STUDY PROTOCOL with STATISTICAL ANALYSIS PLAN

TITLE OF STUDY: Treatment with Romosozumab versus Denosumab to Improve Bone Mineral Density and Architecture in Subacute SCI

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ABSTRACT:

Background: Sublesional bone loss after acute spinal cord injury (SCI) is sudden, progressive, and dramatic. After depletion of bone mass and the loss of architectural integrity, it may be difficult, if even possible, to restore skeletal mass and strength. Romosozumab is a human monoclonal anti-sclerostin antibody bone anabolic agent that recently gained FDA approval to treat osteoporosis in postmenopausal women. This study will test the ability of romosozumab administered in FDA-approved therapeutic doses for 12 months to prevent loss of BMD to regions of interest of the lower extremities in persons with subacute SCI; attention will be focused to the knee region (distal femur), but the proximal tibia and hip regions will also be acquired and analyzed. The ability of denosumab to preserve the gains in BMD attained with romosozumab will be determined.The romosozumab + denosumab group will be compared to a group that receives 24 months of denosumab. **Objective**: In persons with acute/subacute motor-compete SCI (<6 months since SCI):The primary objectives in the intervention group are to maintain baseline values of sublesional distal femur aBMD at 12 months after single drug therapy (12 months of romosozumab) and at 24 months after sequential dual drug therapy (12 months of romosozumab followed by 12 months of denosumab). This dual drug intervention group will be compared to a group that receives denosumab for 24 months; each active drug group will be compared to a historical control (placebo) group. **Setting:** James J. Peters VA Medical Center (JJPVAMC) and Kessler Institute for Rehabilitation (KIR) (each facility will perform patient enrollment and study procedures). **Design:** A prospective, randomized clinical trial. **Participants:** Fourty (40) subjects with subacute SCI (≤6 months) motor complete and incomplete SCI who have been admitted to JJPVAMC or the KIR will be recruited for participation in the study. The age of study participation will be males and females, between the ages of 18 and 55 years old. **Main Outcome Measures:** Primary outcome measure will be areal bone mineral density (aBMD; by DXA) of the distal femur at 12 and 24 months. **Funding Source:** (New York State Department of Health (NYS DOH): Grant # DOH01-C34461GG-3450000)

1. Hypothesis

In persons with subacute motor complete and incomplete SCI who are injured less ≤6 months and have been randomized to receive either 12 months of romosozumab followed by 12 months of denosumab or 24 months of denosumab (comparitor group). Each of the active drug intervention study groups will each be compared to a historical control group that has been composed of participants from the identical study sites who were enrolled in the study with almost identical entrance criteria. The hypothesis is that areal bone mineral density (aBMD) will be maintained for all regions of interest (ROI) at the specified time points post baseline with active drug treatment—that is, romosozumab or denosumab, regardless of which agent is administered, as opposed to marked and progressive loss of BMD in the historical control group:

a. Primary hypotheses:

i. At 12 month, 80% of the intervention (active drug) groups and 7% of the historical control (placebo) group will maintain ≥90% of baseline distal femur aBMD.

ii. At 24 months, 80% of the intervention groups (12 months of romosozumab + 12 months of denosumab or 24 months of denosumab) and 7% of the historical control (placebo) group will maintain ≥90% of baseline distal femur aBMD.

b. Secondary hypotheses:

At 12 and 24 months, ≥90% of baseline aBMD at the other ROIs (e.g., total hip, femoral neck, proximal tibia, and calcaneus) will be maintained in 80% of the intervention (active drug) and 7% at these ROI at 12 and 24 months in the historical control group.

c. Exploratory hypotheses:

i. Microarchitectural changes measured by peripheral quantitative computed tomography (pQCT) by will comparable to the aBMD changes at the distal femur and the other ROIs.

ii. Level of suppression of C-telopeptide (CTx) will correlate with preservation of aBMD, integral volumetric BMD (vBMD) and trabecular BMD (tBMD) at the ROIs at 12 and 24 months.

2. Study Objectives (primary, secondary, exploratory)

In persons with acute/subacute motor complete and incomplete SCI (<6 months since SCI):

The **primary objectives** in the active drug intervention groups are to maintain baseline values of sublesional distal femur aBMD at 12 months (12 months of romosozumab or 12 months of denosumab) and at 24 months after 12 months of romosozumab + 12 months of denosumab administration or 24 months of denosumab administration. The rationale for 90% preservation of aBMD at 12 and 24 months, rather than 100% preservation, is that the dose of romosozumab and/or denosumab may not be sufficient to prevent complete bone resorption in all subjects.

The **secondary objectives** in the active drug intervention groups are to maintain 90% of baseline aBMD values at the femoral neck, total hip, proximal tibia, and calcaneus at 12 and 24 months of drug therapy.

The **exploratory objectives** are to demonstrate that microarchitectural, vBMD and tBMD changes are comparable to changes in aBMD in the ROIs in both groups. Additional exploratory aims are to evaluate the magnitude of changes in bone biomarkers (P1NP & CTx) in relation to the ROI of the sublesional bone. In the romosozumab group, it is hypothesized that the magnitude of increase in P1NP coupled with the suppression of CTx will correlate with the most favorable changes in the skeletal endpoints (aBMD, vBMD, and tBMD at 12 months); those participants who have the greatest suppression in CTx from 12 months to 24 months will have the greatest preservation at the ROIs for aBMD, vBMD, and tBMD at 24 months. In the denosumab group, those participants who have the greatest suppression in CTx from baseline to 24 months will have the greatest preservation at the ROIs for aBMD, vBMD, and tBMD at 24 months.

3. Study Endpoints (primary, secondary, exploratory)

Primary Endpoints: Distal femur aBMD values at 12 and 24 months.

Secondary Endpoints: Total hip, femoral neck, proximal tibia, and calcaneus aBMD at 12 and 24 months.

Exploratory Endpoints: ROIs for microarchitectural, vBMD, and tBMD changes at 12 and 24 months. Sum CTx values (summed up to and including 12 and 24 months) in relationship to aBMD, vBMD, tBMD at the ROI and microarchitectural changes at the distal femur.

The ROIs to be studied are the hip, knee and calcaneus. DXA will be used to measure aBMD at the hip and knee. pQCT will be used to measure knee and ankle vBMD, tBMD and microarchitecture at the distal tibia.

BACKGROUND:

There is no practical treatment to prevent the severe loss of sublesional bone in persons with acute/subacute spinal cord injury (SCI) until recently when our group demonstrated in a randomized, placebo controlled clinical trial that denosumab administration preserved BMD at ROI in the lower extremities. The objective of the proposed work is to determine whether administration for 12 months of romosozumab (the maximal length of treatment approved by the FDA), a recently FDA-approved bone anabolic drug, followed by 12 months of denosumab (a potent anti-resorptive agent) will maintain bone mass at the knee in subjects with subacute SCI compared to 24 months of denosumab administration. It is possible, and even likely, that if romosozumab or denosumab does preserve bone mass that these gains in aBMD will be fairly rapidly lost once treatment is discontinued. As such, any gains achieved in bone mass from active drug administration will be attempted to be preserved with treatment for an additional 12 months with denosumab. Preservation of bone below the level of injury would reduce morbidity associated with fractures and permit safer participation in upright rehabilitation activities.

Immobilization osteoporosis results from unloading of the skeleton, and the magnitude of bone loss is in proportion to time and degree to which the forces of ambulation are interrupted, and it leads to an increased risk of low-impact fractures, especially at the knee (e.g., distal femur and proximal tibia) in a wheelchair-dependent population [1, 2]. The severity of bone loss may exclude individuals with SCI from being eligible to participate in rehabilitation programs designed to increased mobility and increase functional independence, which would be anticipated to result in further worsening of the disuse osteoporosis. **In the VA Cooperative Study entitled "Exoskeletal-assisted Walking in Persons with SCI: Impact on Quality of Life," 69 of 158 (44%) of all screen failures from a total of 254 persons pre-screened for study participation were due to low bone mineral density (BMD) and/or prior fractures** (interim analysis by PI).

Thus, persons with chronic SCI represent a unique population that is permanently immobilized due to partial or complete paralysis of the lower extremities. To characterize the magnitude and specificity of post-SCI bone loss of the lower extremities over the initial years after paralysis, peripheral quantitative computed tomography (pQCT) was performed to monitor loss of trabecular and cortical bone compartments until steady state levels were once again established; investigators observed that the femoral and tibial epiphyses (e.g., predominantly trabecular bone) each declined by about half over the initial 2 to 3 years after SCI, whereas cortical bone in the femoral and tibia shafts decreased by about one-third over the initial 7 to 8 years after SCI [3]. Of note, low BMD of the epiphyseal trabecular region by pQCT has been associated with an increased risk of fracture [4]. In a study of persons with SCI, the risk of fracture at the femoral neck increased 2.2 and 2.8 times for each 0.1 decrement in BMD or for each unit decrease in the standard deviation, respectively [5]. Thus, the vast majority of persons with SCI develop premature and extensive bone loss of the lower extremities, increasing the risk for low-impact fracture, especially in those with greater degrees of neurological impairment [6]. Moreover, these data support the use of BMD of the femoral epiphyseal region as a surrogate for predicting fracture risk. It should also be appreciated that those with SCI will continue to age and may be prescribed medications or make lifestyle modifications that will adversely affect vitamin D and calcium intake/metabolism and/or have endocrine dysfunction (e.g., depressed anabolic hormones: testosterone and growth hormone) [7, 8], which would serve to further aggravate bone loss of immobilization and further increase the risk of fracture [9]; albeit these effects would be anticipated to have a relatively minor impact upon skeletal deterioration than that of profound disuse.

There are two broad classifications of agents that are currently FDA approved for the treatment of osteoporosis: bone anti-resorptive agents and bone anabolic agents. The anti-resorptive agents include bisphosphonates (e.g., alendronate, risedronate, ibandronate, tiludronate, pamidronate, and zoledronate) and an antagonist to receptor activator of nuclear factor kappa-B ligand (RANKL; denosumab). The anabolic agents that are currently FDA approved are 1-34 parathyroid hormone (e.g., 1-34 PTH; teriparatide), an analog of parathyroid hormone-related peptide (PTH-rp; abaloparatide), and a human monoclonal anti-sclerostin antibody (romosozumab). The bone biomarker studies that have been performed in persons who are in the chronic stage of SCI suggest that the skeletal regions are in a state of low bone turnover due to a permanent and marked reduction in the forces applied to the sublesional skeleton from upright posture and ambulation. As such, anti-resorptive agents alone may not prove to be effective in reversing bone loss in the~300,000 persons with chronic SCI in the United States [10]. Work to date has shown that rehabilitation strategies, such as locomotor training and electrical stimulation (ES), are labor-intensive to perform and marginally effective in preventing bone loss (e.g., bone mass will be regained only in the regions of the skeleton to which the forces have been applied, and only for the duration of the ES treatment) or reversing the bone loss that has occurred in persons with chronic SCI (e.g., the degree of improvement is relatively small and limited to the regions exposed to the forces of ES, and the benefit is lost once ES is discontinued).

