Official Title: Zoledronic Acid to Prevent Bone Loss After Acute Spinal Cord Injury

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A. Project Summary and Timeline

Maintenance of bone mass following spinal cord injury (SCI) is essential to fracture prevention and the associated morbidity of bed rest and further secondary complications. Intravenous (IV) zoledronic acid (ZA) has been shown to be more effective than other agents in reducing bone mass resorption and fracture of the legs in post-menopausal women, but has not been studied in acute spinal cord injury. Two previous studies of ZA in persons with subacute SCI, while promising, were inconclusive. As stated in the long range plan of the National Institute on Disability and Rehabilitation Research (NIDRR), one goal in the area of health and function is to "focus on the onset of new conditions…exacerbation of existing conditions, or the development of coexisting conditions" [1]. This study is intended to demonstrate reduction in loss of bone mass at the hip and knee regions in acute SCI in a rigorous study of sufficient size to determine effectiveness of our intervention.

Central Hypothesis: Alteration in levels of osteogenic or osteoclastogenic factors secondary to SCI plays a crucial role in bone loss and that this loss can be prevented by a single dose of IV ZA administered within three weeks following injury in individuals with complete SCI. This will be a randomized, double-blind, placebo-controlled study of ZA enrolling 48 subjects over 4 years, randomized to ZA or placebo in a 2:1 ratio.

P**rimary Outcome:** Change in bone mineral density (BMD) assessed by dual energy X-ray absorptiometry (DXA) at baseline, 4 months, and 12 months post-injury.

This will compare BMD at the hip, distal femur and proximal tibia.

Secondary Outcome: Collection of blood biomarkers of bone formation and resorption at selected time intervals within the first 12 months post-injury.

This will observe the timing of metabolic indexes of bone formation and resorption, and of pro-osteoclastogenic factors promoting bone resorption.

Secondary Outcome: Assessment of the safety and tolerability of ZA in the study population.

Study Timeline: The timeline for the study is depicted in **Table 1** below:

Table 1: Study Timetable

	Year 1				Year 2				Year 3				Year 4				Year 5			
Quarter	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
Set up/confirm DSMB	■	■											■				■			
Subject Enrollment		■	■	■	■	■	■	■	■	■	■	■	■	■	■	■				
Follow up studies		■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
Longitudinal follow up						■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
Reports for DSMB			■		■		■		■		■		■		■		■		■	
Statistical Analysis																■	■	■	■	■
Interpretation of Results																■	■	■	■	■
Progress Reports				■				■				■				■				■
Dissemination of results													■	■	■	■	■	■	■	■

B. Specific Aims

Specific Aim 1: To determine the short and long term effects of ZA on BMD values of the hip, distal femur and proximal tibia in patients with acute SCI relative to patients receiving placebo.

Primary hypothesis: Subjects treated with ZA will have at least a 50% smaller percentage decline in BMD values for the hip, distal femur and proximal tibia at 4 and 12 months post-injury than control subjects**.**

Secondary hypothesis 1: Subjects treated with ZA will maintain original BMD values for the hip, distal femur and proximal tibia at 4 months and lose no more than 5% at12 months post-injury than control subjects.

Secondary hypothesis 2 (Exploratory): Incidence of fractures in the hip, distal femur, and proximal tibia will be at least 50% lower in subjects treated with ZA compared to controls. This final hypothesis is exploratory, since we do not expect to have the numbers to achieve statistical significance given the relatively low frequency of sub-lesional fractures in SCI.

Specific Aim 2: To measure and compare selected blood biomarker levels: 1) pro-osteoclastogenic factor IL-1β; 2) bone formation marker P1NP; and 3) bone resorption marker serum C terminal telopeptide of type I collagen (s-CTX) in subjects treated with ZA to control subjects.

Primary hypothesis: Subjects treated with ZA will have lower levels of serum marker of bone resorption s-CTX at 4 and 12 months, relative to controls. *In terms of absolute marker levels*, we hypothesize that the ZA subjects will have s-CTX values within the reference range at 4 and 12 months, in contrast to control subjects whose resorption marker levels will be significantly above the reference range at all of the time points.

Secondary hypothesis: Levels of serum marker of bone formation P1NP will not be lower than control subjects at 4 and 12 months.

Specific Aim 3: To determine the safety and tolerability of early administration of intravenous ZA in persons with acute SCI. We will test the **hypotheses** that compared to controls, subjects treated with ZA will: 1) have an increased incidence of myalgias, fever, gastrointestinal disturbances, and flu-like symptoms over the three days after infusion of study drug; 2) be less likely to participate in physical and occupational therapy during the first week after infusion, 3) be less likely to want the treatment again.

C. Background and Significance of Bone Loss in SCI

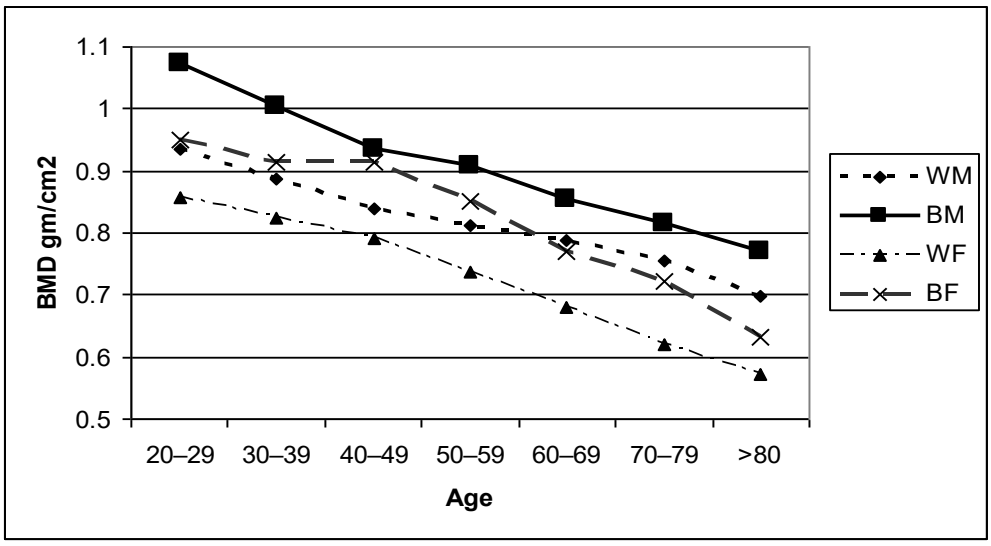
Increased bone turnover and resultant osteoporosis is a prevalent condition following acute traumatic SCI. In the initial weeks following SCI, a process of rapid bone remodeling and bone resorption occurs, with resorption exceeding formation, thereby leading to osteoporosis[2]. Radiographic evidence suggests that an estimated 25% of bone mineral density (BMD) below the level of injury is lost within the first 4 months following acute SCI and progresses to a 33% loss by 16 months post injury[3,4], leaving patients at or near the fracture threshold[3]. Additional investigations extending the time from injury to 2 years estimate BMD reduction of 30-40% at the femoral neck , 37-43% at the distal femur[5], and 50-60% at the proximal tibia[6], with the majority of this loss occurring during the first 12 months. Given that bone loss begins shortly after injury with the bulk of loss in BMD occurring within 4-6 months, aggressive steps to prevent bone loss should be undertaken shortly after injury.

Individuals with SCI specifically develop sub-lesional osteoporosis**,** namely bone loss below the level of paralysis[5]. The regional BMD in the area below the injury, rather than the overall BMD, is among the strongest predictors of future “fragility fractures” or ones that occur under conditions of minimal or low impact in the absence of trauma[7,8]. The most significant risk factor for osteoporosis after SCI is the completeness of injury[9]. This association is particularly strong for regions at and below the knee. A higher

incidence of sub-lesional osteoporosis is also observed with increasing number of years post injury[10,11]; with onset of SCI prior to age 16 when peak bone mass has not yet been achieved[5], with increasing age[9,12], and with female gender[13].
In the patient with SCI, the primary goal of osteoporosis is fracture prevention. Although the annual incidence of lower extremity bone fractures has been reported as 2-6% [3,11,14] the above figures may be underestimated[5,14,15] since many subjects present to local hospitals rather than major spinal cord injury centers for evaluation and treatment[3]. Among those with chronic SCI, 25-49% ultimately experience a fragility fracture[14,16]. The majority of fragility fractures occur in the hip, distal femur and proximal tibia[5,7,14]. Fractures may arise from routine activities such as transfers or low-velocity falls from a wheelchair or commode[3,7,17]. Maintenance of bone mass is essential to fracture prevention given the morbidity that can result: hospitalization, increased spasticity, autonomic dysreflexia, heterotopic ossification, and in select cases, surgical intervention[8]. In those fractures treated conservatively, pressure ulcers may arise from bracing or splints, temporary casts, or bed rest[7]. The consequences invariably lead to reduced level of independence, increased nursing or attendant care, and greater cost to the patient and health care system. With the combined effects of an increased life expectancy after SCI and a greater number of new cases among persons above age 55, who may have already developed early osteoporosis, the cost physically and emotionally to the patient and financially to the health care system will rise in coming years[7,18].

