Official Title:	**A Multicenter Phase 2 Open-Label, Single-Arm, Prospective, Interventional Study of Plasma-Derived Factor VIII/VWF (Alphanate®) in Immune Tolerance Induction Therapy in Subjects with Congenital Hemophilia A**
NCT Number:	NCT03095287
Document Date:	Protocol Version 4.0: 21 March 2019

Clinical Study Protocol

Protocol Title:	A Multicenter Phase 2 Open-Label, Single-Arm, Prospective, Interventional Study of Plasma-Derived Factor VIII/VWF (Alphanate®) in Immune Tolerance Induction Therapy in Subjects with Congenital Hemophilia A
Investigational Product:	Alphanate®
Sponsor's Name and Address:	Grifols Biologicals LLC 5555 Valley Boulevard Los Angeles, CA 90032
Sponsor's Telephone Number:	[redacted] (Medical Monitor)
Study Number/Protocol Version Number/Date:	GBI1406/Version 4.0/21 Mar 2019 Includes Version 3.0/20 Dec 2016, Version 2.0/10 Feb 2016, Version 1.0/13 Nov 2015
EUDRACT Number:	2015-005524-26
IND/CTA Number:	016703
Development Phase:	2

The undersigned confirm that they agree to conduct the study under the conditions described in this protocol:

Medical Monitor:	[redacted], MD
Signature:	[redacted] Date: 28 March 2019

Summary of Changes for Amendment 3

Protocol Version	Date of Approval
4.0 Amendment 3 + Integrated Protocol	21 Mar 2019
3.0 Amendment 2 + Integrated Protocol	20 Dec 2016
2.0 Amendment 1 + Integrated Protocol	10 Feb 2016
1.0 Original	13 Nov 2015

Amendment 3/21 Mar 2019

A Multicenter Phase 2 Open-Label, Single-Arm, Prospective, Interventional Study of Plasma-Derived Factor VIII/VWF (Alphanate®) in Immune Tolerance Induction Therapy in Subjects with Congenital Hemophilia A

The protocol for GBI1406 (Version 3.0, dated 20 Dec 2016) has been amended and reissued as Protocol Amendment 3, Version 4.0, dated 21 Mar 2019. See Appendix 2 for a summary of changes for Amendment 3.

Investigator Signature Page

The undersigned confirms that he/she agrees to conduct the study under the conditions described in this protocol and comply with International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use Good Clinical Practice (ICH GCP) and all applicable regulatory requirements:

_____	_____
INVESTIGATOR NAME (Please Print)	LOCATION
_____	_____
INVESTIGATOR SIGNATURE	DATE

PROTOCOL SYNOPSIS

Title of Study: A Multicenter Phase 2 Open-Label, Single-Arm, Prospective, Interventional Study of Plasma-Derived Factor VIII/VWF (Alphanate®) in Immune Tolerance Induction Therapy in Subjects with Congenital Hemophilia A

Study Number: GBI1406

Phase: 2

Study Objectives:

Primary Efficacy Objectives

- To assess the proportion of subjects who achieve complete immune tolerance within 33 months of initiating Alphanate for immune tolerance induction (ITI)

Secondary Efficacy Objective(s) (if applicable)

- To assess the proportion of subjects who achieve either complete or partial immune tolerance within 33 months of initiating Alphanate for ITI
- To assess the maintenance of complete or partial immune tolerance without relapse for 12 months
- To assess the annualized frequency of bleeding events

Safety Objective

- To assess the safety of Alphanate treatment for ITI

Overall Study Design and Description: This is a multicenter, multinational, prospective, single-arm, nonrandomized, open-label study of approximately 25 male subjects with congenital hemophilia A who will receive their first (primary) ITI treatment with Alphanate. The study will be conducted at approximately 30 study centers.

The study consists of 2 phases:

- A 33-month ITI Treatment Phase in which all eligible subjects will receive ITI treatment with Alphanate for a period of up to 33 months. Upon confirmation of complete immune tolerization, subjects will then enter a 12-month Prophylactic Phase (that includes the protocol-defined dose tapering). If, after 33 months of ITI, a subject has achieved partial immune tolerance, the subject will enter a 12-month Prophylactic Phase (that includes the protocol-defined dose tapering).
- A 12-month Prophylactic Phase for all subjects who meet the criteria for complete or partial success to continue on a prophylactic dosing regimen of Alphanate.

Number of Subjects Planned: 25

Diagnosis and Main Criteria for Inclusion:

Note that any criterion number containing v*X*, was modified in the indicated protocol version *X* (eg, 2v4 indicates that the original criterion #2 was last modified in Protocol Version 4).

Inclusion Criteria:

A subject must meet all of the following inclusion criteria at the time of the Screening/Baseline Visit (as specified below) to be eligible for participation in the study.

1. The subject has a documented diagnosis of severe congenital hemophilia A with factor VIII activity (FVIII:C) <1% of normal.

2v4. The subject is a male <12 years of age at the Baseline Visit.

3v4. The subject's documented historical peak inhibitor titer is ≥5 Bethesda units (BU) and ≤200 BU.

4. The subject has an inhibitor titer >0.6 BU and <10 BU at Screening.
5. The subject has had a delay ≤24 months from the date of diagnosis of the inhibitor to the start of the subject's ITI treatment.
6. The subject has a caregiver willing to participate and comply with requirements of the protocol, including home infusions, blood sampling, and daily diary for the duration of the trial.
7. The subject has provided signed assent, if applicable (per Institutional Review Board or Ethics Committee requirements), and a parent or legal guardian has provided signed informed consent.

Exclusion Criteria:

A subject meeting any of the following exclusion criteria is not eligible for participation in the study:

1. The subject has acquired factor VIII (FVIII) deficiency.
2. The subject has previously received ITI treatment.
3. The subject has a recent (within 1 month) history of central line infection at the time of Screening.
4. The subject has a high risk of cardiovascular, cerebrovascular, or thromboembolic event as judged by the investigator.
5. The subject is currently undergoing treatment with immunosuppressive drugs (eg, systemic corticosteroids), azathioprine, cyclophosphamide, high dose immunoglobulin, interferon, or the use of a protein A column or plasmapheresis and is unwilling to discontinue these treatments starting at the screening visit.
6. The subject has a known infection with human immunodeficiency virus (HIV) or has clinical signs and symptoms consistent with current HIV infection.
7. The subject has a known previous infection with hepatitis B virus (HBV) or hepatitis C virus (HCV) or has clinical signs and symptoms consistent with current HBV or HCV infection.

8. The subject has significant proteinuria, has a history of acute renal failure or severe renal impairment (blood urea nitrogen or creatinine >2 times the upper limit of normal), or is receiving dialysis at Screening.
9. The subject has a value of aspartate transaminase or alanine aminotransferase >2 times the upper limit of normal at Screening.
10. The subject has clinical evidence of any significant acute or chronic disease that, in the opinion of the investigator, may interfere with successful completion of the trial or place the subject at undue medical risk.
11. The subject has a history of anaphylaxis or severe systemic reaction to any plasma-derived or other blood products.
12. The subject has participated in another clinical trial of an investigational product within 30 days prior to Screening—imaging studies without investigative treatments are permitted—or has received any investigational blood product within the previous 3 months.
13. In the opinion of the investigator, the subject or caregiver may have compliance problems with the protocol or the procedures of the protocol.

Investigational Product, Dose and Mode of Administration:

Alphanate is a sterile, lyophilized powder for intravenous injection after reconstitution. Alphanate is to be reconstituted with Sterile Water for Injection (SWFI), United States Pharmacopeia, European Pharmacopeia, or comparable SWFI. The diluent is supplied in a separate vial or prefilled syringe. Instructions for the reconstitution of Alphanate are provided in the Pharmacy Manual/Study Manual.

Subjects will start treatment with Alphanate 100 IU/kg/day with a one-time option to increase to a dosing regimen of 200 IU/kg/day at any time after 90 days of ITI treatment if inhibitor titer has decreased <20% of the level from the initiation of Alphanate ITI treatment ***or*** if there has been an increase in the rate of bleeding events relative to the rate of bleeding events experienced during the first 90 days of treatment ***or*** if the inhibitor titer increases to >500 BU following the initiation of Alphanate ITI treatment. Increased bleeding may be treated with bypass agents; however, the dose of Alphanate may not be increased until after 90 days of ITI treatment. Following this 90-day treatment/observation period, a possible increase in rate of bleeding events relative to the rate during the 90-day observation period will be assessed at each clinic visit, which at any given time will allow for the dosage increase to 200 IU/kg.

The drug is administered by bolus intravenous injection daily until complete immune tolerance is achieved or for 33 months if complete immune tolerance is not achieved.

Complete immune tolerance is defined as the subject achieving all of the following:

- Undetectable inhibitor titer (<0.6 BU); must be confirmed by 2 consecutive assessments performed within approximately 2 weeks of each other
- FVIII:C plasma recovery ≥66% of the predicted normal value

- FVIII:C half-life ≥6 hours after a 72-hour FVIII treatment-free period

Partial immune tolerance is defined as the subject achieving all of the following:

- Reduction of inhibitor titer to <5 BU; must be confirmed by 2 consecutive assessments performed within approximately 2 weeks of each other
- FVIII:C plasma recovery of <66% of the predicted normal value or FVIII:C half-life of <6 hours after a 72-hour FVIII treatment-free period
- Clinical response to FVIII therapy

Treatment failure is defined as:

- Failure to fulfill the criteria for complete or partial immune tolerance within 33 months

Subjects achieving complete immune tolerance will enter a 12-month Prophylactic Phase. Subjects achieving partial immune tolerance after 33 months will also enter the Prophylactic Phase. Treatment failure subjects will be discontinued.

When subjects being treated at 100 IU/kg/day enter the Prophylactic Phase, the dose will be tapered down in a step-wise manner over an 8-week period to reach a final prophylactic dose of 50 IU/kg every other day or 3 times per week, at the investigator's discretion.

When subjects being treated at 200 IU/kg/day enter the Prophylactic Phase, the dose will be tapered down in a step-wise manner over a 12-week period to reach a final prophylactic dose of 50 IU/kg every other day or 3 times per week, at the investigator's discretion.

After completing the taper period, the subject will receive the prophylactic dose regimen of 50 IU/kg every other day or 3 times per week, at the investigator's discretion, for the remainder of the 12-month Prophylactic Phase. Subjects completing the 12-month Prophylactic Phase will have completed the study.

Duration of Treatment:

The duration of a subject's participation could be as long as 46 months for those subjects who achieve partial immune tolerance at the end of the 33-month ITI Treatment Phase and also successfully complete the 12-month Prophylactic Phase. Subjects who attain complete success before the Month 33 Visit may have a shorter study duration.

Reference Therapy, Dose and Mode of Administration: None

Key Study Variables:

Primary Efficacy:

The primary efficacy endpoint is the proportion of subjects achieving complete immune tolerance within 33 months of initiation of ITI treatment.

Secondary Efficacy (if applicable):

- The proportion of subjects who achieve either complete or partial immune tolerance within 33 months of receiving Alphanate for ITI
- The proportion of subjects who maintain complete immune tolerance or partial immune tolerance without relapse for 12 months
- The annualized frequencies of bleeding events during the ITI Treatment Phase and the Prophylactic Phase

Safety:

- Adverse events (AEs), including suspected adverse drug reactions (ADRs), adverse reactions (ARs), serious adverse events (SAEs), and discontinuations due to AEs and SAEs
- Physical examination results
- Vital signs (temperature, respiratory rate, pulse rate, systolic blood pressure, and diastolic blood pressure)
- Clinical laboratory parameters: hematology panel, clinical chemistry panel, and urinalysis
- Bleeding events by severity

Study Assessments and Procedures:

ITI Treatment Phase

Subjects will have study center visits to measure FVIII inhibitor titers at Weeks 2, 4, 6, 8, and monthly thereafter during the ITI Treatment Phase to assess the subject's response to the ITI treatments. Additional unscheduled visits for inhibitor titer measurements may occur during the first 3 months of ITI treatment and throughout the trial as deemed necessary by the investigator. After 2 consecutive undetectable FVIII inhibitor titer measurements have been obtained that are required to qualify the subject as a complete success, the sequential assessment of the FVIII:C in vivo recovery followed by FVIII:C half-life assessment will be performed to confirm that the subject has normal FVIII:C pharmacokinetic parameters.

FVIII inhibitor titer will be measured by the central laboratory using the Nijmegen modification of the Bethesda method for FVIII inhibitor measurements. A negative result will be a value of <0.6 BU. The scheduled inhibitor titer sampling should occur ≥24 hours after the last Alphanate dosing or at the maximum possible interval from the last dose of Alphanate. Upon receipt of a negative inhibitor titer result from the central laboratory, the study center will request the subject/caregiver to return to the study center within approximately 2 weeks after the last assessment for the confirmatory inhibitor titer assessment, along with a simultaneous assessment of FVIII:C in vivo recovery.

For subjects with negative titer levels, if in vivo recovery is <66% of the predicted normal value, the subject will continue ITI treatment, and the site is to assess in vivo recovery monthly until recovery is ≥66% of the predicted normal value. For subjects with negative titer levels, if in vivo recovery is ≥66% of the predicted normal value, the site is to schedule the visit for FVIII:C half-life assessment to occur within approximately 2 weeks, with instructions to caregivers to adhere to a 72-hour FVIII treatment-free period immediately prior to the half-life assessment visit. FVIII:C half-life will be assessed from blood samples collected immediately before a 50 IU/kg dose of Alphanate is administered in the study center, followed by samples at 15 to 30 minutes after Alphanate administration and at 1, 3, 6, 12 (±2), 24 (±4), and 48 (±4) hours after Alphanate administration.

Partial Success Subjects: For subjects with 2 consecutive inhibitor titers <5 BU who are approaching the end of the ITI Treatment Phase (at the Month 33 Visit), the assessment of FVIII:C in vivo recovery and FVIII:C half-life will be performed to obtain FVIII:C pharmacokinetic data.

Prophylactic Phase

The inhibitor titer will be assessed every month for the first 4 months during the Prophylactic Phase and then every 2 months for the remaining 8 months of the Prophylactic Phase to measure FVIII inhibitor titer for relapse and to assess sustainability of immune tolerance. Additional unscheduled visits for inhibitor titer measurements may occur throughout the trial as deemed necessary by the investigator.

Subjects achieving complete immune tolerance and subjects achieving partial immune tolerance at the completion of 33 months of ITI treatment will be monitored for relapse during the 12-month Prophylactic Phase. Inhibitor titer and FVIII:C in vivo recovery will be assayed by the central laboratory at Months 1, 2, 3, 4, 6, 8, 10, and 12 during the Prophylactic Phase. If the inhibitor titer or in vivo recovery indicate the possibility of a recurrence of an inhibitor in a subject with complete immune tolerance, the inhibitor titer assessment and FVIII:C in vivo recovery assessment must be repeated by the central laboratory within approximately 2 weeks to confirm relapse. In a subject with partial immune tolerance, if there is a rise of an inhibitor to ≥5 BU, the inhibitor titer assessment must be repeated along with a FVIII:C in vivo recovery assessment within approximately 2 weeks to confirm relapse by the central laboratory.

Safety assessments: physical examination, hematology, and urinalysis will be assessed at Screening and Baseline, at Months 6, 12, 18, 24, 30, and 33 during the ITI Treatment Phase, at Months 3 (except for physical examination), 6, and 12 during the Prophylactic Phase, and at the Early Withdrawal Visit. Clinical chemistry will be assessed at all of the aforementioned visits except for Baseline. Vital signs will be assessed at all scheduled visits. Samples for viral safety testing will be collected at Screening, Baseline, Week 8, Month 6, Month 15, Month 30, and Month 33 of the ITI Treatment Phase, as well as at the end of the Prophylactic Phase (Month 12) or at the Early Withdrawal Visit. Adverse events and concomitant medications will be assessed at all scheduled visits.

Statistical Methods:

Primary Efficacy Analyses

The proportion of subjects achieving complete immune tolerance receiving Alphanate during the ITI Treatment Phase will be calculated along with the 95% confidence interval (CI). The exact (Clopper-Pearson) method for the binomial proportion will be used to calculate the 95% CI. If the lower bound of the 95% CI is greater than 10%, then it is statistically significant that the proportion of subjects achieving complete immune tolerance receiving Alphanate is greater than the historical spontaneous remission rate of 10%.

Secondary Efficacy Analyses

The proportion of subjects achieving either complete or partial immune tolerance within 33 months of ITI treatment will be summarized with the number, percentage, and 95% CI for the percentage.

