Official Title: A Study of Immunologic Safety for Alphanate® in Previously Treated Patients Diagnosed With severe Hemophilia A.
(formerly ATC 02-01 of Alpha Therapeutic Corporation)

NCT Number: NCT00323856

Document Date: Statistical Analysis Plan Version 1.0: 29 September 2010

GRIFOLS

DOCUMENT: Statistical Analysis Plan

PROTOCOL: GBI 04-01
A Study of Immunologic Safety for Alphanate® in Previously Treated Patients Diagnosed With severe Hemophilia A.
(formerly ATC 02-01 of Alpha Therapeutic Corporation)

SAP VERSION: Version 1.0

SAP DATE: 29 September 2010

PROTOCOL DATE: Original: 26 March 2002
Amendment 1: 11 July 2002
Amendment 2: 14 October 2002
Amendment 3: 17 April 2003
Amendment 4: 16 December 2003 (became GBI 04-01)
Amendment 5: 1 May 2006
Amendment 6: 10 January 2007

SPONSOR: Grifols Biologicals Inc.

PREPARED BY: Clinipace Worldwide

AUTHOR: [redacted] PhD
[redacted]
Clinipace Worldwide

[redacted] MS
Statistician
Clinipace Worldwide

APPROVED:

[redacted]

9-29-2010

[redacted] PhD

Date

[redacted]

Grifols Biologicals Inc.

TABLE OF CONTENTS

ABBREVIATIONS:

AE:	Adverse event
BU:	Bethesda unit
BUN:	Blood urea nitrogen
ALT:	Alanine aminotransferase
AP:	Alkaline phosphatase
AST:	Aspartic aminotransferase
CBC:	Complete blood count
CPWW:	Clinipace Worldwide
CRF:	Case report form (s)
CTM:	Clinical trial material
FDA:	United States Food and Drug Administration
F VIII:	Factor VIII
FVIIIc:	Factor VIII coagulant activity
HAV:	Hepatitis A virus
HBsAg:	Hepatitis B surface antigen
HBcAb:	Hepatitis B core antibody
HBsAb:	Hepatitis B surface antibody
HBV:	Hepatitis B virus
HCV:	Hepatitis C virus
HIV-1:	Human immunodeficiency virus type 1
HIV-2:	Human immunodeficiency virus type 2
LDH:	Lactic acid dehydrogenase
MedDRA:	Medical Dictionary for Regulatory Activities
mL:	Milliliter
SAE:	Serious adverse event
SAP:	Statistical Analysis Plan
SD:	Standard deviation
SOC:	System organ class
TEAE:	Treatment-emergent adverse event
WBC:	White blood cell count
WHO:	World Health Organization

1. INTRODUCTION

This document describes the statistical methods and data presentations to be used in the summary and analysis of safety and efficacy data from Protocol GBI 04-01. Background information is provided for the overall study design and objectives. The reader is referred to the study protocol and case report forms (CRFs) for details of study conduct and data collection.

2. STUDY OBJECTIVE(S), TREATMENTS, AND ENDPOINT(S)

2.1 Study Objectives

The objective of this study is to determine the immunologic and overall safety associated with long-term use of Alphanate® in individuals diagnosed with severe hemophilia A (Factor VIII:C less than 0.01 IU/mL), who have been previously treated with plasma-derived Factor VIII products other than Alphanate® and who have no history of developing either antibody inhibitors to Factor VIII or nonspecific inhibitors of coagulation.

2.2 Treatment Comparisons

Alphanate® is the only study treatment being administered, so this is an open label, non-comparator study.

2.3 Study Endpoints

2.3.1 Efficacy

There are two secondary efficacy endpoints in this study:

- The amount of product used per year as part of at-home prophylaxis and therapy for bleeding episodes, and
- A physician's qualitative assessment of hemostasis (evaluated as "None", "Moderate", "Good", or "Excellent" in response to therapy with Alphanate® received by the subject in a hospital or another location under the physician's direct supervision.

