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Adult Brl/+ Mouse Model of Osteogenesis Imperfecta Demonstrates Anabolic Response to Sclerostin Antibody Treatment with Increased Bone Mass and Strength

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Abstract

Purpose—Osteogenesis imperfecta (OI) is a heritable collagen-related bone dysplasia, characterized by brittle bones with increased fracture risk. Although OI fracture risk is greatest before puberty, adults with OI remain at risk of fracture. Anti-resorptive bisphosphonates are commonly used to treat adult OI, but have shown mixed efficacy. New treatments which consistently improve bone mass throughout the skeleton may improve patient outcomes. Neutralizing antibodies to sclerostin (Scl-Ab) are a novel anabolic therapy that have shown efficacy in preclinical studies by stimulating bone formation via the canonical wnt signaling pathway. The purpose of this study was to evaluate Scl-Ab in an adult 6 mo old Brl/+ model of OI that harbors a typical heterozygous OI-causing Gly>Cys substitution on *Colla1*.

Methods—6mo old WT and Brl/+ mice were treated with Scl-Ab (25mg/kg, 2x/week) or Veh for 5 weeks. OCN and TRACP5b serum assays, dynamic histomorphometry, microCT and mechanical testing were performed.

Results—Adult Brl/+ mice demonstrated a strong anabolic response to Scl-Ab with increased serum osteocalcin and bone formation rate. This anabolic response led to improved trabecular and cortical bone mass in the femur. Mechanical testing revealed Scl-Ab increased Brl/+ femoral stiffness and strength.

Conclusion—Scl-Ab was successfully anabolic in an adult Brl/+ model of OI.

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Disclosures

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Keywords

Osteogenesis imperfecta; sclerostin antibody; osteoporosis; mouse model

Introduction

Osteogenesis imperfecta (OI) is a heritable disorder caused by mutations in type I collagen or collagen-associated proteins. Patients with OI have increased bone fragility, causing fracture susceptibility from minimal force, skeletal deformities and growth deficiency [1]. OI is a disease that most severely affects children, and fracture rate tends to decrease after puberty. Despite this relative improvement, adults remain at an increased risk for fracture compared to a normal population, and commonly have osteoporotic BMD values [2].

Treatment options to improve bone strength in adults with OI include anti-resorptive bisphosphonates (BP) which are considered the current standard of care. However, the limited clinical trials investigating BP use in adult OI have found mixed reports, with some studies citing a reduced fracture incidence with treatment, while others show no therapeutic effect despite increases in bone mass [3–6]. More effective therapies are needed for the treatment of adult OI and several preliminary reports have begun to investigate the use of anabolic teriparatide [7, 8].

Neutralizing antibodies to sclerostin are a novel anabolic osteoporosis treatment strategy presently in clinical trials for the treatment of osteoporosis, and may be useful for increasing bone mass in adult OI. Sclerostin is a negative regulator of bone formation secreted primarily by osteocytes which inhibits anabolic canonical wnt signaling in osteoblasts by binding to the LRP4/5/6 complex [9–11]. Treatment with a neutralizing antibody to sclerostin (Scl-Ab) prevents sclerostin inhibition of canonical wnt signaling and effectively reduces sclerostin's ability to limit bone formation. Scl-Ab has been shown to be highly anabolic in pre-clinical models of osteoporosis and in phase 1 and phase 2 clinical trials of men and postmenopausal women [12–15].

The *Brtl/+* mouse is a knock-in model for moderately severe Type IV OI with a G349C point mutation on *Col1a1* that recreates an identical defect found in an OI patient [16]. The *Brtl/+* mouse recapitulates multiple features of the observed clinical phenotype including short stature, reduced BMD, increased bone brittleness, and impaired remodeling [17–19]. Importantly, the *Brtl/+* phenotype becomes less severe with age [17], making it an appropriate model for testing the anabolic efficacy of Scl-Ab in adult OI.

We have previously reported that short-term Scl-Ab therapy is capable of stimulating bone formation and increasing bone mass in an 8 week old *Brtl/+* model of OI [20]. Importantly, no preclinical studies have analyzed the effects of any anabolic or anti-catabolic agents in an adult model of OI. The purpose of this study was to determine if Scl-Ab could increase bone formation in osteoblasts harboring a classical OI-causing mutation to improve bone mass and whole bone strength in an adult *Brtl/+* model of OI.