Time to achieving inhibitor titer <0.6 BU, time to complete immune tolerance, and time to partial immune tolerance will be summarized using the Kaplan-Meier method.

FVIII inhibitor titer, FVIII:C recovery, and FVIII:C half-life will be summarized with mean, standard deviation, median, and minimum/maximum values by visit.

Frequency of bleeding events will be summarized by severity and annualized for the duration of each phase of the clinical trial. For subjects who achieved complete or partial immune tolerance, a similar analysis will also be performed for the annualized Prophylactic Phase.

Safety Analysis

Physical examination findings will be summarized.

The incidence of AEs, AEs by causality, and AEs by severity will be summarized. Subjects with deaths, SAEs, and AEs leading to premature discontinuation from the study will be listed.

The following AEs of special interest must be reported as related to study drug and will be summarized: central venous access and catheter-related complications for which the device was placed or was used for FVIII administration within the trial, infusion-site reactions, thromboembolic events, and hypersensitivity reactions.

The severity of bleeding events will be summarized.

For all laboratory tests, the Screening and/or Baseline value and the change from Baseline will be summarized for numeric results, and frequency/percentage will be summarized for qualitative results. For those tests with normal ranges, shift tables will be provided. For virus safety test results, a data listing will be provided.

Summary statistics will be provided for the original value and change from baseline values for vital sign parameters.

Determination of Sample Size

The study by Caram et al (22) indicated that among patients with very high maximum inhibitor titers (≥10 BU), the spontaneous remission rate was 3.4% (1 of 29), with the upper limit of the 95% CI being 10%. Considering the various factors in estimating the true

spontaneous remission rate for this population, a 10% spontaneous remission rate is considered a historical control. The Kurth et al publication (3) summarized the retrospective review of Alphanate when used for primary ITI treatment and reported complete success in 3 of 8 primary ITI patients. Using the same criteria for defining "complete success" as those in the current study and an intent-to-treat (ITT) analysis approach, the complete success rate reported in the International Immune Tolerance Study (1) and in the North American Immune Tolerance Registry (14) was in the range of approximately 25 to 40%. Assuming that the rate for achieving immune tolerance is 35% with Alphanate treatment based on these publications, 25 subjects would provide 80% power to demonstrate a statistically significant improvement with a one-sided test at alpha level of 0.025 in an ITT analysis.

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GLOSSARY AND ABBREVIATIONS

ADR	adverse drug reaction
AE	adverse event
ALP	alkaline phosphatase
ALT	alanine aminotransferase
AR	adverse reaction
AST	aspartate transaminase
B19V	parvovirus B19
BU	Bethesda units
BUN	blood urea nitrogen
CI	confidence interval
EC	Ethics Committee
eCRF	electronic case report form
EP	European Pharmacopeia
FDA	Food and Drug Administration
FVIII	factor VIII
FVIII:C	factor VIII activity
HAV	hepatitis A virus
HBV	hepatitis B virus
HCV	hepatitis C virus
HIV	human immunodeficiency virus
ICF	informed consent form
ICH GCP	International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use Good Clinical Practice
IP	investigational product
IRB	Institutional Review Board
ITI	immune tolerance induction
ITT	intent-to-treat
LDH	lactate dehydrogenase
NAT	nucleic acid amplification technology
SAE	serious adverse event
SWFI	Sterile Water for Injection

US	United States
USP	United States Pharmacopeia
VWD	von Willebrand disease
VWF	von Willebrand factor

1 INTRODUCTION

1.1 Background

Alphanate® is an antihemophilic factor/von Willebrand factor (VWF) complex (human) indicated for the control and prevention of bleeding in patients with hemophilia A. Development of an inhibitor, an endogenously produced neutralizing, alloantibody that reacts with and inactivates infused factor VIII (FVIII), represents the most serious complication of treatment for patients with hemophilia A (1-3). Patients more likely to develop an inhibitor are those with null FVIII mutations, large deletions, nonsense mutations, and intron 22 inversions (2). The goal of immune tolerance induction (ITI) is to gradually eliminate the immune response and the production of anti-FVIII antibodies.

VWF is a crucial component of hemostasis and plays a key role in FVIII function, production, and stabilization. Moreover, there are data indicating that VWF competes with the FVIII binding of anti-FVIII antibodies directed against the A3 and C2 domains, which are key binding sites for VWF located in the light chain of FVIII (4,5).

Criteria for a clinically meaningful response to ITI vary among reported studies, but complete ITI success (also referred to as complete immune tolerance) is typically defined as achieving undetectable inhibitor titers and normal pharmacokinetics of infused FVIII.

The available published ITI studies have served to identify which variables influence ITI success, but direct comparison between studies (or registries) is difficult because of the marked differences in products and dosing regimens. These studies also differ widely in patient demographics, data collection methods, and endpoint definitions. Existing clinical data have been primarily observational but deemed sufficient for various independent expert panels to issue ITI management guidelines. These include the European Consensus Panel (6), the International Consensus Panel (7), the United Kingdom Hemophilia Centre Doctors Organization Guidelines (8), and United States (US) Guidelines (9). Although differences exist among panels concerning FVIII dose, supportive care, and treatment duration/discontinuation, considerable consensus has been reached regarding the definition of complete immune tolerance. Additionally, studies have shown that there are various risk factors that increase the risk of failing ITI. These include the initiation of treatment at an age ≥8 years, a historical peak inhibitor titer >200 Bethesda units (BU), a titer at ITI treatment start ≥10 BU, and time between inhibitor detection and ITI ≥2 years (7). Patients who have 3 or more of these factors are considered at high-risk for ITI failure (10). Beginning therapy early after inhibitor diagnosis, preferably when the titer is <10 BU, significantly improves the likelihood of successful development of immune tolerance. However, patients with multiple risk factors have been shown to successfully achieve immune tolerance, albeit at somewhat lower rates (10).

The definition of complete immune tolerance includes achieving nondetectable inhibitors, ≥66% in vivo recovery of FVIII activity (FVIII:C) after FVIII infusion, and ≥6 hours FVIII:C half-life (7). The definition of a partial, clinically meaningful response to ITI typically includes the reduction of inhibitor titer to <5 BU, often with a subnormal in vivo recovery of FVIII:C following FVIII infusion (<66% of the predicted normal value based on

body mass and amount of FVIII infused) and a subnormal FVIII:C half-life (<6 hours). Subjects achieving complete or partial immune tolerance show improved clinical response to FVIII replacement.

In addition, all consensus panels agree that immune tolerance by ITI can be achieved successfully with both recombinant and plasma-derived FVIII concentrates and that the superiority of product type has not yet been established. The switch to a FVIII/VWF product can be considered an option if the response to initial ITI fails with a monoclonal or recombinant FVIII product (6,11). The epitope specificity of the inhibitor and the patient's genetic mutation may also impact whether a VWF-containing product will be more successful in inducing immune tolerance than a recombinant or highly purified product (12).

The International Immune Tolerance Study Group registry (13) and the North American Immune Tolerance Study registry (14) agreed that patients with poor prognostic factors were better tolerized with a high-dose regimen (100 to 200 IU/kg/day); the same unanimity was not reached with respect to low-risk patients. Further, a meta-analysis of both retrospective registries determined that ITI success was not influenced by dose in low-risk patients, defined as patients presenting inhibitor titers <10 BU at the start of ITI and a historical peak titer ≤200 BU (15).

The International Immune Tolerance Study was a randomized clinical trial designed to help determine the appropriate dose in low-risk subjects (1). Severe high-titer inhibitor hemophilia A subjects were randomly assigned to receive either a high-dose FVIII regimen (200 IU/kg/day) or a low-dose FVIII regimen (50 IU/kg 3 times per week). The success rate did not differ between treatment arms; however, low-dose subjects bled more often than did high-dose subjects, and the study was stopped early for futility and safety considerations.

1.2 Clinical Experience with Grifols FVIII/VWF Products and ITI

The first version of this FVIII/VWF concentrate was licensed in the US in 1978. The first formulation to be designated as Alphanate was licensed by the Food and Drug Administration (FDA) in 1994, and the current formulation was approved by the FDA in 1997. Alphanate is approved for the control and prevention of bleeding in patients with congenital hemophilia A (and for the treatment of von Willebrand disease [VWD]).

Alphanate was approved in Italy in 1997 for the treatment and prophylaxis of bleeding in patients with hemophilia A and the prevention and treatment of hemorrhage or surgical bleeding in patients with VWD, followed by the Netherlands in 2001 for the treatment and prophylaxis of bleeding in patients with hemophilia A, and the United Kingdom in 2003 for the treatment and prophylaxis of bleeding in patients with hemophilia A and the prevention and treatment of hemorrhage or surgical bleeding in patients with VWD.

A second Grifols product, Fanhdi®, has been approved in the European Union since 1994 for the treatment of hemophilia A. Both Alphanate and Fanhdi are manufactured by Grifols using very similar processes that include viral inactivation by solvent detergent and heat treatment (80ºC for 72 hours). They contain similar amounts of VWF and have comparable product release specifications. Alphanate and Fanhdi may be considered comparable

products. In addition, in Europe the Summaries of Product Characteristics include information on the use of Alphanate and Fanhdi for ITI.

One retrospective study was performed at 11 US institutions where patients had received Alphanate for ITI (3). Data collection started in 2007 from patients who received ITI between 1997 and 2010. Primary ITI treatment was carried out in 8 patients, and rescue ITI was completed in 25 patients. The dosing regimen was 100 to 200 IU/kg/day for 7 of the primary ITI patients, while one of them received 50 IU/kg/day. All of the primary ITI patients were <16 years of age at the time of inhibitor diagnosis, and the median age at the time of initiation of primary ITI treatment was 41 months. This retrospective study provides the basis for adding prospective, clinical trial data to the existing knowledge base. Similarly, retrospective data were collected in an international (Spain, Italy, and Germany), multicenter, observational study to assess outcomes in patients with moderate to severe hemophilia A who developed inhibitors and were subsequently treated with Fanhdi for primary or rescue ITI (9,16). Adults and children (age <18 years) with moderate to severe congenital hemophilia A (FVIII:C <2%) with inhibitors treated with Fanhdi for primary or rescue ITI were included in the study. Treatment regimens varied widely (38 IU/kg 3 times per week to 300 IU/kg twice a day), although most patients received ≥100 IU/kg/day. All study data were collected via retrospective chart review of patients with known ITI outcomes. A total of 41 patients received primary ITI and 19 patients received rescue ITI. Of the primary ITI patients, 88% achieved complete or partial success, generally following the International Consensus Guidelines definitions for complete and partial success (7). Of the rescue ITI patients, 73% achieved complete or partial success. Median time to complete success was 19 months; median time to partial success was 22 months.

Prospective data for ITI with Fanhdi were obtained in the Rits-Fitnhes study. This study was a prospective, observational, multicenter design conducted in Italy and Spain with financial support from Grifols (17). Subjects were consecutively enrolled from 1999 to 2005. The primary outcome parameters of the Rits-Fitnhes study were success (complete or partial) or failure to induce immune tolerance and time to ITI success. Seventeen subjects (13 primary ITI; 4 rescue ITI) were prospectively enrolled in the study and included adults and children (<18 years) with moderate to severe congenital hemophilia A (FVIII <2%), with high-responding inhibitors and at least one predictive factor of a poor ITI response. Fanhdi was the only FVIII/VWF complex concentrate administered for ITI during the study. Nine subjects (53%) had complete success, 7 (41%) had a partial response, and 1 subject (6%) discontinued early.

Overall, the published retrospective data on Alphanate and the supportive published Fanhdi (retrospective and prospective) data support the current proposed trial to formally evaluate the use of Alphanate in primary ITI subjects.

1.3 Study Rationale

To date, only one randomized, controlled trial in ITI, the International Immune Tolerance Study (1), has been completed. Currently, most supporting data for ITI have come from small cohort studies and international and national ITI registries. Due to the lack of randomized prospective clinical trial data supporting the superiority of any one FVIII product, current

common practice of many clinicians is to attempt to induce tolerance with the product the patient was receiving when the inhibitor was first identified.

The purpose of this prospective, multicenter, open-label, single-arm study is to evaluate the efficacy and safety of a FVIII/VWF product, Alphanate, when used in treating subjects who have developed inhibitors to their current FVIII product.

2 STUDY OBJECTIVES

2.1 Primary Objectives

- To assess the proportion of subjects who achieve complete immune tolerance (as defined in Section 3.5.1.2) within 33 months of initiating Alphanate for ITI
- To assess the safety of Alphanate treatment for ITI

2.2 Secondary Objectives

- To assess the proportion of subjects who achieve either complete or partial immune tolerance within 33 months of initiating Alphanate for ITI
- To assess the maintenance of complete or partial immune tolerance without relapse for 12 months
- To assess the annualized frequency of bleeding events

2.3 [redacted]

- [redacted]
- [redacted]
- [redacted]

3 INVESTIGATIONAL PLAN

3.1 Study Design and Plan

This is a multicenter, multinational, prospective, single-arm, nonrandomized, open-label study of approximately 25 male subjects with congenital hemophilia A who will receive their first (primary) ITI treatment with Alphanate. The study will be conducted at approximately 30 study centers.

Male subjects <12 years of age at the time of the Baseline Visit with severe congenital hemophilia A (FVIII:C levels <1%) who are candidates for ITI and who meet all inclusion criteria (Section 3.2.1.1) and do not meet any exclusion criteria (Section 3.2.1.2) are eligible to participate.

The study consists of 2 phases:

- A 33-month ITI Treatment Phase in which all eligible subjects will receive ITI treatment with Alphanate for a period of up to 33 months. Upon confirmation of complete immune tolerization, subjects will then enter a 12-month Prophylactic Phase (that includes the protocol-defined dose tapering). If, after 33 months of ITI, a subject has achieved partial immune tolerance, the subject will enter a 12-month Prophylactic Phase (that includes the protocol-defined dose tapering).
- A 12-month Prophylactic Phase for all subjects who meet the criteria for complete or partial success to continue on a prophylactic dosing regimen of Alphanate (with a protocol-defined dose tapering).

ITI Treatment Phase up to 33 months: Subjects presenting with an inhibitor titer <10 BU should proceed to the Screening Visit.

The Screening Visit should occur 21 to 30 days before the planned start of ITI treatment to ensure availability of all inclusionary/exclusionary laboratory results prior to the initiation of ITI dosing. Subjects continuing to meet the entrance criteria will enter the ITI Treatment Phase and receive daily doses of Alphanate 100 IU/kg/day for up to 33 months. There is a one-time option to increase the dosing regimen to 200 IU/kg/day. This change in the subject's ITI dosing regimen during the ITI Treatment Phase can occur at any time after 90 days of ITI treatment if there has been less than a 20% decrease in inhibitor titer since the initiation of Alphanate ITI treatment ***or*** if there has been an increase in the rate of bleeding events relative to the rate of bleeding events experienced during the first 90 days of treatment ***or*** if the inhibitor increases to >500 BU following initiation of Alphanate ITI treatment.

To accurately capture the peak inhibitor titer on ITI treatment, subjects will have study center visits at Weeks 2, 4, 6, 8, and monthly thereafter. Additional unscheduled visits for inhibitor titer measurements may occur during the first 3 months of ITI treatment and throughout the trial as deemed necessary by the investigator. Weight will be measured at every study center visit, and dosing will be based on the subject's current weight. Between study center visits when Alphanate will be administered at home by the subject's caregiver, the Alphanate dose will be the same as the dose calculated at the most recent study center visit where weight was measured. Training of caregivers at home may be provided within the first few weeks of initiation of ITI treatment for initial port access and newly implanted devices, upon sponsor approval. Ongoing home health care for IP infusions will be assessed on a case-by-case basis.

Subjects will continue to receive their daily Alphanate dose until the inhibitor assay titer is negative (<0.6 BU) on 2 consecutive assessments and treatment success is confirmed by FVIII:C pharmacokinetic assessments (Section 3.5.1.2), at which time they will enter the 12-month Prophylactic Phase.

All subjects will receive ITI treatment for up to 33 months until they achieve complete immune tolerance (Section 3.5.1.2). Subjects who have achieved partial immune tolerance at the completion of 33 months of ITI treatment will enter the 12-month Prophylactic Phase (that includes the protocol-defined dose tapering). Subjects who do not achieve partial

immune tolerance at the completion of 33 months of ITI treatment will be discontinued as treatment failures.