This study will test the ability of romosozumab for 12 months followed by administration of denosumab for 12 months to prevent loss of BMD to regions of interest of the lower extremities; the romosozumab group will be compared to the group that received 24 months of denosumab administration and a historical control (placebo) group. Attention will be focused to the knee region (distal femur), but the proximal tibia and hip regions will also be acquired and analyzed. In addition, the ability of denosumab to preserve the gains in BMD attained with romosozumab will be determined.

Therapy with Bone Anti-Resorptive Agents: The mechanism responsible for the anti-resorptive action of bisphosphonates is inhibition of farnesyl pyrophosphate synthase, and later generation bisphosphonates interfere with isoprenylation of GTPases at the ruffled border of osteoclasts, preventing attachment of osteoclasts to the bone surface, halting resorption, and initiating cell death [11]. The efficacy of

bisphosphonates has been addressed in various clinical and preclinical models of immobilization [12-18]. However, our experience, as well as work by others, has raised questions of the efficacy of bisphosphonates to prevent bone loss at the regions of interest in persons with neurologically more motor-complete forms of acute SCI [17-19]. It should be appreciated that other investigators have reported varying degrees of success with bisphosphonate administration in patients after acute SCI, but not at the knee in persons with motor-complete lesions, and there exist no reports of success in those with chronic SCI [15, 20-23].

Denosumab, a human monoclonal antibody of the IgG_2 immunoglobulin isotype with a high affinity and specificity for binding RANKL to antagonize its action, represents a novel pharmacological approach to the treatment of osteoporosis. This agent has received FDA approval and is commercially available. While being an anti-resorptive agent, the mechanism of action of denosumab, to inhibit the osteoclast is distinctly different from that of bisphosphonates. Denosumab has been demonstrated to be an effective agent in post-menopausal osteoporosis in the Fracture Reduction Evaluation of Denosumab in Osteoporosis Every 6 Months (FREEDOM) Trial (36 months in duration), with reduction of fracture risk at several sites with associated increases in BMD and a marked and persistent reduction in markers of bone resorption [24]. In another report from the FREEDOM Trial, markers of bone resorption (serum C-telopeptide of type 1 collagen; CTx) were suppressed more rapidly (-80% to 90% at 6 months after each dose) and to a greater degree than those for bone formation, suggesting a tilt toward net bone formation [25]. Administration of denosumab every 6 months for 3 years has been demonstrated to increase BMD, decrease bone remodeling (a marker of bone resorption was suppressed in association with a reduction in a marker of bone formation, findings confirmed by bone histomorphometry), and reduce risk of fractures in postmenopausal women with osteoporosis [26]. Histomorphometic and biochemical bone marker findings suggest that the effects of denosumab on bone remodeling are more potent than those with bisphosphonates [26-29]. Trans-iliac crest bone biopsies performed after 5 years of denosumab therapy in the FREEDOM Trial Extension revealed normal mineralized lamellar bone—that is, normal bone quality; bone turnover was depressed by dynamic remodeling indices [30]. The most recent published follow-up of the FREEDOM Trial, which followed subjects for 10 years (in these subjects, administration of denosumab was begun at the start of the study and continued for the next 7 years) or 7 years [in these subjects, administration of denosumab began <u>after</u> the first 3 years of the study (when these participants received placebo) and then subjects received active agent for the next 7 years], demonstrated continued low bone turnover by circulating metabolic bone markers, a continuous and steady increase in BMD at skeletal sites measured, and low fracture rate for vertebral and non-vertebral fractures [31, 32].

Anti-resorptive agents have been used to preserve and extend the effect of anabolic agents on bone in postmenopausal osteoporosis [33-36]. Prescription of these agents after bone anabolic agents is rapidly becoming the standard of practice. The investigators will test this approach to preserve and, possibly, to extend, gains in BMD in regions of interest (ROI) in the proposed work.

The only published work to date addressing the efficacy of the administration of denosumab in individuals with SCI was performed by Gifre et al. [37]. Fourteen persons were studied who had a mean duration of injury of 15 months (range: 8-21 months); thus, it may be assumed that the sublesional skeleton in this SCI cohort was still in a rapid state of bone resorption [37]. Treatment with denosumab (60 mg at baseline and at 6 months) was for 12 months. Compared to baseline values, a slight increase in BMD at the hip (total hip=2.4±3.6%; femoral neck=3±3.6%) was observed, which is a remarkable finding because a continued loss in BMD would have been anticipated without drug intervention. With treatment, there was a profound reduction in bone turnover, as determined by suppression of biochemical markers of bone resorption and formation. Thus, in individuals who averaged ~1.5 years after acute SCI, the predominant mechanism for the observed treatment benefit with denosumab administration may be assumed to be prevention of further bone deterioration at the hip rather than bone accrual. Our group has recently broken the randomization blind and found that denosumab has successfully prevented sublesional bone loss after acute SCI (treatment initiated within 3 months of acute SCI), which would also suggest that this agent would act to preserve bone gain in those with chronic injury. If the balance of resorption to formation could be tilted to net formation, bone loss in persons with chronic SCI may be favorably affected with an anti-resorptive medication but, because the skeleton below the level of lesion is in a low turnover state, it may take several years to observe an increase in BMD at ROI. Thus, an approach to increase bone formation with an anabolic agent for bone should be considered prior to the administration of an anti-resorptive agent.

Therapy with Bone Anabolic Agents: There are two classes of commercially available anabolic agents (e.g., the ability to stimulate osteoblast activity) indicated for the treatment of osteoporosis: PTH/PTH analogs or sclerostin antagonists. On April 9, 2019, romosozumab, a human monoclonal anti-sclerostin antibody was approved by the FDA to treat postmenopausal osteoporosis.

The anabolic effects of teraparatide occur by two mechanisms, as determined by metabolic bone markers and histomorphometry [38]. One mechanism is the direct stimulation of bone formation that occurs in active remodeling sites and on the surfaces of bone previously inactive, or modeling-based bone formation. The second mechanism is an increase in new remodeling sites. It is appreciated that both these mechanisms are responsible for the increase in BMD observed. The efficacy of teriparatide to prevent bone loss at time of acute SCI, or to restore bone lost in those with chronic injury, has not been adequately addressed in clinical trials, although preliminary work followed by a more comprehensive study by one group of investigators has been reported. A pilot study was essentially negative with teriparatide administration and mechanical stimulation (e.g., robotically-assisted gait training) in 12 chronically injured non-ambulatory subjects [39]. An article by Edwards et al. randomized individuals with chronic SCI into 3 groups for a 1 year intervention clinical trial: teriparatide alone, teriparatide + vibration, or vibration alone, and then a 12 month open-label extension of the study; despite positive BMD changes of the spine, and modest changes to cortical bone at the knee, the authors concluded that the therapeutic effect was not of clinical significance for those with SCI [40]. When used to treat postmenopausal osteoporosis, abaloparatide appears to be more effective to increase BMD than teriparatide. The difference in action between these two drugs is especially evident for the appendicular skeleton [41]. Abaloparatide administration reduces vertebral fractures, similar to teriparatide, but treatment with abaloparatide also appeared to reduce nonvertebral fractures, a benefit which has not been observed with teriparatide therapy [42, 43].

Until April 2019, when the FDA approved romosozumab, an agent which represents a completely new class of medications to treat bone loss, teriparatide and abaloparatide were the only two pharmacological agents commercially available with the ability to stimulate osteoblast activity. Romosozumab was demonstrated to be more potent than teriparatide (i.e., teriparatide 20 μg/day vs. romosozumab 210 mg/month) to increase BMD [44, 45]; treatment for one year with romosozumab significantly increased vBMD and bone mineral content (BMC) at the lumbar spine and total hip from baseline values compared with teriparatide [44]. After 12 months of romosozumab, strength of the proximal femur for a sideways fall significantly increased for romosozumab compared to teriparatide (3.6% versus -0.7%); compartmental analysis showed that that the increases in strength were associated with contributions from both cortical and trabecular bone compartments [45].There have been two Phase 3 clinical trials in postmenopausal women with osteoporosis that have tested the safety and efficacy of romosozumab [46, 47]. In the FRAME study, treatment with romosozumab reduced new vertebral fractures at 12 months compared to placebo. This reduction in risk of fracture was observed for two years in those who received romosozumab during the first year and then continued on denosumab during the second year compared to those who were on placebo the first year and denosumab the second year. At 12 months, romosozumab increased BMD at all sites tested (e.g., lumbar spine, total hip and femoral neck) compared to placebo; BMD progressively increased for two years in participants on active agents [46]. In the ARCH study, treatment with romosozumab for 12 months followed by 12 months of alendronate reduced the incidence of new vertebral fracture at 24 months; at 12 and 24 months, BMD increased at the lumbar spine, total hip and femoral neck to a greater extent on romosozumab than those treated with alendronate [47]. The risk of clinical fracture, which was defined as a composite of symptomatic vertebral fracture and nonvertebral fracture, was also lower in the romosozumab group [47]. After initiating romosozumab administration, propeptide of type 1 procollagen (P1NP), a marker of bone formation, was increased early (50-100% increase compared to baseline values at 1 and 3 months) with a return to pre-treatment values by 6 months; C-telopeptide (CTx), a marker of bone resorption, was suppressed early and remained low for the duration of drug treatment [48].

Our group performed preclinical studies of sclerostin antagonism or genetic ablation in acute and chronic rodent models of SCI [49-51]. In a rodent model, anti-sclerostin antibody (reagent provided by Amgen) was begun 7 days after spinal cord transaction (significant bone loss occurs as early as 7 days after SCI in the rat model) and the agent was then administered weekly over the next 7 weeks; sclerostin antagonism completely prevented and/or reversed the marked bone loss that occurred in the untreated SCI animals [49]. Of note, the osteocyte appeared far more viable in the romosozumab-treated animals than in the control animals, which suggests that long-term bone health may be improved by improving the Wnt signaling pathway, a result over and above that of preservation of aBMD alone. Sclerostin knockout (SOST-KO) mice (animals

were provided by Amgen) that underwent spinal cord transaction were protected from the severe sublesional bone loss that occurred in wild type mice with acute SCI [50]. To test whether sclerostin antagonism could reverse bone loss that occurred after chronic motor-complete SCI, rats were treated with anti-sclerostin antibody or vehicle for 8 weeks 12 weeks after complete spinal transection [51]. In SCI rodents that received normal saline injections, there was significant reduction in BMD, estimated bone strength, and deterioration of bone structure at the distal femoral metaphysis at 20 weeks whereas animals that received anti-sclerostin antibody had remarkably restored BMD, bone structure, and bone mechanical strength [51]. There have been no clinical trials on the effect of sclerostin antagonism in persons with acute or chronic SCI.

