Prevention of Bone Loss after Acute SCI by Zoledronic Acid: Durability, Effect on Bone Strength, and Use of Biomarkers to Guide Therapy

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STUDY SUMMARY

Title	Prevention of Bone Loss after Acute SCI by Zoledronic Acid: Durability, Effect on Bone Strength, and Use of Biomarkers to Guide Therapy
Short Title	Effects of zoledronic acid on bone in acute SCI: Guide to therapy
Protocol Number	A-18350
IND/IND Holder	104662 / Thomas J. Schnitzer, MD, PhD
Phase	Phase 2
Methodology	Randomized, Double-blind, Placebo-controlled
Study Duration	2 years
Study Center(s)	Single center: Northwestern University
Objectives	Identify an effective therapeutic approach to prevent or mitigate the loss of bone mass and bone strength after acute spinal cord injury.
Number of Subjects	60
Diagnosis and Main Inclusion Criteria	Acute spinal cord injury
Study Product(s), Dose, Route, Regimen	Zoledronic acid 5mg (NDC# 42023-0163-01) IV infusion
Duration of administration	15-30 mins, once a year (Total twice for entire study)
Reference therapy	Placebo, IV infusion
Statistical Methodology	A linear mixed-effects model with percentage-change from baseline to endpoint between active and placebo groups

CLINICAL PROTOCOL

Protocol Title: Prevention of Bone Loss after Acute SCI by Zoledronic Acid: Durability, Effect on Bone Strength, and use of Biomarkers to Guide Therapy/A-18350

Study Locations
The study will be conducted at the following locations:

Northwestern University Feinberg School of Medicine
Federalwide Assurance (FWA) Identification Number: FWA 00001549

Shirley Ryan AbilityLab (formerly Rehabilitation Institute of Chicago (RIC))
Federalwide Assurance (FWA) Identification Number: FWA 00001553

University of Calgary
Federalwide Assurance (FWA) Identification Number: FWA 00000810

The principal investigator for the study and at Northwestern University will be:

Thomas J. Schnitzer, MD, PhD
Professor of Physical Medicine and Rehabilitation and Internal Medicine
710 N. Lake Shore Drive, Room 1020, Chicago, IL 60611
Employing institution: Northwestern University

For consortium purposes, the co-investigator at the Rehabilitation Institute of Chicago/Shirley Ryan AbilityLab will be (the PI at RIC/AbilityLab remains Thomas J. Schnitzer, MD, PhD):

David Chen, MD
Professor of Physical Medicine and Rehabilitation
355 E. Erie Street, Chicago, IL 60611
Employing institution: Rehabilitation Institute of Chicago/Shirley Ryan AbilityLab

Analysis of CT images will be performed at the University of Calgary, where the principal investigator will be:

W. Brent Edwards, PhD
Faculty of Kinesiology
2500 University Dr. NW
Calgary, Alberta, Canada
T2N 1N4
Employing institution: University of Calgary

1. Background

This section will describe the clinical problem to be addressed, the scientific rationale for the interventions being proposed, and preliminary data generated to support the methodology being employed.

1.1. Rationale

Spinal cord injury results in acute bone loss which is rapid and profound.

Spinal cord injury (SCI) results in marked acute loss of bone, primarily limited to those bony structures that are below the level of the neurologic lesion and non-weight-bearing, with little or no loss of bone at the spine and in bony structures that are supralesional.1,2 The bone loss occurs rapidly, with the greatest decrease in bone density observed during the first 6-18 months after spinal cord injury, and is profound in magnitude, commonly being in the range of 15-30% at the hip, and potentially even higher at sites of less weight-bearing and of greater trabecular content below the hip.1,2 The degree of bone loss has been shown to be highly dependent on the degree of loading, with individuals having incomplete motor lesions and capable of some degree of weight-bearing having less bone loss than those with motor complete lesions.3 Lower extremity bone loss is similar in paraplegics and quadriplegics with similar motor function loss; upper extremity bone mass is unaffected in paraplegics but variably affected in quadriplegics, dependent on the degree of impairment of upper extremity function4. Age, weight and gender are important determinants of bone loss.3 Initially, bone mineral density (BMD) decline is reported to be most marked in areas rich in trabecular bone, with relative sparing of cortical bone 2,5-8 though recent studies from our group indicate significant acute loss of cortical bone as well 9 This enhanced rate of bone loss can be observed for between 2 and 5 years after acute SCI, at which time a new steady state is reached and bone resorption and bone formation become once again tightly coupled, but now at a new “set point” with bone mass that is 30-50% below the level prior to the injury.2,5-7 After this point in time, there is a much reduced rate of bone loss, with other secondary causes of bone loss, e.g., estrogen withdrawal, endocrinopathies, being potentially important contributors to further changes in bone status.

Loss of bone mass after acute SCI is paralleled by a marked decrease in bone strength.

Although reduction in bone mass after acute SCI has been well documented, there is an even more profound reduction in bone strength,9,10 assessed by finite element (FE) modeling. Bone strength is a multifactorial measure dependent not only on bone mass, but on parameters such as geometry, mineral distribution, material properties, and mode of loading. Unlike basic densitometry measures, FE models capture this information and account for the complex interaction between these factors. Research has consistently illustrated that information derived from FE models is associated with a substantial improvement in fracture strength prediction compared to DXA and QCT.11,12 Evidence from large fracture surveillance studies in able-bodied adults suggests that FE-predicted fracture strength is a better predictor of both prevalent13 and incident14 fractures when compared to DXA. Data from our group that has been published recently10 and detailed below provides further support to the greater magnitude in changes of estimating bone strength after acute SCI compared to either CT or DXA derived bone parameters alone.

Rapid bone loss after acute SCI is due to increased bone resorption and decreased bone formation.

The exact factors contributing to the rapid and profound bone loss after SCI have not been fully defined. Lack of normal weight-bearing is clearly an important component but likely not the sole contributor, with neural as well as endocrine dysregulation being hypothesized to potentially play other important roles. Thus, correction of weight-bearing alone may not be adequate to restore normal bone mass in this setting. Regardless of which factors are at play, when bone metabolism has been studied in the acute setting what has been most striking is the marked increase in bone resorption with elevated levels of bone resorption markers such as CTX and deoxypyridinoline, reflecting increased osteoclast activity.21-23 Additionally, bone formation in down-regulated, undoubtedly due to diminished loading, with evidence of an inadequate bone formation osteoblastic response. 1,23,24 The subsequent decrease in bone mass is coupled with a decrease in bone quality, with both cortical and trabecular bone being affected 5,7,9 which has a major impact on bone strength.

Prevention of bone loss reduces risk of fracture.

Large epidemiologic studies have identified a number of risk factors for fracture in the general population including gender, age, family history of fracture, prevalent fracture, vitamin D status, and current bone mineral density.25-29 Because of the accelerated bone loss occurring in women at the time of menopause and their subsequent high risk of fracture, attention early on focused on looking for interventions to prevent this bone loss with the goal of reducing fracture risk. Initial efforts focused on estrogen30 and other hormonal therapies, including calcitonin31, but over the past decade or more the predominant approach to the prevention and treatment of bone loss and osteoporosis has been the use of bisphosphonates.

Second and third-generation bisphosphonates (alendronate32, risedronate33, ibandronate34 and zoledronate18) have been rigorously tested in post-menopausal women with osteoporosis and demonstrated to be effective at not only reducing bone loss but also increasing bone mass as well as preventing vertebral, and in most instances, non-vertebral fractures in this population.32 Importantly, these studies also confirmed the epidemiologic data that highlighted the importance of both current BMD and pre-existing fracture as risk factors for subsequent fracture, identifying those at highest risk.

In contrast to the extensive data available in post-menopausal women regarding reduction in fracture risk with bisphosphonates, few fracture prevention studies have been undertaken in other high risk populations (older men, people receiving glucocorticoids) with a recent study demonstrating efficacy of zoledronic acid in preventing morphometric vertebral fractures in older men being a notable exception.35 Nevertheless, bisphosphonates have been shown to increase BMD (or prevent bone loss after glucocorticoid use) in these settings and have been approved for the prevention of bone loss and the treatment of osteoporosis in these groups of individuals. No large studies of bisphosphonates have been undertaken in individuals with SCI, but smaller studies cited below have demonstrated the effectiveness of some bisphosphonates, administered immediately after acute SCI, in preventing bone loss and the subsequent development of osteoporosis.