Materials and Methods

Animals

Wildtype (WT) and Brlt/+ mice are maintained on a mixed background of Sv129/CD-1/C57BL/6S, and all Brlt/+ animals were the product of breeding male Brlt/+ with female WT. 6 month old male WT and Brlt/+ mice were randomly assigned to Scl-Ab (Scl-Ab VI, Amgen, Thousand Oaks, CA) treatment or vehicle injection (PBS) with WT Veh n=8, WT Scl-Ab n=9, Brlt/+ Veh n=8, and Brlt/+Scl-Ab =9. Sclerostin antibody was injected subcutaneously at 25mg/kg, two times per week, for five weeks, following the protocol described previously[13]. To facilitate dynamic histomorphometry, calcein (30mg/kg, i.p.) was injected at the start of experiment, 3 weeks before sac and 1 week before sac. Alizarin (30mg/kg, i.p.) was injected 1 day prior to sacrifice. Body weights were recorded with each injection. Blood samples were collected at euthanasia by intracardiac puncture, serum separated by centrifuge, and stored at -80°C until analyzed by ELISA.

Left femurs were collected for microCT and mechanical testing, and right femurs for dynamic histomorphometry. Both were stored at -20°C in lactated ringers solution (LRS) soaked gauze until testing or further specimen preparation. All protocols and procedures involving animals were approved by the University of Michigan's Committee on Use and Care of Animals.

Serum Assays

To measure osteoblast activity, serum osteocalcin (OCN) was quantified with a commercially available ELISA kit (BT-470, BTI, Stoughton, MA). To quantify osteoclast number, serum TRACP5b was measured with a commercially available solid phase immunofixed enzyme activity assay (MouseTRAP, IDS, Fountain Hills, AZ). Both serum tests were performed in duplicate.

MicroCT

Left femora were scanned in water using cone beam computed tomography (eXplore Locus SP, GE Healthcare Pre-Clinical Imaging, London, ON, Canada). Scan parameters included a 0.5 degree increment angle, 4 frames averaged, an 80kVp and 80μA x-ray source with a 0.508mm Al filter to reduce beam hardening artifacts, and a beam flattener around the specimen holder [21]. All images were reconstructed and calibrated at 18μm isotropic voxel size to a manufacturer supplied phantom of air, water and hydroxyapatite. The entire femora was reoriented with the mid-diaphysis parallel to the z-axis, and bone length was measured as the distance between the most proximal and distal transverse plans containing the femur. Regions of interest (ROI) were located for both cortical and trabecular parameters. A diaphyseal cortical ROI spanning 15% of total femur length was located midway between the distal growth plate and third trochanter. Cortical bone was isolated with a fixed threshold of 2000 Hounsfield Units for all experimental groups. Parameters including cortical thickness, cross sectional area, tissue mineral density (TMD), bending moment of inertia in the anterior-posterior direction (about the medial-lateral axis), endosteal perimeter, and periosteal perimeter were quantified with commercially available software (MicroView v2.2 Advanced Bone Analysis Application, GE Healthcare Pre-Clinical Imaging, London, ON,

Canada). A trabecular ROI 10% of total femur length was located approximately 100 microns proximal to the central, and most proximal, portion of the distal femoral growth plate. The inner cortical surface was defined with a splining algorithm. Due to the different morphology induced by Scl-Ab treatment, a fixed threshold could not be utilized without bias. Trabecular metaphyseal bone was isolated with a more conservative autothresholding algorithm for each specimen based on the bimodal distribution between marrow and bone [22]. Parameters including bone volume fraction (BV/TV), trabecular thickness (TbTh), and trabecular number (TbN) were quantified using standard stereology algorithms (MicroView v2.2). A 3D sphere fitting algorithm was also used to confirm the stereology data for TbTh [23].

The 8th caudal vertebrae were dissected and scanned with the same protocol as the femora. Trabecular bone of the caudal vertebrae was analyzed in two ROIs, each spanning 9% of bone length and placed adjacent to the proximal and distal growth plates. The trabecular surface was defined on the inner cortical surface with a splining algorithm. Cortical bone of the 8th caudal vertebrae was analyzed in a ROI spanning 9% of bone length and placed in the middle of bone. Cortical thickness was measured along four lines originating at the center of gravity of the section, and placed 40 and 50 degrees anterior to the medial lateral plane similar to previous reports [24].

Whole Bone Mechanical Four-Point Bending

Following μ CT scanning, left femora were loaded to failure in four-point bending using a servohydraulic testing machine (MTS 858 MiniBionix, Eden Prairie, MN). All specimens were kept hydrated in LRS-soaked gauze until mechanical testing. In the same mid-diaphyseal region analyzed by μ CT, the mid-diaphysis was loaded in four point bending with the posterior surface oriented under tension. The distance between the wide, upper supports was 6.26mm, and span between the narrow, lower supports 2.085mm. The vertical displacement rate of the four-point bending apparatus in the anterior-posterior direction was 0.5mm/sec. Force was recorded by a 50lb load cell (Sensotec) and vertical displacement by an external linear variable differential transducer (LVDT, Lucas Schavitts, Hampton, VA), both at 2000Hz. A custom MATLAB script was used to calculate all parameters measured from the force-displacement data. Elastic energy was defined as the area under the load-displacement curve prior to the yield point, and plastic energy was defined as the area under the load-displacement curve from the yield point until failure. The yield point was defined as a 10% reduction in secant stiffness relative to the initial tangent stiffness. Combining bending moment of inertia data from μ CT with mechanical stiffness from four point bending, the estimated elastic modulus was calculated using standard beam theory as previously described [17].