Considerations Relevant to Study Design: Patients with SCI tend to more frequently fracture at the distal femur and proximal tibia than at the hip or other sublesional regions [1, 2]. As such, pharmacologic intervention studies that have noted preservation of BMD and bone strength at the hip, without regard to BMD changes at the knee, are of lesser relevance to clinicians caring for patients with long-standing SCI [17, 19, 20, 52]. In the work proposed, the primary endpoints are areal BMD (aBMD) at the distal femur after 12 months of romosozumab treatment and after 24 months of sequential dual drug treatment (12 months of romosozumab treatment followed by 12 months of denosumab treatment). Because of the observed efficacy of denosumab to prevent bone loss at the knee in persons with recent SCI (manuscript in preparation), the comparison group will be 12 and 24 months of denosumab administration; a historical control (placebo) group will also be used to compare the two active drug groups for efficacy to preserve aBMD at the knee.

Subjects with subacute SCI are proposed to be studied with a 1:1 romosozumab to denosumab ratio in randomization to test the efficacy and safety of romosozumab to prevent SCI-related changes in aBMD at the distal femur; the proximal tibia, hip regions, and calcaneus will also be captured by DXA. Volumetric BMD (vBMD) and trabecular BMD (tBMD) of the distal femur and the proximal tibia will be acquired as exploratory endpoints, along with trabecular microarchitectural changes of the distal tibia (the only skeletal site that can acquire these skeletal parameters) by pQCT.

BMD was chosen as a surrogate endpoint in the proposed work because BMD predicts fracture in 48 to 89% of cases [53]. It has been reported by Lazo et al. that the risk of fracture at the femoral neck in men with SCI increased 2.2 and 2.8 times for each 0.1 decrement in BMD or for each unit decrease in the standard deviation, respectively [5]; as such, BMD may be considered as a surrogate marker for risk for fracture. Unlike clinical trials that are designed to test the efficacy of anti-osteoporotic agents in women with postmenopausal osteoporosis that have the statistical power to determine differences in fracture incidence as an endpoint, the use of BMD as a surrogate endpoint for fracture is necessary when investigating immobilization osteoporosis in those with chronic SCI due to the markedly smaller, less accessible subject population. Thus, to designate fracture as our primary study endpoint would require a sample size of at least several hundred SCI subjects who would be followed for at least 5-10 years, which is not a practical alternative to obtaining BMD measurements at the distal femur as our endpoint.

Hypercalciuria, and infrequently, hypercalcemia are recognized complications of acute immobilization secondary to SCI. However, hypocalcemia is a recognized potential complication of treatment with romosozumab because calcium is being more rapidly and extensively deposited in the skeleton as new bone. As such, participants will be determined to have sufficient serum levels of vitamin D, and then monitored for serum vitamin D levels and calcium intake during the length of the study.

Because of the regulatory limit of 12 months to treat with romosozumab, and the knowledge that gains in bone health have a likelihood of being lost when therapy is terminated, it is now routine clinical practice to place patients on an anti-resorptive therapy when the bone anabolic agent is discontinued. Several studies have demonstrated that treatment with denosumab after treatment with romosozumab results in positive increases in BMD at the lumbar spine and hip, as well as a continued reduction in risk of fracture [35, 36, 48]. A such, ther proposed prospective, randomized controlled trial will test whether romosozumab transitioned to denosumab can prevent sublesional bone loss in persons with chronic SCI who have lost substantial bone mass yet are deemed still to have sufficient bone mass to respond to anabolic-bone therapy.

The work proposed in this application will attempt to identify a second agent (romosozumab) that will be available as an efficacious clinical option to prevent sublesional bone loss in individuals with subacute SCI.. Sequential addition of anti-resorptive therapy (e.g., with denosumab) will test as to whether lower extremity skeletal ROI will be maintained in those with subacute SCI.

The PI and his collaborators have considerable experience in conducting clinical trials that have evaluated the efficacy of agents to prevent bone loss in patients with acute and chronic SCI [17, 18],

addressing the pathophysiological changes associated with disuse osteoporosis [54-56], and performing bone imaging by employing DXA and pQCT methodologies in the acute and chronic SCI populations [57]. Previous work demonstrated the lack of efficacy of bisphosphonates to preserve BMD at the knee (e.g., BMD of the distal femur and proximal tibia) in persons after acute SCI; however, hip BMD was observed to be better preserved after zoledronic acid administration [17]. Biochemical markers of bone function have also been performed in several past and current investigations conducted by the PI [17, 18].

Replacement and/or Maintenance of Adequate Vitamin D and Calcium Levels: To study the efficacy of an intervention on bone, and also because romosozumab may cause a fall in the serum calcium concentration in vitamin D deficient individuals or those on a calcium restricted diet, it is requisite that calcium intake be adequate and vitamin D levels be maintained within the normal range. By adequately replacing and/or maintaining sufficient intake, the possible confounding effects of calcium/vitamin D deficiency on the skeletal endpoints being tested after intervention will be removed from consideration. Thus, it is vital that vitamin D levels remain in the normal range (≥30 ng/ml) and calcium intake be adequate so as not to impede bone mineralization [58, 59]. Levels of vitamin D will be monitored at baseline, 12, and 24 months.

PRELIMINARY STUDIES AND CURRENT STATUS OF THE FIELD

Studies in our unit showed that sclerostin antagonism in rats or sclerostin knockout mice after acute spinal cord transection prevented the loss of BMD and several of the adverse microarchitectual changes [49, 50]. The work proposed in this application is to prevent the bone loss in persons with subacute SCI. To that aim, rats were studied that underwent complete spinal cord transection (or laminectomy) 12 weeks prior to the administration of anti-sclerostin antibody or placebo for 8 weeks [51]. The findings were dramatic to bone below the level of lesion. Animals that did not receive active treatment had a significant reduction of BMD, bone strength and deterioration of bone structure at the distal femoral metaphysis. The administration of anti-sclerostin antibody to SCI animals restored much of the BMD, bone structure, (Figures 1- 3), and bone mechanical strength. Furthermore, sclerostin antagonism markedly improved osteocyte number, cell spatial orientation, and number of dendritic projections, which strongly suggests an improvement in osteocyte viability, function, and health

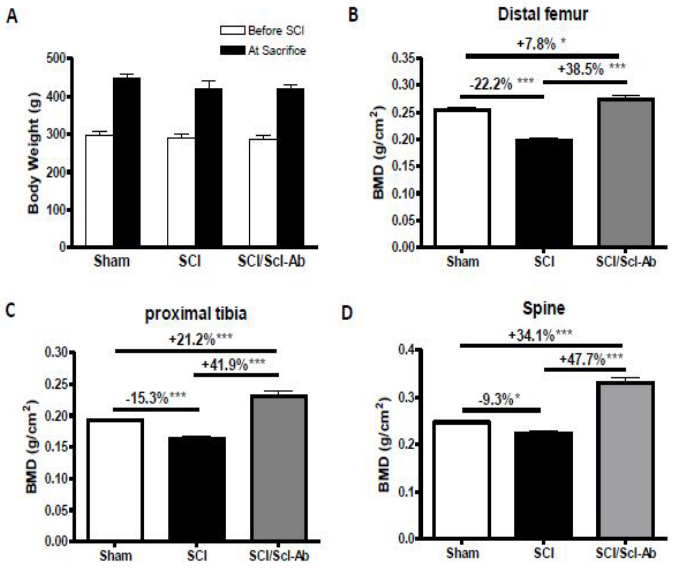


Figure 1. Sclerostin antibody reversed the loss in BMD after chronic SCI. Drug intervention was initiated in rats 12 week post spinal cord transection.

It is our hypothesis, strongly supported by recent literature, that romosozumab treatment in persons with subacute SCI will be the most potent approach to improve sublesional bone mass and mechanical strength. One may also speculate that in addition to the improved aBMD of the lower extremities with sclerostin antagonism after acute immobilization that long-term bone health will be favorably impacted because of the dramatic improvement in osteocyte viability.

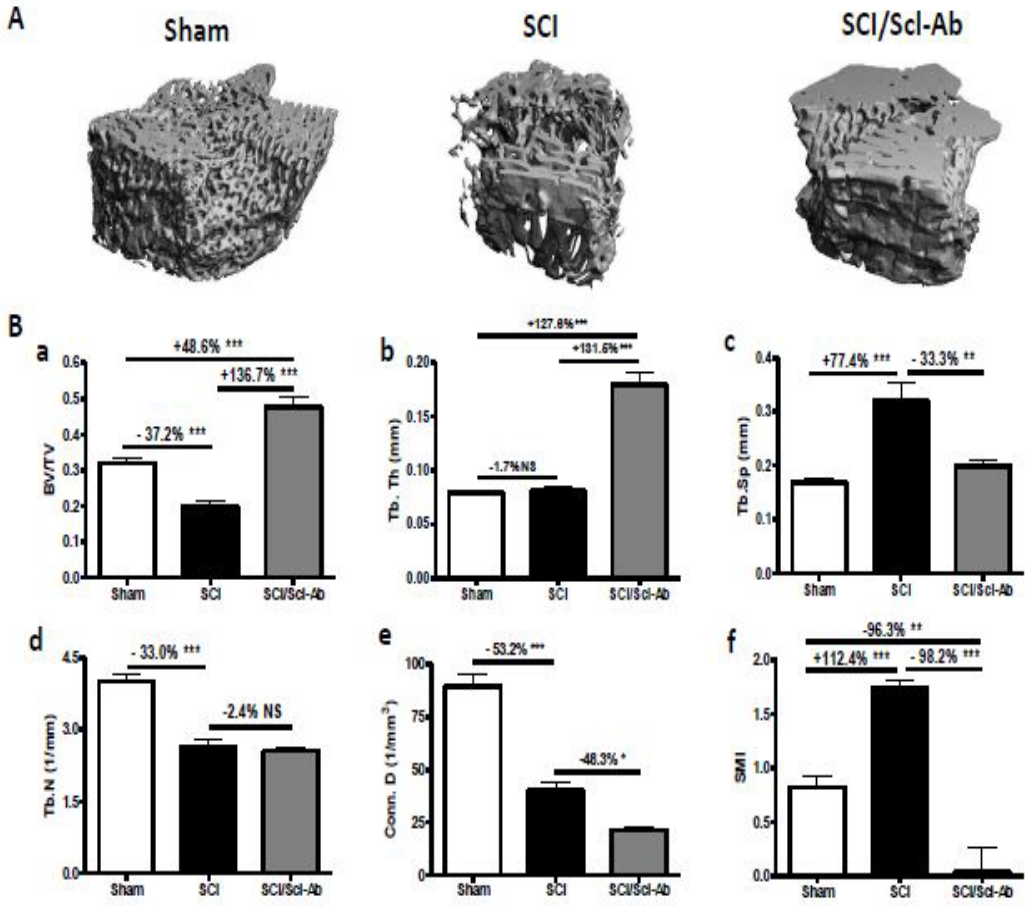


Figure 2. Effects of sclerostin antibody on trabecular bone architecture of the distal femur metaphysis.

