STATISTICAL ANALYSIS PLAN

Prevention of Bone Loss after Acute SCI by Zoledronic Acid: Durability, Effect on Bone Strength, and Use of Biomarkers to Guide Therapy. NCT02325414

Principal Investigator: Thomas J. Schnitzer, MD, PhD

Sites: Northwestern University

Table of Contents

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 protocol). Subjects were 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 was 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 were obtained at baseline, 3 months, 6 months, 12 months, 18 months and 24 months. CT imaging of the hip was obtained at baseline, 6 months, 12 months, and 24 months.

Study Objectives/Statistical Hypotheses

Hypothesis 1a. In participants treated shortly after acute SCI with a single infusion of zoledronic acid, the mean loss of bone after one year at skeletal sites in the lower extremity will be less than that determined for participants who received placebo. Hip areal bone mass density (aBMD) will be assessed as the primary endpoint.

Hypothesis 1b: In participants treated shortly after acute SCI with a single infusion of zoledronic acid (zol/placebo) as well as those receiving a second treatment with zoledronic acid (zol/zol), the mean loss of bone after two years at skeletal sites in the lower extremity will be less than that determined for participants who received placebo for two years (placebo/placebo).

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.

Hypothesis 1d: Initiation of treatment with zoledronic acid at one year after acute SCI in people not previously treated (placebo/zol) will still result in preventing further bone loss compared to no active bone-specific treatment (placebo/placebo); loss of bone mass that has occurred during the initial year without treatment will not be reversed, however.

Endpoints

Primary endpoints. The primary endpoints in this study are the percent-change in aBMD, evaluated by DXA, in the non-dominant (1) total hip and (2) femoral neck skeletal sites. If data on the non-dominant hip are unavailable, we will use aBMD for the dominant hip. Percent-change will be calculated as $((aBMD_{follow\text{-}up} - aBMD_{baseline}) / aBMD_{baseline}) \times 100\%$.

Key secondary endpoints. Secondary endpoints include: (1) integral volumetric bone mineral content (Int.vBMC) distal femoral epiphysis, (2) Int. vBMC tibial epiphysis, and (3) Int.vBMC distal femoral metaphysis measured by QCT. Percent-change will again be calculated for each outcome.

Additional secondary endpoints. CT biomarkers: Trabecular volumetric bone mineral density (Tb.vBMD; g/cm3) and cortical volumetric bone mineral content (Ct.vBMC; g) and bone volume (Ct.vBV; cm3) at epiphyseal and metaphyseal regions. For the diaphyseal region, Ct.vBMC and Ct.vBV. WISCI.

Exploratory endpoints. Torsional strength index (TSI; cm3) for each skeletal region. Stiffness and strength at the hip, distal femur, and proximal tibia determined by CT-based finite element analysis. Serum markers of bone metabolism: P1NP, BSAP (bone formation markers), and CTX (bone resorption marker).

Statistical Methods

Diagnostics

All variables will be plotted to examine distribution shapes as well as percentage of missing values. The mixed modelling proposed below uses both all available observations without deleting cases and imputed data. Residuals will be examined for each model fitted in case of unexpected violations of assumptions. Analysis will be performed as ITT (intention to treat): patients will be analyzed according to the treatment arm to which they were randomized.

Missing Data

Missing data will primarily be inputed by maximum likelihood estimation using a mixed effects model. A sensitivity analysis will be done latter, by using a multiple imputation methodology before modelling. Here, imputation of missing data will be based on sampling from a normal distribution using a mean value of the subjects' last observed value and standard deviation (over treatment groups) of the observed data at the missing endpoint (3, 6, or 12 months). This is a multiple imputation version of Last Observation Carried Forward (LOCF) single imputation method. Note, a subject's last observation may be the Baseline observation. One hundred imputed datasets will be used in this analysis. The linear mixed-effects model described below will be used for each imputation dataset, and the overall results will be calculated to take account of the variability both within and between imputation datasets using standard methods (Little & Rubin, 2002).

Primary analyses

Group comparisons will be tested with a linear mixed-effects model with the percentage-change from baseline to months 6 and 12 in aBMD at the total hip and femoral neck. Baseline aBMD at the total hip and femoral neck, study visit, ambulatory status measured by WISCI and interaction of WISCI with visit will be fitted as random factors. aBMD by group interaction will be considered statistically significant at $p < 0.05$. Raw effect sizes will be calculated as differences between groups at each time point. Multiplicity will be handled by utilizing a fixed-sequence approach to testing, with initial analysis of total hip ($\alpha=0.05$) and if successful followed by femoral neck ($\alpha=0.05$).