2.3.2 Safety

Safety will be evaluated on the basis of numerous assessments. The primary safety assessment will be made on the presence/absence of the development of Factor VIII inhibitors.

Additional secondary safety assessments will be made on:

- The frequency, nature, causality and severity of adverse events,
- The occurrence of changes in biochemical parameters which would indicate renal or hepatic impairment,
- The incidence of seroconversion to human immunodeficiency virus type 1 and 2 (HIV-1/HIV-2), hepatitis A virus (HAV), hepatitis B virus (HBV), hepatitis C virus (HCV) or parvovirus B19 in subjects seronegative for these viruses at the time of enrollment.

3. STUDY DESIGN

3.1 Overall Study Design

This is a phase IV, non-randomized, multi-center study to assess the safety of Alphanate® as the sole source of Factor VIII concentrate for prophylaxis and treatment of all bleeding episodes and surgical procedures for a period of at least two years and a minimum of 50 exposure days, or, if 50 exposure days are not reached, for a maximum of 30 months.

3.2 Overall Schedule of Time and Events

Test	Screening[1]	Enrollment[2]	Seven to 14 days Post Infusion 1	In Hospital and In Clinic Treatments	Every Three Months until Month 30 and/or Off-Study[3]	Unsched
Sign Informed Consent Form	X					
Physical Examination medical & past treatment history, vital signs, height & weight	X					
Abbreviated Physical Exam interim medical history, vital signs, & weight		X			X	X
Hematology Panel CBC with WBC differential and platelet count	X				X	X
Biochemistry Panel ALT, AST, AP, LDH, BUN, total bilirubin, and creatinine	X				X	X
Viral Serology HBsAg, and antibodies to HAV, HBcAb, HBsAb, HCV, HIV1/HIV-2, and parvovirus	X	X[2]			X	X[5]
Parvovirus B19 PCR Assay performed only if seronegative for parvo B19 at enrollment			X			
CD4 Count	X					
Factor VIII:C	X	X[2]			X	X
Factor VIII:C Inhibitor (two sets of samples)	X	X[2]			X[4]	X[6,7]
Collect 2 mL citrated plasma for inhibitor testing			X			
Plasma for inhibitor characterization[7] performed only if subject develops an inhibitor					X[7]	
Serum for storage Draw approx 5 mL of serum and aliquot into three vials of at least 1.5 mL of sample each. Freeze and retain the sample at	X	X			X	X
Patient Diary if the Alphanate® is to be self-administered			X	X	X	X
Physician's Assessment of Hemostasis evaluated as “None,” “Moderate,” “Good,” or “Excellent”				X		

1. Perform at least 72 hours after the most recent administration of any F VIII conc. or any other blood products.
2. These tests are to be performed at enrollment (baseline testing) only if the subject received any Factor VIII, cryoprecipitate, or whole product treatment *after* screening tests were performed.
3. Quarterly testing will be ± 10 days of scheduled testing date. Schedule the visit at least three days after an infusion with Alphanate®.
4. If subject develops an inhibitor, repeat assay at two week intervals until either the inhibitor falls to < 0.6 BU/mL or it is clear that the inhibitor titer will not fall.
5. Perform only if subject exhibits clinical or subjective symptoms indicating possible viral infection.
6. Perform only if a) there is a clinical indication that the normal dosage of Alphanate® is not providing adequate hemostasis or b) surgery.
7. If an inhibitor titer is detected at a titer of greater than or equal to 10 BU/mL, collect a 10 mL sample of citrated plasma. For an inhibitor titer less than 10 BU/mL, collect a 50 mL sample of citrated plasma. If a 50 mL is required, this sample can be obtained in multiple draws within a two-week period.