1.2. Preliminary Data

Pilot study of zoledronic acid after acute SCI

Our recent research has focused on the use of intravenous zoledronic acid to prevent bone loss after acute SCI. Zoledronic acid is felt to represent an ideal anti-resorptive agent for acute SCI because it can be easily given in either an in-patient or out-patient setting (one 15 minute intravenous infusion), ensures compliance for long periods of time, does not require the strict early morning administration of oral bisphosphonates, and can be utilized in people who may not be able to remain sitting for a full half-hour after administration. As noted above, two limited studies have previously evaluated zoledronic acid after acute SCI;69,70 both demonstrated an initial response to treatment, with a significant reduction in bone loss compared to placebo with somewhat discordant results regarding longer-term efficacy. Our own pilot data support the efficacy of zoledronic acid to mitigate the acute loss of BMD that occurs after acute SCI.

We have completed a pilot, double-blind, randomized, controlled trial in which participants after acute SCI received either a single intravenous administration of zoledronic acid 5 mg or placebo and then were followed with DXA and serum bone markers at 3 months, 6 months and one year and at 6 monthly intervals thereafter.

Because of the concern of not treating individuals who demonstrated significant bone loss, we stipulated that if any participant were to demonstrate a decrease in BMD at any DXA site at 6 months after treatment of >10%r, that participant would be eligible for retreatment, in a blinded manner, with whichever treatment they did not receive at baseline, and then continue to be followed as above. This approach meant that if placebo treatment resulted in rapid bone loss of great magnitude, these participants would have the option of receiving active treatment. It also ensured that individuals receiving zoledronic acid at baseline would not receive a second infusion after 6 months, regardless of their change in BMD. This latter assurance was a consequence of an FDA request made upon review of our original protocol when submitting the IND for this study.

Fourteen participants were recruited from among in-patients admitted to the Rehabilitation Institute of Chicago over approximately a one year period. BMD changes determined by DXA demonstrated a rapid loss of bone in all of the placebo participants, with all reaching the 10% threshold of BMD loss at one region of interest by 6 months. All were offered retreatment but only 3 gave consent and these participants were then infused with zoledronic acid 5 mg and followed according to the original protocol. None of the participants initially treated with zoledronic acid reached the 10% threshold at 6 months; four of the seven participants treated initially with zoledronic acid are continuing to be followed beyond the 12 month time point in an observational longitudinal extension study to evaluate the duration of effect of zoledronic acid (Fig. 1).

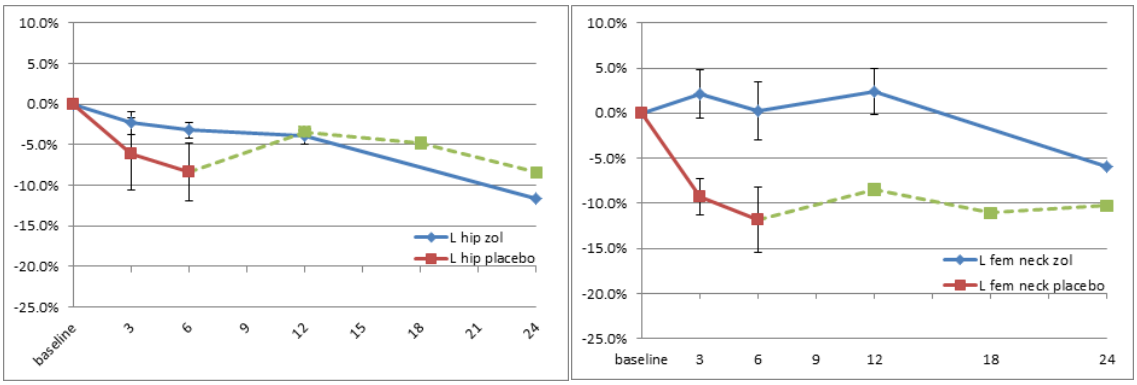


Fig. 1. Subjects treated at baseline with either zoledronic acid (blue line) or placebo (red line); at 6 months, all placebo subjects had lost >10% BMD and were treated with zoledronic acid (dashed green line). BMD determined at times indicated.

Thus, our data add new information to the current literature by providing some, though limited, data regarding duration of the effect of a single infusion of zoledronic acid, and by exploring the consequences of delaying treatment to prevent bone loss. The value of retreatment after 1 year to maintain efficacy and the potential role of serum markers of bone metabolism to guide decisions regarding retreatment are currently unknown and have not been explored in these studies. Additionally, the effect of intervention with zoledronic acid on bone strength, not simply DXA-determined BMD, also has not been evaluated and should provide additional important information regarding efficacy of treatment and its durability.

Change in bone strength after acute SCI.

Bone strength was not evaluated in any of the participants in the pilot zoledronic acid study. However, in a parallel study of 10 SCI individuals, evaluated at the knee (proximal tibia and distal femur) by CT shortly after their acute injury and then 3 months later, we have seen dramatic decreases in both cortical and trabecular bone mineral content and volumetric bone mineral density71. Finite element (FE) analysis of these data has indicated an associated marked decrease in bone strength approximately 2.5 times greater than that observed for bone mineral density. Examination of the distal femur, done as part of the same study, showed very similar results.9 These data support the value of not only obtaining CT data in order to more accurately determine changes in BMD that is possible by DXA, where positioning and the presence of heterotopic ossification both pose significant concerns, but also to provide information which can be used to evaluate changes in bone strength, which may be an important consideration when recommendations regarding initiation of activities such as assisted weight-bearing are being considered during recovery.

2. Objectives and Specific Aims

The overall objective of this study is to define an effective therapeutic approach, using currently available medication, to prevent or mitigate the loss of bone mass and bone strength that occurs after acute spinal cord injury. Zoledronic acid is felt to represent an ideal anti-resorptive agent in this setting for the reasons outlined above. Additionally, treatment with zoledronic acid after SCI has been shown by us and others in studies of limited size and duration to demonstrate short-term efficacy in prevention of bone loss. However, the durability of the response to a single infusion of zoledronic acid is undefined, the need for repeat treatment has not been explored, the

consequences of delayed intervention with zoledronic acid are not known, and effects on bone strength have not been determined. Consequently, treatment after acute SCI to prevent bone loss is rarely, if ever, considered and is not the standard of care.

This study aims to provide a robust base of evidence to guide the use of zoledronic acid after acute SCI. This will be accomplished by undertaking a randomized, controlled clinical trial in which different treatment regimens of zoledronic acid will be compared with each other and with placebo.

The primary aims of the study and the associated hypotheses are:

AIM 1: To define the timing and frequency of administration of zoledronic acid 5mg infusion that will result in the optimal prevention of bone loss after acute SCI.

Hypothesis 1: In participants treated shortly after acute SCI with a single infusion of zoledronic acid, the mean loss of bone after both one year (Hypothesis 1a) and two years (Hypothesis 1b) at skeletal sites in the lower extremity (total hip, femoral neck, proximal tibia, distal femur) will be less than that determined for participants who received placebo. The primary hypothesis will focus on changes at the total hip site and the study powered accordingly.

The following additional hypotheses will also be addressed:

Hypothesis 1c: The effects of a single infusion of zoledronic acid after acute SCI will wane over time; retreatment with zoledronic acid after one year will result in greater maintenance of bone than a single infusion with zoledronic acid.

Hypothesis1d: Initiation of treatment with zoledronic acid at one year after acute SCI in people not previously treated to prevent bone loss will still result in preventing further bone loss compared to no active bone-specific treatment; loss of bone mass that has occurred during the initial year without treatment will not be reversed, however.

To accomplish these objectives, we will undertake a 2 year, double-blinded, randomized, controlled trial. During the first year, participants will be randomized to either zoledronic acid 5 mg or placebo, and evaluated at 3, 6 and 12 months after infusion by DXA and CT imaging to determine changes in bone mass. At the 12 month time point, the participants in each of the treatment groups (zoledronic acid, placebo) will be re-randomized in a 1:1 ratio to receive either an infusion of zoledronic acid 5 mg or an infusion of placebo and followed during a second year of study. During year 2, participants will be evaluated at 6 and 12 months after re-infusion by DXA and CT imaging to determine changes in bone mass. (Subjects weighing less than 40 kg and assigned to receive study drug will be administered a reduced dose of 2 mg instead of 5 mg.)
AIM 2: To evaluate the use of serum markers of bone metabolism to guide therapeutic decisions of timing and need for retreatment with zoledronic acid after acute SCI.

Hypothesis 2: Serum levels of markers of bone resorption will be elevated after acute SCI and suppressed by zoledronic acid infusion. Recovery of bone markers to high levels will correlate

with continued bone loss and provide a signal indicating the need for additional anti-resorptive intervention.