Prophylactic Phase up to 12 months: Upon confirmation of achieving complete immune tolerance, subjects will enter the 12-month Prophylactic Phase (that includes the protocol-defined dose tapering). Subjects achieving partial immune tolerance at the completion of the 33-month ITI Treatment Phase will enter the 12-month Prophylactic Phase (that includes the protocol-defined dose tapering).

The Prophylactic Phase begins with an 8-week taper period for subjects tolerized with 100 IU/kg/day or with a 12-week taper period for subjects tolerized with 200 IU/kg/day to bring the dose down in a step-wise manner to a prophylactic dose of Alphanate 50 IU/kg every other day or 3 times per week, at the investigator's discretion. During the Prophylactic Phase, subjects will be monitored monthly for the first 4 months and then every 2 months for the remaining 8 months to measure FVIII inhibitor titer and FVIII:C in vivo recovery for relapse. No other immunotherapy will be permitted during this phase. Additional unscheduled visits for inhibitor titer measurements may occur throughout the trial as deemed necessary by the investigator.

For subjects who have attained complete tolerization, relapse in the Prophylactic Phase is defined as:

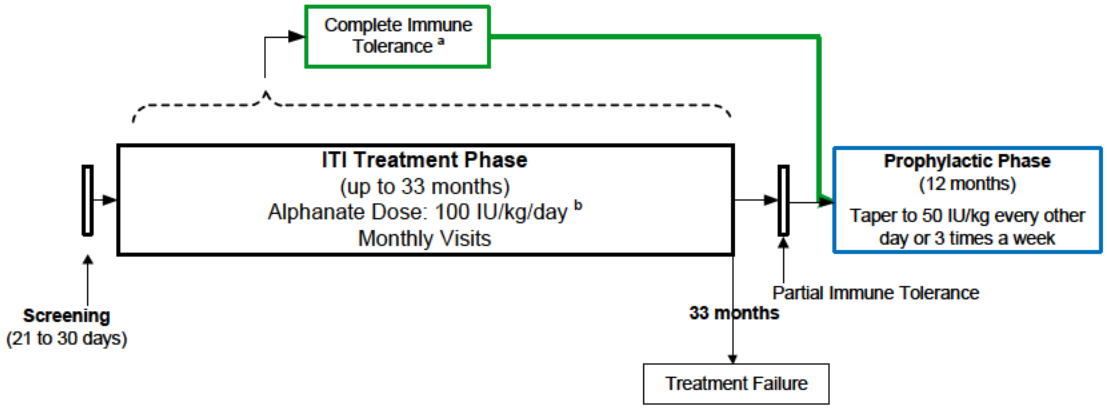
- A return of FVIII inhibitor titer to detectable levels (ie, ≥0.6 BU), FVIII:C recovery <66% of the predicted normal value, or FVIII:C half-life <6 hours, confirmed by repeat assessment within approximately 2 weeks in the Prophylactic Phase

For subjects who have attained partial tolerization, relapse is defined as:

- An increase of FVIII inhibitor titer to ≥5 BU, confirmed by repeat assessment within approximately 2 weeks in the Prophylactic Phase

In the event of a confirmed relapse during the Prophylactic Phase in which there is a concomitant loss of clinical response to FVIII therapy, the subject will be withdrawn from the study.

The duration of a subject's participation could be as long as 46 months for those subjects who achieve partial immune tolerance at the end of the 33-month ITI Treatment Phase and also successfully complete the 12-month Prophylactic Phase. Subjects who attain complete success before the Month 33 Visit may have a shorter study duration. The overall study schema is outlined in Figure 3-1, and the schedule of study procedures is provided in Appendix 1.



[a] Begin assessing for tolerance at the first monthly visit.
[b] After 90 days, dosing regimen can be adjusted once to 200 IU/kg/day if there is <20% decrease in inh bitor titer since the beginning of ITI treatments ***or*** if the rate of bleeding events has increased relative to the rate of bleeding events during the first 90 days of treatment ***or*** if the inhibitor increases to >500 BU.

Figure 3-1 Study Design

3.2 Selection of Study Population

3.2.1 Screening and Baseline Entry Criteria

Subjects who initially fail to meet eligibility criteria at Screening may be rescreened one time. Subjects who fail to meet these eligibility criteria upon rescreening are screen failures and will not be eligible to participate in the study.

Note that any criterion number containing v*X*, was modified in the indicated protocol version *X* (eg, 2v4 indicates that the original criterion #2 was last modified in Protocol Version 4).

3.2.1.1 Inclusion Criteria

A subject must meet all of the following inclusion criteria at the time of the Screening/Baseline Visit (as specified below) to be eligible for participation in the study.

1. The subject has a documented diagnosis of severe congenital hemophilia A with FVIII:C <1% of normal.

2v4. The subject is a male <12 years of age at the Baseline Visit.

3v4. The subject's documented historical peak inhibitor titer is ≥5 BU and ≤200 BU.

4. The subject has an inhibitor titer >0.6 BU and <10 BU at Screening.
5. The subject has had a delay ≤24 months from the date of diagnosis of the inhibitor to the start of the subject's ITI treatment.
6. The subject has a caregiver willing to participate and comply with requirements of the protocol, including home infusions, blood sampling, and daily diary for the duration of the trial.

7. The subject has provided signed assent, if applicable (per Institutional Review Board [IRB] or Ethics Committee [EC] requirements), and a parent or legal guardian has provided signed informed consent.

3.2.1.2 Exclusion Criteria

A subject meeting any of the following exclusion criteria is not eligible for participation in the study:

1. The subject has acquired FVIII deficiency.
2. The subject has previously received ITI treatment.
3. The subject has a recent (within 1 month) history of central line infection at the time of Screening.
4. The subject has a high risk of cardiovascular, cerebrovascular, or thromboembolic event as judged by the investigator.
5. The subject is currently undergoing treatment with immunosuppressive drugs (eg, systemic corticosteroids), azathioprine, cyclophosphamide, high dose immunoglobulin, interferon, or the use of a protein A column or plasmapheresis and is unwilling to discontinue these treatments starting at the screening visit.
6. The subject has a known infection with human immunodeficiency virus (HIV) or has clinical signs and symptoms consistent with current HIV infection.
7. The subject has a known previous infection with hepatitis B virus (HBV) or hepatitis C virus (HCV) or has clinical signs and symptoms consistent with current HBV or HCV infection.
8. The subject has significant proteinuria, has a history of acute renal failure or severe renal impairment (blood urea nitrogen [BUN] or creatinine >2 times the upper limit of normal), or is receiving dialysis at Screening.
9. The subject has a value of aspartate transaminase (AST) or alanine aminotransferase (ALT) >2 times the upper limit of normal at Screening.
10. The subject has clinical evidence of any significant acute or chronic disease that, in the opinion of the investigator, may interfere with successful completion of the trial or place the subject at undue medical risk.
11. The subject has a history of anaphylaxis or severe systemic reaction to any plasma-derived or other blood products.
12. The subject has participated in another clinical trial of an Investigational Product (IP) within 30 days prior to Screening—imaging studies without investigative treatments are permitted—or has received any investigational blood product within the previous 3 months.
13. In the opinion of the investigator, the subject or caregiver may have compliance problems with the protocol or the procedures of the protocol.

3.3 Treatments

3.3.1 Treatments to be Administered

Subjects will start treatment with Alphanate 100 IU/kg/day with a one-time option to increase to a dosing regimen of 200 IU/kg/day at any time after 90 days of ITI treatment if inhibitor titer has decreased <20% of the level from the initiation of Alphanate ITI treatment ***or*** if there has been an increase in the rate of bleeding events relative to the rate of bleeding events experienced during the first 90 days of treatment ***or*** if the inhibitor titer increases to >500 BU following the initiation of Alphanate ITI treatment. Increased bleeding may be treated with bypass agents (Section 3.4); however, the dose of Alphanate may not be increased until after 90 days of ITI treatment. Following this 90-day treatment/ observation period, a possible increase in the rate of bleeding events relative to the rate during the 90-day observation period will be assessed at each clinic visit, which at any given time will allow for the dosage increase to 200 IU/kg.

The drug is administered by bolus intravenous injection daily until complete immune tolerance is achieved (Section 3.5.1.2) or for 33 months if complete immune tolerance is not achieved.

When subjects being treated at 100 IU/kg/day enter the Prophylactic Phase, the dose will be tapered down in a step-wise manner over an 8-week period to reach a final prophylactic dose of 50 IU/kg every other day or 3 times per week, at the investigator's discretion. During this taper period, the subject will receive:

- Alphanate 50 IU/kg/day for the first 4-week period
- Alphanate 50 IU/kg every other day for the second 4-week period

When subjects being treated at 200 IU/kg/day enter the Prophylactic Phase, the dose will be tapered down in a step-wise manner over a 12-week period to reach a final prophylactic dose of 50 IU/kg every other day or 3 times per week, at the investigator's discretion. During this taper period, the subject will receive:

- Alphanate 100 IU/kg/day for the first 4-week period
- Alphanate 50 IU/kg/day for the second 4-week period
- Alphanate 50 IU/kg every other day for the third 4-week period

After completing the taper period, the subject will receive the prophylactic dose regimen of 50 IU/kg every other day or 3 times per week, at the investigator's discretion, for the remainder of the 12-month Prophylactic Phase. Subjects completing the 12-month Prophylactic Phase will have completed the study.

In the event of a confirmed relapse during the Prophylactic Phase in which there is a concomitant loss of clinical response to FVIII therapy, the subject will be withdrawn from the study.

3.3.1.1 Alphanate, Powder for Injection

Alphanate is a sterile, lyophilized powder for intravenous injection after reconstitution. Alphanate is to be reconstituted with Sterile Water for Injection (SWFI), United States Pharmacopeia (USP), European Pharmacopeia (EP), or comparable SWFI. The diluent is supplied in a separate vial or prefilled syringe. Instructions for the reconstitution of Alphanate are provided in the Pharmacy Manual/Study Manual.

3.3.1.2 Labeling of Investigational Product

Grifols Alphanate IP will be labeled according to the requirements of local law and legislation. Label text will be approved according to agreed Grifols procedures, and copies of the labels will be made available to the study center upon request. Alphanate batch numbers will be included on the labels and in the study files.

This open-label study design does not require blinding of study participants or investigative study center personnel to treatment information.

3.3.1.3 Storage of Investigational Product

Alphanate must be stored in a secure area accessible only by study personnel authorized by the investigator, such as the study staff responsible for the preparation and dispensing of IP.

Depending upon the regional location of the investigator, Alphanate will be supplied with diluent either contained in a separate diluent vial or with diluent in a prefilled syringe.

Alphanate supplied with a separate diluent vial is stable for 3 years, up to the expiration date printed on its label, provided that the storage temperature does not exceed 25°C (77°F). Do not freeze.

Alphanate supplied with diluent in a prefilled syringe is stable for 3 years, up to the expiration date printed on its label, provided that the storage temperature does not exceed 30°C (86°F). Do not freeze the diluent.

Investigators, or designees, are responsible for maintaining storage temperature records and for immediately reporting deviations in temperature to the study monitor.

Details for the storage are located in the Pharmacy Manual provided to each study center, and instructions will also be provided to participating subjects.

3.3.1.4 Accountability for Investigational Product

The IP, Alphanate, is to be used only for the study in accordance with the directions given in this protocol. The investigator, or designee such as the study center pharmacist, is responsible for the distribution of the IP in accordance with directions given in the protocol and Pharmacy Manual.

The investigator is responsible for maintaining accurate records of the IP for his/her study center. The IP inventory/dispensing documentation verifying the receipt, dispensing,

destruction, or return must be maintained and kept current by the investigator or designee. The inventory must be made available for inspection by the monitor. The IP supplies must be accounted for by the monitor, and inventory/dispensing logs must be verified by the monitor prior to IP return or destruction. Written documentation of all unused inventory is required. At the end of the study, a copy of the inventory/dispensing log(s) will be retrieved by the monitor and returned to Grifols.

3.3.2 Rationale for Selection of Doses/Timing of Investigational Products in the Study

3.3.2.1 Selection of Doses and Timing of Doses in the Study

Differences continue to exist between national and international panels (European Consensus Panel [6], the International Consensus Panel [7], the United Kingdom Hemophilia Centre Doctors Organization Guidelines [8], and the US Guidelines [9]) concerning FVIII ITI dose and treatment duration/discontinuation. While the International Immune Tolerance Study Group registry and the North America Immune Tolerance Study registry agreed that patients with poor prognostic factors were better tolerized with a high-dose regimen (100 to 200 IU/kg/day), the same unanimity was not reached with respect to low-risk patients. Further, a meta-analysis of both registries determined that ITI success was not influenced by dose in low-risk patients, defined as patients presenting inhibitor titers <10 BU at the start of ITI and a historical peak titer ≤200 BU (15).

The selection of the Alphanate ITI dose and frequency of dosing in this trial is based on a review of reports of previous ITI studies, the dosing regimens evaluated, and the type of patients enrolled (high-risk versus low-risk patients). The International Immune Tolerance Study (1) enrolled subjects with low risk. Complete immune tolerance was achieved in 69.7% of subjects with a dosing regimen of either a high dose (200 IU/kg/day) or a low dose (50 IU/kg 3 times per week) and allowed either recombinant or plasma-derived FVIII. An important outcome of the International Immune Tolerance Study was that the time to achieve a negative inhibitor titer and normal FVIII:C recovery was shorter in the high-dose group than in the low-dose group. Additionally, statistically significantly lower bleeding rates during ITI were observed in the high-dose group. This observation led to the early termination of the study.

A FVIII dose as low as 50 IU/kg 3 times per week was effective in the International Immune Tolerance Study but with higher bleeding rates than with the 200 IU/kg/day regimen, which suggests a dose higher than 50 IU/kg 3 times per week may be more appropriate. For this reason, the dose of 100 IU/kg/day Alphanate has been chosen as the initial primary ITI treatment regimen, with the option to increase the dose to 200 IU/kg/day if the lower dose is not effective or to better control bleeding events. For those subjects entering the Prophylactic Phase, the Alphanate dose will be reduced in a step-wise manner to 50 IU/kg every other day or 3 times per week, at the investigator's discretion.

3.3.2.2 Subject Numbering

Within each study center, subjects in the study will receive a consecutive 7-digit subject number. Subject numbers are assigned beginning with the study center number (3 digits,

assigned by the sponsor) followed consecutively with a unique number for each subject (4 digits, including leading zeros). For example, if the investigator's center number is 301, subject numbers will be 3010001, 3010002, 3010003, etc., in consecutive order. Subject numbers, once assigned, will not be reused at any study center.

3.3.2.3 Randomization

This is a single-arm study with no randomization.

3.3.2.4 Blinding

This is an open-label, single-arm study with no blinding.

3.3.2.5 Treatment Compliance

Subjects/caregivers will be issued a diary to record each dose of Alphanate administered during the study (Section 3.6.2.7). Treatment compliance will be assessed based upon records from the subject's diary. Reasons for a deviation from the administration of less than 100% of the IP dose (see Sections 3.8 and 3.9) must be recorded in the electronic case report form (eCRF) and in the subject's medical records.

3.4 Prior and Concomitant Therapy

Concomitant medications received by the subject during the ITI Treatment Phase and Prophylactic Phase must be recorded in the eCRF, including the trade or generic name of the medication, the dose, the route of administration, the frequency of the dosing, and the date the medication was discontinued. Medications received within 30 days prior to Screening will also be recorded in the eCRF. Vaccinations are to be recorded in the concomitant medication eCRF.

Recombinant factor VIIa, prothrombin complex concentrates, activated prothrombin complex concentrates, emicizumab, and human FVIII (Alphanate) are allowed to be used prophylactically or to treat active bleeding during the ITI Treatment and Prophylactic Phases of the study as directed by the investigator. Alphanate will be the only human FVIII allowed during this study. Use of these types of medications for prophylaxis or to treat a bleeding event should be recorded in the eCRF as concomitant medications for the indication bleeding prophylaxis or for the specific bleeding event. This information will be collected from the time of study entry onwards.

3.4.1 Prohibited Concomitant Medications During the Study

The following medications and treatments are prohibited during the entire study:

- Azathioprine
- Immunosuppressive drugs (ie, cyclophosphamide, rituximab)
- Immunoglobulin
- Treatment with a protein A column or plasmapheresis

- Drugs with immunosuppressive side effects (eg, systemic corticosteroids)

3.5 Study Endpoints

3.5.1 Efficacy Endpoints

3.5.1.1 Primary Efficacy Endpoint

The primary efficacy endpoint is the proportion of subjects achieving complete immune tolerance (Section 3.5.1.2) within 33 months of initiation of ITI treatment.