Pathophysiology of Osteoporosis:
Osteoporosis or "porous bone" is a condition of low bone mass and microarchitectural deterioration of bone tissue that together result in increased bone fragility and susceptibility to fracture[19]. Bone is reduced in quantity per area studied but mineralized normally. Bone is comprised of two types of living bone cells, the osteoclasts that remove weakened sections of bone and the osteoblasts that initially build and continually replace bad bone with new bone throughout the life span. This process leads to thicker and stronger bones until approximately the age of 25; after that bone is essentially maintained or slowly lost until age 50 when effects of aging and hormone changes occur[20]. Bone loss can accelerate rapidly in certain conditions of high bone turnover such as menopause when estrogen levels fall sharply or medical conditions resulting in sudden immobility such as SCI[21]. The typical pattern of bone loss in an able-bodied individual beginning around age 25 is illustrated in figure 1 below.

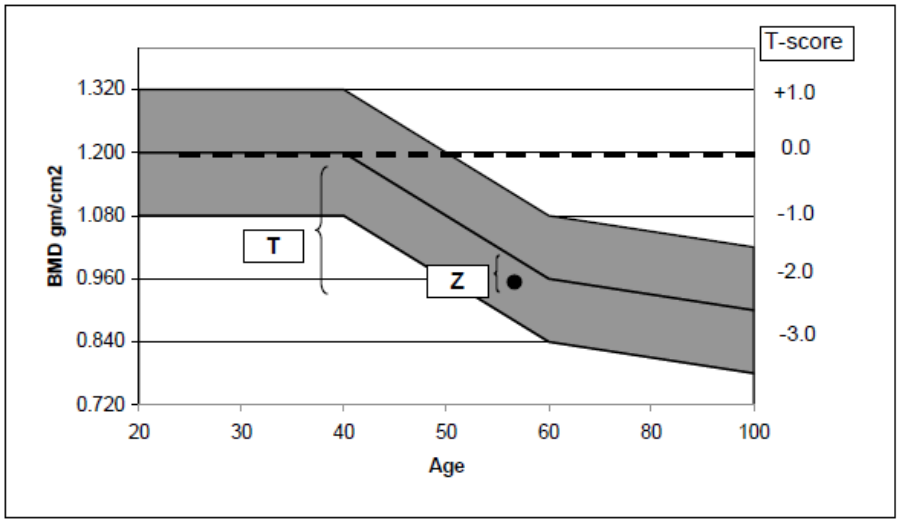
Figure 1: Mean BMD at the femoral neck by age in the US, data from Looker AC[22]



WM = white males; WF = white females; BM = black males; BF = black females

Osteoporosis is traditionally diagnosed with BMD measurements measured by DXA which uses bone mineral content in grams by summing pixels in a given region viewed by the scanner and dividing that number by the bone area examined in cm^2. A patient's results can be interpreted as a standard deviation from the mean of sex-matched peak bone mass (T score).[23] The World Health Organization has defined osteoporosis as a T score at or below 2.5 standard deviations from the mean BMD of a young normal adult of the same gender. It is designed for those who have already reached peak bone mass[24] . Another measure known as the Z score is defined in standard deviations from the mean of age and sex matched bone mass. The Z score is often used for patients below age 25 in which peak bone mass has not been achieved, but is a useful measure for all pre-menopausal women and men < 50 years (International Society for Clinical Densitometry Official Positions. www.iscd.org. Updated 2007). A given patient is assigned a T or Z score for a given location based on established norms illustrated in **Figure 2**.

Figure 2: From ISCD bone densitometry Clinician Course, Lecture 5(2008) accessed at National Osteoporosis Foundation website, www.nof.org.



The Z score is an additional means of identifying high risk patients that may not be osteoporotic but are below expected bone density for age and thus, should be followed more closely[21]. While there is no specific T score that correlates with fracture threshold, the more negative the T score, the greater the risk. In able-bodied post-menopausal females, several prospective studies indicate that half of patients with incident fractures had baseline BMD assessed by DXA above the diagnostic threshold of osteoporosis[25-27]. Our study will calculate both T and Z scores for initial and follow up testing so that results can be compared to other studies that use these values rather than absolute BMD.

Causes of Osteoporosis

In the general population, development of osteoporosis is due to factors such as age, diet, lifestyle (with or without weight-bearing exercise, gender, and ethnicity[21]. However, certain medications, including ones given for secondary conditions related to SCI, also contribute to osteoporosis. Glucocorticoids decrease bone formation by downregulating osteoblasts and contribute to further bone loss by prolonging the life span of osteoclasts[28]. Thiazolidinediones given to persons with diabetes mellitus can lead to osteoporosis by direct action on bone cell differentiation. The "A Diabetes Outcome Progression Trial" (ADOPT) found that patients randomized to rosiglitazone have a higher risk of fractures than those receiving metformin or glyburide[29,30]. Unfractionated heparin given for > 1 year has been associated with decreased bone formation and increased resorption, but the effects are notably less with low molecular weight heparin[31]. Proton pump inhibitors moderately inhibit calcium resorption essential to bone formation,

while H2 blockers have a smaller adverse effect in some studies and a neutral effect in other reports[28]. In addition, long term use of carbamazepine, phenobarbital, phenytoin and valproic acid have been associated with decreased BMD due to elevated vitamin D catabolism; elevated parathyroid hormone or increased osteoclastic activity[32,33].

Inadequate stores of serum 25-hydroxy vitamin D contribute to low BMD and increased risk of fractures[34]. Low levels of serum 25-hydroxy vitamin D occur in substantial numbers of persons with acute and chronic SCI[35,36]. The degree of vitamin D deficiency is also important in development of osteoporosis due to upregulation of osteoclast cells in proportion to the degree of low vitamin D. Work done by this group[35] has demonstrated that only 35% of acute SCI patients injured in summer months and 16% in winter months actually have therapeutic levels of vitamin D (>32 ng/ml). The remainder required supplementation to achieve therapeutic levels. Findings from this study are summarized in **Table 2** below:

Table 2: Seasonal Vitamin D levels in Acutely Injured SCI Subjects

Serum 25-OH vitamin D (ng/ml)	Summer (% total/season)	Winter (% total/season)
Therapeutic (≥ 32 ng/ml)	34.5	15.4
Sub-therapeutic (20-31.99)	31.0	30.8
Insufficient (13-19.99)	27.6	23.0
Deficient (0-12.99)	6.9	30.8

Historical patterns of bone loss following SCI:

Garland[3] compared complete, subacute SCI subjects (average 4 months from SCI, with repeat assessment at 16 months) to complete chronic subjects 5 years from injury. Results demonstrated 24% lower BMD of the distal femur of SCI subjects 4 months after injury and 34% lower BMD 16 months after SCI, relative to controls. Mean BMD in the proximal tibia also declined between the 4 month and 16 month evaluations. A follow up assessment of the subjects 5 years post-injury showed lower BMD than at 16 months, but differences were not statistically significant. Roberts illustrated the sudden loss of BMD in the immediate post-injury period[37] in his longitudinal study where 11 of 30 subjects obtained DXA scans at weeks 8 and 24 post-injury. These subjects demonstrated a decline in the total lower limb but not in hip BMD between scans. A longitudinal study[38] examining BMD at 6 time points during the first year of SCI found impressive losses averaging 4% per month for high turnover areas of trabecular bone and 2%/month for areas of cortical bone. This finding of greater losses in trabecular relative to cortical bone is consistent with several previous investigations[10,39], particularly in the first 4 months following SCI due to its faster turnover rate. The authors found that subjects with motor complete paraplegia lost 40-45% of BMD in the pelvis and 25% in the total leg between 5 and 50 weeks post injury. Moreover, among subjects with partial motor recovery, 30% of pelvic bone mineral and 10% of total leg bone mineral content was lost between 5 and 50 weeks. In a study of chronic SCI subjects, Biering-Sorenson[6] observed loss in bone mineral content in the proximal tibia of 40-50% of control subjects and in the femoral neck 30-40% of controls by 2 years post injury. Although some evidence suggests a steady state is eventually reached with a lower mineral density[6,40], others have found a continued, albeit slower decline with years post injury[41.]