Dynamic Histomorphometry

Right femora were encased in epoxy (Kold Mount, Vernon-Benshoff, Albany, NY), and cut transversely with a low-speed saw (IsoMet, Beuhler, Lake Bluff, IL). The distal section of tissue was polished using progressive grades of silicon carbide abrasive paper (1200, 2400, and 4000 grit) to a plane immediately distal to the third trochanter.

Fluorescent images were acquired using a Zeiss Axiovert 200M inverted microscope equipped with a structured illumination (Apotome) imaging system. This focuses the image on a specific plane, minimizes out-of-plane light, and negates the need for several micron thin sections. Multiple fluorescent images were taken with a 10x objective of the calcein (excitation: 485/20nm, emission: 540/25nm) and alizarin (excitation: 557/55nm, emission: 615nm) labels around the entire cortex. The 10x images of the cortex were merged into a single image (Photoshop, Adobe), and these merged images were analyzed using commercially available software (Bioquant Osteo v7.20.10, Nashville, TN). Bone surface (BS), mineral apposition rate (MAR), mineralizing surface to bone surface (MS/BS), and bone formation rate (BFR) were quantified on the calcein label given 3 weeks before sacrifice and the alizarin label given 1 day before sac on the periosteal surface and analyzed according to ASBMR standards [25].

In order to quantify the level of cortical drift in these mice and contrast the therapeutic response to prior studies analyzing rapidly growing 3 week old *Brtl/+* mice treated with Scl-Ab [26], dynamic histomorphometry outcomes were also assessed in anatomic regions around the cortex with a method similar to Bivi et. al [27]. The fluorescent images were oriented anatomically by using the contralateral microCT femoral 3D images as a reference. The centroid of the fluorescent image was identified in ImageJ, and 110 degree angles from the centroid were used to define the anterior and posterior regions (Fig 4). MAR, MS/BS, and cortical thickness were quantified in each of these anterior and posterior subregions.

In any regions where there was no dual label, MAR was treated as a missing value. For the purpose of calculating BFR/BS, a value of 0.1 μ m/day was assigned to MAR where missing values were present. This value was determined from both looking at the biological limit observed in our specimens as well as values established in the literature [27]. BFR/BS was also analyzed when treated as a missing value. One animal (WT Veh) had a missing fluorescent label, most likely due to injection error, and was excluded from this analysis. Two additional animals (both WT Scl-Ab) displayed all fluorescent labels, but a portion of the endosteal surface had errors in image acquisition which could not be corrected. Both of these animals were excluded from analysis on the endosteal surface only. Final group sizes are shown in the relevant figures and tables.

Statistics

A two-factor multivariate ANOVA was performed for all data (IBM SPSS Statistics v21.0.0.0), the individual factors as genotype (WT or *Brtl/+*) and treatment (Veh or Scl-Ab). The interaction term between genotype and treatment is also presented. Tukey's HSD post-hoc test was used to make comparisons except when unequal variances were observed. Dunnett's T3 post-hoc test was used for variables which displayed unequal variances. In all cases, $p < 0.05$ was considered significant. Values between $p = 0.05$ and $p = 0.10$ are reported in all instances, and are referred to as "trends" in the text. All data are presented as mean $\pm$ S.D.

Results

Body Mass and Bone Length Not Changed by Scl-Ab

At the beginning of the experiment, WT animals (38.4 ± 4.1 g) were significantly heavier than Brl/+ (34.9 ± 4.7 g) when pooling data from Veh and Scl-Ab groups for each genotype. Throughout the 5 weeks of experiment, WT Veh and Brl/+ Veh did not have any significant changes in body mass. Scl-Ab did not induce a change in body mass during the 5 weeks of treatment (not shown).

Brl/+ Veh femurs were shorter than WT Veh as assessed by endpoint microCT (Table 1). Scl-Ab had no effect on femoral length.

Scl-Ab Increased Serum Osteocalcin and Reduced Serum TRACP5b

Scl-Ab significantly increased serum osteocalcin (OCN) levels in both WT (+49%) and Brl/+ (+47%) indicative of increased bone formation and an anabolic response to therapy (Fig 1). Serum OCN levels were not different between Veh treated WT and Brl/+ mice.

Serum TRACP5b, a marker of osteoclast number, was significantly decreased in WT (−78%). Despite a numerical decrease in Brl/+ (−66%) with Scl-Ab treatment, it was not statistically significant ($p=0.064$) (Fig 1). The interaction term between genotype and treatment was not significant, suggesting that Scl-Ab did not have a differential effect on Serum TRACP5b between WT and Brl/+. Similar to OCN data, there were no phenotypic differences observed between WT Veh and Brl/+ Veh in serum TRACP5b.