4. SAMPLE SIZE CONSIDERATIONS

Assuming a maximum biannual rate of inhibitor incidence of three percent in multi-transfused hemophilia patients, the 95% one-sided confidence interval for this incidence rate in a sample of 50 subjects is up to two percent. Thus, with a sample of 50 evaluable subjects monitored for two years, if one subject develops an antibody inhibitor to Factor VIII with a titer higher than 0.6 modified BU/mL, that persists for at least one month, the incidence of inhibitors to Factor VIII will be judged to be higher than that for patients with hemophilia A who are treated with currently marketed Factor VIII concentrates. If the sample size is increased to 80 subjects, no more than 1 subject may develop an antibody inhibitor to Factor VIII.

5. ANALYSIS POPULATIONS

5.1 Safety Population

The safety analysis population consists of all patients who were randomized and received at least one Alphanate® treatment; no treated patients will be excluded from the safety analysis population.

6. CONSIDERATIONS FOR DATA ANALYSIS

6.1 Programming Environment

All analyses will be conducted using SAS® version 9.2.

6.2 Strata and Covariates

There are no planned strata or covariate adjustments planned for in the analysis.

6.3 Subgroups

There are no planned subgroup analyses.

6.4 Multiple Comparisons and Multiplicity

There are no planned adjustments for multiplicity, especially since the analysis is primarily descriptive.

6.5 Significance Level

Unless otherwise noted, all statistical analyses will be conducted with a significance level (α) of 0.05 and utilize two-sided testing.

6.6 Statistical Notation and Methodology

Unless stated otherwise, the term “descriptive statistics” refers to the number of subjects (n), mean, median, standard deviation (SD), minimum, and maximum for continuous data and frequencies and percentages for categorical data. Unless otherwise noted, all data collected during the study will be

included in data listings and will be sorted first by subject number and then by date within each subject number.

7. DATA HANDLING METHODS

7.1 Missing Data

Every effort will be made to obtain required data at each scheduled evaluation from all patients who have been enrolled. There will be no imputation for missing data.

7.2 Visit Windows

Study periods will be windowed as follows:

- Post-Infusion # 1 – 7 to 14 days after infusion
- Quarterly Visits - ± 10 days

7.3 Data Derivations

Baseline values will be considered as the last non-missing assessment prior to first dose of study drug.

On-treatment will be considered as all days between the first dose of study drug and until 1 day post Off-Study.

8. STUDY POPULATION

8.1 Subject Disposition

Subject disposition will be presented for all subjects. Subjects who completed and discontinued from the study will be summarized with descriptive statistics. Reasons for early discontinuation also will be presented with frequencies and percentages for all categories.

8.2 Protocol Deviations

Protocol deviations will be presented in a data listing and include date of violation and description of the event.

8.3 Demographic Characteristics

Demographic and baseline characteristic data will be summarized with descriptive statistics for age and race.

8.4 Medical History

Medical history will be presented in a data listing and include body system, diagnosis and the onset month and year as appropriate.

8.5 Concomitant Medications

Concomitant medications will be summarized with descriptive statistics by drug using the WHO Drug dictionary. Data listings will display all data by preferred term and verbatim name. Prior medications will be excluded from the summary but be included in the listing.

9. STATISTICAL ANALYSIS

9.1 Primary Analysis

Analysis of the primary safety endpoint will be conducted on the Safety Population. The primary safety endpoint, presence/absence of Factor VIII inhibitors, will be summarized with frequencies and percentages.

9.2 Secondary Analyses

There are both efficacy and safety endpoints. All secondary safety analyses will be conducted on the Safety Population.

Secondary efficacy analyses will be summarized with categorical descriptive statistics for the following:

- The amount of product used per year as part of at-home prophylaxis and therapy for bleeding episodes, and
- A physician's qualitative assessment of hemostasis (evaluated as "None", "Moderate", "Good", or "Excellent" in response to therapy with Alphanate® received by the subject in a hospital or another location under the physician's direct supervision).