Bone markers:

In the early weeks following SCI, the rate of bone resorption greatly exceeds that of bone formation due primarily to upregulation of the osteoclast[42]. Hypercalcemia and hypercalciuria, observed in the weeks following paralysis, arise in parallel with elevation of resorption indices such as urine and serum N-telopeptide, hydroxyproline, and urine pyridinoline[42-44] leading to overall bone loss. Trabecular bone is

more commonly affected than cortical bone in the first 4 months following SCI due to its faster turnover rate[10,39]. Roberts[37] observed a rapid rise in urinary values of total and free deoxypyridinoline, total pyridinoline, and NTX in the immediate period following injury. Elevations in bone resorption markers began at one week post injury, peaked at 16-20 weeks post injury, and then gradually declined. At their highest value, some markers measured 10 times the upper limit of normal. Following acute SCI, markers of bone formation range from mildly depressed to slightly elevated during the first 6 months, depending on the study cited[6,37,42]. Even if markers of formation are slightly increased, this compensatory process is not sufficient to counteract the significant bone resorption during this same interval. Since acute SCI involves significant edema and inflammation, animal models examining the appearance of pro-inflammatory mediators in acute SCI have been developed. Interleukin-1β mediates osteoclastogenesis by acting upon osteoclast precursors[45]. Yang[46] has observed upregulation of IL-1β six hours after producing an SCI in a rat. By 24 hours post trauma, the elevations were no longer detectable in that animal species. The timing of comparable elevations of these factors in humans with SCI has not been precisely determined but appears to persist longer than in rodents. Based on the work of Davies[47], interleukin-1β may be one of the earlier factors elevated after SCI 36 but the duration of its presence is unclear. Other interleukins, including IL-6 and TNF-α may be chronically elevated in SCI patients with secondary conditions including pain and pressure ulcers, but IL-1β levels are not detectable chronically in these patients[47]. For these reasons, our study will examine levels of Il-1β only to 1 month post injury.

Non-pharmacologic Interventions for bone loss after SCI

A number of non-pharmacologic interventions have been explored to prevent and treat bone loss secondary to SCI. Effects of gravity, mechanical and electrical stimulation, and other forms of exercise or positioning have been undertaken in an effort to improve BMD. The majority of treatments to enhance bone density have been initiated after the patient leaves acute care-some during inpatient rehabilitation, others during outpatient treatment. Kunkel[48] reported a case series on the effect of a standing frame in 6 SCI patients, all of whom were non-ambulatory and averaged 19 years post injury. After 5 months of 90 minutes daily standing, measurements in the lumbar spine or proximal femur showed no change. Warden[49] examined how pulsed therapeutic ultrasound for 6 weeks, 20 minutes daily, was administered in a double blind manner to two groups of SCI patients. While similar interventions have been successful in post-menopausal osteoporosis, the two groups of SCI patients experienced no benefit. Garland[50] examined use of pulsed electromagnetic field in persons with chronic SCI, with one knee receiving treatment and each subject's contralateral knee serving as a control. After 3 months, DXA results in the lower extremity demonstrated a 5% higher value in the treated leg, yet no difference was found in DXA scores after 6 months and 12 months.

Results using functional electrical stimulation (FES) have been mixed. Needham-Shropshire found no improvement in a study of complete paraplegics after 3 months therapy with a microprocessor-controlled FES unit known as a "Parastep"[51]. Leeds and colleagues also found no BMD change using an FES bicycle ergometry in seven tetraplegic patients 2-7 years after original injury[52]. However, a similar study of FES-induced lower extremity exercise found BMD reductions that were smaller than expected and less than control subjects[53]. Belanger et al. had greater benefits in their use of FES, with 30% recovery seen in BMD of the distal femur and proximal tibia relative to pre-treatment values[54]. But, final BMD values remained significantly lower than in able-bodied controls.

Early investigations of oral and IV bisphosphonates

Several groups have explored the use of various bisphosphonates for prevention of bone loss in acute and chronic SCI. Two studies examined the use of oral alendronate plus calcium. Zehnder[55] studied alendronate plus calcium in 55 motor complete males (AIS A or B) with chronic SCI over a 24 month period and compared them to control subjects who received only calcium supplementation. Vitamin D 400 IU was given only to those with severe deficiency of serum 25-OH vitamin D <6 ng/ml. The mean

time from injury was 9.8 years, when significant amounts of BMD had already been lost. The group that received oral alendronate 10 mg daily plus 500 mg daily calcium was able to maintain their lower pre-treatment level of BMD at the total hip, tibial diaphysis and tibial epiphysis, but BMD continued to decline steadily in the calcium only group serving as a control. The baseline z scores were already in the osteopenic range for the hip (-1.83) and tibial diaphysis (-1.75) and in the osteoporotic range (-3.39) and the tibial epiphysis. In order to prevent the baseline osteoporosis seen in this study, an earlier intervention would be needed.

A double blind, placebo controlled study by Gilchrist[56] examined 31 subjects with neurological levels of injury C4-L2 and AIS grades A-D, 10 days from injury. All were between ages 18-55. Subjects were divided equally into an experimental group given alendronate 70 mg weekly for 12 months and a control group given placebo. Subjects were not given supplemental calcium. Oral vitamin D of unlisted amount was given only to those in the deficient range (<12.5 ng/ml), rather than to all subjects for prevention. Alendronate-treated subjects had a decline in BMD of 5.6% in the total leg and 3.3% in the total hip, but this degree of bone loss was notably less than the decline observed in the placebo group, who lost 12.7% in the total leg and 20.7% in the hip. However, many of these subjects were incomplete injuries, and several patients had regained ambulation, possibly reducing the amount of bone loss in both groups.

Challenges exist to giving oral alendronate in the setting immediately following SCI. Limitations to upright posture include post-operative spinal stability precautions, delay in brace fitting needed to maintain stability in sitting position, autonomic effects of orthostasis, and pain. Erosive esophagitis may occur following consumption of oral bisphosphonates, unless patients are able to sit fully upright after administration of medications. In the previously described study by Gilchrist[56], authors specifically reported no difficulties encountered with GI symptoms. This may have been due to the number of subjects with AIS Grades B-D or with thoracolumbar neurological levels of injury. Severe orthostatic hypotension is less common in these groups, permitting earlier functional mobility and facilitating upright positioning sooner after injury. Because IV formulations can be administered in the supine position, investigators have explored the use of IV bisphosphonates in the setting of acute SCI. An early investigation by Nance[57] examined the use of IV pamidronate, a relatively weak bisphosphonate, within 6 weeks of injury in 24 subjects with traumatic SCI. This non-randomized trial divided the sample into 14 subjects receiving 30 mg of IV pamidronate monthly for 6 consecutive months and 10 controls with comparable degrees of SCI given standard rehabilitation therapy but no drug. Interpretation of results was limited by the heterogeneity of subjects: some had sensory and motor complete injuries (AIS A), while others were AIS D, a subset of whom were ambulatory. Outcomes at 12 months evaluated BMD at the hip, distal femur and proximal tibia by percent change from baseline. Only the pamidronate-treated AIS D subjects demonstrated an increase in BMD and this rise was observed only in the distal femur. The proximal tibia and hip were largely unchanged from subjects' baseline BMD, but control subjects demonstrated BMD losses of .5%, 13.4% and 10.5% respectively. In this study, control subjects with AIS grade A injury lost 14.6% of original BMD, while pamidronate-treated subjects lost only 6.5% of their original BMD. Neither group was able to maintain their pre-injury bone density. A second investigation by Bauman[58] examined the effect of IV pamidronate on sublesional osteoporosis in 11 subjects with acute SCI 22-65 days post injury. Although early mild reduction in the degree of BMD loss was observed, by the end of 12 months both placebo and pamidronate-treated subjects lost statistically similar amounts of BMD below the level of injury: 18 vs. 15% in the pelvis; 28 vs. 17% in the distal femur, and 42 vs. 36% in the proximal tibia.

<u>Zoledronic Acid: Initial trial data and clinical pharmacology</u>

Zoledronic acid demonstrates superior pharmacodynamic properties for suppression of the primary bone resorbing cell, the osteoclast, in comparison to actions of earlier generation oral and IV bisphosphonates[59]. Zoledronic acid has a particularly high affinity for binding to the calcium phosphate bone mineral, hydroxyapatite. Binding localizes to regions of high bone turnover, specifically trabecular

bone. Within the osteoclast, zoledronic acid inhibits a key regulatory enzyme known as farnesyl diphosphate synthetase which is 17 times more effectively than alendronate and 67 times more than pamidronate[59,60]. Zoledronic acid in the 5 mg dose is now an option for treatment of post-menopausal osteoporosis in patients with advanced disease who may have failed less aggressive therapies, including treatment with other bisphosphonate drugs. This medication is administered IV once annually and has the advantage of single time administration.

Side Effects of Zoledronic Acid

Due to the potency of IV ZA, adverse effects of the agent may occur. The most common side effects of ZA are an acute phase reaction involving fever (15%), myalgias (8%), flu-like illness (7%), headache (6%) and arthralgias (5%)[61,62]. In rare cases, patients may experience noncardiac chest pain (1/3%). Because infusion of IV ZA can cause an increase in serum creatinine up to 30 days post treatment, the drug should be avoided in persons with creatinine clearance <35 mL/min. An association between atrial fibrillation and IV ZA use was observed in a large study of over 7000 patients[62]. The FDA subsequently conducted an investigation, comparing results of the prior trial to other studies of IV ZA, and concluded that no clear relationship could be identified between bisphosphonate use and atrial fibrillation[63]. Both oral and IV bisphosphonates have been associated with osteonecrosis of the jaw (ONJ), but further review of the data by the American Dental Association found that incidence of ONJ is rare in post-menopausal women with osteoporosis but more common in those with monthly infusions for cancer treatment[64]. This group recommended no special treatment beyond routine dental care for those using IV ZA for osteoporosis. Finally, several reports of low trauma fractures of the femur have been observed among patients primarily over age 60 who are prescribed chronic bisphosphonate treatment (2.5-8 years)[61,65]. A recent cohort study of over 1.1 million patients by Dell at el. found that while duration of use of bisphosphonates was correlated with incidence of atypical femur fractures, (particularly beyond 5 years of use), no correlation with age and duration of use was found.[66] Because our study will involve only a single dose of ZA, we do not anticipate observing the above effect from the drug alone. Based on these new findings, we also believe that individuals ages 61-65 should not be excluded by age alone.