Scl-Ab Increases Femoral Trabecular Thickness and Trabecular Number

Phenotypically, Brl/+ Veh animals had reduced femoral trabecular BV/TV (Fig 2) compared to WT Veh (−93%). These differences were the result of significant reductions in trabecular number (−67%), but not trabecular thickness.

In WT and Brl/+, five weeks of Scl-Ab therapy significantly increased bone volume fraction (WT +117%; Brl +119%) and trabecular BMD. The significant gains in femoral trabecular bone mass with therapy were a result of significant increases in both trabecular thickness (WT +38%; Brl +29%) and trabecular number (WT +70%; Brl +55%).

Despite these significant gains in the femur, we observed no effect of Scl-Ab on trabecular bone of the 8th caudal vertebrae by microCT (Fig 3)

Scl-Ab Increases Brl/+ Bone Formation on Endosteal and Periosteal Femora Surfaces

On the total periosteal surface of the diaphyseal femur, treatment of Brl/+ with Scl-Ab significantly increased BFR/BS (+205%) as a result of large increases in MAR (+132%) and smaller gains in MS/BS (+38%) (Fig 4). In WT animals, Scl-Ab significantly increased MAR (+114%) which led to an increase in BFR/BS (+169%). Scl-Ab did not significantly increase MS/BS in WT on this surface even though 7 of 9 WT Scl-Ab animals had a MS/BS greater than the largest WT Veh value on the periosteal surface. The interaction term was not significant, suggesting Scl-Ab did not behave differently for periosteal MS/BS between genotypes. On the total endosteal surface of both genotypes, Scl-Ab significantly increased

MS/BS (WT +181%; Brl +155%) and MAR (WT +126%; Brl +180%) to a similar extent. These gains led to large increases in endosteal BFR/BS in both genotypes with Scl-Ab treatment (WT +650%; Brl +683%). No differences were found between WT Veh and Brl Veh on either surface, but overall Brl/+ had greater endosteal MS/BS levels.

Regional analysis in the anterior and posterior regions of the cortex revealed a weak posterior shifting cortical drift pattern in both WT Veh and Brl/+ Veh with more bone formation observed on the posterior periosteal surface compared to the anterior periosteal surface (Fig 4A). No complimentary cortical drift pattern was observed on the endosteal surface, where bone formation was very low in both genotypes.

Analyzing the effect of Scl-Ab on the anterior and posterior sub-regions revealed a similar effect on the periosteal surface in each compartment, with BFR/BS gains primarily driven by increases in MAR (Table 2). On the endosteal anterior and posterior surfaces, Scl-Ab increased MAR as well as MS/BS in Brl/+, leading to increased BFR/BS.

Analyzing BFR/BS data as a missing value when no MAR was present led to an identical statistical conclusion on the periosteal and endosteal surfaces (data not shown). Two small statistical differences are apparent in the regional data on the low-forming endosteal posterior surface. Specifically, when BFR/BS is treated as a missing value, WT Veh vs WT Scl-Ab becomes significant on the endosteal posterior surface, and is no longer significant ($p=0.068$) for Brl/+ Veh vs Brl/+ Scl-Ab.

Scl-Ab Increases Brl/+ Femoral Cortical Bone Mass

Five weeks of Scl-Ab led to large increases in cortical cross-sectional area (WT +36%; Brl +21%) and cortical thickness (WT +42%; Brl +22%) in both genotypes (Table 1) as measured by microCT of the left femora. Consistent with the stronger endosteal vs periosteal response observed by dynamic histomorphometry, Scl-Ab significantly decreased marrow area in both genotypes (WT -11%; Brl -19%), although total area and bending moment of inertia were unchanged. A two-factor ANOVA revealed that overall Scl-Ab significantly increased the Bending Moment of Inertia and TMD, although the Scl-Ab effects were not significant within each genotype for these outcomes.

No geometric phenotypic differences were observed between WT Veh and Brl/+ Veh in the femoral cortex at this time point. However, overall genotype effects from a two-factor ANOVA revealed that relative to WT, Brl/+ had significantly reduced Marrow Area, significantly reduced Total Area, and significantly increased TMD.

Consistent with a lack of effect in caudal vertebral trabecular bone, there was no effect of Scl-Ab on cortical thickness in either WT or Brl/+ 8th caudal vertebrae (Fig 3).