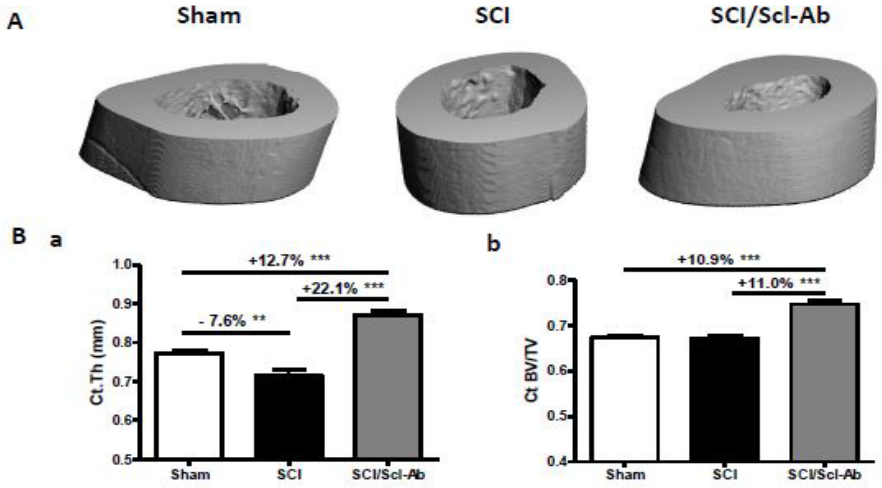


Figure 3. Effects of sclerostin antibody on cortical architecture of the midshaft of the femur.

RESEARCH DESIGN AND METHODS:

A prospective, randomized, open label two drug clinical trial will be performed to test the efficacy and safety of romosozumab administration compared to denosumab for preservation of aBMD at ROI of the lower extremities; 12 months of denosumab will follow each of the initial administration of these agents for maintenance of aBMD. An IND will be obtained from the FDA for the use of these agents in the study protocol.

Study Population:

Forty (40) subjects with acute/subacute SCI are proposed to be studied with a 1:1 ratio of randomization of romosozumab or denosumab for the first 12 months; for the next 12 months, each group will receive denosumab. The anticipated dropout rate is 30%, with approximately 30 subjects completing the entire study protocol. The study will be performed at the National Center for the Medical Consequences of Spinal Cord Injury, JJP James J. Peters VA Medical Center, Bronx, NY, and Kessler Institute for Rehabilitation. A convenience sample of patients with subacute SCI who are affiliated with the SCI Service at the JJP VA Medical Center or the Kessler Institute for Rehabilitation will be potential participants for study enrollment. The initial ~32 months will be used to recruit, enroll, treat subjects with active agents *(**Post study plan**: **Assuming that denosumab successfully retains or increases BMD achieved by 12 months of romosozumab treatment, the subject's primary care provider will be informed of the study outcome and provided the opportunity to consider continuing therapy with denosumab or another anti-resorptive medication after the subject has completed the study in an effort to preserve the gains in BMD achieved with the experimental dual drug intervention.)*. The remaining ~4 months of the study will be used to complete data collection and perform data analysis. The study will require four years to complete.

RESEARCH DESIGN AND METHODS:

A prospective, randomized, two drug clinical trial will be performed to test the efficacy of romosozumab administration for 12 months for preservation of BMD followed by 12 months of denosumab versus 24 months of denosumab administration. A historical control group will be compared to each active drug group. An IND will be obtained from the FDA for the use of these agents in the study protocol.

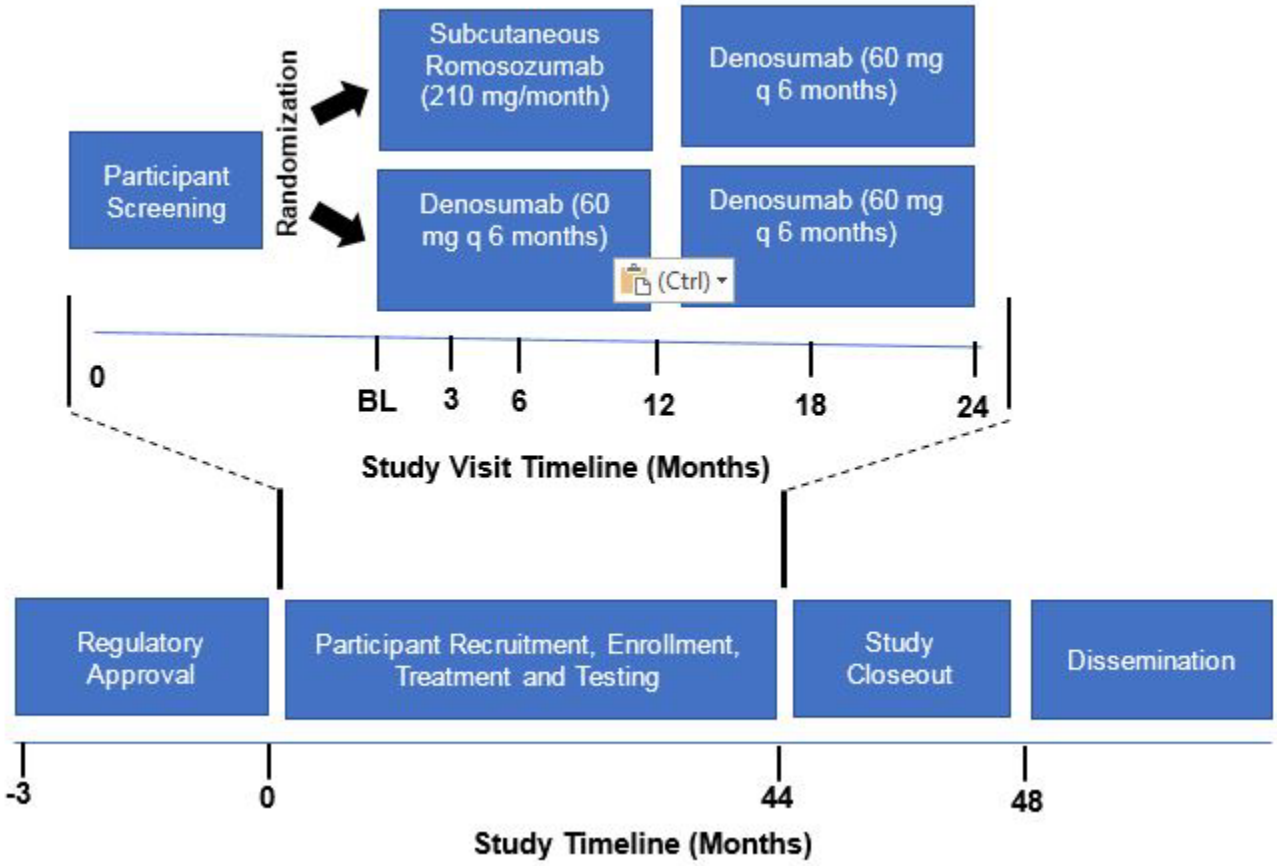
Inclusion Criteria

1. Traumatic motor-complete or incomplete SCI C4-L2 {International Standards for Neurological Classification of Spinal Cord Injury (ISNSCI) grade A-C (wheelchair dependent 100% of the time)};;
2. ≤6 months;
3. Males and females (e.g., premenopausal) between the ages of 18 and 55 years old; and

Exclusion Criteria

1. Active and/or history of coronary heart disease or stroke;
2. Osteosarcoma (bone cancer);
3. Long-bone fracture of the leg within the past year;
4. History of prior bone disease (Paget's hyperparathyroidism, osteoporosis, etc.);
5. Postmenopausal women;
6. Men with known hypogonadism (low functioning testes) prior to SCI;
7. Anabolic therapy (drugs geared towards increasing BMD) longer than six months duration after SCI;
8. Glucocorticoid administration longer than three months duration within the last year;
9. Endocrinopathies (hyperthyroidism, Cushing's disease or syndrome, etc.);
10. Severe underlying chronic disease (e.g., COPD, end-stage heart disease, chronic renal failure);
11. Heterotopic ossification (HO) of the distal femur (the knee end of the thigh bone). HO is a condition where bone tissue forms outside of the skeleton. If HO is found in any other area than the distal femur it will not prevent study participation.
12. History of chronic alcohol abuse;
13. Diagnosis of hypercalcemia (high levels of calciuuum in the blood);
14. Pregnancy;
15. Prescribed a bisphosphonate for heterotopic ossification (HO), or prescribed any other agent to treat osteoporosis other than calcium and vitamin D;

16. Current diagnosis of cancer or history of cancer;
17. Prescribed moderate or high dose corticosteroids (>40 mg/d prednisone or an equivalent dose of other corticosteroid medication) for longer than one week, not including drug administered to preserve neurological function at the time of acute SCI; and
18. Life expectancy less than 5 years.



Methods and Procedures

Subjects will be informed verbally and in writing of the purpose of this study. Informed consent will be obtained from all subjects who agree to participate. If the subject is not able to provide written informed consent because of paralysis of the upper extremities, despite full mental capacity to provide verbal informed consent, written informed consent will be obtained from their legal surrogate. Subjects will be free to withdraw their consent at any time. Subject travel costs and a stipend for participation will be provided by the study. Standard rehabilitation care that is appropriate may be prescribed for patients with motor complete and incomplete neurological classifications, and may include range of motion exercises, mat activities, transfer training, activities of daily living, strengthening maneuvers, and use of a standing frame. Records will be kept confidential by linking subject data identifier numbers; the numeric identifier will be linked to the subject's names using a separate key accessible only to the study coordinator and PI. A study investigator or research coordinator will enroll subjects, schedule patient travel, coordinate densitometry and blood/urine laboratory studies, collect data, and coordinate the responsibilities of all study subjects. Subjects will be contacted by phone or email at least every 2 weeks to assure compliance with drug treatment, confirm the level of upright activity, and as a method to maximize study retention. Because subjects will have motor complete and incomplete (ISNSCI grade A-C) SCI, they will not have the ability to walk without benefit of sophisticated upright rehabilitation modalities that, if engaged in prior to the study, may be continued during the duration of the study. In the absence of electrical stimulation of muscle groups, which is an exclusion criterion, there is no credible evidence that an upright ambulatory activity alone increases BMD of regions of the lower extremity. However, all individuals who are engaged in walking" activities will be recorded and analyzed for a possible effect that may be observed in combination with drug administration post hoc. At each visit, subjects will be interviewed by the research coordinator, and the use of alcohol or cigarettes will be recorded, and this

information will be analyzed post hoc for potential confounding effects; it is speculated that alcohol and/or cigarettes will have a negligible effect on the study endpoints when compared to the relatively large effect exerted by SCI and immobilization.