3.5.1.2 Definition of Complete/Partial Immune Tolerance or Failure

Successful achievement of immune tolerance (complete or partial) and failure of ITI are defined per International Consensus Panel recommendations (7) and are detailed below.

Complete immune tolerance is defined as the subject achieving all of the following:

- Undetectable inhibitor titer (<0.6 BU); must be confirmed by 2 consecutive assessments performed within approximately 2 weeks of each other
- FVIII:C plasma recovery ≥66% of the predicted normal value
- FVIII:C half-life ≥6 hours after a 72-hour FVIII treatment-free period

Partial immune tolerance is defined as the subject achieving all of the following:

- Reduction of inhibitor titer to <5 BU; must be confirmed by 2 consecutive assessments performed within approximately 2 weeks of each other
- FVIII:C plasma recovery of <66% of the predicted normal value or FVIII:C half-life of <6 hours after a 72-hour FVIII treatment-free period
- Clinical response to FVIII therapy

Treatment failure is defined as:

- Failure to fulfill the criteria for complete or partial immune tolerance within 33 months

3.5.1.3 Secondary Efficacy Endpoints

The following are secondary efficacy endpoints:

- The proportion of subjects who achieve either complete or partial immune tolerance within 33 months of receiving Alphanate for ITI
- The proportion of subjects who maintain complete immune tolerance or partial immune tolerance without relapse (Section 3.5.1.4) for 12 months
- The annualized frequencies of bleeding events during the ITI Treatment Phase and the Prophylactic Phase

3.5.1.4 Definition of Relapse

Relapse during the Prophylactic Phase is defined as follows:

- For subjects who have achieved complete immune tolerance, a return of FVIII inhibitor titer to detectable levels (≥0.6 BU) or FVIII:C recovery <66% of the predicted normal value or FVIII:C half-life <6 hours, confirmed by repeat assessment within approximately 2 weeks
- For subjects who have achieved partial immune tolerance, an increase of FVIII inhibitor titer to ≥5 BU, confirmed by repeat assessment within approximately 2 weeks

3.5.1.5 [redacted]

[redacted]

- [redacted]
- [redacted]
- [redacted]

3.5.2 Safety Endpoints

The following safety endpoints will be assessed in this study:

- Adverse events (AEs), including suspected adverse drug reactions (ADRs), adverse reactions (ARs), serious adverse events (SAEs), and discontinuations due to AEs and SAEs. For the subset of local infusion-site reactions where the symptoms/signs lead to infusion interruption or discontinuation, require concomitant medication, or have an impact on the general condition of the subject as judged by the investigator, these will be considered as AEs.
- Physical examination results
- Vital signs (temperature, respiratory rate, pulse rate, systolic blood pressure, and diastolic blood pressure)
- Clinical laboratory parameters: hematology panel, clinical chemistry panel, and urinalysis
- Bleeding events by severity (Section 3.6.2.6)

The following AEs of special interest must be reported as related to study drug:

- Central venous access-related and catheter-related complications for which the device was placed or was used for FVIII administration within the trial (eg, line infections, line thrombosis)
- Infusion site reactions
- Thromboembolic events

- Hypersensitivity reactions, including anaphylaxis and anaphylactic reactions

3.6 Assessments

3.6.1 Assessment Periods

The study consists of 2 phases:

- A 33-month ITI Treatment Phase in which all eligible subjects will receive ITI treatment with Alphanate for a period of up to 33 months. The ITI Treatment Phase begins with a Screening Visit occurring up to 30 days before start of ITI treatment. Upon confirmation of successful immune tolerization, subjects will then enter the Prophylactic Phase (immediately for complete success subjects; after 33 months of ITI for partial success subjects).
- A 12-month Prophylactic Phase for all subjects who meet the criteria for complete or partial success to continue on a prophylactic dosing regimen of Alphanate.

3.6.2 Observations and Measurements

The following is a description of the procedures or assessments to be performed during the study. Refer to the Schedule of Procedures in Appendix 1 and to Section 3.6.4 for the study center visits at which the assessments are performed. During the ITI Treatment Phase, the study center visits occurring after the Baseline Visit should be scheduled at the protocol-specified time relative to the date of the Baseline Visit. During the Prophylactic Phase, the study center visits are to be scheduled relative to the beginning of the Prophylactic Phase. A ±4-day window around each scheduled visit is allowed. Unscheduled visits may be conducted if deemed necessary for the purpose of subject safety.

3.6.2.1 Demography, Baseline Characteristics, and Medical History

Demographic and baseline characteristics of the subject will be collected at the Screening Visit and updated at the Baseline Visit. Baseline data will include date of birth, date of FVIII inhibitor diagnosis, FVIII genotype information, FVIII treatment at the time of inhibitor diagnosis, FVIII products used prior to ITI start, immunosuppressive treatment initiated subsequent to inhibitor diagnosis, bleeding events prior to ITI start (collected from medical records for a period of up to 12 months prior to Screening), peak FVIII inhibitor titer before start of Alphanate ITI (from medical records) and the date of this assessment, and FVIII inhibitor titer at the last measurement prior to start of Alphanate ITI.

For subjects who do not have a documented FVIII genotype, a blood sample should be obtained at the Baseline Visit (or at a later clinic visit if blood volumes at the Baseline Visit are restrictive) and sent to the central laboratory for genotyping analysis.

Medical history will be collected at Screening to determine the subject's eligibility.

3.6.2.2 Physical Examination

A physical examination will be performed at the Screening Visit to determine the subject's eligibility, every 6 months during the ITI Treatment Phase, every 6 months during the Prophylactic Phase, and at the Early Termination Visit. The physical examination will not include a genitourinary examination. Findings will be recorded as normal or abnormal according to the investigator's judgment.

Vital signs (temperature, respiratory rate, pulse rate, and systolic and diastolic blood pressures) will be assessed at all scheduled visits (prior to Alphanate dosing if Alphanate is administered during the visit).

Height will be measured at Screening. Weight will be measured at all scheduled visits.

3.6.2.3 Factor VIII Inhibitor Titer

Factor VIII inhibitor titer will be measured by the central laboratory using the Nijmegen modification of the Bethesda method for FVIII inhibitor measurements. A negative result will be a value of <0.6 BU. The scheduled inhibitor titer sampling should occur ≥24 hours after the last Alphanate dosing or at the maximum possible interval from the last dose of Alphanate. The study center may need to call the subject/caregiver to remind them about this requirement prior to coming in for their regularly scheduled study center visit. Upon receipt of a negative inhibitor titer result from the central laboratory, the study center will request the subject/caregiver to return to the study center within approximately 2 weeks after the last assessment for the confirmatory inhibitor titer assessment, along with a simultaneous assessment of FVIII:C in vivo recovery.

The absence of inhibitor will be confirmed by 2 consecutive negative inhibitor values measured within approximately 2 weeks of each other and normalization of FVIII:C pharmacokinetic parameters.

ITI TREATMENT PHASE

To accurately capture the peak inhibitor titer on ITI treatment, subjects will have study center visits at Weeks 2, 4, 6, 8, and monthly thereafter. Additional unscheduled visits for inhibitor titer measurements may occur during the first 3 months of ITI treatment and throughout the trial as deemed necessary by the investigator.

Complete Success Subjects:

During the ITI Treatment Phase, after 2 consecutive undetectable FVIII inhibitor titer measurements have been obtained that are required to qualify the subject as a complete success, the sequential assessment of the FVIII:C in vivo recovery (which can be obtained at the visit to confirm the negative inhibitor titer) followed by FVIII:C half-life assessment will be performed to confirm that the subject has normal FVIII:C pharmacokinetic parameters.

Partial Success Subjects:

For subjects with 2 consecutive inhibitor titers <5 BU who are approaching the end of the ITI Treatment Phase (at the Month 33 Visit), the assessment of FVIII:C in vivo recovery and FVIII:C half-life will be performed to obtain FVIII:C pharmacokinetic data.

PROPHYLACTIC PHASE

The inhibitor titer will be assessed every month for the first 4 months during the Prophylactic Phase and then every 2 months for the remaining 8 months of the Prophylactic Phase to measure FVIII inhibitor titer for relapse and to assess sustainability of immune tolerance. Additional unscheduled visits for inhibitor titer measurements may occur throughout the trial as deemed necessary by the investigator.

3.6.2.4 Factor VIII:C Pharmacokinetics

Assessment of FVIII:C pharmacokinetics involves an assessment of in vivo recovery and a half-life assessment in order to confirm disappearance of the inhibitor. When the first inhibitor titer result has been received indicating the subject may have attained complete immune tolerance, a second confirmatory inhibitor measurement is to be performed within approximately 2 weeks after the last assessment; at this next visit, a sample will also be collected for measuring FVIII:C in vivo recovery. Upon confirmation of FVIII:C in vivo recovery ≥66% of the predicted normal value, a FVIII:C half-life assessment visit is to be scheduled within the following 2 weeks to occur 72 hours after the last Alphanate dose.

All partial success subjects will also undergo an assessment of FVIII:C pharmacokinetics (assessments of FVIII:C in vivo recovery and a half-life) to begin within approximately 2 weeks of or at their scheduled Month 33 ITI Treatment Phase visit.

GENERAL PROCEDURES

Factor VIII:C in vivo recovery

Factor VIII activity in vivo recovery sampling may occur without a 72-hour FVIII treatment-free period but ≥24 hours after the last FVIII treatment. Sampling for in vivo recovery is performed immediately before in-center dosing with 50 IU/kg Alphanate and is followed by a sample collected 15 to 30 minutes after the Alphanate dosing.

The predicted normal value of FVIII:C in vivo recovery is 2 IU/dL per IU/kg dosed.

For subjects with negative inhibitor titer levels, if in vivo recovery is <66% of the predicted normal value, the subject will continue ITI treatment, and the site is to assess in vivo recovery monthly until recovery is ≥66% of the predicted normal value.

For subjects with negative inhibitor titer levels, if FVIII in vivo recovery is ≥66% of the predicted normal value, the site is to schedule the visit for FVIII:C half-life assessment to occur within approximately 2 weeks, with instructions to caregivers to adhere to a 72-hour FVIII treatment-free period immediately prior to the half-life assessment visit.

Factor VIII:C half-life assessments

A visit to the study center will be scheduled to assess FVIII:C half-life, and the subject/caregiver will be informed to discontinue the daily dose of Alphanate for 72 hours prior to the visit. A reminder telephone call is to be made by the study center staff 4 days prior to the half-life visit date. The staff are also to remind the caregiver regarding the 2 additional study center visits needed (ie, at 24 hours and 48 hours after in-center dosing, respectively) in order to collect the final half-life samples.

Factor VIII activity half-life will be assessed from blood samples collected immediately before a 50 IU/kg dose of Alphanate is administered in the study center, followed by samples at 15 to 30 minutes after Alphanate administration and at 1, 3, 6, 12 (±2), 24 (±4), and 48 (±4) hours after Alphanate administration. Note that 2 additional visits to the study center are required to collect the 24-hour and the 48-hour samples. The subject should not receive additional Alphanate until the final blood samples for FVIII:C half-life have been taken unless treatment is required for a bleeding event. All of the pharmacokinetic samples are to be shipped to the central laboratory.

Subjects should receive their daily dose of Alphanate after all pharmacokinetic blood samples have been taken and will continue to receive their daily dose until it is determined that complete immune tolerance has been achieved. The subject's weight, number of units of FVIII infused, sampling times, and FVIII:C assay results are necessary for FVIII:C half-life calculations.

For subjects with a negative inhibitor titer level and in vivo recovery ≥66% but with FVIII:C half-life <6 hours, the half-life assessment should be repeated approximately every 12 weeks, at the investigator's discretion, until the half-life is ≥6 hours; until then, the subject should continue their ITI dosing regimen.

If the subject's FVIII:C half-life is ≥6 hours (in conjunction with a negative inhibitor titer and in vivo recovery ≥66%), the subject will be considered a complete success and will enter the Prophylactic Phase and begin dose tapering as described in Section 3.3.1.

General procedures pertaining to assessment of FVIII:C pharmacokinetic assessments to monitor for relapse are detailed in Section 3.6.2.5.

3.6.2.5 Monitoring for Relapse

Prophylactic Phase: Subjects achieving complete immune tolerance and subjects achieving partial immune tolerance at the completion of 33 months of ITI treatment will be monitored for relapse during the 12-month Prophylactic Phase. Inhibitor titer and FVIII:C in vivo recovery will be assayed by the central laboratory at Months 1, 2, 3, 4, 6, 8, 10, and 12 during the Prophylactic Phase. If the inhibitor titer or in vivo recovery indicate the possibility of a recurrence of an inhibitor in a subject with complete immune tolerance, the inhibitor titer assessment and FVIII:C in vivo recovery assessment must be repeated by the central laboratory within approximately 2 weeks to confirm relapse. In a subject with partial immune tolerance, if there is a rise of an inhibitor to ≥5 BU, the inhibitor titer assessment must be

repeated along with a FVIII:C in vivo recovery assessment within approximately 2 weeks to confirm relapse by the central laboratory.

Two consecutive tests indicating a return of inhibitor titer, reduced FVIII:C in vivo recovery, or reduced FVIII:C half-life, are required to define a relapse in subjects achieving complete immune tolerance. Two consecutive tests indicating a rise of inhibitor titer to ≥5 BU are required to define a relapse in subjects achieving partial immune tolerance.

In addition, FVIII:C in vivo recovery and half-life will be measured at the completion of the 12-month Prophylactic Phase to confirm that relapse has not occurred in complete success subjects and to obtain pharmacokinetic data in partial success subjects.

3.6.2.6 Bleeding Events

Bleeding events experienced by subjects will be recorded in the eCRF.

- Central nervous system hemorrhage/head trauma, throat and neck hemorrhage, compartment syndrome, and acute gastrointestinal/abdominal hemorrhage will be classified as major bleeding events. Hemoglobin should be monitored along with hemodynamic stability (ie, pulse rate and blood pressure).
- Other bleeding events will be classified as minor bleeding events unless otherwise specified by the investigator or if their treatment requires hospitalization.

TREATMENT OF BLEEDING

Bleeding in patients with hemophilia can occur at different sites, each of which requires specific management.

Recombinant factor VIIa, prothrombin complex concentrates, activated prothrombin complex concentrates, and human FVIII (Alphanate) are allowed to be used to treat active bleeding or prophylactically as directed by the investigator during the entire study. Alphanate will be the only human FVIII allowed during this study. All treatment for bleeding or for bleeding prophylaxis during the study will be recorded in the eCRF.

3.6.2.7 Subject Diary

A diary will be provided to each subject/caregiver at the Baseline Visit. The diary will be used to record details of Alphanate drug administration (date/clock time of start and end of infusion, dose/volume, rate, and treatment interruptions) or other items. The diary may also be used to record days of missed work/school/daily activities due to infections and other complications.

3.6.3 Description of Laboratory Tests and Procedures

Detailed descriptions of laboratory test procedures are located in the study Laboratory Manual. Table 3-1 provides a summary of the laboratory tests conducted for this study.

Table 3-1 Name, Description, and Location of Laboratory Tests and Procedures

Test Panel	Description	Location
Hematology [b]	Hemoglobin, hematocrit, platelets, red blood cell count including red blood cell morphology, white blood cell count with differential, absolute reticulocyte count	Central
Clinical Chemistry [b]	Sodium, potassium, creatinine, chloride, calcium, BUN, bicarbonate, albumin, LDH, AST, ALT, ALP, glucose, total bilirubin, indirect bilirubin	Central
Factor VIII Genotype [a, b]	Identification of specific hemophilia A genetic mutation. Samples will be shipped to a central laboratory and analyses may occur at a specialty laboratory.	Central
Factor VIII Inhibitor [b]	Nijmegen modified Bethesda assay method for inhibitor assay.	Central
Factor VIII:C In Vivo Recovery [b]	Blood collection predose and at 15 to 30 minutes after administration of Alphanate 50 IU/kg.	Central
Factor VIII:C Half-life [b]	Following 72-hour FVIII treatment-free period. Blood collection predose and at 15 to 30 minutes and 1, 3, 6, 12 (±2), 24 (±4), and 48 (±4) hours after administration of Alphanate 50 IU/kg.	Central
Viral Nucleic Amplification Technology [b]	Baseline:[c] HAV RNA, HBV DNA, HCV RNA, HIV-1 RNA, and B19V DNA testing Screening and Postbaseline:[d] Collection of retain samples for HAV RNA, HBV DNA, HCV RNA, HIV-1 RNA, and B19V DNA testing	Central
Viral Serology Testing[b]	Baseline:[c] Hepatitis A antibody differential (IgM/IgG), hepatitis B core antibody differential (IgM/IgG), hepatitis C antibody, HIV-1/-2 + Group O antibody, and B19V antibody differential (IgM/IgG) testing Screening and Postbaseline: [d] Collection of retain samples for hepatitis A antibody differential (IgM/IgG), hepatitis B core antibody differential (IgM/IgG), hepatitis C antibody, HIV-1/-2 + Group O antibody, and B19V antibody differential (IgM/IgG) testing	Central
Urinalysis	Microscopic evaluation is done only with cause. pH, protein, glucose, ketones, bilirubin, nitrites, urobilinogen, blood, leukocyte esterase (with microscopic examination of the urine if abnormal)	Central

[a] For subjects who do not have a documented FVIII genotype, a blood sample should be obtained at the Baseline Visit or at a later clinic visit (if blood volumes at the Baseline Visit are restrictive).

