

STATISTICAL ANALYSIS PLAN

Bone Histomorphometry of the Proximal Femur in Denosumab-treated Subjects Undergoing Total Hip Replacement

Protocol Number: 20140259

Version: 1.0

Date: 6 November 2015

Author: PPD

NCT Number: 02576652

This NCT number has been applied to the document
for purposes of posting on clinicaltrials.gov

Table of Contents

Table of Abbreviations	4
1. Introduction	5
2. Objectives	5
2.1 Primary Objective	5
2.2 Secondary Objectives.....	5
2.3 Exploratory Objectives.....	5
3. Study Overview	5
3.1 Study Design	5
3.2 Sample Size	6
4. Study Endpoints and Covariates	6
4.1 Study Endpoints	6
4.1.1 Primary Endpoint.....	6
4.1.2 Secondary Endpoints	6
4.1.3 Exploratory Endpoints	7
4.1.4 Safety Endpoint	8
4.2 Planned Covariates	8
5. Hypotheses and/or Estimations.....	8
6. Definitions	9
6.1 Basic Definitions	9
6.2 Study Points of Reference	9
6.3 Study Dates	9
6.4 Study Time Intervals.....	10
6.5 Endpoint Definitions.....	10
6.6 Arithmetic Calculations.....	10
7. Analysis Subsets	11
7.1 Full Analysis Set.....	11
7.2 Primary Analysis Set	11
7.3 Safety Analysis Set.....	11
7.4 Subgroup Analyses	11
8. Interim Analysis and Early Stopping Guidelines.....	11
9. Data Screening and Acceptance.....	11
9.1 General Principles	11
9.2 Data Handling and Electronic Transfer of Data.....	11
9.3 Handling of Missing and Incomplete Data	12
9.4 Outliers	12
9.5 Validation of Statistical Analyses.....	12

10. Statistical Methods of Analysis.....	12
10.1 General Principles	12
10.2 Subject Accountability	13
10.3 Important Protocol Deviations	13
10.4 Demographic and Baseline Characteristics.....	13
10.5 Efficacy Analyses	13
10.5.1 Primary Endpoint.....	13
10.5.2 Secondary Endpoint	13
10.5.3 Exploratory Endpoints	14
10.6 Safety Analyses.....	14
10.6.1 Adverse Events	14
11. Changes From Protocol-specified Analyses	14

List of Tables

Table 1. Specialized Histomorphometric Modeling- and Remodeling-based Bone Formation Parameters.....	7
Table 2. Specialized Histomorphometric Modeling- and Remodeling-based Mineralizing Surface Parameters.....	7
Table 3. Conventional Histomorphometric Endpoint Parameters	8

Table of Abbreviations

Please refer to the study glossary in the protocol of Study 20140259.

1. Introduction

The purpose of this Statistical Analysis Plan (SAP) is to provide details of the planned statistical analyses that have been outlined within the protocol amendment 1 for denosumab study 20140259 dated 6 April 2015. The scope of this plan includes the final analysis and will be executed by Global Biostatistical Sciences. This is a phase 4 study in a small group of subjects who have received denosumab in the prior 18 months and have elected to undergo total hip replacement (THR) surgery due to osteoarthritis. Subjects will be exposed to 2 dosing cycles of fluorochrome treatment (tetracycline followed by democloccycline) prior to THR and will not receive any denosumab injection whilst on study. There are no planned visits after the THR surgery.

2. Objectives

2.1 Primary Objective

The primary objective of this study is to determine the incidence of modeling-based bone formation (MBF) at the femoral neck in subjects who have received denosumab and are undergoing THR.

2.2 Secondary Objectives

The secondary objective of this study is to describe the bone formation parameters of the femoral bone in subjects undergoing THR.

2.3 Exploratory Objectives

The exploratory objectives of this study include:

- To determine the extent of modeling- and remodeling-based formation based on morphology of the underlying cement line (smooth = modeling; scalloped = remodeling) as a percentage of bone surface.
- Comparison of histomorphometric parameters of bone formation in the femoral neck of subjects enrolled in this study with those in historical controls.

3. Study Overview

3.1 Study Design

This phase 4 study will enroll approximately 15 subjects who previously received denosumab and are undergoing elective THR due to underlying osteoarthritis.

The study will consist of a screening visit followed by 1 cycle of tetracycline dosing and 1 cycle of demecloccycline. Each cycle will be 3 days in duration, with cycle 2 beginning approximately 10 days after the receipt of the last dose of tetracycline in cycle 1. If tetracycline or demecloccycline is not available, other tetracycline derivatives may be used. The surgery for THR will be at least 5 and no greater than 42 days after the last

dose of demeclocycline received in cycle 2 (ie, from days 22 to 58). The fragment of the femur will be obtained during the THR procedure. There are no visits planned after THR surgery procedure. The total duration of the study for each subject therefore is expected to be approximately 2 months including the screening visit.