The serum 25 hydroxyvitamin D level will be measured at baseline, 12, and 24 months to exclude a vitamin D deficiency/insufficiency. A deficiency/insufficiency of vitamin D will not disqualify a patient from study participation. If an absolute vitamin D deficiency/insufficiency is identified (<30 ng/ml), supplemental vitamin D 4000 IU/d for 4 weeks will be administered (in those who have a vitamin D deficiency, it is anticipated that serum values will be raised on average ~1 ng/ml for each 100 IU/day of vitamin D administered, or ~40 ng/ml for 4000 IU/d [60]; serial testing will be performed monthly until the serum 25 hydroxyvitamin D level is within the normal range, before reducing the dose to 2000 IU/day. If not vitamin D deficient, subjects will receive 2000 IU vitamin D/day. All subjects will be instructed by nutritional evaluation and counseling to consume a normal calcium diet of 700-1,000 mg/day to assure sufficient calcium is available not to impede bone formation.

Study Timeline:

Month	**Baseline (0)**	**1**	**3**	**6**	**12**	**18**	**24**
<u>Studies/Tests</u>							
DXA	**X**			**X**	**X**	**X**	**X**
pQCT	**X**			**X**	**X**	**X**	**X**
Bioelectrical Impedance Spectroscopy (BIS):	**X**			**X**	**X**	**X**	**X**
Markers of Bone Formation/Resorption	**X**	**X**	**X**	**X**	**X**	**X**	**X**
Calcium Metabolism	**X**	**X**	**X**	**X**	**X**	**X**	**X**
General Laboratories	**X**	**X**	**X**	**X**	**X**	**X**	**X**
Endocrine Laboratories	**X**				**X**		**X**

Romosozumab 210 mg SQ will administered at baseline and then each month subcutaneously (SQ) for 12 months followed by denosumab for 12 months; in the comparator group, denosumab 60 mg SQ will be administered at baseline and 6, 12, and 18 months. Bone Biomarkers: serum C-telopeptide, serum osteocalcin, bone alkaline phosphatase, and carboxyterminal propeptide of type 1 procollagen. Calcium Metabolism: serum total and ionized calcium concentrations, 24-hour urine calcium, 25 OH-vitamin D (performed monthly during supplementation therapy), 1,25 $(OH)_2$-vitamin D, and intact PTH. Endocrine Labs: serum thyroid function tests (T_3, T_4, & TSH), cortisol, total testosterone, calculated free testosterone, estradiol, growth hormone, insulin-like growth factor-1.

Romosozumab or denosumab will be administered to enrolled subjects by a designated administrator. Subjects will be asked to visit the JJPVAMC or KIR once a month for 12 months to receive the monthly injection of romosozumab or biannual injection of denosumab. Romosozumab and denosumab will each be administered at the currently recommended dose by the FDA for the treatment of osteoporosis and is anticipated to be well tolerated, with the most frequently reported adverse events being injection-site erythema; arthralgias and headache have been reported to occur at rates ≥5% over that of placebo. Hypocalcemia has occurred, particularly in patients with severe chronic renal disease; during the treatment intervention, vitamin D levels will be checked at baseline, and 12 months with supplementation provided, if indicated, and subjects will be monitored throughout the study for adequate calcium intake. Safety laboratory values (calcium metabolism and general lab studies) will be drawn at 3 and 6 months and then at 6 month intervals for the duration of the study. At 12 months, measurements will be performed for calcium and bone metabolism, tBMD, vBMD, microarchitecture by pQCT at the distal tibia, and aBMD by DXA at the knee and hip ROI. Regardless of group assignment, all subjects will receive denosumab 60 mg SQ at months 12 and 18. The complete battery of imaging studies and blood work will be performed at 24 months.

Participants will be allowed to enroll in other ambulatory rehabilitation clinical trials after the last administration of denosumab at the 18 month time point and pror to the final follow-up visit 24 months after randomization to treatment allocation.

Pitfalls/Limitations

The PI is an authority on bone disease in persons with SCI and highly experienced in performing the work proposed in this application. As such, no technical obstacles are anticipated. All the equipment and resources are, or will shortly be, in place to perform the study (the investigator has recently purchased a Stratec XCT 3000 pQCT scanner which will be delivered in December 2019). The most common hurdle to overcome in any clinical investigation is to meet the subject recruitment target. If there are not sufficient Veterans who satisfy the entrance criteria for recruitment at our VA facility, there are large numbers of potential subjects at the Kessler Institute for Rehabilitation, a premire rehabilitation facility to which the VA has an active Collaborative Research Agreement and maintains a staffed VA research unit. Although romosozumab has been well tolerated in the nondisabled population, it is conceivable that this medication may be less well tolerated in the SCI population. Every effort will be made to retain subjects in the protocol, but a high dropout rate may occur due to medication side effects or intercurrent illness over the length of the 2-year clinical trial, which commonly occurs in clinical trials with subjects with subacute SCI. If termination of study medication should occur, the subject's data will be analyzed with that of their group assignment for the length of time that the participant was compliant with and adhered to drug therapy. An amendment for recruitment of additional subjects for study participation will be considered if a greater subject attrition rate occurs than expected. If no significant net difference between the drug groups is demonstrated, a negative result will suggest that either medication may be used to prevent bone loss after acute SCI, and that further research of each of these medications should be pursued in the chronic SCI population. The possibility that the knee does not respond to drug with preservation of aBMD but the hip exhibits a significant response, this too would be useful information, and stimulate further study with other classes of anti-osteoporotic agents to improve BMD at the knee. A noninferiority analysis will certainly be underpowered with 30 subjects randomized 1:1 to each drug group and an expected efficacy of each agent to preserve aBMD at the distal femur. Realizing this highly likely possibility, the analysis may still provide useful feasibility and proof-of-principle finding for future studies with a larger subject population sample.

The absence of preliminary data in persons with SCI to support the efficacy of romosozumab to prevent bone loss in persons with acute SCI and the anticipated magnitude of the drug effect are the main limitations of the work proposed. Although data regarding the ability of romosozumab to prevent bone loss is not available in persons with SCI, this agent has been investigated extensively in the nondisabled population [46, 47]. In human trials, the efficacy of romosozumab (a bone anabolic agent) appears to exceed that of PTH (another bone anabolic agent, or its analog) at the lumbar spine in its potency to increase BMD and reduce fracture occurrence [44, 45, 61]. In addition, romosozumab has a beneficial effect on the appendicular skeleton, increasing BMD and reducing risk of long-bone fracture, unlike that reported for teriparatide, making romosozumab the preferred agent to test in the SCI population. As previously discussed, the femoral epiphysis and metaphysis have a relatively large component of trabecular bone, which one may anticipate would respond to romosozumab treatment in a similar manner as that of the lumbar spine, a skeletal region in post-menopausal women that had a gain on average of ~10% BMD after treatment for 12 months [31, 35]. If romosozumab does prove to prevent bone loss, postponing this work will invariably result in further bone deterioration in persons with SCI, possibly rendering romosozumab therapy ineffective if initiated at a future date (e.g., it is the belief of the investigator that there must be sufficient bone mass/structure to permit meaningful improvement in bone because bone re-models appositionally). Because progressive lower extremity bone loss in the chronic SCI population will invariably limit weight-bearing rehabilitation options and place persons at increased risk of fracture even while performing activities of daily living, strong preclinical and clinical evidence exists that the approach proposed in this application has merit. Thus, the argument to obtain preliminary clinical data prior to funding this clinical trial is a not reasonable request because of the high cost of the medication and the length of time required to adequately test the dual drug intervention.

Measurements:

Dual Energy X-ray Absorptiometry (iDXA, GE Lunar): aBMD of ROI in the lower extremity (distal femur and proximal tibial; total hip & its subregions; calcaneus) are the study outcome measures. BMD will be performed at baseline, 6, 12, 18, and 24 months (the final scan will be performed after completing denosumab administration). The same GE Lunar iDXA bone densitometers with the same software packages are available at all three study sites (VA, Mount Sinai & Kessler). Cross calibration has been performed and demonstrated that the two devices are essentially identical. Dedicated ISCD certified technicians with more than 15 years of experience will acquire DXA images. Imaging will be performed with the subjects lying on a padded tabletop to

acquire regional BMD of the knee (e.g., distal femoral and proximal tibial epiphyses using the orthopedic knee software commercially available from GE Lunar) and hip (total dual hip and subregions). The hip has been investigated by prior groups and is a region routinely acquired; the hip also has accepted values for T-score and Z-scores, which have not yet been reported for the knee. The starting point to acquire the knee is set on the tibia approximately 10 cm distal from the edge of the patella, with the scan field extending to the epiphysis and metaphysis of the distal femur. This software acquisition and analysis method has established reliability and validity in persons with SCI [62] and has been used previously by our group to successfully monitor changes in BMD at the knee [17] (Figure 5). In accordance with ISCD guidelines, serial DXA scans for precision error were performed and expressed as the least significant change (LSC–CV%) at the 95% level of confidence to assess and quantify for changes attributed to random machine error and technician variability [63, 64]. The LSC for the femoral neck, total hip, distal femur epiphysis, and proximal tibia epiphysis was obtained by performing two scans on 30 SCI subjects with SCI using an on-and-off-the-table method, yielded a LSC–CV % as follows: femoral neck = 4%; total hip = 3%; femoral epiphysis = 4%; and tibial epiphysis = 5% [17]. DXA requires a very low dose of radiation. It is estimated that all of the DXA measurements combined will be approximately 30-45 μSv of radiation exposure per visit (for comparison, a routine chest x-ray is an approximately 60μSv).

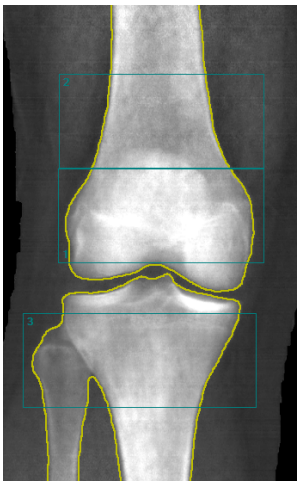


Figure 5. Image of the knee using the manufacturer Orthopedic Knee software and the validated method to capture aBMD of the (1) distal femoral epiphysis, (2) distal femoral metaphysis, and the (3) proximal tibia epiphysis.