[b] Any potential extra serum/plasma samples (including virus safety retain samples) will be retained by a certified laboratory for up to an additional 5 years after completion of the study for [redacted]

[c] Specimens will be collected prior to Alphanate administration.

[d] Virus safety retain samples will be collected at Screening, Week 8, Month 6, Month 15, Month 30, and Month 33 of the ITI Treatment Phase, as well as at the last visit of the Prophylactic Phase or at the Early Termination visit for virus testing in the event that a subject exhibits clinical signs and/or symptoms of viral infection. For subjects who achieve complete immune tolerance before Month 33, a viral retain sample will be collected at their last visit during the ITI Treatment Phase, before entering the Prophylactic Phase.

Samples collected for laboratory analyses that are non-analyzable due to various factors (ie, lost, quantity not sufficient, laboratory error) or that give a false positive result will need to be recollected by contacting the subject and arranging for resampling.

3.6.3.1 Virus Safety Testing

Blood samples for viral nucleic acid amplification technology (NAT) and viral serology will be collected at Baseline, prior to Alphanate administration, and analyzed for information only. If necessary, in order to minimize total blood volume drawn per day, virus safety samples may be drawn the day prior to the Baseline Visit instead of combining with other baseline blood draws on a single day. Virus safety retain samples will be collected, but only tested if subjects show clinical signs and/or symptoms of viral infection, at the following time points:

- At the Screening Visit
- At the Week 8 Visit of the ITI Treatment Phase
- At the Month 6 Visit of the ITI Treatment Phase
- At the Month 15 Visit of the ITI Treatment Phase
- At the Month 30 Visit of the ITI Treatment Phase
- At the Month 33 Visit of the ITI Treatment Phase (unless a subject achieves complete immune tolerance prior to Month 33, in which case a viral retain sample will be collected at his/her last visit during the ITI Treatment Phase, before entering the Prophylactic Phase)
- At the Early Termination Visit for treatment failures and early withdrawals
- At the last visit of the Prophylactic Phase

Virus safety retain samples will be stored for up to an additional 5 years after all analyses in support of the study are complete. Additional blood samples for viral NAT and viral serology testing may be collected and tested during the study ***only*** if the subject exhibits clinical signs and symptoms consistent with hepatitis A virus (HAV), HBV, HCV, HIV, or parvovirus B19 (B19V) infection while participating in the study.

Any potential extra serum/plasma samples (refer to Table 3-1, footnote 'b') will be stored for up to an additional 5 years after completion of the study for [redacted] No poststudy genetic testing will be performed.

3.6.4 Procedures by Visit

3.6.4.1 Screening Visit (21 to 30 Days Prior to Baseline Visit)

The following procedures and assessments will be performed at the Screening Visit, which is to occur 21 to 30 days prior to initiation of Alphanate ITI treatment:

- Informed consent
- Entrance criteria (Section 3.2.1) to determine subject eligibility
- Medical history/demography
- Prior and concomitant medications

- Vital signs (temperature, pulse rate, respiratory rate, systolic and diastolic blood pressure)
- Height and weight
- Physical examination, excluding genitourinary examination
- Blood and urine samples for clinical laboratory assessments at central laboratory
  - Hematology: Hemoglobin, hematocrit, platelets, red blood cell count including red blood cell morphology, white blood cell count with differential, absolute reticulocyte count
  - Virus safety retain samples
  - Clinical chemistry: sodium, potassium, creatinine, chloride, calcium, BUN, bicarbonate, albumin, lactate dehydrogenase (LDH), AST, ALT, alkaline phosphatase (ALP), glucose, total bilirubin, indirect bilirubin
  - FVIII inhibitor titer blood sample
  - Urinalysis: pH, protein, glucose, ketones, bilirubin, nitrites, urobilinogen, blood, leukocyte esterase (with microscopic examination of urine sediment if abnormal)

Subjects are permitted to be rescreened once. Subjects who fail to meet eligibility criteria upon rescreening are screen failures and will not be eligible to participate in the study.

3.6.4.2 Baseline Visit

The following procedures and assessments will be performed at the Baseline Visit:

- Entrance criteria (Section 3.2.1) to confirm subject eligibility (eg, Screening inhibitor titer is <10 BU)
- Update medical history
- Adverse events
- Prior and concomitant medications
- Vital signs (temperature, pulse rate, respiratory rate, systolic and diastolic blood pressure)
- Weight
- Physical examination, excluding genitourinary examination
- Blood and urine samples for clinical laboratory assessments at central laboratory
  - Hematology: hemoglobin, hematocrit, platelets, red blood cell count including red blood cell morphology, white blood cell count with differential, absolute reticulocyte count
  - Virus safety testing blood samples
    - If necessary, in order to minimize total blood volume drawn per day, virus safety samples may be drawn the day prior to the Baseline Visit instead of combining with other baseline blood draws on a single day. However, these samples ***must*** be obtained prior to first dose of Alphanate.
  - FVIII inhibitor titer blood sample
  - Genotyping sample: only for subjects with no documented FVIII genotyping results available in their medical charts

    - The genotyping sample may be obtained at the next scheduled clinic visit, if needed.
  - Urinalysis: pH, protein, glucose, ketones, bilirubin, nitrites, urobilinogen, blood, leukocyte esterase (with microscopic examination of urine sediment if abnormal)
- Train and document training of subject/caregiver on Alphanate storage requirements, preparation instructions, and self/caregiver-administration of Alphanate.
- Issue diary and train subject/caregiver on its use.
- Administer first Alphanate treatment based on subject's current weight measured in the study center.
- Dispense Alphanate IP sufficient until next scheduled visit.

3.6.4.3 Week 2, Week 4, Week 6, Week 8, and Subsequent Monthly Visits During ITI Treatment Phase

The following procedures and assessments will be performed at scheduled visits at Weeks 2, 4, 6, 8, and monthly thereafter during the ITI Treatment Phase:

- Adverse events
- Concomitant medications
- Vital signs (temperature, pulse rate, respiratory rate, systolic and diastolic blood pressure)
- Weight
- FVIII inhibitor titer blood sample (assayed by central laboratory)
- Virus safety retain samples (Week 8 and Month 15 only)
- Review subject diary, assess treatment compliance, and retrain subject or caregiver as needed.
- Dispense Alphanate IP sufficient until next scheduled visit.

3.6.4.4 Additional Assessments at Months 6, 12, 18, 24, and 30 During ITI Treatment Phase

In addition to the procedures and assessments to be performed during the scheduled semimonthly and monthly visits during the ITI Treatment Phase, the following procedures and assessments will be performed at Months 6, 12, 18, 24, and 30 during the ITI Treatment Phase:

- Physical examination, excluding genitourinary examination
- Blood and urine samples for clinical central laboratory assessments
  - Hematology: hemoglobin, hematocrit, platelets, red blood cell count including red blood cell morphology, white blood cell count with differential, absolute reticulocyte count
  - Virus safety retain samples (Months 6 and 30 only)

- Clinical chemistry: sodium, potassium, creatinine, chloride, calcium, BUN, bicarbonate, albumin, LDH, AST, ALT, ALP, glucose, total bilirubin, indirect bilirubin
- Urinalysis: pH, protein, glucose, ketones, bilirubin, nitrites, urobilinogen, blood, leukocyte esterase (with microscopic examination of urine sediment if abnormal)

3.6.4.5 Factor VIII:C Pharmacokinetic Assessment Visits

When the first inhibitor titer result has been received indicating the subject may have attained complete immune tolerance, a second confirmatory inhibitor measurement is to be performed within approximately 2 weeks after the last assessment; at this next visit, a sample is also collected for measuring FVIII:C in vivo recovery.

At FVIII:C in vivo recovery visit, the following assessments will be performed:

- Adverse events
- Concomitant medications
- Vital signs (temperature, pulse rate, respiratory rate, systolic and diastolic blood pressure)
- Weight
- Review subject diary, assess treatment compliance, and retrain subject or caregiver as needed.
- FVIII inhibitor titer blood sample (assayed by central laboratory)
- FVIII:C in vivo recovery blood samples (assayed by central laboratory) as detailed below:
  - Immediately prior to Alphanate infusion
    - Administer Alphanate 50 IU/kg.
  - 15 to 30 minutes after the completion of the Alphanate infusion

Upon confirmation that FVIII:C in vivo recovery is ≥66% of the predicted normal value, an additional visit will be scheduled to occur within approximately the following 2 weeks to determine FVIII:C half-life (Section 3.6.2.4).

Prior to the FVIII:C half-life assessment visit:

- Call the subject/caregiver to schedule the pharmacokinetic assessment visit and instruct subject/caregiver to stop Alphanate treatments for 72 hours prior to the FVIII:C half-life assessment visit. Also remind the caregiver of the need for 2 additional visits to obtain the 24-hour and the 48-hour pharmacokinetic samples.

At the FVIII:C half-life visit, the following procedures and assessments will be performed:

- Adverse events
- Concomitant medications

- Vital signs (temperature, pulse rate, respiratory rate, systolic and diastolic blood pressure)
- Weight
- Review subject diary, assess treatment compliance, and retrain subject or caregiver as needed.
- FVIII inhibitor titer blood sample immediately before Alphanate infusion (assayed by central laboratory)
- FVIII:C in vivo recovery and half-life blood samples (assayed by central laboratory) as detailed below:
  - Immediately prior to Alphanate infusion
    - Administer Alphanate 50 IU/kg.
  - 15 to 30 minutes after the completion of Alphanate infusion
  - 1 hour after completion of Alphanate infusion
  - 3 hours after completion of Alphanate infusion
  - 6 hours after completion of Alphanate infusion
  - 12 hours (±2 hours) after completion of Alphanate infusion
  - 24 hours (±4 hours) after completion of Alphanate infusion
  - 48 hours (±4 hours) after completion of Alphanate infusion

  Note: Two additional visits to the study center are required to collect the 24-hour sample and the 48-hour sample, and the subject is not to receive additional Alphanate until the 48-hour blood sample has been taken unless treatment is required for a bleeding event.

Subjects should receive their daily dose of Alphanate after all pharmacokinetic blood samples have been taken, and subjects will continue to receive their daily dose until it is determined that complete immune tolerance has been achieved.

Upon receipt of the result of the FVIII:C half-life assessment:

The results of FVIII inhibitor titer, FVIII:C in vivo recovery, and FVIII:C half-life will be provided to the investigator. If the subject's FVIII:C half-life is ≥6 hours (in conjunction with negative inhibitor titers and FVIII:C in vivo recovery ≥66%), the subject is considered a complete success and is to enter the Prophylactic Phase and begin dose tapering as described in Section 3.3.1. If the subject has not met the criteria for complete immune tolerance, the subject will continue ITI treatments up to 33 months until complete immune tolerance is achieved.

In addition, all subjects achieving partial immune tolerance at the completion of 33 months of ITI will have FVIII:C in vivo recovery and half-life assessments. These assessments can begin at the Month 33 Visit or begin at a separate visit scheduled within approximately 2 weeks of the scheduled Month 33 Visit (following a 72-hour FVIII treatment-free period).

3.6.4.6 Month 33 During ITI Treatment Phase

Subjects who are treatment failures at Month 33 should be scheduled for the Early Termination Visit (Section 3.6.4.8) instead of the Month 33 Visit.

For subjects who have achieved partial immune tolerance, the subject will undergo FVIII:C pharmacokinetic assessments at the Month 33 Visit. Alternatively, the pharmacokinetic assessments can be scheduled to begin at a separate visit following the Month 33 Visit. If FVIII:C pharmacokinetic assessments are to begin at the Month 33 Visit, a telephone call is to be made by the study center staff 4 days prior to the visit to instruct the partial success subject/caregiver to stop Alphanate treatments for 72 hours prior to the visit.

The following procedures and assessments will be performed at Month 33 during the ITI Treatment Phase for subjects who have achieved partial immune tolerance:

- Adverse events
- Concomitant medications
- Vital signs (temperature, pulse rate, respiratory rate, systolic and diastolic blood pressure)
- Weight
- Physical examination, excluding genitourinary examination
- Blood and urine samples for clinical central laboratory assessments
  - Hematology: hemoglobin, hematocrit, platelets, red blood cell count including red blood cell morphology, white blood cell count with differential, absolute reticulocyte count
  - Virus safety retain samples
  - Clinical chemistry: sodium, potassium, creatinine, chloride, calcium, BUN, bicarbonate, albumin, LDH, AST, ALT, ALP, glucose, total bilirubin, indirect bilirubin
  - Urinalysis: pH, protein, glucose, ketones, bilirubin, nitrites, urobilinogen, blood, leukocyte esterase (with microscopic examination of urine sediment if abnormal)FVIII inhibitor titer blood sample (assayed by central laboratory)
- Review subject diary, assess treatment compliance, and retrain subject or caregiver as needed.
- Perform FVIII inhibitor titer assessment and begin FVIII:C pharmacokinetic assessments (Section 3.6.4.5) or schedule a separate visit for the FVIII:C pharmacokinetic assessments.
- Remind subject to not receive Alphanate until completion of the pharmacokinetic assessments at 24 hours and 48 hours after in-clinic Alphanate administration.

3.6.4.7 Prophylactic Phase Visits

All subjects who have confirmed complete tolerization and have had FVIII:C pharmacokinetic assessments at the completion of the ITI Treatment Phase will enter the 12-month Prophylactic Phase and continue in this phase through completion. Subjects who have confirmed partial tolerization at the completion of 33 months of ITI treatment and have had FVIII:C pharmacokinetic assessments at the completion of the ITI Treatment Phase will enter the Prophylactic Phase (that includes dose tapering) and continue in this phase through completion. If relapse during the Prophylactic Phase is suspected based on FVIII inhibitor titer or FVIII:C recovery results, confirmatory assessments of FVIII inhibitor titer and

FVIII:C recovery must be performed (assayed by central laboratory) within approximately 2 weeks to confirm relapse (Section 3.6.2.4).

The following procedures and assessments will be performed as detailed below during the Prophylactic Phase:

MONTHS 1, 2, 4, 8, AND 10 VISITS DURING PROPHYLACTIC PHASE

- Adverse events
- Concomitant medications
- Vital signs (temperature, pulse rate, respiratory rate, systolic and diastolic blood pressure)
- Weight
- Review subject diary, assess treatment compliance, and retrain subject or caregiver as needed
- FVIII inhibitor titer blood sample (assayed by central laboratory)
- FVIII:C in vivo recovery blood samples (assayed by central laboratory) as detailed below:
  - Immediately prior to Alphanate infusion
    - Administer Alphanate 50 IU/kg.
  - 15 to 30 minutes after the completion of Alphanate infusion
- Instruct subject and caregiver on tapering the dose regimen (during the taper period only) (Section 3.3.1).
- Dispense Alphanate sufficient until the next scheduled visit.

