Official Title:	**A STUDY OF IMMUNOLOGIC SAFETY FOR ALPHANATE® IN PREVIOUSLY TREATED PATIENTS DIAGNOSED WITH SEVERE HEMOPHILIA A**
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ANTIHEMOPHILIC FACTOR (HUMAN) ALPHANATE®
SOLVENT-DETERGENT, HEAT-TREATED

PHASE IV PROTOCOL: GBI 04-01
(formerly ATC 02-01 of Alpha Therapeutic Corporation)

A STUDY OF IMMUNOLOGIC SAFETY FOR ALPHANATE® IN PREVIOUSLY TREATED PATIENTS DIAGNOSED WITH SEVERE HEMOPHILIA A

GRIFOLS INC.
2410 Lillyvale Avenue
Los Angeles, CA 90032

10 January 2007
(Incorporating Protocol Amendment Number Six)

Contains Confidential and Proprietary Information of Grifols Inc.

PROTOCOL NUMBER: GBI 04-01 (formerly ATC 02-01)

Amendment Tracking

Please indicate any amendments to this protocol by writing the Amendment Number and the Date of the Amendment in the space below. This page will be used to track original protocols and their amendments. This is the original protocol if no amendment number is listed below.

Original Protocol: Title: A STUDY OF IMMUNOLOGIC SAFETY FOR ALPHANATE® IN PREVIOUSLY TREATED PATIENTS DIAGNOSED WITH SEVERE HEMOPHILIA A (OF ALPHA THERAPEUTIC CORPORATION)

DATE: 26 MARCH 2002

NO.: ATC 02-01

Amendment Number Six

Date of Amendment: 10 January 2007

Section and/or Item	Amendment 6
Section 4 Study Design	Delete: All subjects will have CD4 counts greater than or equal to 400 cells/µL at baseline. Add: All subjects will have adequate immune system function (CD4 count must be greater than or equal to 400 cells/µL and subject must not have an immune system impairment or damage by disease or treatment).
Section 5.2 Exclusion Criteria	Add: The subject is immunocompromised (including HIV+ status or has an impaired immune system due to disease or treatment)**.**
Section 7.1 Study Procedures	Delete: CD4 count will be determined. Add: Immune system function will be assessed, including a CD4 count.. Any patient with suspicion of a compromised immune system must be approved by the sponsor prior to enrollment.
Section 10.2 Serious Adverse Events	Add: … [redacted], who can be contacted during working hours (8:00 a.m. to 5:00 p.m. Pacific Time) at [redacted] or [redacted] during non-working hours.

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PROTOCOL NUMBER: GBI 04-01
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A STUDY OF IMMUNOLOGIC SAFETY FOR ALPHANATE® IN PREVIOUSLY TREATED PATIENTS DIAGNOSED WITH SEVERE HEMOPHILIA A

Study Sponsor:	Grifols Inc. 2410 Lillyvale Avenue Los Angeles, CA 90032
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Medical Safety Officer	[redacted], R.N. [redacted] Telephone: [redacted] Cellular: [redacted]
Date of Protocol:	26 March 2002 (as ATC 02-01)
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Proprietary Notice:	The concepts and information contained herein are considered proprietary and shall not be disclosed in whole or in part without the expressed consent of Grifols Inc.

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LIST OF ABBREVIATIONS AND DEFINITIONS OF TERMS

AHF	Antihemophilic Factor
ALT	Alanine aminotransferase
AP	Alkaline phosphatase
AST	Aspartic aminotransferase
ATC	Alpha Therapeutic Corporation
BU	Bethesda unit
BUN	Blood urea nitrogen
CBC	Complete blood count
CEDs	Cumulative exposure days
CFR	Code of Federal Regulations (U.S.)
CPMP	European Commission Committee for Propriety Medicinal Products
CRF	Case report form (s)
FDA	United States Food and Drug Administration
FVIII	Factor VIII
FVIII:C	Factor VIII coagulant activity
GI	Grifols Inc.
HAV	Hepatitis A virus
HBsAg	Hepatitis B surface antigen
HBcAb	Hepatitis B core antibody
HBsAb	Hepatitis B surface antibody
HBV	Hepatitis B virus
HCV	Hepatitis C virus
HIV-1	Human immunodeficiency virus type 1
HIV-2	Human immunodeficiency virus type 2
IDUR	Investigational drug utilization record
IRB	Institutional review board
LDH	Lactic acid dehydrogenase
PCR	Polymerase chain reaction
PEG	Polyethylene glycol
PTP	Previously-treated patients
PUP	Previously-untreated patients
SD/HT	Solvent Detergent/Heat Treated
seronegative	non-reactive in an ELISA test for antibody to the virus in question
seropositive	reactive in an ELISA test for antibody to the virus in question
SWI	sterile water for injection
USP	United States Pharmacopeia
VWF	von Willebrand Factor