Table 2. Outcome Measurements for Bone Mineral Density Regions of Interest for DXA and pQCT

Regions of Interest	DXA	pCT		
	aBMD	vBMD	tBMD	Micro-architectural
Hip				
Femoral neck	x			
Total hip	x			
Knee				
Distal femur	x	x	x	
Proximal tibia	x	x	x	
Ankle				
Distal tibia				x
Calcaneus				
	x			

Table 2 Legend. DXA = dual photon x-ray absorptiometry; pQCT = peripheral quantitative computed tomography.

Peripheral Quantitative Computed Tomography (pQCT): Imaging will be performed at baseline and then at 6 month intervals for the duration of the study protocol. Imaging in the proposed study will be performed at either the JJP VA Medical Center and/or Kessler Institute for Rehabilitation; both sites will have the identical device with identical software packages (Stratec XCT 3000 scanner; STIM designs, Carmel, CA). A scout view will be obtained for locating the desired scan positions. Measurements of bone geometry and vBMD will be made at the epiphyseal region at the 4% femoral length proximal to the knee joint; three additional tibial sites will be obtained by imaging proximal (e.g., superior ankle joint) to distal: 4%, 38%, (for cortical BMD), 66% and 96% of tibial length. Slice thickness of 2.4 mm and default voxel size of 0.5 mm will be acquired, as previously described [65, 66]. The root mean square-coefficient of variation (RMS-CV%) for pQCT are 1 to 2.3% for vBMD, tBMD and geometric measures at the distal femur [67]. The following parameters will be obtained: total BMC, total vBMD (mg/cm^3), trabecular BMC (mg), trabecular BMD (mg/cm^3), total area (mm^2), cortical BMC (mg), cortical volumetric BMD (mg/cm^3), cortical area (mm^2), cortical thickness (mm), polar moment of inertia (mm^4), and stress-strain index (mm^3). Radiation exposure from pQCT is <1.0 μSv. In addition to acquire these parameters, the higher resolution mode of the Stratec pQCT enables the calculation of the trabecular microarchitecture at the distal tibia region using a custom software program. Through the use of a custom software package (pQCT OsteoQ, Inglis Software Solutions Inc., Hamilton, ON) combined threshold-based and region-growing algorithms will be used to measure trabecular microarchitecture [trabecular separation (Tb.Sp), bone volume fraction (BV/TV), trabecular number (Tb.N), and trabecular thickness (Tb.Th)]. Short term validity of bone microstructure measurements have demonstrated a precision error of less than 5% [68].

Bioelectrical Impedance Spectroscopy (BIS): Bioelectrical-Impedance Spectroscopy (BIS) is another method of measuring body composition. The BIS measures fat mass, fat free mass, intra and extracellular fluids via a small, insensible (cannot be felt), electrical current that is sent through the hands and feet. The electrical current sends a frequency between 4 and 1000 kHz, which will collect approximately 256 data points. The BIS also allows us to take segmental records that include the right arm, left arm, right leg or left leg and determine the hydration in each of these segments. The device has a tetra polar set of leads, which are attached to self-adhesive skin electrodes on the hands and feet by means of alligator clips. The subject will have this measurement performed while lying on the DXA table for ten minutes prior to the start of this test. The duration of this procedure is less than 5 minutes.

Biochemical Bone Markers: The levels of the circulating biochemical markers of bone resorption and formation before (baseline) and after initiating romosozumab therapy (1, 3, 6, and 12 months), and then after 6 and 12 months of denosumab administration. These biochemical bone markers will be determined employing methods that have been used previously, as described by our investigators [17, 18]. Levels of serum C-telopeptide (CTx) (ABclonal. 86 Cummings Park, Woburn, MA) will be measured as the biomarker of bone resorption. Serum osteocalcin (Alpco Diagnostics, Salem, NH), bone alkaline phosphatase (MyBiosource, Inc., San Diego, CA) and propeptide of type 1 procollagen (P1NP) (MyBiosource, Inc., San Diego, CA) will be measured as biomarkers of bone formation [17, 18]. Romosozumab should increase biomarkers of bone formation, representing an increase in activity and number of osteoblasts, and suppress the biomarker of bone resorption.

Calcium Metabolism Studies: Serum total and ionized calcium concentration, 24-hour urine calcium, 25 OH-vitamin D level (DiaSorin Inc. Stillwater, MN), 1,25 $(OH)_2$-vitamin D level (Quest Diagnostics), and intact PTH level (ALPCO Diagnostics, Salem, NH) will be measured employing methods used previously by our investigators [17, 18]. The serum 25 OH-vitamin D level will be measured at baseline and 12 and 24 months; more frequent measurement will be performed, if indicated, as previously described. Serum total and ionized calcium concentrations will be measured at baseline, 1, 3 months, and at 6-month intervals until month 24.

General Endocrine Studies: Serum thyroid function (T_3, T_4, & TSH; DiaSorin Inc. Stillwater, MN) will be determined by kit assay. Cortisol, total testosterone (T), free testosterone (calculated from total T, albumin and SHBG)[69], estradiol (E_2), growth hormone (GH) will be determined by kit assays (MP Biomedicals, Orangeburg, NY); insulin-like growth factor-1 (IGF-I) will be measured by kit assay (ALPCO Diagnostics, Salem, NH). Sex-hormones (T & E_2) play a significant role in promoting bone health, with low levels causing increased bone resorption. Hypogonadal states generally result in bone loss, which may confound interpretation of study endpoints. GH and IGF-1 have anabolic effects on bone; stress may reduce levels of GH/IGF-1, which may blunt the effect of any therapeutic intervention. As such, it is important to confirm that these hormones (T_3, T_4, TSH, cortisol, T, and GH/IGF-1) are within the normal range and not significantly different between the experimental and control groups. General endocrine studies will be performed at baseline, 12 and 24 months.

General Laboratory Studies: To ensure that the general health of the subject is acceptable for study, CBC and comprehensive chemistry panels will be obtained at each study visit.

Data, Safety and Monitoring Plans:

A member of the study team will be present at all times with the subject. Subjects will be monitored continuously throughout the study visits. All subjects enrolled will be under the direct care and supervision of the principal investigator, the study coordinator, and the study physician. Subjects will be interviewed at each study visit and medication administration visit to determine any side effects they may have experienced. These symptoms will be recorded, and the study investigators will meet on a monthly basis to review the data and adverse events for any identifiable trends. Results of blood tests will also be reviewed by the study team after every study visit to ensure the subject is in good health. A minimum number of tests will be performed while still ensuring that study outcomes are met. All AEs and SAE's will be reported without exception to the local IRB. Serious adverse events will be reported immediately. If any significant trend is detected, subject recruitment and testing will be discontinued for project evaluation and modified as necessary.

To ensure confidentiality, data and the VA subjects' records will be stored on a VA server located at the JJP VAMC behind a VA firewall and not on any individual computer hard drive. Access to this computer

storage system is password protected with access to shared project data files limited to individually-authorized project staff members. Further, remote access is, and will continue to be, limited to authorized users of the VA Virtual Private Network (VPN) controlled by the NSOC and authenticated by the JJP VAMC Bronx Information Security Officer in compliance with VA policy (VA Directive and Handbook 6500). The database for computation and analysis will be stored on this VA server so that these raw data files will remain unchanged if there are computational errors or computer problems. Once this study has been completed, the de-identified research records will be retained in accordance with the record control schedule. Access to specific data files will be protected by strong passwords (numbers, special symbols, upper and lower case letters), provided only to project staff members authorized to access to the data. For research subjects at Kessler Institute of Rehabilitation signing a VA consent form, PHI information and consent forms will be temporarily stored in a locked cabinet at the Kessler Institute of Rehabilitation Room L-050. VA consent forms will be hand delivered to the VA in a locked briefcase at approximately monthly intervals, and are stored behind locked doors in a locked file cabinet at the VA Room 7A-13. Case report forms and data collected will be kept in a separate locked cabinet at the Kessler Institute of Rehabilitation before being transferred to the VA for permanent storage.In addition, incremental back-ups of data on servers will be performed weekly with full back ups completed on a monthly basis. Back up media will be removed and stored in a physically secure location within the Center of Excellence (JJP VAMC). External access to systems via an enterprise gateway already is and will continue to be strictly controlled and monitored by the VA Network Security Operations Center (NSOC). Hard copies of subjects intake and raw data will be stored in locked files in the investigator's VAMC Office in room 7A-13.

Statistical Considerations and Analyses:

Sample size

A sample size of 14 per group is needed to complete the study. Due to the 24-month length of the study, 20 per group (total enrollment = 40 subjects) will be enrolled to account for as much as 30% attrition (Table 3).

Sample size justification

Assuming the proportion in the historical control group achieving a clinically meaningful aBMD retention (≥90%) at the distal femur to be 7% (0.07; n=1) for both the 12- and 24-month distal femur aBMD outcome measures, the needed sample sizes range from 11 (80% power) per group to 14 (90% power) per group. [Estimating a 20% attrition and rounding up, 15 (80% power) per group and 18 (90% power) per group will be required.] Being more conservative (estimating a 30% attrition and also rounding up), 16 (80% power) per group and 20 (90% power) per group will be required. The targeted enrollment will be 20 per group (Table 3).

Sample Size Calculations

There are two primary outcome measures in this study: the first is the 12-month value for the distal femur aBMD and the second is the 24-month value for the distal femur aBMD for each of the active study drugs. The primary study hypotheses are: 1) at 12 months, 80% of the intervention group and 7% of

Table 3. Sample Size / Power Calculations for 90% and 80% Power for the Primary Outcomes

Two group continuity corrected X^2 test of equal proportions (odds ratio=1) (equal n's per group)	Primary outcomes for 12 and 36 months with two power calculations			
	Maintenance of ≥80% of Baseline Distal Femur aBMD at 12 months	Maintenance of ≥80% of Baseline Distal Femur aBMD at 36 Months	Maintenance of ≥80% of Baseline Distal Femur aBMD at 12 months	Maintenance of ≥80% of Baseline Distal Femur aBMD at 36 Months
Test significance level, α	0.01	0.01	0.01	0.01
1 or 2 sided test?	2	2	2	2
Hypothesized Control proportion, π_1	0.07	0.07	0.07	0.07
Hypothesized Intervention proportion, π_2	0.80	0.80	0.80	0.80
Allocation Ratio	1.00	1.00	1.00	1.00
Power (%)	90	90	80	80
n per group (rounded up)	14 (15)	14 (15)	11 (12)	11 (12)
n per group with 20% attrition (rounded up)	16.8 (18)	16.8 (18)	13.2 (15)	13.2 (15)
n per group with 30% attrition (rounded up)	18.2 (20)	18.2 (20)	14.3 (16)	14.3 (16)

the control group will maintain ≥90% of baseline distal femur aBMD and 2) at 24 months, 80% of the intervention group and 7% of the historical control group will maintain ≥90% of baseline distal femur aBMD. For each of the outcomes, the proportion of participants successful at achieving a clinically significant improvement will be compared between the two groups (intervention vs. historical control) using a chi-square analysis.