MONTHS 3 AND 6 VISITS DURING PROPHYLACTIC PHASE

- Adverse events
- Concomitant medications
- Vital signs (temperature, pulse rate, respiratory rate, systolic and diastolic blood pressure)
- Weight
- Physical examination at Month 6 visit only, excluding genitourinary examination
- Review subject diary, assess treatment compliance, and retrain subject or caregiver as needed
- FVIII inhibitor titer prior to in-center Alphanate dosing (assayed by central laboratory)
- FVIII:C in vivo recovery blood samples (assayed by central laboratory) as detailed below:
  - Immediately prior to Alphanate infusion
    - Administer Alphanate 50 IU/kg.
  - 15 to 30 minutes after the completion of Alphanate infusion
- Blood and urine samples for clinical central laboratory assessments

- Hematology: hemoglobin, hematocrit, platelets, red blood cell count including red blood cell morphology, white blood cell count with differential, absolute reticulocyte count
- Clinical chemistry: sodium, potassium, creatinine, chloride, calcium, BUN, bicarbonate, albumin, LDH, AST, ALT, ALP, glucose, total bilirubin, indirect bilirubin
- Urinalysis: pH, protein, glucose, ketones, bilirubin, nitrites, urobilinogen, blood, leukocyte esterase (with microscopic examination of urine sediment if abnormal)

- Dispense Alphanate sufficient until next scheduled visit.

MONTH 12 VISIT DURING PROPHYLACTIC PHASE

For subjects who have maintained complete or partial immune tolerance, this visit will include the pharmacokinetic assessment of FVIII:C recovery and half-life (analyses performed at central laboratory) to confirm absence of relapse and to obtain final FVIII:C pharmacokinetic data. Therefore, all subjects must be instructed to not receive Alphanate for 72 hours prior to the visit.

- Adverse events
- Concomitant medications
- Vital signs (temperature, pulse rate, respiratory rate, systolic and diastolic blood pressure)
- Weight
- Physical examination, excluding genitourinary examination
- Review subject diary and assess compliance.
- Virus safety blood samples and retains
- Blood and urine samples for clinical central laboratory assessments
  - Hematology: hemoglobin, hematocrit, platelets, red blood cell count including red blood cell morphology, white blood cell count with differential, absolute reticulocyte count
  - Clinical chemistry: sodium, potassium, creatinine, chloride, calcium, BUN, bicarbonate, albumin, LDH, AST, ALT, ALP, glucose, total bilirubin, indirect bilirubin
  - Urinalysis: pH, protein, glucose, ketones, bilirubin, nitrites, urobilinogen, blood, leukocyte esterase (with microscopic examination of urine sediment if abnormal
- FVIII inhibitor titer blood sample immediately before Alphanate infusion (assayed by central laboratory)
- FVIII:C in vivo recovery and half-life blood samples (assayed by central laboratory) as detailed below:
  - Immediately prior to Alphanate infusion
    - Administer Alphanate 50 IU/kg
  - 15 to 30 minutes after the completion of Alphanate infusion
  - 1 hour after completion of Alphanate infusion

- 3 hours after completion of Alphanate infusion
- 6 hours after completion of Alphanate infusion
- 12 hours (±2 hours) after completion of Alphanate infusion
- 24 hours (±4 hours) after completion of Alphanate infusion
- 48 hours (±4 hours) after completion of Alphanate infusion

Note: Two additional visits to the study center are required to collect the 24-hour sample and the 48-hour sample, and the subject is not to receive additional Alphanate until the 48-hour blood sample has been taken unless treatment is required for a bleeding event.

- Collect unused Alphanate before sending subject home after in-clinic Alphanate dosing.

3.6.4.8 Early Termination Visit

The following assessments and procedures will be performed for subjects who are withdrawn from the study for treatment failure or subjects who discontinue the study prematurely for any reason:

- Adverse events
- Concomitant medications
- Vital signs
- Weight
- Physical examination, excluding genitourinary examination
- Blood and urine samples for clinical laboratory assessments to be sent to the central laboratory
  - Hematology: hemoglobin, hematocrit, platelets, red blood cell count including red blood cell morphology, white blood cell count with differential, absolute reticulocyte count
  - Clinical chemistry: sodium, potassium, creatinine, chloride, calcium, BUN, bicarbonate, albumin, LDH, AST, ALT, ALP, glucose, total bilirubin, indirect bilirubin
  - FVIII inhibitor titer
  - FVIII:C in vivo recovery (Note: If FVIII:C in vivo recovery is ≥66%, separate visits must be scheduled at which time the assessment of FVIII:C half-life will be performed.)
  - Virus safety retain samples
  - Urinalysis: pH, protein, glucose, ketones, bilirubin, nitrites, urobilinogen, blood, leukocyte esterase (with microscopic examination of urine sediment if abnormal)
- Collect unused Alphanate

3.6.4.9 Unscheduled Visits

Monitoring the safety of the subject by the investigator may require additional unscheduled visits. The sponsor should be consulted prior to performing assessments or procedures

outside the scope of the types of assessments that are performed at scheduled visits (ie, for assessments or procedures other than repeat physical examination, hematology, clinical chemistry, urinalysis, FVIII inhibitor titer, and FVIII:C recovery).

3.7 Screen Failures

Screening evaluations will be used to determine the eligibility of each subject for enrollment. Subjects who fail to meet eligibility criteria at Screening may be rescreened once. Subjects who fail to meet eligibility criteria upon rescreening will be considered screen failures and will not be eligible to participate in the study.

3.8 Interruption of ITI Treatment

Interruptions of ITI treatments should be avoided whenever possible because they could have an adverse effect on the outcome of the ITI. If central venous access is temporarily lost because of line infection, ITI should continue through peripheral veins as long as possible until central venous access can be restored.

3.9 Removal of Subjects

Subjects may withdraw or be withdrawn from the study for the following reasons:

- At their own request or at the request of their legally acceptable representative
- If, in the investigator's opinion, continuation in the study would be detrimental to the subject's well-being
- At the specific request of the sponsor

Also subjects must be withdrawn for the following reasons:

- Subject is determined to be a treatment failure at the completion of 33 months of ITI treatment (Section 3.5.1.2).
- Subject has as a confirmed relapse in the Prophylactic Phase in which there is a concomitant loss of clinical response to FVIII therapy.
- Subject develops an infection with HAV, HBV, HCV, or HIV during the study.
- Subject develops a concomitant disease which, either because of its severity or duration or because it requires a change in treatment, contravenes the condition of the study.
- Subject is noncompliant with the protocol per the investigator's discretion.

In all cases, the reason for withdrawal must be recorded in the eCRF and in the subject's records.

3.10 Premature Termination of Study/Closure of Center

The sponsor, IRB/EC, and regulatory authorities have the right to close this study or a study center, and the investigator/sponsor has the right to close a center at any time, although this should occur only after consultation among involved parties. The IRB/EC must be informed.

Should the study or study center be closed prematurely, all study materials (except documentation that has to remain stored at the study center) must be returned to the sponsor. The investigator will retain all other documents until notification is given by the sponsor for destruction.

A study center can be closed for the following reasons:

- Lack of enrollment
- Noncompliance with the requirements of the study protocol
- Noncompliance with ICH GCP

4 ADVERSE EVENTS

4.1 Warnings and Precautions

For complete information on Alphanate, refer to the Investigator's Brochure.

4.2 Adverse Event Monitoring

Subjects must be carefully monitored for AEs. This monitoring includes clinical and laboratory tests and physical signs. Adverse events should be assessed in terms of their seriousness, severity, and relationship to Alphanate.

4.3 Adverse Event Definitions

4.3.1 Adverse Events

An AE is defined as any untoward medical occurrence in a subject or clinical investigation subject administered a medicinal product or study treatment and which does not necessarily have a causal relationship with this administration. An AE can therefore be any unfavorable and unintended sign (including any abnormal laboratory finding, for example), symptom, or disease temporally associated with the use of a medicinal product, whether or not considered related to the medicinal product.

Any AE that occurs at any time between the time of the signature of the informed consent form (ICF) and the last day of the subject's participation in the clinical trial must be reported and recorded on the AE eCRF entry.

All local infusion-site reactions will be recorded in the eCRF. The subset of local infusion-site reactions where the symptoms/signs lead to infusion interruption or discontinuation, require concomitant medication, or have an impact on the general condition of the subject as judged by the investigator will be considered as AEs.

4.3.2 Suspected Adverse Drug Reactions/Adverse Reactions

All noxious and unintended responses to a medicinal product or study treatment related to any dose should be considered suspected ADRs. The phrase "responses to a medicinal

product" means that a causal relationship between a medicinal product or study treatment and an AE is at least a reasonable possibility, that is, the relationship cannot be ruled out. An AR is defined as a suspected ADR with a causal relationship of "definite."

4.3.3 Causality of Adverse Event

The investigator is required to provide a causality assessment for each AE reported to the sponsor. The sponsor will consider the investigator's causality assessment and also provide its own assessment. Assessment of the causal relationship to the study drug will be made according to the following classifications based on Karch et al (18):

- **Definite**: an event that follows a reasonable temporal sequence from administration of the treatment or in which the treatment level has been established in body fluids or tissues, that follows a known response pattern to the suspected treatment, and that is confirmed by improvement on stopping the treatment (dechallenge) and reappearance of the event on repeated exposure (rechallenge)
- **Probable**: an event that follows a reasonable temporal sequence from administration of the treatment, that follows a known response pattern to the suspected treatment, that is confirmed by dechallenge, and that could not be reasonably explained by the known characteristics of the subject's clinical state
- **Possible**: an event that follows a reasonable temporal sequence from administration of the treatment, that follows a known response pattern to the suspected treatment, but that could have been produced by the subject's clinical state or other modes of therapy administered to the subject
- **Doubtful/Unlikely**: an event that follows a reasonable temporal sequence from administration of the treatment, that does not follow a known response pattern to the suspected treatment, but that could not be reasonably explained by the known characteristics of the subject's clinical state
- **Unrelated**: any event that does not meet the criteria above

The operational tool to decide the AE causal relationship is based on algorithms by Karch et al (19) and Naranjo et al (20).

When an AE is classified assessing causal relationship by the investigator, as "definitive," "probable," "possible," or "doubtful/unlikely," the event will be defined as a suspected ADR. When the causal relationship is labeled "unrelated," then it will be considered that the AE is not imputable to the study treatment and it is not a suspected ADR.

In addition, when a causal relationship between the study treatment and the AE cannot be ruled out by the investigator and/or sponsor, it means that the AE cannot be labeled "unrelated."

For any subject, all AEs that occur at any time from the beginning of Alphanate administration until the final visit of the clinical trial will be considered as treatment-emergent AEs.

4.3.4 Severity of Adverse Event or Suspected Adverse Drug Reaction

Adverse events and suspected ADRs will be classified depending on their severity according to the following definitions:

- **Mild**: an AE which is well tolerated by the subject, causing minimum degree of malaise and without affecting normal activities
- **Moderate**: an AE that interferes with the subject's normal activities
- **Severe**: an AE that prevents the subject from performing their normal activities

Adverse event and suspected ADR severity gradation must be distinguished from AE and suspected ADR seriousness gradation, which is defined according to event consequence. For example, headache can be mild, moderate, or severe but not necessarily serious in all these cases.

The investigator will be responsible for assessing the AE and suspected ADR intensity during the clinical trial, taking into account current criteria included in this section.

4.3.5 Expectedness of Adverse Event or Suspected Adverse Drug Reaction

An AE or suspected ADR is considered "unexpected" if the nature, seriousness, severity, or outcome of the reaction(s) is not consistent with the reference information. The expectedness shall be determined by the sponsor according to the reference document (ie, the Investigator's Brochure) for any serious ADRs (potentially related SAEs) for expedited safety reporting purposes.

4.3.6 Seriousness of Adverse Event or Suspected Adverse Drug Reaction; Serious Adverse Event

An AE or suspected ADR is considered "serious" if, in the view of either the investigator or sponsor, it results in any of the following outcomes:

- Death
- Life-threatening AE
  (Life-threatening in the definition of "serious" refers to an event in which the subject was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe.)
- In-patient hospitalization (when a patient stays more than 24 hours in hospital) or prolongation of existing hospitalization
- A persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions
- A congenital anomaly/birth defect
- An important medical event
  (Important medical event in the definition of "serious" refers to those events which may not be immediately life-threatening or result in death or hospitalization but from medical

and scientific judgment may jeopardize the subject or may require medical or surgical intervention to prevent one of the other outcomes listed above.)

Only hospital stays of at least 24 hours in duration are to be considered hospitalizations in the definition of an SAE. The following hospitalizations do not qualify an AE as an SAE:

- Hospitalization or prolongation of hospitalization needed for procedures required by the clinical trial protocol.
- Hospitalization or prolongation of hospitalization as part of a routine procedure followed by the center.
- Hospitalization for a survey visit, annual physicals, or social reasons.
- Elective or preplanned hospitalizations for a pre-existing condition that had not worsened from Baseline (eg, elective or scheduled surgery arranged prior to start of the study).
- Admissions not associated with an AE (eg, social hospitalization for purposes of respite care).

This definition permits either the sponsor or the investigator to decide whether an event is "serious." If either the sponsor or the investigator believes that the event is serious, the event must be considered "serious" and evaluated by the sponsor for expedited reporting.

A distinction should be drawn between serious and severe AEs. The term "severe" is used to describe the intensity (severity) of a specific event; the event itself, however, may be of relative minor medical significance (such as severe headache). This is not the same as "serious," which is defined on subject/event outcome or action criteria usually associated with events that pose a threat to a subject's life or functioning. Seriousness (not severity) is a medical term while severity is a subjective term.

4.3.7 Adverse Event Documentation

All AEs and SAEs occurring after the subject has signed the ICF through the final visit (ie, end of study) must be fully recorded in the subject's eCRF and SAE form (if serious) as well as in the medical record. If no AE has occurred during the study period, this should also be indicated in the eCRF.

It is the responsibility of the investigator to ensure that AEs are appropriately recorded.

At each visit, AEs will be elicited by asking the individual a nonleading question such as, "Do you feel different in any way since the last visit?" Moreover, AEs will also be collected through directly observed events or spontaneously volunteered by the subject. Clearly related signs, symptoms, and abnormal diagnostic procedures should preferably be grouped together and recorded as a single diagnosis or syndrome wherever possible.

The following variables must be recorded on the AE eCRF entry:

- The verbatim term (a diagnosis is preferred)
- Date/time of onset

- Date/time of resolution
- Severity (mild, moderate, severe)
- Causality (unrelated, doubtful/unlikely, possible, probable, definite)
  - Causality assessment will be made only when the AE occurs after the subject has initiated at least one administration of Alphanate. An AE occurring before the subject's exposure to Alphanate will be always labeled as "unrelated."
- Seriousness (yes, no)
- Action taken (with regard to Alphanate)
- Other action (to treat the event)
- Outcome and sequelae (follow-up on AE)

For AEs that occur during Alphanate administration, the infusion rate in effect at the time of onset of the AE, the time of onset of the AE, and the time of AE change materially in intensity and/or resolve will be captured in the eCRF entry.

In addition to the investigator's own description of the AEs, each AE will be coded according to the Medical Dictionary for Regulatory Activities (MedDRA).

For example, a laboratory test abnormality considered clinically relevant, eg, causing the subject to withdraw from the study, requiring treatment or causing apparent clinical manifestations, or judged relevant by the investigator, should be reported as an AE. Each event must be adequately supported by documentation as it appears in the subject's medical or case file.

4.3.8 Events of Special Interest

The following AEs during ITI with Alphanate are of special interest, will be intentionally solicited from the subject or subject's caregiver, and will be reported as related to study drug:

- Central venous access and catheter-related complications for which the device was placed or was used for FVIII administration within the trial (eg, line infections, line thrombosis)
- Infusion-site reactions
- Thromboembolic events
- Hypersensitivity reactions, including anaphylaxis and anaphylactoid reactions

4.3.9 Type and Duration of the Follow-Up of Subjects After Adverse Events

Insofar as is possible, all individuals will be followed up until the AE or suspected ADR has been resolved. If an AE/suspected ADR/SAE is present when the subject has completed the study, the course of the event must be followed until the final outcome is known or the event has been stabilized, no further change is expected, and the investigator decides that no further follow-up is necessary.

4.4 Reporting of Serious Adverse Events

4.4.1 Reporting Serious Adverse Event

Any SAE (see Section 4.3.6) that occurs after signing the study ICF through the final visit (ie, end of study) must be expeditiously reported whether or not considered attributable to the study drug. Each SAE must be fully recorded in the subject's eCRF and SAE Report Form.

Serious adverse events will be reported using the designated SAE Report Form. When the investigator becomes aware of an SAE, she/he must submit a completed, signed, and dated SAE Report Form (in English) ***within 24 hours*** to the sponsor by email/fax. The date of this SAE discovery by the site staff should be documented in the source documents (ie, medical records).

Each SAE must be followed up until resolution or stabilization. After the initial report, all relevant information for SAE follow up, and for the outcome, must also be supplied to the sponsor in a timely manner (within 3 days from its identification or within 24 hours for relevant new information) by means of the SAE Report Form. In addition, the sponsor or contract research organization may request additional information and/or reports.