Serum will be obtained at each of the imaging visits and biomarkers of bone metabolism will be measured and correlated with bone changes determined by DXA and CT.

AIM 3: To evaluate the effects of zoledronic acid in mitigating loss of bone strength that occurs after acute SCI.

Hypothesis 3: Changes in bone strength determined by finite element analysis will be greater than those seen in measurement of bone mass by either DXA or CT, with particular loss of strength noted at the distal femur and proximal tibia.

FE analysis utilizing data from the CT imaging will be undertaken to provide estimates of bone strength.

2.2. Projected Outcomes and Endpoints

<u>One year efficacy outcome results.</u>
Mean change in BMD from baseline after one year, evaluated by DXA at the total hip, in the group of participants treated with zoledronic acid will be statistically significantly less than the mean change in BMD in the group receiving placebo (primary endpoint). It is also anticipated that there will be a statistically significant difference in mean change in BMD at one year at the femoral neck and at the knee (distal femur, proximal tibia) between these two groups.

<u>Two year retreatment outcome efficacy results.</u>
Retreatment of those participants who received zoledronic for the first year of the study with zoledronic acid again (zol/zol) is expected to result in a continued inhibition of bone loss, such that when compared to individuals who had received zoledronic acid for the first year and then placebo (zol/placebo), a statistically significant difference between mean changes from baseline in BMD at the hip and knee skeletal sites will be observed after 2 years.

Observation of the zol/placebo group during the second year of the study will permit definition of the duration of a single infusion of zoledronic acid. It is anticipated that at the end of 2 years there will remain a statistically significant difference in mean change in BMD at hip and knee skeletal sites in this group compared to the group receiving placebo throughout the 2 years of the study (placebo/placebo).

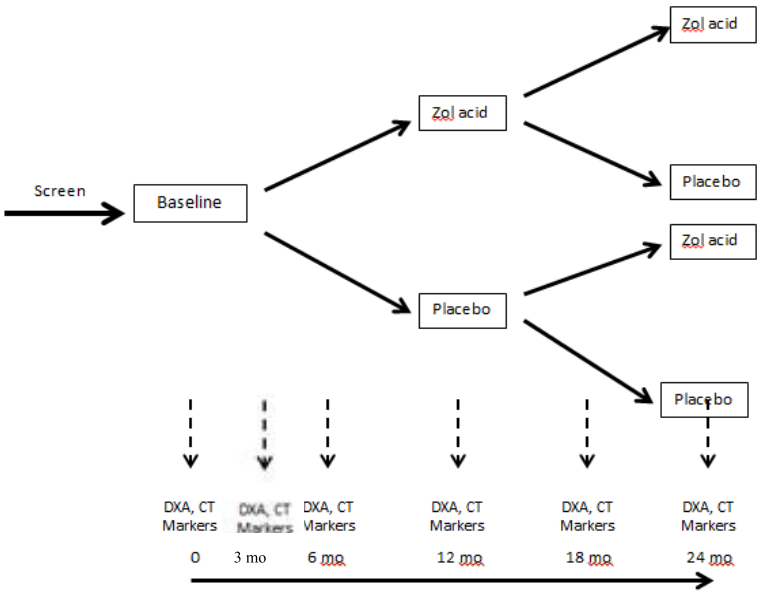
The group of individuals receiving zoledronic acid after a first year on placebo (placebo/zol) will be expected to show rapid bone loss during year 1 after which further bone loss will be prevented or slowed by treatment with zoledronic acid. By the end of year 2, the mean change in BMD from baseline at hip and knee skeletal sites in the placebo/zol group will be less than the mean change seen in the placebo/placebo group but greater than observed in the zol/zol group.

Markers of bone metabolism.
Levels of serum markers of bone metabolism will provide useful information regarding appropriate timing for retreatment. Mean levels of serum markers of bone resorption will be significantly reduced after each infusion of zoledronic acid and will increase again at the end of one year but will not reach baseline. Retreatment with zoledronic acid will reduce mean serum marker levels of bone resorption again whereas the group given placebo will have a return of mean serum levels of resorption markers close to the baseline value. Increases in bone resorption markers will presage further loss of bone.

CT analyses and bone strength.
CT analysis will provide additional information regarding differential bone loss at trabecular and cortical sites at both hip and knee, and FE analysis of bone strength will demonstrate greater differences between and among groups than seen with DXA and CT evaluation at all skeletal sites.

3. Study Design

3.1. Overview
This is a 2 year, randomized, double-blind placebo-controlled study of zoledronic acid to evaluate its efficacy and safety for the prevention of bone loss and maintenance of bone strength in individuals with recent onset SCI (see diagram below). Subjects will be randomized at the baseline visit to receive either zoledronic acid or placebo. At the end of the first year of the study, each treatment group will be re-randomized to either zoledronic acid or placebo to evaluate the durability of response to zoledronic acid and the utility of serum bone markers to guide therapeutic decision making. DXA imaging, CT imaging of knee and bone markers will be obtained at baseline, 3 months, 6 months, 12 months, 18 months and 24 months. CT imaging of hip will be obtained at baseline, 6 months, 12 months, and 24 months.



3.2. Centers and enrollment goal:
This will be a single center study. 60 subjects meeting inclusion and exclusion criteria will be enrolled at Northwestern University. University of Calgary (UC) will act as a site for de-identified data analysis of CT scans. No subjects will be enrolled and no visits will take place at UC.

3.3. Interventions

Zoledronic acid
Zoledronic acid is a potent, 3rd generation aminobisphosphonate that has been shown to be effective at preventing bone loss in post-menopausal women as well as reducing fracture risk, including hip, vertebral and non-vertebral fractures in this population.[18] It has demonstrated efficacy and has FDA approval for use in the prevention and treatment of post-menopausal osteoporosis, male osteoporosis and in the treatment of bone loss associated with the use of long-term glucocorticoids.[18,35,72] When studied in people treated immediately post hip fracture compared to placebo, zoledronic acid not only reduced subsequent fracture risk but also resulted in reduced all-cause mortality during the mean 1.9 year follow-up.[59]

Zoledronic acid is unique in being able to be given once yearly and retain efficacy in the above conditions compared to more frequent dosing with other existing bisphosphonates. Recent studies have demonstrated that efficacy for the prevention of bone resorption and bone loss for many post-menopausal women may extend considerably beyond 12 months, with the optimal dosing frequency still being debated.[63-65] When used in Paget's disease, a single treatment results in a markedly superior response compared to oral bisphosphonates with less than 1% of zoledronic acid-treated participants having a loss of therapeutic response vs 26% of those treated with oral risedronate.[73] In addition to its demonstrated superior efficacy, zoledronic acid is administered as in intravenous infusion that can be given over a 15 minute period, thus eliminating the vigilance needed with oral bisphosphonates, both in regard to the problem of interference when taken with food or other medications as well as the need to maintain an upright posture to prevent erosive esophagitis. These issues are particularly important for the acute SCI population, where positioning may be an issue and concomitant medication use is the rule.

Zoledronic acid has been shown by our group in a pilot study as well as by others in similar studies with limited numbers of participants after acute SCI to be able to prevent bone loss, at least over a 6 month period[69] and possibly as long as 12 months,[70] when changes in bone at the hip were analyzed. There has been no study of the effects of zoledronic acid beyond 12 months, nor has any study evaluated retreatment with this drug. Additionally, no studies have reported the effects of zoledronic acid on bone loss or bone strength at the knee, the site most frequently involved with fractures in people with chronic SCI.

Placebo
A matching solution containing the same diluent as the zoledronic acid will be available for infusion in those participants randomized to placebo treatment. The appearance and properties of

this solution will be identical to that containing the active treatment and all labeling will be identical other than participant identifier.

Calcium and vitamin D

Supplemental calcium and vitamin D will be provided to all participants. At study entry, all participants will have to have a normal serum 25-hydroxyvitamin D level, and we will provide cholecalciferol 1000 IU/day to all participants to assure normal levels during the course of the study. At this time, 1000 IU of vitamin D3 appears to be an adequate dose for maintenance of normal vitamin D levels.[12] If recommendations appear that indicate higher doses would be appropriate, treatment will be altered accordingly. For individuals who are already taking vitamin D supplements, the dosage of study cholecalciferol may be adjusted so as not to exceed 2000 IU per day, unless prescribed as such by the subject's primary physician.

