple treatments that was not seen in our previous acute study. Furthermore, mechanically, SclAb alone can significantly improve maximum load, but minimal PAM dosing may help amplify the effects of SclAb to further improve vertebral rigidity, a beneficial outcome for children with OI.

Significance: SclAb has been proposed as a novel anabolic intervention to treat the low bone mass and fragility phenotype present in patients with OI. The present data provides key pre-clinical results to support a synergistic treatment plan to first stabilize retention of trabecular bone during growth with anti-resorptive therapy, facilitating a larger baseline bone mass upon which SclAb can subsequently induce an anabolic response.

Figure 1. Dumbbell plots show PAM alone (open markers) and PAM+SclAb combination treatment (solid markers) for both Brtl/+ (blue and circles) and WT (black and squares). Standard deviations are shown as shadows behind the dot plots. A) Microstructural properties of the vertebral trabecular bone reveal overall preservation of trabecular number with PAM and increased trabecular thickness with SclAb. Though monotherapy results in bone volume fraction gains, combination therapy revealed a synergistic anabolic response. Gains in cortical thickness were realized with SclAb and not PAM. B) Mechanical properties of the vertebral body reveals that both PAM and SclAb induced a dose-dependent increase on stiffness, however, maximum load was increased only when combined with SclAb. A synergistic effect was observed when SclAb was combined with a higher dose of PAM. Results of Two-Way ANOVA factors for PAM and SclAb are shown: * p<0.05; ** p<0.01; **** p<0.0001.