Primary Analyses
There will be two primary analyses. To test the hypotheses for the primary outcome measures, each randomized participant will be deemed a success or failure at attaining: maintenance of ≥90% of baseline distal femur aBMD at 12 months. The second primary analyses will test the hypotheses for the primary outcome measures for each randomized participant who completes the 24-month study for success or failure at attaining: maintenance of ≥90% of baseline distal femur aBMD at 24 months. Because of the expected 20 to 30% dropout rate, most of which may occur during the first year due to heterotopic ossification, an intent-to-treat model will not be employed. Drop outs will be included up to the last time point or removed fully from the data analyses.

To test the hypotheses for the secondary outcome measures, each randomized participant who has completed up to the 12- and 24-month timepoints will be deemed a success or failure at attaining: 1) maintenance of ≥90% of baseline distal femur aBMD at 24 months; 2) maintenance of ≥90% of the other ROIs of total hip, femoral neck and proximal tibia aBMD at 12 and 24 months. For the control group, two separate analysis using a Wilcoxon Signed Rank test will be performed to test the hypothesis that compared with baseline, at 24 months, ≥90% of 12-month distal femur aBMD will be achieved.

For each of the outcomes, the proportion of participants successful at achieving a clinically significant retention of ≥90% distal femur aBMD at 12 and 24 months will be compared between the two groups (intervention vs. historical control) using a chi-square analysis. These two time points will constitute the primary efficacy analyses.

Noninferiority Analysis
The efficacy of treatment with romosozumab will be compared to treatment with denosumab. Because both agents are anticipated to successfully retain aBMD at the distal femur, it is hypothesized that no significant difference will be found between the administration of each of these agents for retention of aBMD at the distal femur. If a small, nonsignificant difference between these agents is observed, a far larger sample size will be required to confirm a significant difference in efficacy between the two drug treatments.

Secondary Analyses
Several secondary analyses will be performed that include only those participants who complete the study (excluding drop-outs). Chi-square analyses of the primary and major secondary outcome measures (proportion of successes) will be repeated for only those participants who complete the 24-month intervention phase. Analyses of all the ROI aBMD and other secondary outcome measures will include comparisons of the mean difference scores (change from baseline to the 12- and 24-month intervention phase) of these outcome variables using t-tests.

Other Statistical Considerations
An analysis to determine the characterization of the drop-outs will be performed by using descriptive and correlation statistics. The reasons for study termination and the number of time points completed will be described. A correlation analysis will be performed with the reasons for study termination and the demographic variables and other potential variables to identify characteristics of persons who dropped out of the study. Knowledge of the reasons for termination will be important for clinical administration of the sequential dual drug administration if this approach is found efficacious at preventing bone loss in persons with motor complete and incomplete SCI.

Statistical Analysis Plan
Baseline comparability between the treatment groups will be evaluated with respect to such variables as demographics (e.g., age, gender, race, and level of SCI) and baseline values of outcome measures (ROIs aBMD). Chi-square and analysis of variance techniques, as appropriate, will be used to determine any differences in distribution of the variables across the treatment groups. Any variable that appears to be different between the groups ($p<0.10$) will be considered as a potential covariate in statistical analyses.

All statistical tests will be 2-sided. The two primary outcome measures will be tested at a 0.01 level of significance. Because of the large number of secondary outcomes to be analyzed, all other secondary outcomes will also be tested at a significance level of 0.01 to maintain control over Type I error. A variety of analytic methods will be used for the primary endpoints, secondary endpoints and other analyses.
For each of the outcomes, the proportion of participants successful at achieving a clinically significant improvement will be compared between the two groups (Intervention vs. historical controls) using a chi-square analysis. These will constitute the primary and secondary efficacy analyses. Participants who drop out will be treated as failures. As such, by design, there will be no missing data: participants either meet the outcome criteria (successes), do not meet these criteria (failures) or they drop out (also failures).

BIBLIOGRAPHY:

1. Morse, L.R., R.A. Battaglino, K.L. Stolzmann, et al., *Osteoporotic fractures and hospitalization risk in chronic spinal cord injury.* Osteoporos Int, 2009. **20**(3): p. 385-92.
2. Akhigbe, T., A.S. Chin, J.N. Svircev, et al., *A retrospective review of lower extremity fracture care in patients with spinal cord injury.* J Spinal Cord Med, 2015. **38**(1): p. 2-9.
3. Eser, P., A. Frotzler, Y. Zehnder, et al., *Relationship between the duration of paralysis and bone structure: a pQCT study of spinal cord injured individuals.* Bone, 2004. **34**(5): p. 869-80.
4. Eser, P., A. Frotzler, Y. Zehnder, et al., *Fracture threshold in the femur and tibia of people with spinal cord injury as determined by peripheral quantitative computed tomography.* Arch Phys Med Rehabil, 2005. **86**(3): p. 498-504.
5. Lazo, M.G., P. Shirazi, M. Sam, et al., *Osteoporosis and risk of fracture in men with spinal cord injury.* Spinal Cord, 2001. **39**(4): p. 208-14.
6. Garland, D.E., Z. Maric, R.H. Adkins, et al., *Bone mineral denisty about the knee in spinal cord injured patients with pathologic fractures.* Contemp Orthop, 1993. **26**: p. 375-379.
7. Bauman, W.A., C.M. Cirnigliaro, M.F. La Fountaine, et al., *A small-scale clinical trial to determine the safety and efficacy of testosterone replacement therapy in hypogonadal men with spinal cord injury.* Horm Metab Res, 2011. **43**(8): p. 574-9.
8. Bauman, W.A., M.F. La Fountaine and A.M. Spungen, *Age-related prevalence of low testosterone in men with spinal cord injury.* J Spinal Cord Med, 2014. **37**(1): p. 32-9.
9. Jiang, S.D., L.Y. Dai and L.S. Jiang, *Osteoporosis after spinal cord injury.* Osteoporos Int, 2006. **17**(2): p. 180-92.
10. *Spinal Cord Injury (SCI) 2016 Facts and Figures at a Glance.* J Spinal Cord Med, 2016. **39**(4): p. 493-4.
11. Fisher, J.E., M.J. Rogers, J.M. Halasy, et al., *Alendronate mechanism of action: geranylgeraniol, an intermediate in the mevalonate pathway, prevents inhibition of osteoclast formation, bone resorption, and kinase activation in vitro.* Proc Natl Acad Sci U S A, 1999. **96**(1): p. 133-8.
12. Kurokouchi, K., T. Ito, S. Ohmori, et al., *Changes in the markers of bone metabolism following skeletal unloading.* Environ Med, 1995. **39**(1): p. 21-4.
13. Kurokouchi, K., T. Ito, S. Ohmori, et al., *Effects of bisphosphonate on bone metabolism in tail-suspended rats.* Environ Med, 1996. **40**(1): p. 39-42.
14. Williams, C.J., R.A. Smith, R.J. Ball, et al., *Hypercalcaemia in osteogenesis imperfecta treated with pamidronate.* Arch Dis Child, 1997. **76**(2): p. 169-70.
15. Gilchrist, N.L., C.M. Frampton, R.H. Acland, et al., *Alendronate prevents bone loss in patients with acute spinal cord injury: a randomized, double-blind, placebo-controlled study.* J Clin Endocrinol Metab, 2007. **92**(4): p. 1385-90.
16. Poole, K.E., N. Loveridge, C.M. Rose, et al., *A single infusion of zoledronate prevents bone loss after stroke.* Stroke, 2007. **38**(5): p. 1519-25.
17. Bauman, W.A., C.M. Cirnigliaro, M.F. La Fountaine, et al., *Zoledronic acid administration failed to prevent bone loss at the knee in persons with acute spinal cord injury: an observational cohort study.* J Bone Miner Metab, 2015. **33**(4): p. 410-21.
18. Bauman, W.A., J.M. Wecht, S. Kirshblum, et al., *Effect of pamidronate administration on bone in patients with acute spinal cord injury.* J Rehabil Res Dev, 2005. **42**(3): p. 305-13.
19. Shapiro, J., B. Smith, T. Beck, et al., *Treatment with zoledronic acid ameliorates negative geometric changes in the proximal femur following acute spinal cord injury.* Calcif Tissue Int, 2007. **80**(5): p. 316-22.
20. Bubbear, J.S., A. Gall, F.R. Middleton, et al., *Early treatment with zoledronic acid prevents bone loss at the hip following acute spinal cord injury.* Osteoporos Int, 2011. **22**(1): p. 271-9.
21. Pearson, E.G., P.W. Nance, W.D. Leslie, et al., *Cyclical etidronate: its effect on bone density in patients with acute spinal cord injury.* Arch Phys Med Rehabil, 1997. **78**(3): p. 269-72.
22. Nance, P.W., O. Schryvers, W. Leslie, et al., *Intravenous pamidronate attenuates bone density loss after acute spinal cord injury.* Arch Phys Med Rehabil, 1999. **80**(3): p. 243-51.
23. Bryson, J.E. and M.L. Gourlay, *Bisphosphonate use in acute and chronic spinal cord injury: a systematic review.* J Spinal Cord Med, 2009. **32**(3): p. 215-25.
24. Cummings, S.R., J. San Martin, M.R. McClung, et al., *Denosumab for prevention of fractures in postmenopausal women with osteoporosis.* N Engl J Med, 2009. **361**(8): p. 756-65.
25. Eastell, R., C. Christiansen, A. Grauer, et al., *Effects of denosumab on bone turnover markers in postmenopausal osteoporosis.* J Bone Miner Res, 2011. **26**(3): p. 530-7.
26. Reid, I.R., P.D. Miller, J.P. Brown, et al., *Effects of denosumab on bone histomorphometry: the FREEDOM and STAND studies.* J Bone Miner Res, 2010. **25**(10): p. 2256-65.
27. Kendler, D.L., C. Roux, C.L. Benhamou, et al., *Effects of denosumab on bone mineral density and bone turnover in postmenopausal women transitioning from alendronate therapy.* J Bone Miner Res, 2010. **25**(1): p. 72-81.