Transverse sections of the femoral neck specimen will be obtained and then fixed and embedded. The cancellous, periosteal, and endocortical regions will be evaluated in the stained sections for any pathological findings and evidence of modeling-based and remodeling-based bone accretion. Variables of histomorphometry will be measured to assess structural and dynamic parameters. Modeling-based (evidenced by a smooth cement line) and remodeling-based (evidenced by a scalloped cement line) bone formation units will be described separately.

3.2 Sample Size

Approximately 15 subjects who are scheduled to undergo an elective THR procedure and have received at least 3 doses of denosumab over the previous 18 months with the last dose within the prior 6 months will be enrolled in this study. This sample size is typical for studies investigating bone biopsies to evaluate bone histology and histomorphometry variables. The actual sample size, however, will be largely determined (and limited) by the availability of qualified subjects due to the eligibility criteria of the study as well as the nature of the study procedures.

4. Study Endpoints and Covariates

4.1 Study Endpoints

4.1.1 Primary Endpoint

The primary endpoint is subject incidence of fluorochrome labeling (Yes/No) in the cancellous, periosteal or endocortical surfaces of the femoral neck indicative of MBF (ie, associated with smooth underlying cement lines).

4.1.2 Secondary Endpoints

The secondary endpoints are modeling-based bone formation parameters and remodeling-based bone formation parameters in the cancellous, periosteal or endocortical surfaces of the femoral neck (see [Table 1](#)).

Table 1. Specialized Histomorphometric Modeling- and Remodeling-based Bone Formation Parameters

Histomorphometric parameter	Abbreviation
Cancellous, Periosteal, Endocortical	
Modeling-based formation unit (#/mm)	MF.U/BS
Overfilled remodeling-based formation unit (#/mm)	oRmF.U/BS
Remodeling-based formation unit including overfilled units (#/mm)	RmF.U/BS

4.1.3 Exploratory Endpoints

The exploratory endpoints for this study are as follows:

- extent of fluorochrome-labeled bone surface with smooth or scalloped underlying cement lines as a percentage of total bone surface ([Table 2](#))
- histomorphometric parameters of bone formation in femoral neck ([Table 3](#))

Table 2. Specialized Histomorphometric Modeling- and Remodeling-based Mineralizing Surface Parameters

Histomorphometric parameter	Abbreviation
Cancellous, Periosteal, Endocortical	
Modeling-based single label surface (%)	SL.MF / BS
Modeling-based double label surface (%)	DL.MF / BS
Modeling-based mineralizing surface (%)	MS.MF / BS
Extended remodeling-based single label surface (%)	SL.oRmF / BS
Extended remodeling-based double label surface (%)	DL.oRmF / BS
Extended remodeling-based mineralizing surface (%)	MS.oRmF / BS
Remodeling-based single label surface (%)	SL.RmF / BS
Remodeling-based double label surface (%)	DL.RmF / BS
Remodeling-based mineralizing surface (%)	MS.RmF / BS

Table 3. Conventional Histomorphometric Endpoint Parameters

Histomorphometric parameter	Abbreviation
Cancellous	
Mineralizing Surface/Bone Surface (%)	MS/BS
Mineral Apposition Rate – Corrected ($\mu\text{m}/\text{day}$)	MAR
Bone Formation Rate/Bone Surface – Corrected ($\mu\text{m}^3/\mu\text{m}^2/\text{year}$)	BFR/BS
Bone Formation Rate/Bone Volume – Corrected (%/year)	BFR/BV
Activation Frequency – Corrected (N/year)	Ac.f
Eroded Surface/Bone Surface (%)	ES/BS
Endocortical	
Mineralizing Surface/Bone Surface (%)	MS/BS
Mineral Apposition Rate – Corrected ($\mu\text{m}/\text{day}$)	MAR
Bone Formation Rate/Bone Surface – Corrected ($\mu\text{m}^3/\mu\text{m}^2/\text{year}$)	BFR/BS
Activation Frequency – Corrected (N/year)	Ac.f
Eroded Surface/Bone Surface (%)	ES/BS
Periosteal	
Mineralizing Surface/Bone Surface (%)	MS/BS
Mineral Apposition Rate – Corrected ($\mu\text{m}/\text{day}$)	MAR
Bone Formation Rate/Bone Surface – Corrected ($\mu\text{m}^3/\mu\text{m}^2/\text{year}$)	BFR/BS
Intracortical	
Activation Frequency – Corrected (N/year)	Ac.f
Eroded Surface/Bone Surface (%)	ES/BS

In addition, each of the parameters in [Table 1](#), [Table 2](#), and [Table 3](#) will also be assessed in the 4 quadrants of the femoral neck, namely, the anterior, posterior, inferior and superior sections as exploratory endpoints.