All SAE Report Forms must be reported by email to:

Grifols Global Pharmacovigilance for Reporting SAEs
Email: [redacted]
FAX (back-up only): [redacted] (US)
[redacted] (International)

5 STATISTICAL METHODS AND DETERMINATION OF SAMPLE SIZE

5.1 Statistical and Analytical Plans

Data handling and evaluation procedures will be described in the Statistical Analysis Plan.

5.1.1 Subject Populations for Analysis

The subject populations for analysis include the following:

- The Safety Population includes all subjects who receive any amount of Alphanate.
- The Treated Population includes all subjects who are enrolled and treated for ITI with at least 1 dose of Alphanate. In this study, the Treated Population is the same as the Safety Population; however, for efficacy analyses, the term "Treated Population" will be used.
- The Efficacy Evaluable Population includes all subjects who were determined to have achieved complete immune tolerance, to have achieved partial immune tolerance, or to be

treatment failures based on meeting the protocol-specified definitions of these categories of response.

5.1.2 Demographic and Baseline Characteristics

The demographic and baseline characteristics will be summarized based on the Safety Population. For quantitative variables, mean, standard deviation, median, and minimum/maximum values will be provided. For qualitative variables, the frequency and percentage will be provided.

5.1.3 Efficacy Analyses

Efficacy data and analyses will be performed on the Treated Population. As a sensitivity analysis, the analysis of the primary efficacy endpoint will also be performed on the Efficacy Evaluable Population.

5.1.3.1 Analysis of Primary Efficacy Endpoint

The primary efficacy endpoint is the proportion of subjects achieving complete immune tolerance during the ITI Treatment Phase and will be compared to the historical spontaneous remission rate of 10%.

The proportion of subjects achieving complete immune tolerance receiving Alphanate during the ITI Treatment Phase will be calculated along with the 95% confidence interval (CI). The exact (Clopper-Pearson) method for the binomial proportion will be used to calculate the 95% CI (21). If the lower bound of the 95% CI is greater than 10%, then it is statistically significant that the proportion of subjects achieving complete immune tolerance receiving Alphanate is greater than the historical spontaneous remission rate of 10%.

5.1.3.2 Analysis of Secondary Efficacy Endpoints

The proportion of subjects achieving either complete or partial immune tolerance within 33 months of ITI treatment will be summarized with the number, percentage, and 95% CI for the percentage.

Duration of maintenance of complete and partial immune tolerance will be summarized with mean, standard deviation, median, and minimum/maximum values.

Time to achieving inhibitor titer <0.6 BU, time to complete immune tolerance, and time to partial immune tolerance will be summarized using the Kaplan-Meier method.

Factor VIII inhibitor titer, FVIII:C recovery, and FVIII:C half-life will be summarized with mean, standard deviation, median, and minimum/maximum values by visit.

Frequency of bleeding events will be summarized by severity and annualized for the duration of each phase of the clinical trial. For subjects who achieved complete or partial immune tolerance, a similar analysis will also be performed for the annualized Prophylactic Phase.

5.1.3.3 [redacted]

[redacted]

[redacted]

[redacted]

5.1.4 Safety Analyses

Safety analyses will be performed on the Safety Population.

Physical examination findings will be summarized.

The incidence of AEs, AEs by causality, AEs by severity, ARs, and suspected ADRs will be summarized. Subjects with deaths, SAEs, and AEs leading to premature discontinuation from the study will be listed.

The incidence of AEs of special interest (ie, central venous access and catheter-related complications for which the device was placed or was used for FVIII administration within the trial, infusion-site reactions, thromboembolic events, and hypersensitivity reactions) will be reported as related to study drug and will be summarized.

The severity of bleeding events will be summarized.

For all laboratory tests, the Screening and/or Baseline value and the change from Baseline will be summarized for numeric results, and frequency/percentage will be summarized for qualitative results. For those tests with normal ranges, shift tables will be provided. For virus safety test results, a data listing will be provided.

Summary statistics will be provided for the original value and change from baseline values for vital sign parameters.

5.2 Determination of Sample Size

The study by Caram et al (22) indicated that among patients with very high maximum inhibitor titers (≥10 BU), the spontaneous remission rate was 3.4% (1 of 29), with the upper limit of the 95% CI being 10%. Considering the various factors in estimating the true spontaneous remission rate for this population, a 10% spontaneous remission rate is considered a historical control. The Kurth et al publication (3) summarized the retrospective

review of Alphanate when used for primary ITI treatment and reported complete success in 3 of 8 primary ITI patients. Using the same criteria for defining "complete success" as those in the current study and a Treated Population analysis approach, the complete success rate reported in the International Immune Tolerance Study (1) and in the North American Immune Tolerance Registry (14) was in the range of approximately 25 to 40%. Assuming that the rate for achieving immune tolerance is 35% with Alphanate treatment based on these publications, 25 subjects would provide 80% power to demonstrate a statistically significant improvement with a one-sided test at alpha level of 0.025 in a Treated Population analysis.

6 ADMINISTRATIVE

6.1 Investigators, Other Study Personnel, and External Committees

Information regarding additional key personnel involved in the conduct of the study, including names and contact details of participating investigators, monitors, clinical laboratories, technical departments and/or institutions, as well as information on members of additional study committees, will be found in the study files of the sponsor and at the investigator study center within the study reference manual/file.

Investigators and staff will receive training via an investigators' meeting, study center initiation visit or other appropriate individual study center training session(s).

6.2 Data Quality

Monitoring and auditing procedures defined/agreed by the sponsor will be followed to comply with ICH GCP guidelines. Each study center will be visited at regular intervals by a monitor to ensure compliance with the study protocol, ICH GCP and legal aspects. The on-site verification of the eCRF for completeness and clarity will include cross checking with source documents and clarification of administrative matters. Query verification of data will be described in the Data Management Plan.

6.3 Documentation

The study data will be recorded and kept current in the eCRF by the study center personnel directly responsible for the information. Entries made in the eCRF must be verifiable against source documents or have been directly entered into the eCRF, in which case the entry in the eCRF will be considered the source data.

The data in the eCRF will be monitored at the study center by Grifols representatives at regular intervals, reviewed for completeness, and compared with the source documents. Examples of source documents include individual subject medical records, which are separate from the eCRFs.

All AEs and SAEs must be recorded. All SAEs must be recorded on the SAE form. The SAE form must be kept in study center records with a copy provided to the designated person as detailed in the study file.

6.3.1 Record Retention

At study completion, all study data will be transferred to Grifols according to ICH GCP guidelines, local laws, regulations, and Grifols requirements. The study file and all source data should be retained until notification is given by the sponsor for destruction.

An investigator is required by ICH GCP guidelines to retain the study files. If an investigator moves, withdraws from an investigation, or retires, the responsibility for maintaining the records may be transferred to another person (eg, other investigator). Grifols must be notified in writing of the person responsible for record retention and the notification will be retained in the sponsor study file and the investigator study center file.

6.3.2 Access to Information for Monitoring

The data will be recorded and kept current in the eCRFs by the center personnel directly responsible for the information and reviewed for completeness by the monitor. Grifols personnel or designee can review the records.

In accordance with ICH GCP guidelines, the monitor must have direct access to the investigator's source documentation to verify the data recorded in the eCRFs for completeness, consistency, and accuracy and to verify adherence to the protocol. "Source documentation" includes individual subject files, separate from the eCRFs. Source documentation should be maintained and include visit dates, laboratory results, concomitant treatments, vital signs, medical history, examinations, AEs, IP dispensing logs, and other notes as appropriate. The investigator agrees to cooperate with the monitor to ensure that any problems noted during the course of these monitoring visits are resolved.

6.3.3 Access to Information for Audits or Inspections

Representatives of regulatory authorities or of Grifols may conduct audits or inspections or audits of the investigator's study center. If the investigator is notified of an audit or inspection by a regulatory authority, the investigator agrees to notify the Grifols Medical Monitor immediately. The investigator agrees to provide to representatives of a regulatory agency or Grifols access to records, facilities, and personnel for the effective conduct of an audit or inspection.

7 ETHICAL AND LEGAL ASPECTS

7.1 Institutional Review Board/Ethics Committee

Documented approval from the appropriate IRB/EC will be obtained for each participating center/country prior to study start at that center or in that country, according to ICH GCP guidelines, local laws, regulations, and organizations. When necessary, an extension, amendment or renewal of the IRB/EC approval must be obtained and also forwarded to the sponsor. The IRB/EC must supply to the sponsor, upon request, a list of the IRB/EC members involved in the vote and a statement to confirm that the IRB/EC is organized and operates according to ICH GCP guidelines and applicable laws and regulations.

7.2 Ethical Conduct of the Study

The procedures set out in this protocol pertaining to the conduct, evaluation, and documentation of this study are designed to ensure that the sponsor and investigator abide by ICH GCP guidelines. The study will also be carried out in keeping with applicable local law(s) and regulation(s). This may include an audit by the sponsor representatives and/or an inspection by regulatory authority representatives at any time. The investigator must agree to the audit or inspection of study-related records by the sponsor representatives and/or regulatory authority representatives and must allow direct access to source documents to the sponsor and/or regulatory authority representatives.

Modifications to the study protocol will not be implemented by either the sponsor or the investigator without agreement by both parties. However, the investigator may implement a deviation from, or a change to, the protocol to eliminate an immediate hazard(s) to the study subjects without prior IRB/EC/sponsor approval/favorable opinion. As soon as possible, the implemented deviation or change, the reasons for it, and, if appropriate, the proposed protocol amendment should be submitted to the IRB/EC/sponsor. Any deviations from the protocol must be fully explained and documented by the investigator.

7.3 Regulatory Authority Approvals/Authorizations

Regulatory authority approvals/authorizations/notifications, where required, must be in place and fully documented prior to study start. Study information including contact information for investigator study centers responsible for conducting the study will be posted on a publicly accessible clinical registry(ies) as required by local law.

7.4 Subject Information and Consent

Subject information/ICF will be provided to investigator study centers. Prior to the beginning of the study, the investigator must have the IRB/EC written approval/favorable opinion of the written ICF and any other written information to be provided to subjects. The written approval of the IRB/EC, together with the approved subject information/ICF, must be filed in the study files, and a copy of the documents must also be provided to the sponsor by the investigator study center.

Written informed consent must be obtained before any study specific procedure takes place. Participation in the study and date of informed consent given by the subject should be documented appropriately in the subject's files. A signed copy of the subject ICF will be provided to the subject or subject's authorized representative.

7.5 Insurance

All subjects participating in the study will have insurance coverage by the sponsor, which is in line with applicable laws and/or regulations.

7.6 Confidentiality

All records identifying the subject will be kept confidential and, to the extent permitted by the applicable laws and/or regulations, will not be made publicly available.

Subject names will not be supplied to the sponsor. Only the subject number will be recorded in the eCRF, and if the subject's name appears on any other document (eg, pathologist report), it must be obliterated before a copy of the document is supplied to the sponsor. Study findings stored on a computer will be stored in accordance with local data protection laws. Subjects will be informed in writing that representatives of the sponsor, IRB/EC, or regulatory authorities may inspect their medical records to verify the information collected and that all personal information made available for an audit or inspection will be handled in strictest confidence and in accordance with local data protection laws.

If the results of the study are published, the subject's identity will remain confidential.

The investigator will maintain a list to enable subjects' records to be identified.

8 USE OF DATA AND PUBLICATION

The sponsor is committed to honoring the principles of academic freedom while, at the same time, protecting its confidential information, the subjects, the integrity of the study, and the study documentation in compliance with applicable law. The institution and/or the investigator recognize that, with respect to any study that is part of a multicenter study, there is a need for a coordinated approach to any publication or presentation of results from the study centers. Accordingly, the institution/investigator shall not publish or present any results from this study to any third parties until: (1) the sponsor publishes the results, (2) the institution and/or the investigator receives written notification from the sponsor that publication of the results is no longer planned, or (3) 12 months following the close of study, whichever occurs first.

The institution and/or the investigator shall submit to the sponsor for its review a copy of any proposed publication at least 30 calendar days prior to the planned date of submission for publication or presentation. The institution and the investigator shall consider in good faith all comments received from the sponsor during the review period and shall delete the sponsor's confidential information (other than study results).

If the sponsor determines that the publication contains patentable subject matter which requires protection, the sponsor may require the delay of submission for publication or presentation for an additional period of time for the purpose of filing patent applications or otherwise take measures to protect such information.

The institution and/or the investigator shall acknowledge the sponsor's support in all publications and presentations.

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10 APPENDICES

Appendix 1 Schedule of Procedures

Procedures/Assessments	Screening Visit	ITI Treatment Phase						Prophylactic Phase			Early Withdrawal
		Baseline Visit	Weeks 2, 4, 6, 8, and Then Monthly After ITI Start	Months 6, 12, 18, 24, and 30 After ITI Start	Pharmacokinetic Assessment Visits		Month 33 After ITI Start Pharmacokinetic Visit [a]	Months 1, 2, 4, 8, and 10	Months 3 and 6	Month 12	
	Day –30 to –21 From ITI Start	Day of ITI Start			FVIII:C in vivo Recovery Visit	FVIII:C Half-life Visit [a]					
Informed consent	X										
Entrance criteria	X	X									
Medical history/demography [b]	X	X									
Adverse events		X	X	X	X	X	X	X	X	X	X
Prior [c]/concomitant medications	X	X	X	X	X	X	X	X	X	X	X
Vital signs	X	X	X	X	X	X	X	X	X	X	X
Height	X										
Weight	X	X	X	X	X	X	X	X	X	X	X
Physical examination	X	X		X			X		X [d]	X	X
Hematology	X	X		X			X		X	X	X
Clinical chemistry	X			X			X		X	X	X
FVIII genotype sample		X [e]									
FVIII inhibitor titer sample [f]	X	X	X [g]	X	X	X	X	X	X	X	X
Virus safety testing samples	X [h]	X [h,i]	X [h]	X [h]			X [h]			X [h]	X [h]
Urinalysis	X	X		X			X		X	X	X
Issue/review diary and retrain as needed [j]		X	X	X	X	X	X	X	X	X	
Administer Alphanate in the study center		X			X	X	X	X	X	X	
FVIII:C in vivo recovery [k]					X	X	X	X [l]	X [l]	X	X
FVIII:C half-life [m]						X	X			X	X[n]
Collect unused Alphanate										X	X
Dispense Alphanate		X	X	X			X	X [o]	X [o]		

[a] This visit will be scheduled within approximately 2 weeks following the confirmation of FVIII:C in vivo recovery ≥66% of the predicted normal value. A reminder telephone call is to be made by the study center staff 4 days prior to the half-life visit date. The staff are also to remind the caregiver regarding the 2 additional study center visits needed (ie, at 24 hours and 48 hours, respectively) in order to collect half-life samples.

[b] Up to last 12 months of bleeding history will be captured.

[c] Up to last 12 months of treatments for bleeding events will be captured.

[d] Physical examination is only performed at Month 6 of the Prophylactic Phase.

[e] For subjects who do not have a documented FVIII genotype, a blood sample should be obtained at the Baseline Visit, or later clinic visit (if blood volumes at the Baseline visit are restrictive), and sent to the central laboratory for genotyping analysis.

[f] The scheduled inhibitor titer sample should occur ≥24 hours after the last Alphanate dosing or at the maximum possible interval from the last dose of Alphanate.

[g] To accurately capture the peak inhibitor titer on ITI treatment, additional unscheduled visits for inhibitor titer measurements may occur during the first 3 months of ITI treatment.

[h] Samples for hepatitis A, hepatitis B, hepatitis C, HIV, and parvovirus B19 viral NAT and serology will be collected at Baseline, prior to Alphanate administration, and analyzed for information only. Virus safety retain samples will be collected at Screening, Week 8, Month 6, Month 15, Month 30, and Month 33 of the ITI Treatment Phase, as well as at the last visit of the Prophylactic Phase, or at the Early Termination Visit for virus testing in the event that a subject exhibits clinical signs and/or symptoms of viral infection; additional samples for viral NAT and serology testing may be collected ***only*** if there are clinical signs and symptoms of viral infection during the study. For subjects who achieve complete immune tolerance before Month 33, a viral retain sample will be collected at their last visit during the ITI Treatment Phase, before entering the Prophylactic Phase.

[i] If necessary, in order to minimize total blood volume drawn per day, virus safety samples may be drawn the day prior to the Baseline Visit instead of combining with other baseline blood draws on a single day.