Brl/+ Whole Bone Mechanical Properties Improved by Scl-Ab

Significant gains in whole bone mass with Scl-Ab led to significant improvements in whole bone mechanical properties as measured by mechanical four-point bending of the femoral mid-diaphysis (Fig 5, Table 1). Specifically, five weeks of Scl-Ab caused large increases in Yield Load (WT +79%; Brl +77%), Ultimate Load (WT +129%; Brl +127%) and Bending

Stiffness (WT +70%; Brtl +38%) in both genotypes (Table 1). While increases in Energy-to-Failure with Scl-Ab treatment were expected based on the effects on Ultimate Load, the magnitude of the increases were unexpected (WT +526%; Brtl +509%). These observed increases in Energy-to-Failure with Scl-Ab were primarily a result of significantly increased plastic energy (WT +1498%; Brtl +1660%) and in lesser part a result of increased elastic energy (WT +96%; Brtl +153%). The increased plastic energy with Scl-Ab was a result of an increase in Post-Yield Displacement with therapy in both WT (+539%) and Brtl/+ (+541%) as demonstrated in the representative load-displacement curves in Figure 5. Yield Displacement was increased in Brtl/+ with Scl-Ab, but not WT. The estimated elastic modulus was increased with Scl-Ab in WT, but there was no effect in Brtl/+.

Similar to cortical microCT data, there was no significant whole bone mechanical property phenotype of Brtl Veh vs WT Veh at this age. However, overall genotype effects from a two-factor ANOVA revealed that relative to WT, Brtl/+ had significantly reduced Energy to Failure, and significantly increased Elastic Modulus.

Discussion

In this study, we demonstrated that five weeks of Scl-Ab therapy was able to stimulate bone formation in an adult Brtl/+ mouse model of Type IV OI, leading to increased long bone mass and improved mechanical properties. No preclinical studies to date have analyzed the effect of pharmaceutical intervention for an adult mouse model of OI. In the context of existing anabolic and anti-resorptive treatment options, Scl-Ab is a novel drug with strong therapeutic potential for adult OI patients. While current treatment options to improve bone strength in adults with OI focus on bisphosphonates (BP), the functional benefit of antiresorptive therapy is appears modest. A randomized control trial of intravenous neridronate in adults with Type IV OI found increased BMD at the spine and hip with treatment as well as reduced total fracture incidence [4]. In a randomized placebo-controlled trial of oral alendronate in patients with Type I OI, increased BMD at the spine and hip was observed with treatment, but the study was not powered to detect fracture risk [3]. Two more recent clinical trials have noted similar increases in bone mass with BP treatment, but equivocal effects at reducing fractures in adults with OI [5, 6]. Moreover, BP efficacy may be dependent upon the type and severity of OI [5] as well as the dose. In light of these observations, strong motivation exists for the exploration of alternative therapies for the treatment of adult OI. To address this need, recent studies have begun to investigate the use of anabolic intermittent parathyroid hormone as a therapy for adult OI. An uncontrolled clinical trial indicates promising teriparatide efficacy in adult OI [7], and randomized double-blind placebo controlled trial also shows a benefit in Type I OI patients[8].

Here, we noted suggestions of age-specific differences in response to Scl-Ab treatment, emphasizing the importance of examining adult OI mice separately from young OI mice. Rapidly growing 3 week Brtl/+ mice treated for 5 weeks with Scl-Ab showed a similar gain in femoral cortical bone area (WT +31%; Brtl/+ 25%) when compared to the adult data (WT +36%; Brtl/+ +21%). Furthermore, in the present study, we observed significant gains in femoral BV/TV in both WT and Brtl/+ with 5 weeks of Scl-Ab (Fig 2). This contrasts with treatment of young Brtl/+ mice for an equivalent 5 weeks with Scl-Ab where we observed a

more modest femoral trabecular response based on distance from the growth plate [28]. This data is suggestive of a differential trabecular response with age that may be related to differential turnover, altered sclerostin expression with growth, and perhaps the absence of confounding linear growth effects.

While we expected Scl-Ab to increase femoral BV/TV in adult WT and *Brtl/+* mice in this study by increasing trabecular thickness, we did not expect to see large changes in trabecular number. Unexpectedly, we observed a large increase in trabecular number in the distal femoral metaphysis with five weeks of Scl-Ab (Fig 2). Decreased bone resorption could explain this finding by preserving trabeculae that might otherwise be lost to increased primary trabecular turnover. In this study we found decreased serum TRACP5b with Scl-Ab, consistent with reports from others showing decreased bone resorption in humans [12] as well as preclinical rodent data [13]. Alternatively, the large differences in morphology induced by Scl-Ab could affect quantification of trabecular number. If Veh animals contain trabeculae that were too thin to be detectable by microCT at the applied resolution, thickening of these structures to a detectable level by Scl-Ab could result in the increased trabecular number observed. While our conservative specimen-specific thresholding method helps guard against these effects, it does not eliminate them.