Previous Clinical Trials of Zoledronic Acid

Zoledronic acid has been used in several studies of able-bodied individuals, most commonly in post-menopausal females. The HORIZON Pivotal Fracture Trial[62] examined 7765 subjects with established clinical osteoporosis over 3 years. Findings demonstrated 70% and 41% reductions in vertebral and hip fractures respectively. Reduction of markers of bone resorption and bone formation were also observed, with levels reduced from baseline by 59% for β-CTX, 58% for PINP, and 30% for bone specific alkaline phosphatase (ALP) at 12 months without progressive decline in subsequent years. A direct comparison of a single 5 mg IV ZA dose and 70 mg weekly oral alendronate in post-menopausal females[67] found more striking and rapid declines in markers of bone resorption at weeks 1, 12, and 24 of the study in the ZA group as well as greater reduction in bone formation markers at 12 weeks. The nadir in all bone resorption markers was reached at 1 week for the ZA group but took at least 12 weeks to be achieved in the alendronic acid group, demonstrating a more rapid impact of ZA in suppressing bone resorption.

Two small investigations have explored the use of ZA in subacute SCI. In a double-blind placebo-controlled trial, Shapiro et al[68]. examined the effect of IV ZA in 17 patients with AIS grade A or B SCI given at 10-12 weeks post injury. Four subjects received 4 mg IV ZA, four received 5 mg ZA, and the remaining nine received placebo. Both the intertrochanteric region and the cortical shaft in the ZA treated group maintained BMD near baseline values up to 12 months, while BMD declined in the control group. In contrast, the femoral neck had shown an increased BMD from baseline at 6 months, but fell back to baseline by 12 months in the ZA group. At 12 months, the BMD at the femoral neck did not differ significantly between groups. Bubbear[69] completed a randomized, open-label study of 14 patients with acute SCI, all of whom received either 4 mg IV ZA or standard medical treatment within 3 months (mean of 58 days of injury). Neurological levels of participants ranged from C4 to L3 and included 11 complete

(5 experimental, 6 control) and 3 incomplete subjects (2 experimental, 1 control). Differences between ZA-treated subjects and controls were 3% for the lumbar spine ($p<.05$); 12% for the total hip ($p<.05$), 5% for the femoral neck (NS), and 11% ($p<.05$) at the greater trochanter 12 months after study drug administration. In the ZA group, BMD at the total hip and greater trochanter were maintained from baseline, but the area of the femoral neck still lost 10% of the original BMD. In both of the above studies, pharmacologic intervention did not occur until 2-3 months post injury, when a notable amount of bone mass is already lost. Furthermore, neither study evaluated BMD at the distal femur and proximal tibia, two high risk areas for sublesional fractures after SCI.

Areal Bone Mineral Density

Since marked sub-lesional bone loss after SCI is found consistently[5,6], our study will provide new information on the effectiveness of ZA in preventing bone loss after SCI because it differs from prior studies in the following important aspects:

1. We will administer ZA within 21 days of SCI, when bone resorption is just beginning. This is sooner than the prior studies on ZA in SCI.
2. We will limit enrollment to patients with complete injuries, minimizing potential confounding between ambulatory and non-ambulatory subjects.
3. We will monitor calcium and vitamin D levels periodically throughout the study, with aggressive supplementation of all patients noted to be low, **and** we will supply supplemental calcium and vitamin D to all subjects to prevent anticipated losses in the initial year of injury[35].
4. We will evaluate the effect of ZA on BMD at the hip, the distal femur, and proximal tibia.

D. Study Design

This will be a prospective, double-blind, randomized, placebo-controlled trial with a 2:1 ratio of ZA to placebo in 48 persons with complete traumatic SCI.

Study Location and SCI Population

This study will be conducted at the Regional Spinal Cord Injury Center of the Delaware Valley (RSCICDV), located at Thomas Jefferson University and Hospital (TJU/H) in Philadelphia, PA. TJUH, in affiliation with the Magee Rehabilitation Hospital, is designated as one of the United States' 16 Model Spinal Cord Injury Centers, by the National Institute on Disability and Rehabilitation Research, Department of Education's Office of Special Education and Rehabilitative Services. It is the only such facility in the Delaware Valley. The RSCIC has treated more than 3,000 persons with spinal cord injury and provides for multidisciplinary coordination of emergency and acute medical/surgical care, rehabilitation at the onset of acute care, with vocational evaluation and training, and lifetime follow-up care for persons with SCI. Over 50 percent of the SCI patient population is admitted to the RSCIC within three days of injury and the RSCIC has demonstrated a mortality rate of 5 percent. Over the past 5 years the RSCIC has annually admitted an average of 28-30 patients with complete SCI within 7 days of injury.

D.1. Subjects

Forty-eight subjects evaluated to have ASIA impairment scale Grade A injuries (motor and sensory complete) will be identified, screened, and approached to participate in the study. Persons who have recently experienced traumatic complete SCI and are within 21 days of injury are eligible to participate. The sample size calculation allows for reduced enrollment rates and drop outs. The ASIA impairment scale is detailed in **Table 3** below.

Table 3: ASIA Impairment Scale (ISNCSCI, 2011)

SCI Injury Grade	Description
A = Complete	No motor or sensory function is preserved in the sacral segments S4-S5.
B = Incomplete	Sensory but not motor function is preserved below the neurological level and includes the sacral segments S4-S5.
C = Incomplete	Motor function is preserved below the neurological level, and more than half of key muscles below the neurological level have a muscle grade less than 3.
D = Incomplete:	Motor function is preserved below the neurological level, and at least half of key muscles below the neurological level have a muscle grade of 3 or more.
E = Normal	Motor and sensory functions are normal.

D.2. Inclusion/Exclusion Criteria

Inclusion criteria:

- Ages 18- 65, male or female
- Traumatic SCI with Neurological level C4-T10, AIS A, Serum calcium level >7.0 mg/dL) at time of study drug administration
- Screening baseline serum 25OH vitamin D of at least 13 ng/ml
- No medical contraindication to supplemental vitamin D for participants whose levels are >13 ng/ml but sub-therapeutic (<32ng/ml)
- No medical contraindication to supplemental calcium
- Weight under 300 pounds, which is the maximum permitted on the DXA scanner

Exclusion criteria:

- Ventilator dependent individuals who are not in weaning phase and are unable to provide consent through alternative communication
- Chronic steroid use (defined as >6 months)
- Rheumatoid disease with use of prior disease modifying anti-rheumatic drugs (DMARDs) affecting bone density
- History of osteoporosis or of treatment for osteopenia or osteoporosis with bisphosphonates, or selective reuptake estrogen modifying agents
- Current use of medications* including bisphosphonates to treat osteoporosis (*note that prior calcium or vitamin D use is not an exclusion criteria)
- History of more than one lower extremity osteoporosis-related fracture
- Chronic renal insufficiency, creatinine clearance < 35 ml/min, during screening
- End stage liver or kidney disease
- Medical conditions resulting in hypogonadal states that affect bone density
- Uncontrolled thyroid disease/thyrotoxicosis
- Hereditary or acquired metabolic bone disorder
- History of use of unfractionated heparin for >1 year
- History of selected antiseizure medications, specifically phenobarbital, phenytoin, carbamazepine, sodium valproate >1 year
- Acute or chronic bilateral lower extremity fractures involving tibia or femur, with placement of surgical hardware in any areas of above locations
- Severe hypotension requiring use of IV blood pressure agents such as dopamine, norepinephrine, or phenylephrine. Exception may allow for patients on pressors who are experiencing hypotension as they acclimate to upright posture
- Inability to provide informed consent and understand the consent process
- Facial fractures requiring oral surgery

- Dental surgery or oral maxillofacial surgery within 2 weeks of anticipated study drug administration
- Pregnancy present on admission
- Vitamin D deficiency on admission testing (serum 25-OH D reported as < 13 ng/mL)
- Patients with an established reaction to, or history of, anaphylactic shock to aspirin

D.3. Methods

1. Sample Size Calculations

The sample size calculation is based on the primary outcome of BMD loss at the proximal tibia at 12 months. A set target of at least 50% reduction in percent BMD loss between treatment and control groups is projected. Expected BMD loss is taken from the placebo group of Bauman[58].