All participants will be given supplemental calcium (1000 mg/day), provided as calcium carbonate, to be taken as 500 mg tablets, one tablet twice daily. For individuals who are already taking calcium supplements or obtain sufficient calcium through their diet, the dosage of study calcium may be reduced in order not to exceed 1000 mg of exogenous calcium per day. We recognize that there may be a preference for calcium citrate due to the lack of interference with absorption with concomitant use of proton-pump inhibitors, but our experience has been that calcium carbonate is well tolerated and does provide adequate calcium intake in most clinical settings.

3.4. Randomization Method

This study contains two randomization points – one in the beginning of the study, and a re-randomization after 12 months. For the first randomization, participants will be assigned to treatment versus placebo in blocks of 2, 4, and 6. The order of the blocks of 2, 4 and 6 will be random. Within each block of sequential participants enrolled, half will be assigned to treatment and half will be assigned to the control condition, and the order of assignment will be at random. The randomization will be determined by a predetermined list, which will be created by an unblinded study personnel using a random number generator. The remainder of the study team and the participants will remain blinded to treatment throughout the course of the study. A copy of this list will be provided in a sealed envelope to the principal investigator in the event that treatment assignment needs to be known for a participant for safety reasons.

The second randomization will determine which treatment participants receive during the second year of their participation. The goal of this second randomization is to have 50% of the treatment group be randomized to continued treatment and 50% to be switched to placebo. Within the control group, 50% will be randomized to treatment and the other 50% will receive continued placebo. To achieve this, the unblinded personnel will create a random list of treatment versus control assignment using a random number generator. Participants will be assigned to treatment versus placebo in blocks of 2 and 4. With 60 subjects planning to be randomized, up to 15 subjects will be enrolled subjects in each of the following groups: zol/zol, placebo/zol, zol/placebo, and placebo/placebo.

3.6. Study Variables

Clinical and Demographic Information

Clinical and demographic information will be obtained by personal interview and, if necessary, review of medical records. This information will include a complete medical history, including demographic information, with specific emphasis on bone-related issues (history of fracture, endocrinopathy, vitamin D intake, etc.) utilizing a bone health questionnaire used by our group to acquire baseline data for our in-house bone registry (Spinal Cord Injury & Lifestyle Information Form). They will also complete the Spinal Cord Independence Measure[77,78](SCIM) prior to randomization. In order to assess ambulatory ability and status, participants will be asked to complete the Walking Index for Spinal Cord Injury[79] at each visit. This is a validated instrument to assess activity levels that affect bone loading and may be expected to have an impact on the effects of treatment that participants will be receiving.

DXA BMD

BMD will be determined by DXA at various skeletal sites (bilateral total hip, bilateral femoral neck, spine, non-dominant forearm, non-dominant knee (distal femur, proximal tibia), total body). The DXA scans will be performed at Baseline, 3 mos, 6 mos, 12 mos, 18 mos, and 24 mos by a trained DXA technician from our study team using a Hologic QDR 4500A densitometer. DXA scans performed at SRAlab will be done on the Hologic Horizon DXA scanner and will be performed by a SRAlab DXA technician, with oversight by our study team. All subjects who have their baseline scan at SRAlab will continue their follow up on the same machine. Whole body and forearm scans will be optional scans as they do not contribute to the primary or secondary study endpoints. Standard acquisition and analysis protocols will be used to quantify areal bone mineral density (aBMD) of all skeletal sites.

We have had extensive experience in DXA imaging of individuals with SCI. There have been no technical problems with DXA imaging though positioning has been challenging for some participants due to contractures and spasticity, and orthopedic hardware has also prevented the full set of images from being obtained. For those individuals with obstacles in obtaining images of the non-dominant knee and/or forearm, the dominant extremity will be used. As much as possible, positioning is reproduced at subsequent visits to allow appropriate comparisons.

CT determined bone parameters.

CT imaging will permit determination of volumetric integral BMD at the knee (distal femur and proximal tibia) and hip as well as definition of compartmental BMC and BMD (trabecular and cortical). The non-dominant knee and hip will be scanned (based on handedness), unless metal or other artifacts are present. We also have extensive experience in this technique, with >200 knees imaged and analyzed. The CT scans will be performed using a Siemens Sensation 64 machine or a Siemens Somatom Definition AS (120kVp, 280 mAs, pixel resolution 0.352 mm, slice thickness 1 mm). Each CT scan will include a phantom – placed on the side of, or underneath, the subjects' knee (for knee scans) or hip (for hip scans) – with known calcium hydroxyapatite concentrations of 0, 0.4, and 0.8 g/cm^3 (QRM, Moehrendorf, Germany). The phantom will serve as an interscan calibration, allowing for the conversion of CT Hounsfield units to bone equivalent density.

Three regions of bone will be analyzed corresponding to 0-10%, 10-20%, and 20-30% of segment length, as measured from the distal end of the femur or proximal end of the tibia. These regions were chosen based on their anatomical correspondence to epiphyseal (0-10%), metaphyseal (10-20%), and diaphyseal (20-30%) locations. The CT Hounsfield units will be converted to bone equivalent density and femora and tibiae will be segmented from CT images using a 0.15 g/cm^3 threshold to identify the periosteal surface boundary. Methodology for CT measurement of the hip has been previously published.[1]

Determinants of bone strength.
Measurements of distal femur and proximal tibia geometry and strength indices will be calculated along the longitudinal axis of the bone and subsequently averaged within each region. Cross-sectional area will be calculated as the cumulative sum of voxel area within the periosteal surface boundary. Bone volumes of integral and cortical bone will be quantified for each region and used as surrogate measures of periosteal and endosteal expansion. Both a compressive strength index and a torsional strength index will be calculated. Measurement of bone strength at the hip will utilize methodology previously published.[2]

Clinical chemistry evaluation (bone markers).
At Baseline, 3 months, 6 months, 12 months, 18 months and 24 months, 20 ml of blood will be obtained between 8AM and 10AM from each participant (if possible) for determination of serum bone markers. Serum bone markers will include P1NP, osteocalcin (bone formation markers), CTX (bone resorption marker) and testosterone and sclerostin, as well as other bone-related serum markers to be determined. Serum will be separated and aliquots placed in vials individually labeled with the subject's study number and date; vials will be stored at -80^{o}C in a locked freezer in a University maintained freezer repository.

At the end of the trial, samples will be sent for assay to the Maine Medical Center Research Institute Laboratory, Scarborough, Maine. This laboratory has extensive experience in performing these assays. Shipping will meet all federal, state and local regulations; all individuals involved in shipping biologic samples will have completed a training course provided the Northwestern University Office of Sponsored Research and will have received certification from them that they are aware of IATA/DOT regulations. Specimens will not be maintained for non-bone-related use.

Adverse events.
Adverse events will be obtained by asking participants regarding changes in their health status during the course of the study. There will be specific attention to events occurring immediately after the infusions of study medication as an acute phase response to zoledronic acid is recognized as a common accompaniment, despite the fact that all participants will be pre-treated with acetaminophen and treatment maintained for 24-72 hours after infusion. A MedDRA database will not be used, but all adverse events will be classified by organ system in a safety database generated for this study. A similar safety database has been used successfully in previous long-term longitudinal SCI studies undertaken by our group. Reports of safety outcomes will be generated for use by the data and safety monitoring committee (DSMC) to

review. DSMC meetings will occur at least every 6 months or more frequently depending on the activity of the protocol. All SAEs will be reported as required to the local IRB.

4. Study Population

Participants will be recruited from patients admitted to the in-patient spinal cord unit at the Rehabilitation Institute of Chicago (RIC)/Shirley Ryan AbilityLab (SRAlab). RIC is one of 14 national NIDRR-supported Spinal Cord Injury Model Systems Centers and provides extensive in-patient and out-patient services.

4.1. Patient Eligibility

Subjects must meet all of the inclusion and none of the exclusion criteria to be registered to the study. Study treatment may not begin until a subject is registered.

4.1.1. Inclusion Criteria

- In-patient at RIC/SRAlab or an outpatient who was recently discharged from RIC/SRAlab
- Males and females
- Age ≥18 years
- Medically stable in the opinion of subject's physiatrist
- SCI within 120 days of screening
- SCI with inability to ambulate independently (defined as walking more than 15-50 feet to perform normal activities of daily living such as walking to/from the bathroom with or without any assistive devices such as a walker and/or braces) or ASIA Impairment Scale (AIS) A, B, or C, at time of study entry
- Capable of positioning to have DXA performed
- Able to tolerate acetaminophen
- No known endocrinopathies (diabetes type 1 or 2, treated thyroid conditions can be included)
- Normal serum TSH and/or T4 levels
- Normal 25-OH vitamin D levels (≥ 20 ng/ml while not on supplemental vitamin D or ≥ 15 ng/ml while taking at least 1,000IU vitamin D daily) at baseline (subjects may be repleted)
- Normal calcium levels
- Normal renal function (creatinine <2.0 mg/dl) and estimated creatinine clearance >35 mL/min
- Well hydrated with adequate intake of liquids
- Able to return for all follow-up visits depending on ease of access to SRAlab post-discharge
- Capable of reading and understanding informed consent document
- Females of childbearing potential must be willing and able to use an effective method of contraception or practice abstinence throughout the course of the study.