Interestingly, we observed no effect of Scl-Ab on either the trabecular bone or cortical thickness of the 8th caudal vertebrae in this study. One previous study in adult female rats has observed an anabolic response with Scl-Ab in the caudal vertebrae [29]. Specifically, increases in BFR/BS and BV/TV were measured in sections by histomorphometry at this site of yellow marrow. Interestingly, other studies of anabolic PTH have found reduced effects in the caudal vertebrae relative to the distal femur or lumbar vertebrae in ovariectomized rats with doses 20% of the present study [30]. Additional studies have observed a robust response to anabolic PTH in caudal vertebrae [31] while others have suggested that an anabolic PTH response in caudal vertebrae may be supported by mechanical loading [32]. Our lack of difference in BV/TV may reflect differences in local sclerostin expression or drug delivery in the caudal vertebrae, as differences in the mouse compared to the rat, or dose of Scl-Ab.

The femoral cortical dynamic histomorphometry response to Scl-Ab was also qualitatively different between the adult mice analyzed in this study and our treatment of rapidly growing 3wk *Brtl/+* mice. In the present study, Scl-Ab was able to consistently increase MAR on the periosteal surface. On the endosteal surface, where lower levels of bone formation were found in control animals, Scl-Ab was able to increase both MS/BS and MAR in most cases. Previously, we reported that a short-term two week Scl-Ab therapy in 8 week old *Brtl/+* mice stimulated femoral periosteal BFR by increasing mineralizing surface, but not mineral apposition rate [20]. In addition, we have also treated rapidly growing 3wk old *Brtl/+* mice for 5 weeks with Scl-Ab and similarly found that Scl-Ab was able to increase bone formation by primarily increasing MS/BS, with no effect on MAR on both the endosteal and periosteal surfaces [26]. Although the cortical drift pattern we observed was weak in these 6 month old mice, the posterior periosteal surface remained a location of high bone formation (MS/BS) similar to our observation in rapidly growing 3 week old mice. The regional dynamic histomorphometry analysis allows us conclude that Scl-Ab can increase MAR in

regions that have high MS/BS in adult animals, but is unable to increase MAR in these regions in rapidly growing animals. These findings may be related to different sclerostin expression levels with age, and a strong underlying cortical drift pattern in growing mice. In addition, rapidly growing 3wk mice have a high Veh MAR (~1.25–2.5µm/day) and may be operating at near maximum output, leaving little room for further stimulation via Scl-Ab. In contrast, lower Veh MAR levels in adult 6mo (~0.3µm/day) leave room for Scl-Ab to have a potent effect, and may explain the age-dependent effect of Scl-Ab on MAR.

Increased tissue brittleness is a hallmark feature of the OI phenotype. The *Brtl/+* model demonstrates increased brittleness at young ages when mechanically tested to failure, and remains brittle through 6 months [17]. In this study, we observed that mid-diaphyseal cortical bone of the *Brtl/+* femur had increased TMD, decreased energy to failure, and increased elastic modulus. Previously, we found an unexpected result that a short-term treatment with Scl-Ab significantly increased Post-Yield Displacement (indicating decreased tissue brittleness) in WT animals, but not *Brtl/+* [20]. In the present study, we found exceptionally large gains in PYD with 5 weeks of Scl-Ab treatment in both WT and *Brtl/+*. While we remain aware of the numerous violations of beam theory assumptions and loading differences that may result from Scl-Ab induced geometrical differences, the magnitude of the changes in PYD (WT +539%; *Brtl* +541%) is difficult to ignore. The cause of this difference could be related to the addition of new, more ductile bone, to a mature skeleton. Alternatively, it could reflect that bone formed under the influence of Scl-Ab may be of a different material independent of tissue age. It will be important to determine if these reductions in brittleness are persistent changes, or transient changes that disappear quickly after cessation of therapy. While the beneficial material differences of Scl-Ab on PYD are suggestive, these data require corroboration with detailed composition and mechanical techniques. Interestingly, we observed an increase in the estimated elastic modulus with Scl-Ab in WT, but not *Brtl/+*. This finding was not accompanied by similar gains in WT tissue mineral density, suggesting that changes at a level of organization below that of overall cortical size, but larger than tissue ultrastructural characteristics may be at play. Alternatively, the robust changes in cortical thickness found with sclerostin antibody may induce additional artifact related to beam theory calculations, as has been previously described [33].

There are several limitations of this study. First, osteogenesis imperfecta is a heterogeneous disease with a wide range of phenotypes caused by many different mutations. In this study, we used a mouse model harboring a mutation found in a patient with moderately severe Type IV OI. The findings of this study in the *Brtl/+* mouse may not apply to all mutations and types of OI, including recessive forms as well as those with null *coll* alleles. In addition, while Scl-Ab was potentially anabolic in adult *Brtl/+* mice in this study, it is unclear how long these gains will persist after cessation of therapy. The Scl-Ab dose chosen for these studies was consistent with prior pre-clinical studies ovariectomy studies modeling post-menopausal osteoporosis [15]. As drug pharmacokinetics can vary greatly between human vs rodent, the human “equivalent” dose is not known, but we recognize that the 25mg/kg twice per week dose is likely higher than the currently proposed doses for humans.