Subjects in this study lost 8% +/- 3% BMD at 3 months and **29% +/- 13%** at 12 months in the proximal tibia. The effect size (difference in mean percent reduction divided by standard deviation) for a 50% reduction in BMD loss is 1.3 at 3 months and 1.1 at 12 months. Assuming 2:1 randomization, two-sample t-test, 80% power and two-sided type-I error rate of 5%, we would need a total sample size of 24 for an effect size of 1.3, 33 for an effect size of 1.1, and 39 for an effect size of 1.0. We plan to enroll 48 subjects to allow for potential attrition. With a dropout rate of 18% we would reach a final sample of 39; a dropout rate of 30% would result in a final sample of 33. The enrollment of 10 subjects/year (total = 40) would still allow an 18% drop out to achieve a final sample of 33.

2. Randomization Method

The randomization schedule will be generated by the TJU Division of Biostatistics. Randomization will use the method of random permuted blocks. The block size will not be disclosed to the investigators. The TJU Division of Biostatistics will prepare a randomization scheme document to be kept by the TJUH Investigational Drug Service (IDS). Once a subject is confirmed to be eligible and the informed consent is completed, the IDS will be alerted to assign a randomization and prepare for the subsequent medication preparation and delivery. A completed randomization assignment log will be returned to Biostatistics so they can monitor randomization and accrual.

3. Management of Study Drug Infusion

The TJUH Investigational Drug Service is a well-established service within the Pharmacy Department that participates in numerous inpatient and outpatient research protocols. The IDS reviews all inpatient investigational drug protocols and assists with patient enrollment (randomization), staff education and maintenance of study records, including protocol budgets. The IDS maintains all investigational drug records in accordance with FDA regulations as well as with the policies and procedures of the Institutional Review Board. The IDS provides the procurement, storage, dispensing and maintenance of records for all investigational drugs.

4. Study Activities and Assessments

A. Screening: Post injury days 0-17

- Subjects admitted to the RSCIC with suspected complete SCI will be approached for participation shortly after admission. After providing informed consent, subjects will be screened between 0 and 17 days post injury. The screening will review of the medical record medical/surgical history and physical/neurological examination performed according to ISNCSCI guidelines to ensure an AIS Grade A SCI is present, or post-menopausal) and social or lifestyle factors that could alter ability to participate.
- A serum pregnancy test (for females of child bearing age).
- Standard of Care laboratory tests of kidney (TJUH Basic Metabolic Panel) and liver function (TJUH Hepatic Panel) will be reviewed to ensure the patient does not have a history of chronic liver or kidney failure, unknown to family or subject or not revealed on subject history interview.

- Serum calcium measurement. If initial serum calcium is <7.0 mg/dl, the subject will be given correctional calcium doses in accordance with standard hospital treatment protocol for subjects with critically low calcium levels. The subject may still be able to participate if the calcium level is corrected by the day prior to study drug infusion.
- Serum Vitamin D measurement. Subjects with initial vitamin D levels in the deficient range (<13ng/ml) will not be eligible for participation. If subject's 25-OH vitamin D is between 13 and 20, the subject will be supplemented with high potency oral ergocalciferol 50,000 International Units (IU) per week until retesting occurs at 4 weeks. At the time the screening value is returned, subjects with 25-OH vitamin D levels between 20 and 32 will be started on 2000 IU vitamin D daily and those will levels 32 and higher will be initiated on 1000 IU daily. Supplemental oral calcium of 1200 mg daily will be started on the same day as the vitamin D, except in cases of low values as described above.
- Intact Parathyroid Hormone (iPTH) measurement.

B. Baseline Assessments: Post-injury days 8-21

A Baseline Assessment will be completed between 8 and 21 days post-injury, and include the following:

- Completion of the World Health Organization (WHO) Fracture Risk Assessment Tool (FRAX)[73]. The FRAX asks for information about age, date of birth, sex, weight, height, previous fracture history, parental fracture history, smoking, steroid use, history of rheumatoid arthritis, presence of a medical condition related to osteoporosis, alcohol use, and current hip bone mineral density (see data collection forms in Appendix B). All data are then entered into the FRAX website (available through the WHO) to obtain the 10 year probability of a fracture. No personal identifiers are used and no data is saved on the website.
- Collection of blood sample for pre-treatment levels of PINP as measures of bone formation, s-CTX as marker of bone resorption, IL-1β as a pro-osteoclastogenic factor, CPK (Creatinine Phosphokinase) as a marker of muscle tissue and kidney function, and serum calcium. DXA scan, to be obtained as soon as the subject is medically stable for testing off the intensive/acute care floor (may be done up to 7 days after the infusion of study drug).

C. Study drug infusion: Post injury day 21

Within day 21 post injury, subjects will be administered study drug and assessed as follows:

- Measurement of serum calcium level to ensure therapeutic levels.
- A single IV infusion of ZA 5 mg or a placebo drug with the same dilution and duration of administration (prepared and dispensed by the TJUH IDS).
- The single IV infusion will be administered over a 2 hour period (2.5 mg per hour)
- Supplemental hydration as needed to keep urine output > 2000 cc per day from the day prior to study drug administration until 3 days post-infusion.
- Acetaminophen 650 mg every 4 hours for the 24 hour period following drug administration. This is intended to reduce the frequency and severity of fever or myalgias associated with ZA administration[61].

D. Days 1-7, Post Drug Infusion

- After the first day, acetaminophen will continue to be offered as needed every 4 hours through day 3 post-infusion.
- Supplemental hydration will be continued as needed to keep urine output > 2000 cc per day until 3 days post-infusion
- Subjects will be assessed daily for 3 days, then weekly for one month after study drug administration for known side effects of the drugs, including myalgias, fever, gastrointestinal disturbances, and flu-like symptoms.

- Subjects will be assessed for participation in physical therapy and occupational therapy since receiving the study drug, as well as willingness to get study drug again via interview held 7 days following infusion.

E. Days 1, 3, 7, 14, Post-Drug Infusion

- Subjects will be assessed weekly for one month after study drug administration for known side effects of the drugs, including myalgias, fever, gastrointestinal disturbances, and flu-like symptoms.
- Laboratory values will be drawn for serum calcium, phosphate, and magnesium.

F. Month 1 Post Injury (+/-4 days)

- Subjects will be assessed weekly for one month after study drug administration for known side effects of the drugs, including myalgias, fever, gastrointestinal disturbances, and flu-like symptoms.
- Blood samples* for laboratory values will be drawn for s-CTX, IL-1β, 25-OH vitamin D, serum calcium, intact parathyroid hormone, and Serum P1NP. *The total amount of blood drawn for all laboratory tests will be < 25 ml at each data collection session.

G. Month 2, 6 & 10 Post Injury

A patient questionnaire will be administered either in-person or by telephone. The questions cover:

- Any evidence or knowledge of bone fractures since the previous contact.
- Information about exercises and standing, including use of functional electrical stimulation (FES), such as how often and average daily duration.
- Supply of vitamin D and calcium tablets with reminder to bring pill bottles with them to clinic for follow up assessments and study sponsored refill of medications. At these follow up visits, pill counts will be done to assess their compliance with medication use.

H. Months 4 & 12 Post Injury

- Blood sample will be drawn for s-CTX, 25-OH vitamin D, serum calcium, intact parathyroid hormone. Serum P1NP will also be drawn at 4 months. The total amount of blood drawn for all laboratory tests will be < 25 ml at each data collection session.
- Complete neurological exam (at 12 months only)
- A patient questionnaire will be administered.
- DXA scan

I. Month 8 Post Injury

- Blood sample will be drawn for 25-OH vitamin D and serum calcium. The total amount of blood drawn for all laboratory tests will be < 25 ml at each data collection session.

J. Serum 25-OH Vitamin D Monitoring and Supplements

Since serum 25OH vitamin D represents a storage level of vitamin D accumulated over the preceding months, subjects with therapeutic values immediately after injury may have normal vitamin D stores which decline in subsequent months. For this reason, vitamin D levels in all subjects will be monitored not only at baseline but also at months 1, 4, 8 and 12. Subjects will be supplemented with 50,000 IU oral ergocalciferol weekly, if insufficient (serum level 13-20 ng/ml); 2000 IU cholecalciferol or ergocalciferol daily, if sub-therapeutic (serum vitamin D level 21-31ng/ml). Those subjects in the therapeutic range for serum 25-OH vitamin D will be given a maintenance dose of 1000 IU oral vitamin D daily.

Table 4: Study Events and Visit Schedule

	Screening (Day 0 -17)	Baseline (Day 8-21)	Study Drug and Acetaminophen Administration (within 21 days of injury)	1 month + / - 4 days	4 months + / - 7 days	8 months + / - 14 days	1 year + / - 14 days
Medical History **Standard Care**	X						
Physical Exam **Standard Care**	X						
Basic Metabolic and Hepatic Panel) **Standard Care**	X						
FRAX Score		X					
Study Drug Infusion			X				
Acetaminophen Administration			X [a] X [b]				
Serum calcium, magnesium, phos **Standard Care**	X	X		X	X	X	X
Serum 25OH-D **Standard Care**	X			X	X	X	X
Serum iPTH [d] **Study Specific**	X			X	X		X
P1NP [e] **Study Specific**		X		X	X		
IL-1 beta [f] **Study Specific**		X		X			
Serum CTX [g] **Study Specific**		X		X	X		X
CPK [h] **Study Specific**		X					
DXA** **Study Specific**		X [c]			X		X

a) Administered every 4 hours for the first 24 hours following study drug infusion
b) Administered every 4 hours PRN through day 3 following study drug infusion
c) To be obtained as soon as medically stable to undergo DXA testing

d) iPTH Blood Test Intact parathyroid hormone
e) P1NP Blood Test N-terminal propeptide of Type I collagen
f) IL-1 beta Blood Test Interleukin 1 beta
g) S-CTX Blood Test Serum carboxy-terminal telopeptide of type I collagen
h) CPK Blood Test Creatinine Phosphokinase

Table 5 Schedule of follow up contacts for **Years 2 through 4** of the study.