4.1.2. Exclusion Criteria

- Have Paget's disease of the bone
- Malignancy as a cause of acute SCI
- Have abnormal laboratory values that in the judgement of the investigator would put the participant at increased risk of treatment
- Any active gastrointestinal condition that results in malabsorption
- Poor dental hygiene or requirement for invasive dental procedure within two months prior to or post enrollment
- History of bone metastasis and skeletal malignancies
- History of alcoholism or drug abuse within the 2 years prior to study screening, which in the opinion of the investigator may affect subject's health and/or study commitment
- Other medical conditions that in the opinion of the investigator would preclude the subject from completing the study
- Currently being prescribed anticonvulsants at a dose or frequency that is determined to interfere with bone metabolism as determined by the investigator
- Currently being prescribed glucocorticoids, other than inhaled glucocorticoids
- Current or recent use any bone-active agents, including any bisphosphonate, raloxifene, hormone therapy (estrogen and estrogen/progestin), calcitonin or strontium-containing compounds within 60 days of screening.
- Pregnant, planning to become pregnant, or lactating

5. Recruitment Process

Recruitment at RIC/SRAlab will be coordinated and overseen by Dr. David Chen, medical director of the spinal cord service at RIC and program director of the model systems center at this institution. Approximately 140 patients with acute SCI are admitted per year to RIC. Our group has had previous success at recruiting this population for two previous studies in acute SCI: the pilot study referenced above as well as a parallel study evaluating and comparing CT to DXA in quantifying and characterizing bone changes at the knee after acute SCI.[9,71] In these two studies we had successfully recruited >30 participants in less than 12 months without having a dedicated recruitment coordinator at RIC. We feel confident that with a dedicated part-time recruitment coordinator, working in parallel with the attending physicians on the SCI service and with Dr. Chen, the director, that we will be able to be able to enroll at this rate or greater for the this study.

We plan to apply a similar approach to recruitment for the current study as for our previous studies in acute SCI. All patients who are medically stable and meet the remainder of the inclusion/exclusion criteria will be approached by a study investigator and/or the recruitment coordinator for an initial discussion regarding the importance of maintaining bone health and told about the study. This initial approach may take place at SRAlab. There will be other reading materials on the unit regarding bone health that the patients will have access to. Patients will be seen again by the recruitment and/or study coordinator and either Dr. Chen, their attending physician, or Dr. Schnitzer, the principal investigator, to discuss the study in more detail and, if interested, will be offered the opportunity to participate assuming they continue to meet all the inclusion and exclusion criteria.

6. Informed Consent Process

The study coordinator will speak to the potential participant in person or over the phone and do a brief screening to be certain that the person could potentially qualify for the study and then schedule a time for a screening visit. A consent form may be given at the bedside or sent to the potential participant if there is time prior to his/her scheduled appointment.

At the screening session, which generally will take approximately 1.5-2.5 hours, the participant will be given a consent form to read or review again if they had already received one. After they have had adequate time to read the form completely, the study coordinator will review all the elements of the study again with the participant. The study coordinator will assess whether the potential participant demonstrates the ability to provide informed consent by asking him/her to describe the goals of the study and what is expected of them, explore their motivation for being involved in the study and their expectations, and ask about any logistical issues and determine how they can be dealt with (transportation to the clinic, travel out of town for long periods of time). Questions will be answered by the study coordinator if possible; for those questions that cannot be answered by the study coordinator, the principal investigator will be available to provide them or suggest how an answer can be obtained. The consent process, occurring in the consult room of the clinic or at the patient bedside, is expected to take approximately 30-45 minutes. Potential participants will be offered the opportunity to have family members or others with them during this process or to postpone making a decision if they feel they wish to get advice from another person not immediately available. Participants are asked to sign two copies of the consent form (a witness may watch subjects document permission to study participation by making his/her mark on the consent form if unable to sign their name due to paralysis); one copy is given to the subject and the other is put into the study chart.

7. Study Procedures

Subjects will all be in-patients or recently discharged from RIC/SRALab. Patients are not transferred to RIC/SRAlab unless medically stable, but as the acute phase of their illness may have associated complications as well as requiring psychological adjustment; patients will be approached for inclusion into this study when deemed appropriate by their attending physiatrist and/or nurse practitioner. If patients express an interest in the study, the study procedures will be explained, their questions answered, and the informed consent process as described in the preceding section will take place. Their medical record will be reviewed to assure they meet all inclusion and exclusion criteria. Assessments performed exclusively to determine eligibility for this study will be done only after obtaining informed consent. Assessments performed for clinical indications (not exclusively to determine study eligibility) may be used for baseline values even if the studies were done before informed consent was obtained.

There will be 10 visits for this study: Screening, Baseline, and 8 follow up visit at 3, 6, 9, 12, 15, 18, 21, and 24 months after baseline. It is desirable but not required for research subjects attend visits at 3, 9, 15, and 21 months after baseline. It is understood that based on where research subjects live and available transportation, visits every 3 months may not be possible. If a research subject cannot attend the follow-up visits at 3, 9, 15, or 21 months, the visit will be conducted over the phone and study supplements will be mailed to the research subject.

All patients will have their 25-hydroxyvitamin D levels measured at the screening visit if a recent test has not been done within the past 3 months. Those patients whose levels are lower than 20 ng/ml and are currently not on vitamin D will be dispensed a 7 day supply of vitamin D 50,000 IU. Infusion of study drug at baseline will only take place after a repeat 25-hydroxyvitamin D serum level meeting the study criteria will have been documented. Those patients whose levels are lower than 20 ng/ml but greater than or equal to 15 ng/ml and are currently on at least vitamin D 1,000 IU daily will be eligible for the study and will not need a repeat 25-hydroxyvitamin D serum level prior to infusion.

Baseline Visit: Prior to infusion, it will be verified that all subjects are well-hydrated and have acceptable renal function and serum calcium levels (and a negative pregnancy test if appropriate). Subjects will be pre-medicated with acetaminophen, 650 mg orally, up to 4 hours prior to the infusion.

The study medication for the infusion will be provided by either the in-patient pharmacy or the study team who will have received blinded medication and will allocate active drug or placebo as indicated by the randomization scheduled in a sequential manner. The subject and the investigator/ investigator's staff will be blinded to treatment allocation. Study drug will be given at minimum over 15 minutes as an intra-venous infusion according to standard instructions in the package insert. Subjects weighing less than 40 kg will receive a reduced dose of study drug or placebo. Those assigned to the active study arm will received 2 mg of study drug diluted in 40 mL of saline. Those assigned to the placebo arm will receive 40 mL of saline. Subjects will be allowed to self-medicate post-infusion with 650 mg acetaminophen every 6 hours, up to 2600 mg per day, for up to 3 days.

Screening Visit

- Informed consent process
- Medical history
- Concomitant medications
- Urinalysis
- Pregnancy test (if applicable)
- Labs (CBC, Comprehensive Chemistry Panel, 25-OH vitamin D, TSH)
- Questionnaires (SCIM and SCILI)
- Walking Index
- Physical exam (if done at baseline visit, must be done prior to infusion)
- Vital signs
- Serum bone markers (can also be done at the baseline visit, but prior to study drug infusion)
- DXA (can be done at baseline visit)
- CT of knee and hip (can be done at baseline visit)

Randomization/Infusion (Baseline)

- Provide calcium and vitamin D
- Collect adverse events

- Review concomitant medications
- Vital signs (must be done prior to infusion)
- Physical Exam, with an oral examination (must be done prior to infusion)
- Labs (CBC, Chemistry Panel, 25-OH vitamin D, TSH) to be done prior to infusion. It should be verified that subject has an acceptable renal function, serum calcium concentration and 25-OH vitamin D prior to infusion.
- Urinalysis (must be done prior to infusion)
- Negative pregnancy test, if applicable (must be done prior to infusion)
- Serum bone markers (must be done prior to infusion)
- DXA and CT of knee and hip (must be done prior to infusion)
- Pre-treatment with acetaminophen (0-4 hours prior to infusion)
- Verification that subject is well-hydrated (subject should drink 1-2 full glasses of water 0-4 hours prior to infusion)
- IV infusion of study drug

Follow-up Phone Call (or Visit if still inpatient at RIC/Shirley Ryan AbilityLab) (1-7 days after IV infusion)

- Collect adverse events

Follow-up Visits (Months 3, 6, and 18)

- Provide calcium and vitamin D
- Collect adverse events
- Review concomitant medications
- SCILI
- Walking Index
- Vital signs
- Serum bone markers
- DXA and CT of knee
- CT of hip at Month 6
- Month 6: Subjects with DXA BMD loss >/= 20% from baseline on at least two sites will be offered infusion with opposite treatment.