In summary, we have demonstrated that five weeks of Scl-Ab therapy was capable of increasing bone formation in an adult model of OI with a classic Gly->Cys substitution in *coll1a1*. OI is a disease that most severely affects children, and fracture rate tends to decrease after puberty. Despite this relative improvement, adults remain at an increased risk for fracture compared to a normal population, and commonly have osteoporotic BMD values. While bisphosphonate therapy is often used in adult OI patients, there is a need for more effective anabolic therapeutics to improve patient outcomes. Scl-Ab is a unique and promising anabolic therapy which may be particularly useful for the treatment of adult OI by stimulating bone formation, adding bone mass, and increasing bone strength.

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Figure 1.

Serum OCN is elevated in both WT and *Brtl*^{+/+} with Scl-Ab. Serum TRACP5b is reduced with Scl-Ab therapy in WT and shows a trend ($p=0.064$) toward reduced levels in *Brtl*^{+/+}, suggesting a decoupled effect on bone formation and bone resorption. * $p<0.05$ Scl-Ab vs. Veh.

Figure 2.

Micro computed tomography of the distal femur metaphysis reveals anabolic changes with Scl-Ab therapy. **A:** Representative median BV/TV isosurface images reveal a reduction in Brtl/+ bone mass and the anabolic effect of Scl-Ab therapy. **B:** BV/TV, Tb.Th, Tb.N, and Tb. BMD were increased with Scl-Ab in both WT and Brtl/+. A conservative specimen specific threshold is applied as described in the methods. * $p < 0.05$ Scl-Ab vs. Veh; # $p < 0.05$ WT Veh vs. Brtl/+ Veh.

Figure 3. MicroCT data from the 8th caudal vertebra demonstrate no effect of sclerostin antibody on trabecular (A–C) or cortical (D) bone structure in Btl/+ or WT mice.

Figure 4.

Dynamic histomorphometry at the femoral mid-diaphysis reveals anabolic effect of Scl-Ab in WT and Brlt/+ (A). The centroid of the bone was located, and 110 deg angles drawn to define the anterior and posterior regions of bone. Femoral cortical mid-diaphyseal periosteal BFR (B) increases with Scl-Ab are a result of increased periosteal MAR in WT and increased MAR and MS/BS in Brlt/+. Endosteal BFR (C) increases with Scl-Ab as a result of increased endosteal MAR and MS/BS in both WT and Brlt/+. * p<0.05 Scl-Ab vs. Veh.

Figure 5.
Representative mechanical 4 point bending force-displacement plots.

Table 1

Cortical MicroCT and Mechanical Properties

Femoral mid-diaphyseal cortical microCT and mechanical four point bending data.

	WT Veh	WT Scl-Ab	Brtl Veh	Brtl Scl-Ab	Genotype p-value	Treatment p-value	Interaction p-value
	n=8	n=8	n=7	n=9			
Cortical Micro CT							
Total Femur Length (mm)	16.71 ± .40	16.58 ± .28	15.51 ± 0.78 [#]	15.91 ± .48	<0.001	0.454	0.150
Thickness (mm)	0.201 ± .016	0.286 ± .028 [*]	0.226 ± .028	0.277 ± .029 [*]	0.412	<0.001	0.068
Cross Sectional Area (mm ²)	0.97 ± .07	1.33 ± .14 [*]	1.00 ± .19	1.21 ± .16 [*]	0.413	<0.001	0.162
Marrow Area (mm ²)	1.42 ± .16	1.15 ± .10 [*]	1.16 ± .26	1.02 ± .11 [*]	<0.01	<0.01	0.254
Total Area (mm ²)	2.39 ± .17	2.48 ± .16	2.16 ± .41	2.24 ± .22	<0.05	0.386	0.959
Bending Moment of Inertia (mm ⁴)	0.19 ± .03	0.25 ± .04	0.17 ± .07	0.22 ± .06	0.185	<0.01	0.742
Tissue Mineral Density (mg/cm ³)	1.122 ± .40	1.152 ± .33	1.159 ± .38	1.183 ± .32	<0.05	<0.05	0.806
Mechanical Four Point Bending							
Yield Load (N)	19.9 ± 4.6	35.6 ± 7.5 [*]	18.2 ± 6.7	32.2 ± 7.2 [*]	0.286	<0.001	0.720
Ultimate Load (N)	22.4 ± 3.5	51.5 ± 13.6 [*]	19.4 ± 5.8	44.3 ± 4.9 [*]	0.082	<0.001	0.458
Stiffness (N/mm)	274 ± 39	467 ± 75 [*]	311 ± 90	429 ± 66 [*]	0.945	<0.001	0.139
Energy to Failure (mJ)	1.1 ± 0.3	6.9 ± 3.5 [*]	0.7 ± 0.3	4.2 ± 2.2 [*]	<0.05	<0.001	0.138
Elastic Energy (mJ)	0.77 ± .32	1.51 ± 0.53 [*]	0.53 ± .30	1.35 ± .55 [*]	0.229	<0.001	0.807
Plastic Energy (mJ)	0.33 ± .32	5.43 ± 3.26 [*]	0.16 ± .33	2.90 ± 2.30 [*]	0.073	<0.001	0.117
Post Yield Displacement (mm)	0.019 ± .018	0.124 ± .07 [*]	0.013 ± .024	0.083 ± .058 [*]	0.183	<0.001	0.329
Yield Displacement (mm)	0.066 ± .015	0.076 ± .012	0.052 ± .015	0.077 ± .017 [*]	0.207	<0.01	0.161
Estimated Elastic Modulus (GPa)	5.41 ± .78	7.07 ± 1.1 [*]	7.30 ± 2.34	7.70 ± 1.98	0.042	0.091	0.293