Secondary analyses

Secondary analysis will be divided into three main sections:

a) Effect of functional capacity (WISCI) on aBMD of total hip and femoral neck percent-change; A secondary interaction analysis will be performed for the primary endpoint, exploring the effect of WISCI. The interaction of treatment with WISCI score will be fitted as a fixed effect (in addition to the WISCI score itself), with the resulting estimated treatment differences being shown for continuous scores of the WISCI scale.

b) Secondary endpoint analysis at 12 months;

The primary analysis described above will be applied for the all the three key secondary endpoints at 6, and 12 months visits, with the fixed sequence order described above. Appropriate p-values adjustments to compensate for a possible increased risk in Type I errors due to multiple secondary outcomes measurements will be done using a stepwise Dunnett procedure. All reporting will include both adjusted p values and raw effect sizes.

c) Primary and secondary endpoint analysis at 24 months.

Primary endpoint (total hip and femoral neck aBMD, measured by DXA) and secondary endpoints will be analyzed at 24 months. Here, we will study 4 groups of patients: 24 months of treatment, 24 months of no-treatment, switch from treatment to placebo at 12 months, switch from placebo to treatment at 12 months. A linear mixed model with the four groups, controlling for Baseline aBMD at the hip and ambulatory status measured by WISCI will be fitted to predict percent-change from baseline for each endpoint.

Exploratory Analyses

Serum bone markers (i.e., P1NP, BSAP, CTX) and additional CT bone biomarkers, measured at 3, 6, 12 and 24 months will be evaluated using a similar approach as for the primary analyses – linear mixed effects model. A compressive strength index (CSI; g2/cm4) and a torsional strength index (TSI; cm3) will be computed for each skeletal region. Finally, CT-based finite element analysis will be used to predict stiffness and strength at the hip, distal femur, and proximal tibia.

Sensitivity analyses

This analysis will assess the robustness of the efficacy conclusions regarding the choice of maximum likelihood estimation as the primary method for accounting for missing data. The primary mixed effects model described above will be repeated after multiple imputation, as described above.

Analysis of Safety

Analysis of safety will include all participants who underwent randomization and received at least one dose of the study drug. Safety endpoints will include incidence and severity of adverse events and laboratory blood chemical values. Adverse events will be characterized by system, organ, and class designation. Raw number of events and number of participants experiencing reported serious adverse events and adverse events will be reported.

Analysis Plan Summary

Endpoint	Statistical Method	Model/Covariates	Missing Data	Objective
% Change from Baseline at 6,12 months with total hip and femoral neck aBMD, DXA	MEM	Baseline total hip/ femoral neck aBMD, visit, WISCI score; (subjects as random effects)	MLE	Primary Analysis
% Change from Baseline at 6,12 months with total hip aBMD, DXA	MEM	Baseline total hip aBMD, visit, WISCI score; (subjects as random effects)	Multiple Imputation	Sensitivity Analysis
% Change from Baseline at 6,12 months with total hip aBMD, DXA (a)	MEM	Baseline total hip aBMD, visit, WISCI score, (subjects as random effects)	MLE	Secondary, Interaction Analysis
% Change from Baseline at 6, 12 months with femoral neck aBMD, DXA (b)	MEM	Baseline total hip aBMD, visit, WISCI score; (subjects as random effects)	MLE	Secondary Analysis
% Change from Baseline at 6,12, months with QCT Int.vBMC Femoral Epiphysis (b)	MEM	Baseline total hip aBMD, visit, WISCI score; (subjects as random effects)	MLE	Secondary Analysis
% Change from Baseline at 6,12, QCT Int.vBMC Femoral Methaphysis (b)	MEM	Baseline total hip aBMD, visit, WISCI score; (subjects as random effects)	MLE	Secondary Analysis
% Change from Baseline at 24 months with total hip aBMD, DXA (c)	MEM	Baseline total hip aBMD, visit, WISCI score; (subjects as random effects)	MLE	Secondary Analysis
% Change from Baseline at 24 months with	MEM	Baseline total hip aBMD, visit, WISCI	MLE	Secondary Analysis

femoral neck aBMD, DXA (c)		score; (subjects as random effects)		
% Change from Baseline at 24 months with QCT Int.vBMC Femoral Epiphysis (c)	MEM	Baseline total hip aBMD, visit, WISCI score; (subjects as random effects)	MLE	Secondary Analysis
% Change from Baseline at 24 months with QCT Int.vBMC Femoral Methaphysis (c)	MEM	Baseline total hip BMD, visit, WISCI score; (subjects as random effects)	MLE	Secondary Analysis

(a) Secondary analysis phase 1: Interaction Analysis for primary endpoint with WISCI; (b) Secondary analysis phase 2: Secondary endpoints at 12 months; (c) Secondary analysis phase 3: Primary and Secondary endpoints at 24 months. aBMD (areal bone mineral density); MEM (mixed effects model); MLE (maximum likelihood estimates); WISCI (walking index for spinal cord injury).

References

Senn, S., & Julious, S. (2009). Measurement in clinical trials: a neglected issue for statisticians? Statistics in medicine, 28(26), 3189-3209.

Little RJ & Rubin DB (2002). *Statistical Analysis with Missing Data*. New Jersey:Wiley.