2nd Infusion (Month 12, or potentially Month 6)

- Provide calcium and vitamin D
- Collect adverse events
- Review concomitant medications
- Vital signs (must be done prior to infusion)
- Physical Exam, with an oral examination (must be done prior to infusion)
- Chemistry Panel (or at minimum serum calcium and creatinine) and if applicable, a negative pregnancy test (must be done prior to infusion). Lab workup done at an outside institution/lab is acceptable as long as it is completed within 3 months prior to the infusion.
- Serum bone markers (must be done prior to infusion)
- DXA and CT of knee and hip (can be done after the infusion)
- Pre-treatment with acetaminophen (0-4 hours prior to infusion)

- Verification that subject is well-hydrated (subject should drink 1-2 full glasses of water 0-4 hours prior to infusion)
- IV infusion of study drug

Follow-up Visits (Months 9, 15, and 21)

- Provide calcium and vitamin D
- Collect adverse events
- Review concomitant medications
- Vital signs
- Walking Index
- SCILI

Final Study Visit (Month 24)

- Collect adverse events
- Review concomitant medications
- Vital signs
- Walking Index
- SCILI
- Serum bone markers
- Labs (Chem Panel, CBC, and urinalysis)
- DXA
- CT of knee and hip
- Physical Exam

Event	Screening	Baseline	Follow-Up							
Study Day		0	Mo 3	Mo 6	Mo 9	Mo 12	Mo 15	Mo 18	Mo 21	Mo 24
Visit #	V1	V2	V3	V4	V5	V6	V7	V8	V9	V10
Procedures:										
Informed Consent	X									
Demographic Data	X									
Questionnaires	X		X	X	X	X	X	X	X	X
Walking Index	X		X	X	X	X	X	X	X	X
Physical Exam	X			X*		X				X
Height	X									
Weight	X									
Vital Signs	X	X	X	X	X	X	X	X	X	X
AE Assessment		X	X	X	X	X	X	X	X	X
Incl/Excl Criteria	X									
Review con. meds.	X	X	X	X	X	X	X	X	X	X
Pre-treatment		X		X*		X				
Study Drug Infusion		X		X*		X				
Dispense calcium		X	X	X	X	X	X	X	X	
Dispense vitamin D		X	X	X	X	X	X	X	X	
DXA		X	X	X		X		X		X
CT of Knee		X	X	X		X		X		X

CT of Hip		X		X		X				X
Labs:										
CBC	X									X
Chemistry Panel	X			X*[a]		X[a]				X
25-OH Vitamin D	X									
TSH	X									
Urinalysis	X									X
Pregnancy Test	X			X*		X				
Bone Markers		X	X	X		X		X		X

X* - Month 6: Subjects with DXA BMD loss >/= 20% from baseline on at least two sites will be offered infusion with opposite treatment. In such cases, infusion-related assessments to be done at the 12 month visit will be done at the 6 month visit instead.
X[a] – At minimum, a serum calcium and creatinine is required before an infusion.

8. Compensation for Participants

Visit #	Amount of Stipend	Payment Type
1 – Screening	$0	Cash
2 – Baseline	$50	Cash
3 – Month 3	$50 + $25 (travel)	Cash
4 – Month 6	$50 + $25 (travel)	Cash
5 – Month 9	$25 (travel)	Cash
6 – Month 12	$50 + $25 (travel)	Cash
7 – Month 15	$25 (travel)	Cash
8 – Month 18	$50 + $25 (travel)	Cash
9 – Month 21	$25 (travel)	Cash
10 – Month 24	$50 + $25 (travel)	Cash
Unscheduled Visit	$25 (travel)	Cash
TOTAL	**$525**	

Study payments will be given to participants in cash at the time of each study visit. Participants will only be paid for study visits that are completed. Once discharged from RIC/Shirley Ryan AbilityLab, a travel stipend of $25 will be given at each completed study visit on site. This travel stipend is meant to cover the expenses of travel to and from site. For incurred travel expenses greater than $25, subject will be reimbursed via check instead of $25 cash.

In addition, when necessary, travel arrangements will be made to assist participants in returning to the site for important study time points (Month 6, Month 12, Month 18, and Month 24 visits). This may include reimbursement for mileage, rental car services, taxis, airfare, etc. In these cases, the additional travel stipend of $25 will not be given to subject.

9. Data Management

Data management will be coordinated by the PI, with direct management by the study data manager and guidance from the study biostatistician (Dr. Griffith). A REDCap database will be developed for the study and maintained on a secure server. Whenever possible, study results will be electronically uploaded. Double entry will be used for data that must be manually entered to track entry errors. The database provides transaction logs (i.e., records of who accesses the database) and audit trails (i.e., records of all changes to the database, at the cell level), thereby maintaining the highest level of data security. Confidentiality of data will be maintained by using only patient identification numbers for data entry and in most tables within the database. The master table linking patient names and other Protected Health Information (PHI) with identification numbers will be maintained in a separate password-protected file, accessible only by designated members of the research team. Hard copies of participants' records will be kept in individual study charts that are stored either in locked cabinet or in a locked office. Data records may be destroyed by deleting them from electronic media and by shredding paper documents, but some of the deidentified data records may be retained indefinitely.

10. Risks/Benefits Assessment

Foreseeable risks

Excessive Bone Loss: Participants receiving placebo may experience significant bone loss during the course of the study. Although the current standard of care in acute SCI is to not treat individuals, in this study where treatment is an option, it is not felt to be ethical to withhold treatment from individuals who have experienced extensive bone loss that would put them at risk for the development of osteoporosis. Whether such treatment in this setting would be effective is unknown, and the focus of this study, but participants should have the benefit of making an informed decision regarding their wish for intervention. It is acknowledged that this decision may be controversial, but it is felt to be in the best interests of the participants and has been supported on review by both the FDA and our institutional review board. Therefore, during this study, individuals who experience a loss of 20% or more of bone mass from baseline, detected on at least two sites on repeat testing at the 6 month study visit, will be offered the opportunity to be treated with whatever medication they did not receive at their baseline visit. The participants and the research team will continue to be blinded to study treatment; the decision regarding treatment allocation will be made by the unblinded pharmacist. These participants will be followed for additional 18 months according to the study schedule, but will not be re-randomized at the one year visit. All participants will continue to be followed in the study, regardless of whether they choose to be retreated or not, and all efforts will be made to collect data through 2 full years of follow-up. For those individuals who reach this safety cut-off, all further data will be censored at that point for the purposes of data analysis.

Zoledronic acid: The most serious risks associated with the infusion of zoledronic acid are the possibility of acute renal failure and hypocalcemia. Other risks associated with zoledronic acid, seen also with other bisphosphonates, include osteonecrosis of the jaw and the possibility of severe bone, joint and muscle pains. The most common adverse reactions (greater than 10%) reported with the use of zoledronic acid are pyrexia, myalgia, headache, arthralgia, and pain in extremity. Other important adverse reactions were flu-like illness, nausea, vomiting, diarrhea,

and eye inflammation. An acute flu-like response is seen in greater than 10% of people within 24 hours of infusion.

Radiation Exposure: Risks of radiation exposure will be listed in the study consent form per the Radiation Safety Officer's assessment based on DXA and CT imaging.

Vitamin D and calcium. The doses administered in this study are significantly below the safe upper limit defined by the Institute of Medicine's recent report.[3,4] Overdose of either can result in hypercalcemia, which is associated with nausea and vomiting, loss of appetite, excessive thirst, constipation, abdominal pain, muscle weakness and pain, confusion, lethargy and fatigue.