* p<0.05 Scl-Ab vs. Veh;

[#] p<0.05 WT Veh vs. Brtl/+ Veh.

Table 2

Dynamic Histomorphometry on Femoral Cortical Subregions

Regional anterior-posterior femoral cortical dynamic histomorphometry analysis. Dual label was not present on all surfaces preventing MAR measurement in all samples.

	WT Veh	WT Scl-Ab	Brtl/+ Veh	Brtl/+ Scl-Ab	Genotype p-value	Treatment p-value	Interaction p-value
Anterior							
Cortical Thickness (µm)	192 ± 13	251 ± 27*	200 ± 17	232 ± 25*	0.485	<0.001	0.096
Periosteal MS/BS	0.69 ± .33	0.91 ± .10	0.57 ± .32	0.89 ± .16	0.425	<0.01	0.524
Periosteal MAR	0.16 ± .11	0.59 ± .14*	0.20 ± .09	0.52 ± .17*	0.710	<0.001	0.247
Periosteal BFR/BS	0.13 ± .12	0.53 ± .12*	0.11 ± .09	0.46 ± .15*	0.305	<0.001	0.517
Endosteal MS/BS	0.28 ± .16	0.79 ± .16*	0.36 ± .17	0.87 ± .10*	0.135	<0.001	0.939
Endosteal MAR	0.16 ± .01	0.53 ± .08*	0.14 ± .02	0.47 ± .12*	0.425	<0.001	0.717
Endosteal BFR/BS	0.04 ± .03	0.42 ± .13*	0.04 ± .02	0.41 ± .11*	0.837	<0.001	0.721
Posterior							
Cortical Thickness (µm)	216 ± 25	294 ± 46*	235 ± 40	309 ± 52*	0.262	<0.001	0.878
Periosteal MS/BS	0.92 ± .09	0.99 ± .02	0.83 ± .17	0.98 ± .02	0.133	<0.01	0.189
Periosteal MAR	0.44 ± .24	0.93 ± .16*	0.35 ± .23	0.90 ± .16*	0.422	<0.001	0.644
Periosteal BFR/BS	0.41 ± .21	0.91 ± .15*	0.31 ± .25	0.89 ± .17*	0.373	<0.001	0.604
Endosteal MS/BS	0.24 ± .10	0.67 ± .22*	0.33 ± .12	0.79 ± .16*	0.084	<0.001	0.825
Endosteal MAR	0.22 ± .07	0.80 ± .34	0.29 ± .07	0.61 ± .14*	0.601	<0.01	0.303
Endosteal BFR/BS	0.04 ± .03	0.38 ± .37	0.05 ± .05	0.47 ± .10*	0.420	<0.001	0.590

Anterior Periosteal MAR: WT Veh n=7, WT Scl-Ab n=9, Brtl/+ Veh n=7, Brtl/+ Scl-Ab n=9.

Anterior Periosteal MS/BS, BFR/BS: WT Veh n=7, WT Scl-Ab n=9, Brtl/+ Veh n=8, Brtl/+ Scl-Ab n=9.

Anterior Endosteal MAR: WT Veh n=2, WT Scl-Ab n=7, Brtl/+ Veh n=2, Brtl/+ Scl-Ab n=9.

Anterior Endosteal MS/BS, BFR/BS: WT Veh n=7, WT Scl-Ab n=7, Brtl/+ Veh n=8, Brtl/+ Scl-Ab n=9.

Posterior Periosteal MAR, MS/BS, BFR/BS: WT Veh n=7, WT Scl-Ab n=9, Brtl/+ Veh n=8, Brtl/+ Scl-Ab n=9.

Posterior Endosteal MAR: WT Veh n=2, WT Scl-Ab n=4, Brtl/+ Veh n=2, Brtl/+ Scl-Ab n=9.

Posterior Endosteal MS/BS, BFR/BS: WT Veh n=7, WT Scl-Ab n=7, Brtl/+ Veh n=8, Brtl/+ Scl-Ab n=9.

*p<0.05 Scl-Ab vs. Veh, no phenotypic differences were observed.

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