[j] Issue and train subject/caregiver on diary and importance of ongoing collection of Alphanate drug administration (date/clock time of start and end of infusion, dose/volume, rate, and treatment interruption) or other items.

[k] When the first inhibitor titer result has been received indicating the subject may have attained complete or partial immune tolerance, a second confirmatory inhibitor measurement is to be performed within approximately 2 weeks after the last assessment; at this next visit, a sample is also collected for measuring FVIII:C in vivo recovery. FVIII:C in vivo recovery is determined from blood samples collected before dosing and at 15 to 30 minutes after completion of dosing of 50 IU/kg Alphanate.

[l] FVIII:C in vivo recovery is determined by the central laboratory during the Prophylactic Phase for subjects who achieved complete immune tolerance. Suspected relapse must be confirmed by a complete pharmacokinetic assessment (FVIII:C in vivo recovery and half-life) at a separate scheduled visit within approximately 2 weeks.

[m] FVIII:C half-life will be performed after a 72-hour FVIII treatment-free period at the following times during the study:

- During the ITI Treatment Phase within approximately 2 weeks after the second consecutive inhibitor titer of <0.6 BU is achieved and FVIII:C in vivo recovery is confirmed to be ≥66% of the predicted normal value
- At the end of the ITI Treatment Phase in subjects who have achieved partial immune tolerance
- At the completion of the Prophylactic Phase at the Month 12 visit

- At separately scheduled visits after the Early Termination Visit, but only for subjects who have a FVIII:C in vivo recovery ≥66% of the predicted normal value

FVIII:C half-life is determined from blood samples collected before dosing and at 15 to 30 minutes and 1, 3, 6, 12, 24 (±4), and 48 (±4) hours after completion of dosing of 50 IU/kg Alphanate.

For subjects with a negative inhibitor titer level and FVIII:C in vivo recovery ≥66% but with FVIII:C half-life <6 hours, the half-life assessment should be repeated approximately every 12 weeks, at the investigator's discretion, until the half-life is ≥6 hours.

[n] FVIII:C half-life is to be performed at separately scheduled visits after the Early Termination Visit, but only for subjects who have a FVIII:C in vivo recovery ≥66% of the predicted normal value.

[o] For complete and partial success subjects, the ITI dose will be tapered down in a step-wise manner over an 8- to 12-week period to reach a final prophylactic dose of 50 IU/kg every other day or 3 times per week.

Appendix 2 Amendment 3 – Summary of Changes

Sections	Change From: (Strikethrough is added to highlight deleted text)	Change To: (Underline is added to highlight new text)	Rationale
Protocol Synopsis; Section 3.1, Study Design and Plan	Overall Study Design and Description: This is a multicenter, multinational, prospective, single-arm, nonrandomized, open-label study of approximately 25 male subjects with congenital hemophilia A who will receive their first (primary) ITI treatment with Alphanate. The study will be conducted at approximately ~~20~~ study centers.	Overall Study Design and Description: This is a multicenter, multinational, prospective, single-arm, nonrandomized, open-label study of approximately 25 male subjects with congenital hemophilia A who will receive their first (primary) ITI treatment with Alphanate. The study will be conducted at approximately 30 study centers.	The number of participating sites has increased.
Protocol Synopsis; Section 3.2.1, Screening and Baseline Entry Criteria	Section 3.2.1 No previous text. Section 3.2.1.1 2. The subject is a male <~~8~~ years of age at the Baseline Visit. 3. The subject's documented historical peak inhibitor titer is ≥~~10~~ BU and ≤200 BU.	Section 3.2.1 Note that any criterion number containing v*X*, was modified in the indicated protocol version *X* (eg, 2v4 indicates that the original criterion #2 was last modified in Protocol Version 4). Section 3.2.1.1 2v4. The subject is a male <12 years of age at the Baseline Visit. 3v4. The subject's documented historical peak inhibitor titer is ≥5 BU and ≤200 BU.	Increasing the maximum age of study participants as well as decreasing the threshold of historical peak inhibitor titer should increase the population of eligible subjects that can enroll in the study.
Protocol Synopsis	Safety assessments: physical examination, ~~clinical chemistry,~~ hematology, and urinalysis will be assessed at Baseline, at Months 6, 12, 18, 30, and 33 during the ITI Treatment Phase, at Months 3, 6, and 12 during the Prophylactic Phase, and at the Early Withdrawal Visit. Vital signs will be assessed at all scheduled visits. Samples for viral safety testing will be collected at Baseline ~~and~~ at the end of the Prophylactic Phase (Month 12) or at the Early Withdrawal Visit. Adverse events and concomitant medications will be assessed at all scheduled visits.	Safety assessments: physical examination, hematology, and urinalysis will be assessed at Screening and Baseline, at Months 6, 12, 18, 24, 30, and 33 during the ITI Treatment Phase, at Months 3 (except for physical examination), 6, and 12 during the Prophylactic Phase, and at the Early Withdrawal Visit. Clinical chemistry will be assessed at all of the aforementioned visits except for Baseline. Vital signs will be assessed at all scheduled visits. Samples for viral safety testing will be collected at Screening, Baseline, Week 8, Month 6, Month 15, Month 30, and Month 33 of the ITI Treatment Phase, as well as at the end of the Prophylactic Phase (Month 12) or at the Early Withdrawal Visit. Adverse events and concomitant medications will be assessed at all scheduled visits.	Edited to fix an error stating that clinical chemistry is collected at baseline. Clarified that virus safety retain samples will also be included at additional time points.

Sections	Change From: (Strikethrough is added to highlight deleted text)	Change To: (Underline is added to highlight new text)	Rationale
Section 1.2, Clinical Experience with Grifols FVIII/VWF Products and ITI	The first version of this FVIII/VWF concentrate was licensed in the US in 1978. The first formulation to be designated as Alphanate was licensed in 1994, and the current formulation was approved by the FDA in 1997. Alphanate is approved for the control and prevention of of bleeding in patients with congenital hemophilia A (and for the treatment of von Willebrand disease). Alphanate was ~~also~~ approved in Italy in 1997, followed by the Netherlands in 2001 and the United Kingdom in 2003~~ for the control and prevention of bleeding in patients with congenital hemophilia A~~.	The first version of this FVIII/VWF concentrate was licensed in the US in 1978. The first formulation to be designated as Alphanate was licensed by the Food and Drug Administration (FDA) in 1994, and the current formulation was approved by the FDA in 1997. Alphanate is approved for the control and prevention of bleeding in patients with congenital hemophilia A (and for the treatment of von Willebrand disease [VWD]). Alphanate was approved in Italy in 1997 for the treatment and prophylaxis of bleeding in patients with hemophilia A and the prevention and treatment of hemorrhage or surgical bleeding in patients with VWD, followed by the Netherlands in 2001 for the treatment and prophylaxis of bleeding in patients with hemophilia A, and the United Kingdom in 2003 for the treatment and prophylaxis of bleeding in patients with hemophilia A and the prevention and treatment of hemorrhage or surgical bleeding in patients with VWD.	Edits added to clarify in which countries Alphanate was approved.
Section 3.1, Study Design and Plan	Male subjects <~~8~~ years of age at the time of the Baseline Visit with severe congenital hemophilia A (FVIII:C levels <1%) who are candidates for ITI and who meet all inclusion criteria (Section 3.2.1.1) and do not meet any exclusion criteria (Section 3.2.1.2) are eligible to participate.	Male subjects <12 years of age at the time of the Baseline Visit with severe congenital hemophilia A (FVIII:C levels <1%) who are candidates for ITI and who meet all inclusion criteria (Section 3.2.1.1) and do not meet any exclusion criteria (Section 3.2.1.2) are eligible to participate.	Updated text based on changes to inclusion criteria.
Section 3.1, Study Design and Plan	Between study center visits when Alphanate will be administered at home by the subject's caregiver, the Alphanate dose will be the same as the dose calculated at the most recent study center visit where weight was measured. Training of caregivers at home may be provided within the first few weeks of initiation of ITI treatment for initial port access and newly implanted devices, upon sponsor approval.	Between study center visits when Alphanate will be administered at home by the subject's caregiver, the Alphanate dose will be the same as the dose calculated at the most recent study center visit where weight was measured. Training of caregivers at home may be provided within the first few weeks of initiation of ITI treatment for initial port access and newly implanted devices, upon sponsor approval. Ongoing home health care for IP infusions will be assessed on a case-by-case basis.	Home health care has been deemed necessary and approved for select subjects on a case-by-case basis.

Sections	Change From: (Strikethrough is added to highlight deleted text)	Change To: (Underline is added to highlight new text)	Rationale
Section 3.4, Prior and Concomitant Therapy	Recombinant factor VIIa, prothrombin complex concentrates, activated prothrombin complex concentrates, and human FVIII (Alphanate) are allowed to be used prophylactically or to treat active bleeding during the ITI Treatment and Prophylactic Phases of the study as directed by the investigator.	Recombinant factor VIIa, prothrombin complex concentrates, activated prothrombin complex concentrates, emicizumab, and human FVIII (Alphanate) are allowed to be used prophylactically or to treat active bleeding during the ITI Treatment and Prophylactic Phases of the study as directed by the investigator.	Subjects taking emicizumab are now eligible to enroll in the study.
Table 3-1: Name, Description, and Location of Laboratory Tests and Procedures	Viral Nucleic Amplification Technology [b]: Postbaseline:[d] Collection of retain samples for HAV RNA, HBV DNA, HCV RNA, HIV-1 RNA, and B19V DNA testing	Viral Nucleic Amplification Technology [b]: Screening and Postbaseline:[d] Collection of retain samples for HAV RNA, HBV DNA, HCV RNA, HIV-1 RNA, and B19V DNA testing	Virus safety retain samples will also be included at Screening.
Table 3-1: Name, Description, and Location of Laboratory Tests and Procedures	Viral Serology Testing[b] Postbaseline: [d] Collection of retain samples for hepatitis A antibody differential (IgM/IgG), hepatitis B core antibody differential (IgM/IgG), hepatitis C antibody, HIV-1/-2 + Group O antibody, and B19V antibody differential (IgM/IgG) testing	Viral Serology Testing[b] Screening and Postbaseline: [d] Collection of retain samples for hepatitis A antibody differential (IgM/IgG), hepatitis B core antibody differential (IgM/IgG), hepatitis C antibody, HIV-1/-2 + Group O antibody, and B19V antibody differential (IgM/IgG) testing	Virus safety retain samples will also be included at Screening.
Table 3-1: Name, Description, and Location of Laboratory Tests and Procedures	[b] Any extra serum/plasma samples (including virus safety retain samples) will be retained by a certified laboratory for up to an additional 5 years after completion of the study for [redacted]	[b] Any potential extra serum/plasma samples (including virus safety retain samples) will be retained by a certified laboratory for up to an additional 5 years after completion of the study for [redacted]	Clarified that extra serum/plasma samples collected may be retained and analyzed up to 5 years after study completion.
Table 3-1: Name, Description, and Location of Laboratory Tests and Procedures	[d] Virus safety retain samples will be collected at the last visit of the Prophylactic Phase or at the Early Termination visit.	[d] Virus safety retain samples will be collected at Screening, Week 8, Month 6, Month 15, Month 30, and Month 33 of the ITI Treatment Phase, as well as at the last visit of the Prophylactic Phase or at the Early Termination visit for virus testing in the event that a subject exhibits clinical signs and/or symptoms of viral infection. For subjects who achieve complete immune tolerance before Month 33, a viral retain sample will be collected at their last visit during the ITI Treatment Phase, before entering the Prophylactic Phase.	Virus safety retain samples will also be included at additional time points of the ITI Phase.

Sections	Change From: (Strikethrough is added to highlight deleted text)	Change To: (Underline is added to highlight new text)	Rationale
Section 3.6.3, Description of Laboratory Tests and Procedures	~~Any extra serum/plasma samples will be stored for up to an additional 5 years after completion of the study for [redacted]. No poststudy genetic testing will be performed.~~	No new text.	This text is not applicable in this section.
Section 3.6.3.1, Virus Safety Testing	Virus safety retain samples will be collected: • At the Early Termination Visit for treatment failures and early withdrawals • At the last visit of the Prophylactic Phase	Virus safety retain samples will be collected, but only tested if subjects show clinical signs and/or symptoms of viral infection, at the following time points: • At the Screening Visit • At the Week 8 Visit of the ITI Treatment Phase • At the Month 6 Visit of the ITI Treatment Phase • At the Month 15 Visit of the ITI Treatment Phase • At the Month 30 Visit of the ITI Treatment Phase • At the Month 33 Visit of the ITI Treatment Phase (unless a subject achieves complete immune tolerance prior to Month 33, in which case a viral retain sample will be collected at his/her last visit during the ITI Treatment Phase, before entering the Prophylactic Phase) • At the Early Termination Visit for treatment failures and early withdrawals • At the last visit of the Prophylactic Phase	Virus safety retain samples will also be included at additional time points during the ITI Treatment Phase.
Section 3.6.3.1, Virus Safety Testing	Virus safety retain samples ~~collected after the Baseline Visit~~ will be stored for up to an additional 5 years after all analyses in support of the study are complete. Additional blood samples for viral NAT and viral serology testing may be collected and tested during the study only if the subject exhibits clinical signs and symptoms consistent with hepatitis A virus (HAV), HBV, HCV, HIV, or parvovirus B19 (B19V) infection while participating in the study.	Virus safety retain samples will be stored for up to an additional 5 years after all analyses in support of the study are complete. Additional blood samples for viral NAT and viral serology testing may be collected and tested during the study only if the subject exhibits clinical signs and symptoms consistent with hepatitis A virus (HAV), HBV, HCV, HIV, or parvovirus B19 (B19V) infection while participating in the study.	Virus safety retain samples are collected at time points both before and after the Baseline Visit.

Sections	Change From: (Strikethrough is added to highlight deleted text)	Change To: (Underline is added to highlight new text)	Rationale
Section 3.6.3.1, Virus Safety Testing	No previous text.	Any potential extra serum/plasma samples will be stored for up to an additional 5 years after completion of the study for [redacted] No poststudy genetic testing will be performed.	This text was moved from another section, as it is more applicable here.
Section 3.6.4.1, Screening Visit (21 to 30 Days Prior to Baseline Visit)	No previous text.	- Virus safety retain samples	Virus safety retain samples will be collected at Screening.
Section 3.6.4.3, Week 2, Week 4, Week 6, Week 8, and Subsequent Monthly Visits During ITI Treatment Phase	No previous text.	- Virus safety retain samples (Week 8 and Month 15 only)	Virus safety retain samples will be collected at Week 8 and Month 15 of the ITI Treatment Phase.
Section 3.6.4.4, Additional Assessments at Months 6, 12, 18, 24, and 30 During ITI Treatment Phase	No previous text.	- Virus safety retain samples (Months 6 and 30 only)	Virus safety retain samples will be collected at Months 6 and 30 of the ITI Treatment Phase.
Section 3.6.4.6, Month 33 During ITI Treatment Phase	No previous text.	- Virus safety retain samples	Virus safety retain samples will be collected at the Month 33 Visit.

Sections	Change From: (Strikethrough is added to highlight deleted text)	Change To: (Underline is added to highlight new text)	Rationale
Appendix 1, Schedule of Procedures	[h] Samples for hepatitis A, hepatitis B, hepatitis C, HIV, and parvovirus B19 viral NAT and serology will be collected at Baseline, prior to Alphanate administration, and analyzed for information only. Virus safety retain samples will be collected at the last visit of the Prophylactic Phase or at the Early Termination Visit; additional samples for viral NAT and serology testing may be collected ***only*** if there are clinical signs and symptoms of viral infection during the study.	[h] Samples for hepatitis A, hepatitis B, hepatitis C, HIV, and parvovirus B19 viral NAT and serology will be collected at Baseline, prior to Alphanate administration, and analyzed for information only. Virus safety retain samples will be collected <u>at Screening, Week 8, Month 6, Month 15, Month 30, and Month 33 of the ITI Treatment Phase, as well as</u> at the last visit of the Prophylactic Phase, or at the Early Termination Visit <u>for virus testing in the event that a subject exhibits clinical signs and/or symptoms of viral infection</u>; additional samples for viral NAT and serology testing may be collected ***only*** if there are clinical signs and symptoms of viral infection during the study. <u>For subjects who achieve complete immune tolerance before Month 33, a viral retain sample will be collected at their last visit during the ITI Treatment Phase, before entering the Prophylactic Phase.</u>	Virus safety retain samples will also be included at additional time points.