	Year 1 Every 2 Months	**Year 2 Month 6**	**Year 2 Month 12**	**Year 3 Month 6**	**Year 3 Month 12**	**Year 4 Month 6**	**Year 4 Month 12**
Questions about fractures	X	X	X	X	X	X	X
Questions about Vitamin D/calcium supplements	X	X	X	X	X	X	X
Questions about exercise and other treatments	X	X	X	X	X	X	X

Questions about functional status	X	X	X	X	X	X	X

Information on Physical Examinations and Clinical Assessments

Objective Evaluations: To ensure the patient has a complete spinal cord injury (AIS Grade A), investigators will perform a complete neurological exam according to ISNCSCI guidelines as part of initial screening (days 2-17 post injury). A confirmatory exam will be conducted in the 24 hours prior to study drug delivery. At the end of the study when the patient returns for final laboratory tests and DXA scan, a repeat neurological examination will be performed.

Subjective reports: Interviews will be conducted with patients every 2 months during the first year, either in person if testing is scheduled, or by phone between testing sessions. The first phone call will occur 2 months from injury if the subject is not an inpatient or not available for in-person interview. During these interviews, subjects will be asked about any evidence or knowledge of bone fractures since the previous contact. We will obtain information about exercises and standing, (including use of FES), such as how often and average daily duration.

Follow up for Subsequent Years of Grant Cycle

During the subsequent years of the grant cycle, patients will be contacted every 6 months for information on fractures sustained in the prior 6 months. As part of the consent form, we will be requesting permission to receive x-ray reports of any bone fracture experienced and mechanism of injury between years 1 and 4 post injury and collect information on participation in standing, lower extremity exercise and functional electrical stimulation (FES), and current calcium and vitamin D use.

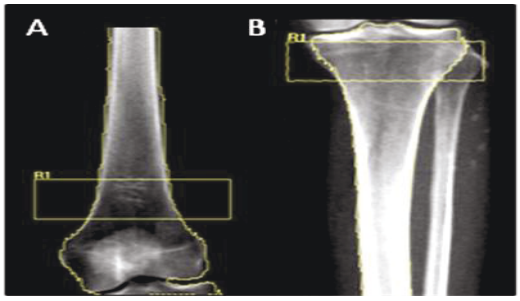
Early Termination

Should a subject gain neurological function prior to initial drug administration and no longer have a complete SCI, that potential subject is withdrawn from the study. Should a subject recover neurological function and the SCI be deemed incomplete any time after the study drug has been administered, that participant will continue with all assessments and follow up studies without exception.

5. DXA Scan Analyses

Analyses of the distal femur and proximal tibia will be conducted as described by Modlesky [70-74] and Shields[75] . For the distal femur analysis, the distal edge of the DXA region of interest box will be placed at 13% of femur length and the proximal edge will be placed at 20% of femur length. For the proximal tibia, the same sized region of interest box will be placed immediately above the fibular head and extend distally (**Figure 3**).

Figure 3: **DXA scans and region of interest for distal femur (A) and proximal tibia (B)**



6. Safety Monitoring

Subjects will be interviewed daily for 3 days, then weekly for one month after study drug administration for any side effects of treatment, in particular, fever and myalgias, using a standardized interview form. Adverse events will be recorded and followed until resolution. A serious adverse event will constitute any event that is life-threatening, requires hospitalization, results in death or disability, requires intervention to prevent permanent impairment or damage, or results in a congenital anomaly. Important medical events that may not result in death, be life threatening or require hospitalization also may be considered to be a serious adverse event when, based on appropriate medical judgment, they may require medical or surgical intervention to prevent one of the outcomes listed in this definition. The PI will report all serious adverse events to the Safety Monitoring Board, the Jefferson IRB, and the project officer at NIDRR.

7. Data and Safety Monitoring Board (DSMB)

The DSMB will be recruited from Jefferson Medical College (JMC) faculty members and charged with monitoring study compliance. They will review screening and enrollment procedures, data collection and analysis, reporting of adverse events, and subject safety. The DSMB members will meet every 6 months or more frequently as needed. The study investigators will meet with the DSMB chair every 6 months to review recommendations or concerns.
The DSMB will be composed of the following members:

John F. Ditunno Jr, MD (Chair)	Professor of Rehabilitation Medicine
Edward B. Ruby, MD	Assistant Professor of Medicine, Division of Endocrinology
Edward J. Filippone, MD	Clinical Assistant Professor of Medicine, Division of Nephrology

E. Data Analysis

1. Evaluation of Baseline Characteristics

We will compare the treatment and control groups for baseline characteristics using t-tests and Chi-square tests as appropriate. Specifically, we will compare age at injury, baseline FRAX fracture risk, sex and injury level (tetraplegia/paraplegia) distribution. The level of significance for all analyses will be 0.05.

2. Evaluation of Hypotheses

Specific Aim 1: To determine the short and long term effects of zoledronic acid on BMD values of the hip, distal femur, and proximal tibia in patients with acute SCI relative to patients receiving placebo.
*The **primary hypothesis** is that subjects treated with zoledronic acid will have at least a 50% smaller percentage decline in BMD values for the hip, distal femur and proximal tibia at 4 and 12 months post-injury than control subjects.*

Analysis: For each of the bone sites to be evaluated, a percent change from baseline ([baseline BMD – follow-up BMD]/baseline BMD) will be modeled using mixed effects linear regression. Fixed effects will be included for treatment assignment, time, treatment by time interaction, and baseline BMD level. A random intercept term will be included to account for correlation among repeated measurements from the same subject. Within this regression model, we will estimate the difference between treatment groups at 4 and 12 months and test for significance of this difference. If the outcome of percent change is not normally distributed, we will apply an appropriate transformation (e.g., log, logit). If the necessary transformation is too complex for interpretation, we will consider using rank-based methods.

***Secondary hypothesis 1** is that subjects treated with zoledronic acid will maintain original BMD values for the hip, distal femur and proximal tibia at 4 months and lose no more than 5% at12 months post-injury.*

Analysis: We will estimate the mean percent change at 4 and 12 months for the active treatment group along with associated 95% confidence intervals from the results of the mixed effects regression. BMD values at 4 months will be considered equivalent to those at baseline if the bounds of the confidence interval are within +/-2%. At 12 months, we will test the one-sided null hypothesis that the percentage decline is greater than 5% vs. the alternative that it is less than or equal to 5%.

***Secondary hypothesis 2** is that fractures in the hip, distal femur, and proximal tibia will be at least 50% lower in the ZA group relative to controls over the course of the grant cycle*. This final hypothesis is exploratory, since we do not expect to have the numbers to achieve statistical significance given the relatively low frequency of sublesional fractures in SCI.

Analysis: The relative risks of all fractures and site-specific fractures will be calculated along with exact 95% confidence intervals. Statistical significance will be evaluated using Fisher's exact test.

Specific Aim 2: To measure and compare levels of pro-osteoclastogenic factor IL-1β, bone formation marker P1NP, and bone resorption marker serum C terminal telopeptide of type I collagen (s-CTX) in

acute traumatic motor complete SCI subjects who receive intravenous zoledronic acid in the first 21 days post injury to the same markers in a similar group of spinal cord injury subjects receiving placebo.

*The **primary hypothesis** is that subjects treated with zoledronic acid will have lower levels of serum marker of bone resorption s-CTX at 4 and 12 months, relative to controls. In terms of absolute marker levels, we hypothesize that the ZA subjects will have s-CTX values within the reference range at 4 and 12 months, in contrast to control subjects whose resorption marker levels will be significantly above the reference range at all of the time points.*

***The secondary hypothesis** is that levels of serum marker of bone formation P1NP will not be lower than controls at 4 and 12 months.*

Analysis: We will again use mixed effects linear regression to model each marker at 1, 4 and 12 months with fixed effects for time, treatment, time by treatment interaction, and baseline biomarker level and a random subject-specific intercept. At months 4 and 12, we will estimate mean group-specific biomarker levels and associated 95% confidence intervals and test for treatment group differences. Since IL-1β will only be measured at baseline and one month, we will use analysis of covariance to compare group averages at one month adjusted for baseline measures.