28. Brown, J.P., R.L. Prince, C. Deal, et al., *Comparison of the effect of denosumab and alendronate on BMD and biochemical markers of bone turnover in postmenopausal women with low bone mass: a randomized, blinded, phase 3 trial.* J Bone Miner Res, 2009. **24**(1): p. 153-61.
29. McClung, M.R., E.M. Lewiecki, S.B. Cohen, et al., *Denosumab in postmenopausal women with low bone mineral density.* N Engl J Med, 2006. **354**(8): p. 821-31.
30. Brown, J.P., I.R. Reid, R.B. Wagman, et al., *Effects of up to 5 years of denosumab treatment on bone histology and histomorphometry: the FREEDOM study extension.* J Bone Miner Res, 2014. **29**(9): p. 2051-6.
31. Papapoulos, S., K. Lippuner, C. Roux, et al., *The effect of 8 or 5 years of denosumab treatment in postmenopausal women with osteoporosis: results from the FREEDOM Extension study.* Osteoporos Int, 2015. **26**(12): p. 2773-83.
32. Bone, H.G., R.B. Wagman, M.L. Brandi, et al., *10 years of denosumab treatment in postmenopausal women with osteoporosis: results from the phase 3 randomised FREEDOM trial and open-label extension.* Lancet Diabetes Endocrinol, 2017. **5**(7): p. 513-523.
33. Leder, B.Z., J.N. Tsai, A.V. Uihlein, et al., *Denosumab and teriparatide transitions in postmenopausal osteoporosis (the DATA-Switch study): extension of a randomised controlled trial.* Lancet, 2015. **386**(9999): p. 1147-55.
34. Ebina, K., J. Hashimoto, M. Kashii, et al., *The effects of switching daily teriparatide to oral bisphosphonates or denosumab in patients with primary osteoporosis.* J Bone Miner Metab, 2017. **35**(1): p. 91-98.
35. Cosman, F., D.B. Crittenden, S. Ferrari, et al., *FRAME Study: The Foundation Effect of Building Bone With 1 Year of Romosozumab Leads to Continued Lower Fracture Risk After Transition to Denosumab.* J Bone Miner Res, 2018. **33**(7): p. 1219-1226.
36. Lewiecki, E.M., R.V. Dinavahi, M. Lazaretti-Castro, et al., *One Year of Romosozumab Followed by Two Years of Denosumab Maintains Fracture Risk Reductions: Results of the FRAME Extension Study.* J Bone Miner Res, 2019. **34**(3): p. 419-428.
37. Gifre, L., J. Vidal, J.L. Carrasco, et al., *Denosumab increases sublesional bone mass in osteoporotic individuals with recent spinal cord injury.* Osteoporos Int, 2016. **27**(1): p. 405-10.
38. Lindsay, R., J.H. Krege, F. Marin, et al., *Teriparatide for osteoporosis: importance of the full course.* Osteoporos Int, 2016. **27**(8): p. 2395-410.
39. Gordon, K.E., M.J. Wald and T.J. Schnitzer, *Effect of parathyroid hormone combined with gait training on bone density and bone architecture in people with chronic spinal cord injury.* Pm r, 2013. **5**(8): p. 663-71.
40. Edwards, W.B., N. Simonian, I.T. Haider, et al., *Effects of Teriparatide and Vibration on Bone Mass and Bone Strength in People with Bone Loss and Spinal Cord Injury: A Randomized, Controlled Trial.* J Bone Miner Res, 2018. **33**(10): p. 1729-1740.
41. Leder, B.Z., L.S. O'Dea, J.R. Zanchetta, et al., *Effects of abaloparatide, a human parathyroid hormone-related peptide analog, on bone mineral density in postmenopausal women with osteoporosis.* J Clin Endocrinol Metab, 2015. **100**(2): p. 697-706.
42. Cosman, F., G. Hattersley, M.Y. Hu, et al., *Effects of Abaloparatide-SC on Fractures and Bone Mineral Density in Subgroups of Postmenopausal Women With Osteoporosis and Varying Baseline Risk Factors.* J Bone Miner Res, 2017. **32**(1): p. 17-23.
43. Miller, P.D., G. Hattersley, B.J. Riis, et al., *Effect of Abaloparatide vs Placebo on New Vertebral Fractures in Postmenopausal Women With Osteoporosis: A Randomized Clinical Trial.* Jama, 2016. **316**(7): p. 722-33.
44. Genant, H.K., K. Engelke, M.A. Bolognese, et al., *Effects of Romosozumab Compared With Teriparatide on Bone Density and Mass at the Spine and Hip in Postmenopausal Women With Low Bone Mass.* J Bone Miner Res, 2017. **32**(1): p. 181-187.
45. Keaveny, T.M., D.B. Crittenden, M.A. Bolognese, et al., *Greater Gains in Spine and Hip Strength for Romosozumab Compared With Teriparatide in Postmenopausal Women With Low Bone Mass.* J Bone Miner Res, 2017. **32**(9): p. 1956-1962.
46. Cosman, F., D.B. Crittenden, J.D. Adachi, et al., *Romosozumab Treatment in Postmenopausal Women with Osteoporosis.* N Engl J Med, 2016. **375**(16): p. 1532-1543.
47. Saag, K.G., J. Petersen, M.L. Brandi, et al., *Romosozumab or Alendronate for Fracture Prevention in Women with Osteoporosis.* N Engl J Med, 2017. **377**(15): p. 1417-1427.
48. McClung, M.R., J.P. Brown, A. Diez-Perez, et al., *Effects of 24 Months of Treatment With Romosozumab Followed by 12 Months of Denosumab or Placebo in Postmenopausal Women With Low Bone Mineral Density: A Randomized, Double-Blind, Phase 2, Parallel Group Study.* J Bone Miner Res, 2018. **33**(8): p. 1397-1406.
49. Qin, W., X. Li, Y. Peng, et al., *Sclerostin antibody preserves the morphology and structure of osteocytes and blocks the severe skeletal deterioration after motor-complete spinal cord injury in rats.* J Bone Miner Res, 2015. **30**(11): p. 1994-2004.
50. Qin, W., W. Zhao, X. Li, et al., *Mice with sclerostin gene deletion are resistant to the severe sublesional bone loss induced by spinal cord injury.* Osteoporos Int, 2016. **27**(12): p. 3627-3636.

51. Zhao, W., X. Li, Y. Peng, et al., *Sclerostin Antibody Reverses the Severe Sublesional Bone Loss in Rats After Chronic Spinal Cord Injury.* Calcif Tissue Int, 2018.
52. Schnitzer, T.J., K. Kim, J. Marks, et al., *Zoledronic Acid Treatment After Acute Spinal Cord Injury: Results of a Randomized, Placebo-Controlled Pilot Trial.* PM R, 2016. **8**(9): p. 833-43.
53. Nelson, H.D., E.M. Haney, T. Dana, et al., *Screening for osteoporosis: an update for the U.S. Preventive Services Task Force.* Ann Intern Med, 2010. **153**(2): p. 99-111.
54. Bauman, W.A. and C.P. Cardozo, *Osteoporosis in individuals with spinal cord injury.* Pm r, 2015. **7**(2): p. 188-201; quiz 201.
55. Cardozo CP, B.W., *Spinal Cord Injury: Pathophysiology and Clinical Issues.* Primer on the Metabolic Bone Diseases and Disorders of Mineral Metabolism 8th Edition. American Society of Bone and Mineral Research. John Wiley & Sons, 2012.
56. Bauman, W.A., F. Biering-Sorensen and A. Krassioukov, *The international spinal cord injury endocrine and metabolic function basic data set.* Spinal Cord, 2011. **49**(10): p. 1068-72.
57. Cirnigliaro, C.M., M.J. Myslinski, M.F. La Fountaine, et al., *Bone loss at the distal femur and proximal tibia in persons with spinal cord injury: imaging approaches, risk of fracture, and potential treatment options.* Osteoporosis International, 2016: p. 1-19.
58. Dawson-Hughes, B., R.P. Heaney, M.F. Holick, et al., *Estimates of optimal vitamin D status.* Osteoporos Int, 2005. **16**(7): p. 713-6.
59. Holick, M.F., N.C. Binkley, H.A. Bischoff-Ferrari, et al., *Evaluation, treatment, and prevention of vitamin D deficiency: an Endocrine Society clinical practice guideline.* J Clin Endocrinol Metab, 2011. **96**(7): p. 1911-30.
60. Institute of Medicine, F.a.N.B., *Dietary Reference Intakes for Calcium and Vitamin D*. 2010, National Academy Press: Washington, DC.
61. Langdahl, B.L., C. Libanati, D.B. Crittenden, et al., *Romosozumab (sclerostin monoclonal antibody) versus teriparatide in postmenopausal women with osteoporosis transitioning from oral bisphosphonate therapy: a randomised, open-label, phase 3 trial.* Lancet, 2017. **390**(10102): p. 1585-1594.
62. McPherson, J.G., W.B. Edwards, A. Prasad, et al., *Dual energy X-ray absorptiometry of the knee in spinal cord injury: methodology and correlation with quantitative computed tomography.* Spinal Cord, 2014. **52**(11): p. 821-5.
63. Baim, S., C.R. Wilson, E.M. Lewiecki, et al., *Precision assessment and radiation safety for dual-energy X-ray absorptiometry: position paper of the International Society for Clinical Densitometry.* J Clin Densitom, 2005. **8**(4): p. 371-8.
64. Morse, L.R., A.A. Lazzari, R. Battaglino, et al., *Dual energy x-ray absorptiometry of the distal femur may be more reliable than the proximal tibia in spinal cord injury.* Arch Phys Med Rehabil, 2009. **90**(5): p. 827-31.
65. Pop, L.C., D. Sukumar, K. Tomaino, et al., *Moderate weight loss in obese and overweight men preserves bone quality.* Am J Clin Nutr, 2015. **101**(3): p. 659-67.
66. Pop, L.C., D. Sukumar, S.H. Schneider, et al., *Three doses of vitamin D, bone mineral density, and geometry in older women during modest weight control in a 1-year randomized controlled trial.* Osteoporos Int, 2017. **28**(1): p. 377-388.
67. Cervinka, T., L. Giangregorio, H. Sievanen, et al., *Peripheral Quantitative Computed Tomography: Review of Evidence and Recommendations for Image Acquisition, Analysis, and Reporting, Among Individuals With Neurological Impairment.* J Clin Densitom, 2018. **21**(4): p. 563-582.
68. Wong, A.K., K.A. Beattie, K.K. Min, et al., *A Trimodality Comparison of Volumetric Bone Imaging Technologies. Part II: 1-Yr Change, Long-Term Precision, and Least Significant Change.* J Clin Densitom, 2015. **18**(2): p. 260-9.
69. Vermeulen, A., L. Verdonck and J.M. Kaufman, *A critical evaluation of simple methods for the estimation of free testosterone in serum.* J Clin Endocrinol Metab, 1999. **84**(10): p. 3666-72.