4.1.4 Safety Endpoint

The safety endpoint is subject incidence of adverse events reported during the study.

4.2 Planned Covariates

No covariate analysis is planned.

5. Hypotheses and/or Estimations

No formal hypothesis will be tested in this study.

6. Definitions

6.1 Basic Definitions

Treatment-emergent Adverse Event

Any adverse events that occur on or after the date of first administration of fluorochrome treatment (tetracycline or democlocycline or their derivatives)

Evaluable Biopsy

An Evaluable Biopsy is defined as a lab result with at least one nonmissing histomorphometric parameter.

6.2 Study Points of Reference

Study Day 1

The first day of fluorochrome administration

Study Day

The number of days from Study Day 1, defined as:

$$\text{Study Day} = (\text{Date of interest} - \text{Date of Study Day 1}) + 1$$

For the screening visit and any unscheduled visit prior to Study Day 1, study day is defined as:

$$\text{Study Day} = (\text{Date of interest} - \text{Date of Study Day 1})$$

Study Baseline

Study baseline is defined as Study Day 1.

End of Study Date for Individual Subject

The last day that protocol-specified procedures are conducted for an individual subject, that is, the date when the individual subject is last assessed as recorded on the End of Study eCRF.

End of Study

The latest End of Study Date among all subjects

Total Hip Replacement Surgery Visit Window

Surgery for THR will be performed 5 to 42 days after the last dose of the second cycle of fluorochrome treatment.

6.3 Study Dates

Informed Consent Date / Study Enrollment Date

The date on which the subject signed the informed consent form

First Dose Date

The date of administration of the first dose of fluorochrome (tetracycline) treatment; this date may or may not be the same as the informed consent date/study enrollment date.

6.4 Study Time Intervals

Study Treatment and Duration of Therapy

Two cycles of fluorochrome treatment that consist of a total oral dose of 1000 mg tetracycline daily for a total of 3 days followed by an approximate 10-day break, then a total oral dose of 600 mg demeclocycline daily for a total of 3 days.

On-study Period

The time period from the enrollment date to the end of study date, inclusive.

AE Collection Period

The time period for AE collection is from the first dose of fluorochrome (tetracycline) treatment

SAE Collection Period

The time period for SAE collection is from the informed consent date until the end of study through the last date of subject assessment or THR surgery, whichever is longer.

6.5 Endpoint Definitions

Subject Incidence of Fluorochrome Labeling for Primary Endpoint

The subject incidence of fluorochrome labeling is defined as the number of subjects with smooth underlying cement lines in the cancellous, periosteal, or endocortical surfaces of the femoral neck (numerator) divided by the total number of subjects with an evaluable bone biopsy (denominator).

Subject Incidence for Adverse Events

The subject incidence for a given event in a given time period is defined as the number of subjects with at least one reported occurrence of the event divided by the number of subjects who are at risk for having the event in the beginning of the given time period.

For subjects with multiple occurrences of the same event, the event will only be counted once per subject.

6.6 Arithmetic Calculations

Body Mass Index (BMI)

$$\text{BMI} = \text{Weight (kg)} / \text{Height (m}^2\text{)}$$

7. Analysis Subsets

7.1 Full Analysis Set

The full analysis set includes all enrolled subjects

7.2 Primary Analysis Set

The primary analysis set includes all enrolled subjects who have an evaluable biopsy for fluorochrome labeling.

7.3 Safety Analysis Set

The safety analysis subset includes all enrolled subjects who have received at least one dose of tetracycline, demeclocycline, or other tetracycline derivative.

7.4 Subgroup Analyses

There are no planned subgroup analyses.

8. Interim Analysis and Early Stopping Guidelines

No interim analysis is planned for this study.

9. Data Screening and Acceptance

9.1 General Principles

The objective of the data screening is to assess the quantity, quality, and statistical characteristics of the data relative to the requirements of the planned analyses. Any questionable values or situations will be reported to Amgen Global Study Operations-Data Management (GSO-DM) for review or confirmation.

As part of the data acceptance procedure, all datasets, planned tables, listings, and graphs will be generated and reviewed to identify any additional data issues. Any critical issues identified must be resolved with GSO-DM before final acceptance of the data.

9.2 Data Handling and Electronic Transfer of Data

The Amgen Global Study Operations-Data Management (GSO-DM) department will provide all data to be used in the planned analyses. This study will use the RAVE database.