Specific Aim 3: To determine the safety and tolerability of early administration of intravenous zoledronic acid in persons with acute SCI. We expect to see only minor, transient side effects from zoledronic acid. *To evaluate this we will test the **hypotheses** that compared to controls, subjects treated with zoledronic acid will have (3a) an increased incidence of myalgias, fever, gastrointestinal disturbances, and flu-like symptoms over the three days after infusion of study drug; (3b) be less likely to participate in physical and occupational therapy during the first week after infusion, and (3c) be less likely to want the treatment again.*

Analysis: For each outcome, we will estimate group-specific rates (proportions) along with exact binomial confidence intervals. Comparisons between groups will be performed using Fisher's exact test.

References Cited

1. National Institute on Disability and Rehabilitation Research. Notice of Final Long-Range Plan for Fiscal Years 2005-2009. *Federal Register.* 2006;71(31):8166-8200.

2. Chantraine A, Heynen G, Franchimont P. Bone metabolism, parathyroid hormone, and calcitonin in paraplegia. *Calcified Tissue International.* Jul 3 1979;27(3):199-204.

3. Garland DE, Stewart CA, Adkins RH, et al. Osteoporosis after spinal cord injury. *Journal of Orthopaedic Research* May 1992;10(3):371-378.

4. Minaire P, Neunier P, Edouard C, Bernard J, Courpron P, Bourret J. Quantitative histological data on disuse osteoporosis: comparison with biological data. *Calcified Tissue Research.* 1974;17(1):57-73.

5. Craven BC, Robertson LA, McGillivray CF, Adachi JD. Detection and treatment of sublesional osteoporosis among patients with chronic spinal cord injury: proposed paradigms. *Topics in Spinal Cord Injury Rehabilitation.* 2009;14(4):1-22.

6. Biering-Sorensen F, Bohr HH, Schaadt OP. Longitudinal study of bone mineral content in the lumbar spine, the forearm and the lower extremities after spinal cord injury. *European Journal of Clinical Investigation.* Jun 1990;20(3):330-335.

7. Morse LR, Battaglino RA, Stolzmann KL, et al. Osteoporotic fractures and hospitalization risk in chronic spinal cord injury. *Osteoporosis International.* Mar 2009;20(3):385-392.

8. Ashe MC. Bone Health Following Spinal Cord Injury. Spinal Cord Injury Rehabilitation Evidence. Ed. J. J. Eng et al. 2010. 1-26.
9. Garland DE, Adkins RH, Kushwaha V, Stewart C. Risk factors for osteoporosis at the knee in the spinal cord injury population. *Journal of Spinal Cord Medicine.* 2004;27(3):202-206.
10. Eser P, Frotzler A, Zehnder Y, Denoth J. Fracture threshold in the femur and tibia of people with spinal cord injury as determined by peripheral quantitative computed tomography. *Archives of Physical Medicine and Rehabilitation.* Mar 2005;86(3):498-504.
11. Zehnder Y, Luthi M, Michel D, et al. Long-term changes in bone metabolism, bone mineral density, quantitative ultrasound parameters, and fracture incidence after spinal cord injury: a cross-sectional observational study in 100 paraplegic men. *Osteoporosis International* Mar 2004;15(3):180-189.
12. Frisbie JH. Fractures after myelopathy: the risk quantified. *Journal of Spinal Cord Medicine.* Jan 1997;20(1):66-69.
13. Garland DE, Adkins RH, Stewart CA, Ashford R, Vigil D. Regional osteoporosis in women who have a complete spinal cord injury. *The Journal of Bone and Joint Surgery. American Volume.* Aug 2001;83-A(8):1195-1200.
14. Vestergaard P, Krogh K, Rejnmark L, Mosekilde L. Fracture rates and risk factors for fractures in patients with spinal cord injury. *Spinal Cord.* Nov 1998;36(11):790-796.
15. Hitzig SL, Tonack M, Campbell KA, et al. Secondary health complications in an aging Canadian spinal cord injury sample. *American Journal of Physical Medicine and Rehabilitation / Association of Academic Physiatrists.* Jul 2008;87(7):545-555.
16. Comarr AE, Hutchinson RH, Bors E. Extremity fractures of patients with spinal cord injuries. *Topics in Spinal Cord Injury Rehabilitation.* 2005;11(1):1-10.
17. Ragnarsson KT, Sell GH. Lower extremity fractures after spinal cord injury: a retrospective study. *Archives of Physical Medicine and Rehabilitation.* Sep 1981;62(9):418-423.
18. Sweet MG, Sweet JM, Jeremiah MP, Galazka SS. Diagnosis and treatment of osteoporosis. *American Family Physician.* Feb 1 2009;79(3):193-200.
19. Harvey N, Dennison E, Cooper C. Epidemiology of Osteoporotic Fractures. *Primer on the Metabolic Bone Diseases and Disorders of Mineral Metabolism.*Ed. C. J. Rosen, J.E. Compston, and J.B. Lian. 7th ed. John Wiley & Sons, Inc., 2008:198.
20. Boning Up on Osteoporosis: A Guide to Prevention and Treatment. *Washington, D.C.: National Osteoporosis Foundation, 2008.*
21. Clinician's Guide to Prevention and Treatment of Osteoporosis. *Washington, D.C.: National Osteoporosis Foundation, 2008.*
22. Looker AC, Wahner HW, Dunn WL, et al. Updated data on proximal femur bone mineral levels of US adults. *Osteoporosis International.* 1998;8(5):468-489.
23. Adams J, Bishop N. DXA in Adults and Children. *Primer on the Metabolic Bone Diseases and Disorders of Mineral Metabolism.*Ed. C.J. Rosen, J.E. Compston, and J. B. Lian. 7th ed. John Wiley & Sons, Inc., 2008:154.
24. Kanis JA. Clinician's Guide to Prevention and Treatment of Osteoporosis. Washington, D.C.: National Osteoporosis Foundation, 2008. 2008:35.

25. Schuit SC, van der Klift M, Weel AE, et al. Fracture incidence and association with bone mineral density in elderly men and women: the Rotterdam Study. *Bone.* Jan 2004;34(1):195-202.
26. Miller PD, Siris ES, Barrett-Connor E, et al. Prediction of fracture risk in postmenopausal white women with peripheral bone densitometry: evidence from the National Osteoporosis Risk Assessment. *Journal of Bone and Mineral Research.* Dec 2002;17(12):2222-2230.
27. Stone KL, Seeley DG, Lui LY, et al. BMD at multiple sites and risk of fracture of multiple types: long-term results from the Study of Osteoporotic Fractures. *Journal of bone and mineral research : the official journal of the American Society for Bone and Mineral Research.* Nov 2003;18(11):1947-1954.
28. Pitts CJ, Kearns AE. Update on medications with adverse skeletal effects. *Mayo Clinic Proceedings. Mayo Clinic.* Apr 2011;86(4):338-343; quiz 343.
29. Kahn SE, Haffner SM, Heise MA, et al. Glycemic durability of rosiglitazone, metformin, or glyburide monotherapy. *The New England Journal of Medicine.* Dec 7 2006;355(23):2427-2443.
30. Kahn SE. Glycemic durability of monotherapy for diabetes. *New England Journal of Medicine.* 2007;356(13):1378-1380.
31. Le Templier G, Rodger MA. Heparin-induced osteoporosis and pregnancy. *Curr Opin Pulm Med.* Sep 2008;14(5):403-407.
32. Verrotti A, Coppola G, Parisi P, Mohn A, Chiarelli F. Bone and calcium metabolism and antiepileptic drugs. *Clinical Neurology and Neurosurgery.* Jan 2010;112(1):1-10.
33. Lee RH, Lyles KW, Colon-Emeric C. A review of the effect of anticonvulsant medications on bone mineral density and fracture risk. *The American Journal of Geriatric Pharmacotherapy.* Feb 2010;8(1):34-46.
34. Heaney RP. Functional indices of vitamin D status and ramifications of vitamin D deficiency. *The American Journal of Clinical Nutrition.* Dec 2004;80(6 Suppl):1706S-1709S.
35. Oleson CV, Patel PH, Wuermser LA. Influence of season, ethnicity, and chronicity on vitamin D deficiency in traumatic spinal cord injury. *Journal of Spinal Cord Medicine.* 2010;33(3):202-213.
36. Bauman WA, Zhong YG, Schwartz E. Vitamin D deficiency in veterans with chronic spinal cord injury. *Metabolism: Clinical and Experimental.* Dec 1995;44(12):1612-1616.
37. Roberts D, Lee W, Cuneo RC, et al. Longitudinal study of bone turnover after acute spinal cord injury. *The Journal of Clinical Endocrinology and Metabolism.* Feb 1998;83(2):415-422.
38. Wilmet E, Ismail AA, Heilporn A, Welraeds D, Bergmann P. Longitudinal study of the bone mineral content and of soft tissue composition after spinal cord section. *Paraplegia.* Nov 1995;33(11):674-677.
39. Shields RK, Dudley-Javoroski S, Boaldin KM, Corey TA, Fog DB, Ruen JM. Peripheral quantitative computed tomography: measurement sensitivity in persons with and without spinal cord injury. *Archives of Physical Medicine and Rehabilitation.* Oct 2006;87(10):1376-1381.
40. Biering-Sorensen F, Bohr H, Schaadt O. Bone mineral content of the lumbar spine and lower extremities years after spinal cord lesion. *Paraplegia.* Oct 1988;26(5):293-301.