Risk management and emergency response

To mitigate the risks associated with the infusion of zoledronic acid, individuals with renal insufficiency (estimated creatinine clearance of ≤ 35 ml/min) and those having low serum calcium levels will be excluded from the study. Additionally, normal serum levels of vitamin D will also be required prior to the baseline infusion and all infusions will be done over 15 minutes to reduce the possibility of exposure to high serum levels of zoledronic acid (15 minutes is the minimum recommended time for infusion). All participants will be pre-medicated with acetaminophen 650 mg at 0-4 hours prior to infusion of zoledronic acid and then every 6 hours thereafter for up to 3 days as needed to reduce the incidence of the acute flu-like response (low grade fever, myalgias, joint aches) that can occur. An oral examination will be performed prior to study entry to exclude individuals with poor dental hygiene or in need of invasive dental procedures. Female patients will have to have a negative pregnancy test to be enrolled in the study and agree to use effective contraception (or abstinence) during the course of the study.

Participants will be told to call the research coordinator or principal investigator if at any time in the study they have a change in their medical condition, particularly if they believe this may be related to the study interventions. They will be given a telephone number to call that will reach a study member at any time (24 hours/day) during the course of the study.

Potential benefits

The possible benefits include reduction in the loss of bone mass and bone strength that occurs during the months after acute SCI; this may result in a reduction in the future risk of bone fractures. Taking part in this study may also help scientists to better understand bone loss after acute SCI and may help inform future research studies about bones.

11. Unblinding Procedure

Unblinding of participants will only be performed when knowledge of the treatment allocation will influence participant management, for example, after overdose of the study treatment or placebo. Individuals not otherwise involved in the day-to-day conduct of the study will perform unblinding. In cases of emergency unblinding, each affected participant will be explained the reason for the unblinding and the potential risks incurred, and all communications will be

documented in affected participants' source documents within 24 hours of notification. The IRB will be notified immediately if such unblinding should occur.

Female patients must have a negative pregnancy test and agree to use effective contraception (or abstinence) during the course of the study. If a female subject becomes pregnant while taking part in this study, the subject will be withdrawn from the study. The subject's physician will be notified and the subject will be followed closely in conjunction with the subject's OB patient schedule through delivery or termination of pregnancy. Unblinding may occur by a member of the research staff so that the subject's physician and/or OB might know whether or not the active drug was in use.

12. Withdrawal from the Protocol

All participants will be informed as part of the consent process that their participation in the protocol is entirely voluntary and that they are free to discontinue at any time. Furthermore, such discontinuation will in no way prejudice any medical care they may be receiving from the institution or any clinician involved, or interfere in any way from their receiving on-going or future care.

If individuals elect to withdraw from the study, a termination visit will be scheduled, if possible, at which time the reason for their discontinuation will be solicited to allow appropriate action. If withdrawal is due to medical reasons, related or unrelated to the protocol, appropriate medical referral and follow-up will be recommended, which could include further safety visits until the event has passed. If individuals decline re-randomization/infusion of study drug at the 12 month visit, they will be allowed to remain in the study and asked to return for their regularly scheduled visits. Participants may be withdrawn from the study by the principal investigator if in his judgment continuation in the protocol would be detrimental to the health or safety of the participant, if the participant is not compliant, or if funding no longer exists for the project.

Although every attempt will be made to retain subjects in this study, we do not plan to replace withdrawn subjects in order to reach the enrollment of 60 subjects.

Additionally, unless a subject revokes his/her consent to use their health information (revokes HIPAA Authorization), any data collected prior to study withdrawal will be used in data analysis.

Female subjects who become pregnant will be withdrawn from the protocol and will not be allowed to continue participation.

13. Study Personnel

Roles and responsibilities of Key Study Personnel

Thomas J. Schnitzer, MD, PhD: Principal investigator is responsible for overall management of all activities for this project. Dr. Schnitzer will provide supervision and direction to the study coordinator for all clinical and regulatory aspects of the study, assist in recruitment, coordinate the randomization, do physical examinations and review the clinical status of participants with Dr. David Chen, be available to answer questions and evaluate changes in clinical status,

evaluate adverse events and report serious adverse events to appropriate authorities, prepare annual reports and protocol updates/amendments, interact and coordinate with co-investigators, interact with DXA technician to assure quality of DXA scans, interact with the other site investigators to assure recruitment and quality of CT evaluations, interact with Research Monitor on regular basis, help in preparation of safety reports with the study statistician, interact with data manager to assure quality and integrity of data, evaluate data with the statistician and remainder of research staff, prepare manuscript and submit for publication.

James W. Griffith, PhD: Dr. Griffith is a faculty member and a clinical psychologist at Northwestern University in the Department of Medical Social Sciences. Dr. Griffith has extensive experience in research biostatistics and will be the primary statistician working with faculty in the Department of Physical Medicine and Rehabilitation. He will be responsible for all the statistical input for this project, including sample size determination, power analysis, development of randomization scheme, data management plan, overseeing the data management process including masters-level statistician and data entry individuals, establishment of data tables, quality control procedures to assure data integrity and accuracy, assurance of data entry and data analysis, development of final data analysis plan including designating all imputation methodologies, implementation of all elements of the data analysis plan, participating in evaluation and interpretation of results of the study and preparation of all resulting manuscripts.

David Chen, MD: Dr. Chen, a co-investigator, is the PI and Director of the Model Systems Spinal Cord Injury Program at RIC and has had over 20 years experience managing people with SCI. He will assist in recruitment of participants for the study and will also provide clinical input and assistance in assessing potential participants. He will interact with the other faculty in the PM&R department and at RIC/Shirley Ryan AbilityLab to provide access to their patients for this study. He will make available the registry/database of the Model Systems Program and work with the clinical study coordinator to be in contact with these individuals regarding entry to the program. Dr. Chen will be available to assess any safety issues that may arise during the course of the study.

W. Brent Edwards, PhD (postdoctoral research fellow). Dr. Edwards will be responsible for ensuring that CT data is collected according to a standard protocol. Dr. Edwards will be responsible for processing and analyzing all CT data. This includes segmenting images, obtaining architectural parameters and generating and running subject-specific finite element models. Working with Drs. Schnitzer, Dr. Edwards will intellectually contribute to the dissemination of this data.

Roles and responsibilities of other study personnel

Study Coordinator: The study coordinator will be responsible for all interactions with study participants, including recruitment, obtaining informed consent, scheduling all DXA, CT and follow-up clinical visits, collecting adverse event information at all visits from the participants, being available to answer queries and concerns from participants, providing telephone contact to all participants, reporting all safety reports, maintaining all regulatory documents and entering clinical data into the appropriate databases. He/she will work closely with all the key personnel

to assure that everyone is aware of each participant's study status and any changes that occur during the study will be communicated to the PI.

Research Monitor: Dr. Elliot Roth has agreed to be the independent Research Monitor for this study. Dr. Roth is the Chairman of the Department of Physical Medicine and Rehabilitation at Northwestern University Feinberg School of Medicine and has significant clinical and administrative experience overseeing research projects.

The Research Monitor will be responsible for the safety oversight of the study. The Research Monitor will work with the PI and the biostatistician to 1) define a charter for the Research Monitor, 2) define the specific safety elements to be tracked, how often these elements should be reported, and in what format, and 3) define a means to document and approve these regular reports.

The Research Monitor will be responsible for reviewing all safety reports generated during the study on a regular basis, and at least every 6 months. Study reports will also be reviewed on a real-time basis. The Research Monitor may ask for assistance from the biostatistician or other study personnel. After each such meeting, the Research Monitor will make a recommendation to the PI to either: 1) continue the study in its current form, 2) consider amending the protocol to include specific actions to enhance safety or safety monitoring, or 3) discontinue the protocol with provisions for orderly discontinuation in accord with good medical practice.

The Research Monitor will review on an individual basis all unanticipated problems involving risk to participants, serious adverse events, and all participant death associated with the protocol, and provide an unbiased written report of the event to the IRB. For any event determined to be an unanticipated problem involving risk to subjects or others (UPIRTSO) will be promptly reported by telephone, email, or facsimile to the HRPO. A complete written report will follow the initial notification.

The Research Monitor may discuss the research protocol with the investigators, interview human subjects, and consult with others outside of the study about the research. The Research Monitor shall have authority to stop the research protocol in progress, remove individual human subjects from the study, and take whatever steps are necessary to protect the safety and well-being of human subjects until the IRB can assess the monitor's report. The Research Monitor is responsible for promptly reporting their observations and findings to the IRB.

14. STATISTICAL PLAN and DATA ANALYSIS

14.1. Overview

Please refer to document titled, "Statistical Analysis Plan version 2.2, dated 17Jun2020," which supersedes the analysis plan described in this section.