Secondary safety analyses will be summarized with categorical descriptive statistics for the following:

- The frequency, nature, causality and severity of adverse events,
- The occurrence of changes in biochemical parameters which would indicate renal or hepatic impairment,
- The incidence of seroconversion to human immunodeficiency virus type 1 and 2 (HIV-1/HIV-2), hepatitis A virus (HAV), hepatitis B virus (HBV), hepatitis C virus (HCV) or parvovirus B19 in subjects seronegative for these viruses at the time of enrollment,

9.2.1 Adverse Events

All reported terms (Investigator descriptions) for adverse events (AEs) will be coded using Version 13.0 of the Medical Dictionary for Regulatory Activities (MedDRA). The incidence of treatment-emergent AEs (TEAEs) will be summarized as those events that occur on or after the date of the first dose of study drug.

AE data will be listed individually and summarized by body system organ class (SOC) and preferred terms within system organ class. Each AE will be counted only once for a given subject. If the same AE occurs on multiple occasions, the highest severity and least favorable relationship will be assumed. If two or more AEs are reported as a unit, the individual terms will be reported as separate experiences.

All AEs will be included in the data listings.

9.2.2 Serious Adverse Events and Death

A data listing of all Serious Adverse Events (SAEs), including treatment-emergent SAEs, will be presented. SAEs with onset prior to receiving study medication or after discontinuation of study medication will be excluded from the descriptive summaries.

A separate listing will be presented for all deaths as well as events leading to discontinuation. Complete narratives will be included for deaths.

9.3 Additional Analyses

9.3.1 Exposure to Study Drug

Total exposure through the number of Factor VIII units infused will be summarized with descriptive statistics by dosing. Frequencies and percentages will also be used to summarize the number of infusions at each dosing.

Exposure to study drug will be listed for each subject to indicate the complete usage pattern including number of exposures at home and in clinic, reason for infusion, whether hemostasis was achieved (if evaluation available) and physician comments.

9.3.2 Laboratory Evaluations

Descriptive summaries of quantitative clinical laboratory results will be presented by nominal study visit for change from baseline. Laboratory results will include Factor VIII findings, biochemistry, hematology, immunological status and viral serology.

A data listing will be included that displays all laboratory panels and test values.

9.3.3 Vital Signs

Vital signs will be summarized by nominal study visit and change from baseline.

10. INTERIM AND END-OF-STUDY ANALYSES

10.1 Interim Analysis

No interim efficacy analysis is planned for this study.

10.2 End-of-Study Analysis

A final analysis is planned after the last patient completes or discontinues the study and the resulting clinical database has been cleaned, quality checked, and locked.

11. ATTACHMENTS

11.1 Table of Contents for Data Displays

Study Population

Tables

Listings

Safety

Tables

12.5. Summary of Serious Adverse Events by System Organ Class and Preferred Term
12.6. Summary of Factor VIII: C Data
12.7. Summary of Factor VIII Inhibitor Data
12.8. Summary of Biochemistry Data
12.9. Summary of Hematology Data
12.10. Summary of Immunological Data
12.11. Summary of Viral Serology Data
12.12. Summary of Vital Signs
12.13. Summary of Positive Factor VIII Inhibitor Tests
12.14. Summary of Viral infection

Listings

12.15. Listing of Exposure to Study Drug
12.16. Listing of Adverse Events
12.17. Listing of Serious Adverse Events
12.18. Listing of Deaths
12.19. Listing of Factor VIII Results
12.20. Listing of Abnormal Factor VIII Results
12.21. Listing of Biochemistry Results
12.22. Listing of Abnormal Biochemistry Results
12.23. Listing of Hematology Results
12.24. Listing of Abnormal Hematology Results
12.25. Listing of Immunological Status Results
12.26. Listing of Abnormal Immunological Status Results
12.27. Listing of Viral Serology Results
12.28. Listing of Abnormal Serology Results
12.29. Listing of Inhibitor Monitoring and Viral Infection
12.30. Listing of Vital Signs
12.31. Listing of Physical Exams
12.32. Listing of Abnormal Physical Exams