41. Bauman WA, Spungen AM, Wang J, Pierson RN, Jr., Schwartz E. Continuous loss of bone during chronic immobilization: a monozygotic twin study. *Osteoporosis International* 1999;10(2):123-127.
42. Reiter AL, Volk A, Vollmar J, Fromm B, Gerner HJ. Changes of basic bone turnover parameters in short-term and long-term patients with spinal cord injury. *European Spine Journal.* Jun 2007;16(6):771-776.
43. Hayes KC, Hull TC, Delaney GA, et al. Elevated serum titers of proinflammatory cytokines and CNS autoantibodies in patients with chronic spinal cord injury. *J Neurotrauma.* Jun 2002;19(6):753-761.
44. Bauman WA, Spungen AM. Metabolic changes in persons after spinal cord injury. *Physical Medicine Rehabilitation Clinics of North America.* Feb 2000;11(1):109-140.
45. Wei S, Kitaura H, Zhou P, Ross FP, Teitelbaum SL. IL-1 mediates TNF-induced osteoclastogenesis. *The Journal of Clinical Investigation.* Feb 2005;115(2):282-290.
46. Yang L, Jones NR, Blumbergs PC, et al. Severity-dependent expression of pro-inflammatory cytokines in traumatic spinal cord injury in the rat. *Journal of Clinical Neuroscience.* Apr 2005;12(3):276-284.
47. Davies AL, Hayes KC, Dekaban GA. Clinical correlates of elevated serum concentrations of cytokines and autoantibodies in patients with spinal cord injury. *Archives of Physical Medicine and Rehabilitation.* Nov 2007;88(11):1384-1393.
48. Kunkel CF, Scremin AM, Eisenberg B, Garcia JF, Roberts S, Martinez S. Effect of "standing" on spasticity, contracture, and osteoporosis in paralyzed males. *Archives of Physical Medicine and Rehabilitation.* Jan 1993;74(1):73-78.
49. Warden SJ, Bennell KL, Matthews B, Brown DJ, McMeeken JM, Wark JD. Efficacy of low-intensity pulsed ultrasound in the prevention of osteoporosis following spinal cord injury. *Bone.* Nov 2001;29(5):431-436.
50. Garland DE, Adkins RH, Matsuno NN, Stewart CA. The effect of pulsed electromagnetic fields on osteoporosis at the knee in individuals with spinal cord injury. *Journal of Spinal Cord Medicine.* Winter 1999;22(4):239-245.
51. Needham-Shropshire BM, Broton JG, Klose KJ, Lebwohl N, Guest RS, Jacobs PL. Evaluation of a training program for persons with SCI paraplegia using the Parastep 1 ambulation system: part 3. Lack of effect on bone mineral density. *Archives of Physical Medicine and Rehabilitation.* Aug 1997;78(8):799-803.
52. Leeds EM, Klose KJ, Ganz W, Serafini A, Green BA. Bone mineral density after bicycle ergometry training. *Archives of Physical Medicine and Rehabilitation.* Mar 1990;71(3):207-209.
53. Hangartner TN, Rodgers MM, Glaser RM, Barre PS. Tibial bone density loss in spinal cord injured patients: effects of FES exercise. *J Rehabil Res Dev.* 1994;31(1):50-61.
54. Belanger M, Stein RB, Wheeler GD, Gordon T, Leduc B. Electrical stimulation: can it increase muscle strength and reverse osteopenia in spinal cord injured individuals? *Archives of Physical Medicine and Rehabilitation.* Aug 2000;81(8):1090-1098.
55. Zehnder Y, Risi S, Michel D, et al. Prevention of bone loss in paraplegics over 2 years with alendronate. *Journal of Bone and Mineral Research.* Jul 2004;19(7):1067-1074.
56. Gilchrist NL, Frampton CM, Acland RH, et al. Alendronate prevents bone loss in patients with acute spinal cord injury: a randomized, double-blind, placebo-controlled study. *The Journal of Clinical Endocrinology and Metabolism.* Apr 2007;92(4):1385-1390.

57. Nance PW, Schryvers O, Leslie W, Ludwig S, Krahn J, Uebelhart D. Intravenous pamidronate attenuates bone density loss after acute spinal cord injury. *Archives of Physical Medicine and Rehabilitation.* Mar 1999;80(3):243-251.

58. Bauman WA, Wecht JM, Kirshblum S, et al. Effect of pamidronate administration on bone in patients with acute spinal cord injury. *Journal of Rehabilitation Res Dev.* May-Jun 2005;42(3):305-313.

59. Dunford JE, Thompson K, Coxon FP, et al. Structure-activity relationships for inhibition of farnesyl diphosphate synthase in vitro and inhibition of bone resorption in vivo by nitrogen-containing bisphosphonates. *The Journal of Pharmacology and Experimental Therapeutics.* Feb 2001;296(2):235-242.

60. Coxon FP, Helfrich MH, Van't Hof R, et al. Protein geranylgeranylation is required for osteoclast formation, function, and survival: inhibition by bisphosphonates and GGTI-298. *Journal of Bone and Mineral Research.* Aug 2000;15(8):1467-1476.

61. Hamdy RC. Zoledronic acid: clinical utility and patient considerations in osteoporosis and low bone mass. *Drug Des Devel Ther.* 2010;4:321-335.

62. Black DM, Delmas PD, Eastell R, et al. Once-yearly zoledronic acid for treatment of postmenopausal osteoporosis. *The New England Journal of Medicine.* May 3 2007;356(18):1809-1822.

63. US Food and Drug Administration. The FDA safety information and adverse event reporting program. 2008.

64. Grbic JT, Landesberg R, Lin SQ, et al. Incidence of osteonecrosis of the jaw in women with postmenopausal osteoporosis in the health outcomes and reduced incidence with zoledronic acid once yearly pivotal fracture trial. *Journal of the American Dental Association.* Jan 2008;139(1):32-40.

65. Neviaser AS, Lane JM, Lenart BA, Edobor-Osula F, Lorich DG. Low-energy femoral shaft fractures associated with alendronate use. *Journal of Orthopaedic Trauma.* May-Jun 2008;22(5):346-350.

66. Dell RM, Adams AL, Greene DF, Funahashi TT, Silverman SL, Eisermon, EO, Zhou, H, Burchette RJ, Ott SM. Incidence of atypical nontraumatic diaphyseal fractures of the femur. *Journal of Bone and Mineral Research* Dec 2012;27:2544-2550.

67. Saag K, Lindsay R, Kriegman A, Beamer E, Zhou W. A single zoledronic acid infusion reduces bone resorption markers more rapidly than weekly oral alendronate in postmenopausal women with low bone mineral density. *Bone.* May 2007;40(5):1238-1243.

68. Shapiro J, Smith B, Beck T, Ballard P, Dapthary M, BrintzenhofeSzoc K, Cannis J. Treatment with zoledronic acid ameliorates negative geometric changes in the proximal femur following acute spinal cord injury. *Calcif Tissue Int.* May 2007;80(5):316-22.

69. Bubbear JS, Gall A, Middleton FR, Ferguson-Pell M, Swaminathan R, Keen RW. Early treatment with zoledronic acid prevents bone loss at the hip following acute spinal cord injury. *Osteoporosis International.* Jan 2011;22(1):271-279.

70. Modlesky CM, Cavaiola ML, Smith JJ, Rowe DA, Johnson DL, Miller F. A DXA-based mathematical model predicts midthigh muscle mass from magnetic resonance imaging in typically developing children but not in those with quadriplegic cerebral palsy. *The Journal of Nutrition.* Dec 2010;140(12):2260-2265.

71. Modlesky CM, Subramanian P, Miller F. Underdeveloped trabecular bone microarchitecture is detected in children with cerebral palsy using high-resolution magnetic resonance imaging. *Osteoporosis International.* Feb 2008;19(2):169-176.

72. Modlesky CM, Bickel CS, Slade JM, Meyer RA, Cureton KJ, Dudley GA. Assessment of skeletal muscle mass in men with spinal cord injury using dual-energy X-ray absorptiometry and magnetic resonance imaging. *Journal of Applied Physiology.* Feb 2004;96(2):561-565.

73. Modlesky CM, Majumdar S, Narasimhan A, Dudley GA. Trabecular bone microarchitecture is deteriorated in men with spinal cord injury. *Journal of Bone and Mineral Research.* Jan 2004;19(1):48-55.

74. Modlesky CM, Slade JM, Bickel CS, Meyer RA, Dudley GA. Deteriorated geometric structure and strength of the midfemur in men with complete spinal cord injury. *Bone.* Feb 2005;36(2):331-339.

75. Shields RK, Schlechte J, Dudley-Javoroski S, et al. Bone mineral density after spinal cord injury: a reliable method for knee measurement. *Archives of Physical Medicine and Rehabilitation.* Oct 2005;86(10):1969-1973.