The main goal of the analytic strategy is to determine whether active treatment versus placebo influences the trajectory of change over time in bone density. This is a clinical trial, so we

will use an endpoint analysis as the primary means to determine whether treatment has been effective. The primary dependent variable will be change in BMD from baseline. The primary dependent variable will be change in BMD from baseline. We will retain all cases in the analysis using an intent-to-treat methodology. In order to ensure that treatment efficacy is not overestimated by participants who worsen dropping out of treatment, we will use the last-observation- carried-forward (LOCF) methodology to impute any data that are missing at the end of treatment. We understand that this method has limitations and may be overly conservative, but we will also have the ability to employ growth curve modeling, which can describe change over time even when some data are missing, and when assessment timing is different across participants. We plan to study 30 participants in each group (overall $N = 60$).

14.2. Data screening

Before analyses begin, all variables will be plotted using histograms to inspect the shape of distributions and to check for outliers. Because this is a clinical trial, we will make an a priori decision to not drop cases nor transform variables. Nonetheless, these issues can be highly relevant to the accuracy of statistical inference. If we identify any possibility that our assumptions have been violated, we will employ alternative approaches to determine whether our findings still hold. As part of data screening, we will describe the amount of data that are missing, and we will compare participants with and without missing data.

14.3. Sample size determination

Determination of the sample size for this study is based on 2 factors: the data we have accumulated of BMD changes at various skeletal sites from several of our preliminary studies and from the practical issues related to the number of participants who can be recruited. Both of these approaches provide congruent results. Our preliminary DXA data from participants followed for one year after infusion of zoledronic acid and a comparable group of acute SCI participants in a parallel study who were only observed and not treated for 12 months are presented in Table 2. If one focuses simply on the total hip skeletal site, which is the primary outcome measure, the data suggest a standardized effect size of Cohen's d of ≥ 3.9 (based on either right or left hip). This effect size would yield virtually 100% power with our planned sample size of $N = 60$. We will also, however, have enough power to detect effect sizes as small as $d = .74$ with 80% power with 30 participants within each group. Another way to express these data is to consider that the largest standard deviation in the BMD measurement at any of the hip sites was 9.02%. If we assume that this will be the standard deviation for all skeletal sites, with 30 participants/group, we will have 80% power to detect a difference of 6.5% between groups.

Region	Zoledronic Acid	Placebo
Spine	3.31 ± 2.11%	-8.04 ± 4.41%
Left Total Hip	-3.40 ± 3.51%	-13.83 ± 2.01%
Left Femoral Neck	4.60 ± 9.02%	-17.60 ± 3.42%
Right Total Hip	-2.80 ± 5.08%	-18.21 ± 2.39%
Right Femoral Neck	-1.10 ± 7.56%	-17.20 ± 4.58%
Left Distal Femur	-3.84 ± 21.99%	-26.68 ± 14.29%

Table 2: BMD changes 12 mo after SCI at various skeletal site

For the knee skeletal sites, with greater variance in measurements observed, the data support Cohen's d of ≥ 1.0, so that with 30/group at one year, there will be 98% power to detect a change as large as is seen in our preliminary data and 80% power to detect a change as small as a 16% BMD difference between groups at BMD at a knee site. We anticipate the CT data to have much smaller variance, given our current experience from published studies, and therefore expect to be able to have adequate power to detect even smaller differences. At year 2, we will have 15 participants/group, and making the conservative assumption that the differences between treatment and placebo groups observed at one year above will not become greater after a second year, and maintaining the maximum variance, we calculate 88% power to detect a change as large or larger than seen at one year at any hip skeletal site between the treatment group (zol/zol) and the placebo group (plac/plac). There will be 80% power to identify differences between groups of 9% or more at year 2. At the knee, there will be 81% power at the end of year 2 to detect a difference as large as that observed after one year (d = 1.0) between the zol/zol and plac/plac groups. Therefore, a total sample size of 60 participants will permit scientifically rigorous outcomes to be achieved, and will also be clinically achievable. We do recognize that there will be some drop-outs. From our previous experience, we feel that this number will be less than 20% (our current study with 60 SCI participants has 3 who have not returned for one year visit with the remainder continuing for a second year). As data from all participants will be utilized in an ITT analysis, the drop-out rate will not directly affect sample size but will lead to lack of a greater degree of uncertainty due to need for imputation and will therefore be kept to a minimum. Even with a 20% drop-out rate, with the sample size of 60, we will have ample power to detect changes considerably smaller than those seen in our preliminary studies. Only 60 subjects will be randomized in this study, however to account for screen failures, we anticipate consenting up to 120 subjects.

15. Modifications to the Protocol

15.1. All modifications to the protocol will be handled as protocol amendments and will be submitted to the Northwestern University IRB for review. Major modifications to the protocol and any modifications that could increase risk to the participants will be submitted to the HRPO prior to implementation. Other modifications and amendments will be submitted to the HRPO at the time of the regular continuing review.

15.2. Protocol Deviations. All deviations from the protocol will be documented by the study coordinator and brought to the attention of the principal investigator. All deviations which could affect the integrity of the study and/or the safety of the participants will be reported to the Northwestern University IRB immediately and if they are UPIRTSOS to the HRPO immediately. Deviations which are minor in nature and do not affect the integrity of the study or safety of the participants (e.g., study visit occurs one week late as participant is on vacation) will be documented but not reported to the IRB. Deviations which result from misinformation due to either the participant providing it incorrectly or incompletely (e.g., concomitant medications) or failure to notice (e.g., laboratory results), will be reported to the principal investigator immediately and, if in his estimation it poses no safety threat to the participant, reported to the Research Monitor during the next DSMC meeting.

16. Reporting of Serious Adverse Events and Unanticipated Problems

The reporting of adverse events, serious adverse events and unanticipated problems will be done according to the guidelines of the Northwestern University IRB, the HRPO, and the FDA.

16.1 Expedited Reporting

- The Principal Investigator must be notified within 24 hours of learning of any serious adverse events, regardless of attribution, occurring during the study.
- The Northwestern University IRB must be notified within 10 business days of "any unanticipated problems involving risk to subjects or others" (UPIRSO).
  NU IRB considers UPIRSOs to include any incident, experience, or outcome that meets all of the following criteria:

  (1) Unexpected (in terms of nature, severity, or frequency) given (a) the research procedures that are described in the protocol-related documents, such as the IRB-approved research protocol and informed consent document; and (b) the characteristics of the subject population being studied;

  (2) Related or possibly related to participation in the research (in this guidance document, possibly related means there is a reasonable possibility that the incident, experience, or outcome may have been caused by the procedures involved in the research); and

  (3) Suggests that the research places subjects or others at a different or greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognized.
- Only unanticipated problems involving risk to subjects or others (UPIRTSOs) will be promptly reported to the HRPO.
- The FDA will be notified within 7 business days of any unexpected fatal or life-threatening adverse event with possible relationship to study drug, and 15 business days of any event that is considered: 1) serious, 2) unexpected, and 3) at least possibly related to study participation.

16.2 Routine Reporting

- All other adverse events- such as those that are expected, or are unlikely or definitely not related to the study participation- are to be reported annually as part of regular data submission.

The Research Monitor is required to review all unanticipated problems involving risk to volunteers or others, associated with the protocol and provide an unbiased written report of the event to the USAMRMC Office of Research Protections (ORP) Human Research Protection Office (HRPO). The Research Monitor will comment on the outcomes of the event or problem and in the case of a serious adverse event or death comment on the relationship to participation in the study. The Research Monitor will also indicate whether he/she concurs with the details of the report provided by the study investigator.

17. Continuing Review and Final Report

A copy of the approved continuing review report and the local IRB approval notification will be submitted to the HRPO as soon as these documents become available. A copy of the approved final study report and local IRB approval notification will be submitted to the HRPO as soon as these documents become available.

1. Edwards WB, Schnitzer TJ, Troy KL. Bone mineral loss at the proximal femur in acute spinal cord injury. Osteoporos Int 2013.
2. Edwards WB, Schnitzer TJ, Troy KL. The mechanical consequence of actual bone loss and simulated bone recovery in acute spinal cord injury. Bone 2014;60:141-7.
3. Ross AC. The 2011 report on dietary reference intakes for calcium and vitamin D. Public Health Nutr 2011;14:938-9.
4. Ross AC, Manson JE, Abrams SA, et al. The 2011 report on dietary reference intakes for calcium and vitamin D from the Institute of Medicine: what clinicians need to know. J Clin Endocrinol Metab 2011;96:53-8.