9.3 Handling of Missing and Incomplete Data

Incomplete start dates of adverse events or concomitant medications will be imputed as follows.

Missing	Impute	Exception
Day	01	Default to Study Day 1 Date if the event started in the same year and month as Day 1
Day / Month	01JAN	Default to Study Day 1 Date if the event started in the same year as Day 1
Day / Month / Year	First Dose Date	Use enrollment date for subjects who did not receive fluorochrome administration

If a death date (ie, End of Study date) is incomplete and missing only the day field, it will be imputed as the first day of the month if the latest assessment date from other data is before the month of the death. However, if the latest assessment date is during the same month as the death, the partial death date will be imputed using the latest assessment date.

No other date variables will be imputed.

9.4 Outliers

Extreme histomorphometric values will be examined to look for questionable values. Before data freeze, the validity of any questionable values will be verified and observations found to be due to data entry errors will be corrected by the study team. Potential outliers that are not due to data entry error will be included in the analysis. No valid measurement will be purposely excluded from the analyses.

9.5 Validation of Statistical Analyses

Programs will be developed and maintained, and outputs will be verified in accordance with current risk-based quality control procedures.

Tables, figures, and listings will be produced with validated standard macro programs where standard macros can produce the specified outputs.

The production environment for statistical analyses consists of Amgen-supported versions of statistical analysis software; for example, the SAS System version 9.3 or later.

10. Statistical Methods of Analysis

10.1 General Principles

No formal hypothesis will be tested in this study. All analyses will be descriptive in nature. Frequencies and percentages will be presented for all categorical variables.

Continuous variables will be summarized descriptively using mean, standard deviation, minimum, maximum, median, 1st quartile, and 3rd quartile.

10.2 Subject Accountability

The disposition of all enrolled subjects will be tabulated. Subject enrollment and disposition of the number of subjects enrolled, successfully completing fluorochrome administration, and completing the study will be included. The disposition of subjects will also include the number of subjects who withdrew from the study and their reasons for withdrawal.

10.3 Important Protocol Deviations

Important Protocol Deviations (IPDs) categories are defined by the study team before the first subject's visit and updated during the IPD reviews throughout the study prior to database lock. These definitions of IPD categories, sub-category codes, and descriptions will be used during the course of the study. Eligibility deviations are defined in the protocol.

10.4 Demographic and Baseline Characteristics

Subject demographics, including sex, age, race, ethnicity, baseline disease characteristics, body mass index (BMI), prior osteoporosis medication, historical body mass density (BMD) and fracture history will be summarized. These analyses will be performed using the primary analysis set.

10.5 Efficacy Analyses

No formal hypothesis will be tested and no formal comparative efficacy analyses will be conducted. The primary analysis set will be used for all the primary, secondary and exploratory efficacy analyses.

10.5.1 Primary Endpoint

Number and the proportion of subjects with modeling-based fluorochrome labeling present in the cancellous, periosteal, or endocortical surfaces (ie, modeling-based formation unit [MF.U/BS] > 0 in any of these bone surfaces) of the femoral neck indicative of MBF out of all evaluable subjects will be provided.

10.5.2 Secondary Endpoint

The actual values of modeling-based bone formation parameters and remodeling-based bone formation parameters will be summarized using descriptive statistics for each analyzed surface of the overall femoral neck (see [Table 1](#)).

10.5.3 Exploratory Endpoints

Extent of fluorochrome-labeled bone surface with smooth or scalloped underlying cement line expressed as a percentage of total bone surface for each of the analyzed bone surfaces of the overall femoral neck using descriptive statistics (see [Table 2](#)).

In addition, a list of conventional dynamic and static histomorphometric parameters will be summarized descriptively for each of the analyzed bone surfaces of the overall femoral neck (see [Table 3](#)).

Also, all the histomorphometric parameters presented in [Table 1](#), [Table 2](#) and [Table 3](#) will be summarized descriptively for each of the 4 femoral neck quadrants.

The histomorphometry laboratory will identify approximately the same number of historical bone biopsy samples and re-analyze them using the same methodology as the current study samples. This will allow an informal comparison of histomorphometric parameters between subjects enrolled in this study and a control group.

10.6 Safety Analyses

10.6.1 Adverse Events

The Medical Dictionary for Regulatory Activities (MedDRA) version 18.0 or later will be used to code all adverse events (AEs) to a system organ class and a preferred term.

Treatment-emergent adverse events are events with an onset after the administration of the first dose of tetracycline treatment in cycle 1. Subject incidence of all treatment-emergent AEs, serious AEs, and fatal AEs will be tabulated by system organ class and preferred term in descending order of frequency.

11. Changes From Protocol-specified Analyses

There are no changes to the protocol-specified analyses.