PROTOCOL SUMMARY

Protocol Number:	GBI 04-01 (formerly ATC 02-01 of Alpha Therapeutic Corporation)
Title:	A Study of Immunologic Safety for Alphanate® in Previously Treated Patients Diagnosed with Severe Hemophilia A
Study Phase:	Phase IV
Study Duration:	At least 2 years
Name of Drug:	Antihemophilic Factor (Human), Alphanate® Solvent Detergent/ Heat Treated
Drug Dosage:	An appropriate dosage for the prevention and/or control of bleeding
Concurrent Control:	None
Route and Form:	Intravenous
Objectives:	To determine the immunologic and general safety of long-term use of Alphanate® in individuals with severe hemophilia A.
Subject Population:	Males diagnosed with severe hemophilia A who have been previously treated with Factor VIII concentrates, cryoprecipitate, or whole blood for a total of 150 cumulative exposure days; at least six years and not more than 65 years of age, who have no history of exposure to Alphanate® and who have never been diagnosed with antibody inhibitors to Factor VIII or to nonspecific inhibitors of coagulation.
Structure:	Multi-center study
Blinding:	Unblinded
Method of Subject Assignment:	Non-randomized
Total Sample Size:	At least fifty evaluable subjects
Pharmacokinetics:	Not Applicable
Efficacy Endpoints	Not Applicable

Primary Endpoint	1. The incidence of Factor VIII inhibitor development.
Secondary Endpoints:	1. The frequency, nature, causality and severity of adverse events. 2. The occurrence of changes in biochemical parameters which would indicate renal or hepatic impairment. 3. The incidence of seroconversion to human immunodeficiency virus type 1 and 2 (HIV-1/HIV-2), hepatitis A virus (HAV), hepatitis B virus (HBV), hepatitis C virus (HCV) or parvovirus B19 in subjects seronegative for these viruses at the time of enrollment. 4. The amount of product used per year as part of at-home prophylaxis and therapy for bleeding episodes. 5. A physician's qualitative assessment of hemostasis (evaluated as "None", "Moderate", "Good", or "Excellent" in response to therapy with Alphanate® received by the subject in a hospital or another location under the physician's direct supervision.

1 INTRODUCTION

The development of an immune response to Factor VIII (*i.e.*, the generation of antibody inhibitors of Factor VIII) by patients diagnosed with hemophilia A and treated with Factor VIII concentrates is a serious complication in the treatment of hemophilia A.[1,2] Estimates of inhibitor formation from retrospective and prospective clinical trials vary from as low as four percent to as high as 28 percent. All studies agree that prevalence of antibody inhibitors is higher among individuals with severe hemophilia A (Factor VIII:C less than 0.01 IU/mL) than among individuals with mild or moderate disease, with estimates ranging from eight to 28 percent.[3,4]

In clinical trials in which previously-untreated or minimally-treated patients (PUPs) were treated with recombinant Factor VIII concentrates, the incidence of inhibitors to Factor VIII was measured as a function of cumulative exposure days (CEDs). In a clinical trial conducted by Bray *et al.*[5], inhibitors developed in 17 of 71 (24%) PUPs treated with Recombinate® (Baxter Bioscience); 5 of the 71 (7%) patients developed high-responder inhibitors. The median number of CEDs was 11 for all patients and nine for patients who developed inhibitors.

Similarly, in a clinical trial conducted by Lusher *et al.* of subjects receiving Kogenate® (Bayer), inhibitor formation occurred in 16 of 62 (27%) subjects.[6] Seven of the 62 (11%) patients developed high-responder inhibitors. The median number of CEDs was nine for patients who developed inhibitors and 28 for subjects who exhibited no immune response to Kogenate®.

Several clinical trials have been conducted in which PUPs were enrolled and then treated with plasma-derived Factor VIII concentrates of varying purity and produced using differing virucidal treatments (e.g., terminal dry heat, pasteurization, solvent-detergent).[7-9] The rates of incidence for antibody inhibitors ranged from 21 to 28 percent, a range similar to that observed with recombinant Factor VIII products. Consequently, the investigators for these trials concluded that recombinant DNA products do not pose any greater risk for inhibitor formation in PUPs than do plasma-derived products.

However, a substantially increased incidence of Factor VIII inhibitors (a 4.5-fold increase) was reported by Peerlinck *et al.*[10] and Rosendaal *et al*[11] in clinical trials of previously treated individuals (PTPs) treated with pasteurized factor VIII concentrate of an intermediate purity that is available in Europe. The increase in inhibitor formation was observed even in individuals who had been previously treated with multiple Factor VIII products (“multitransfused” patients). The authors suggested that pasteurization might have compromised the integrity or conformation of Factor VIII, which in turn could have resulted in increased immunogenicity.

Guérois *et al.*[12] conducted a clinical trial involving 56 PUPs treated with HPSD-VIII® (a plasma-derived, high-purity, solvent-detergent treated concentrate). The incidence of immune response was monitored over a period of 76 months (median = 29 months) and for patients experiencing 100 CEDs (median = 26 CEDs). The median number of CEDs prior to detection of inhibitors was 16 days. Inhibitors developed in five patients (9 percent), only one of whom was categorized as a high responder (2 percent). Therefore, it appeared that the use of HPSD-VIII® was associated with a lower incidence of antibody inhibitor than was observed with the administration of recombinant DNA products or a combination of plasma-derived concentrates. Additionally, inhibitors appeared later (16 CEDs) in individuals receiving HPSD-VIII® than in those treated with the recombinant DNA products (nine CEDs).

Addiego *et al*.[13] investigated the use of Hemofil M®, a plasma-derived Factor VIII concentrate treated with a solvent-detergent process, in 23 PUPs. Only two of 23 (9%) patients developed inhibitors after 14 to 26 CEDs. Although the number of subjects was limited in this trial, the median number of CEDs experienced by this population, 32 days, exceeded those in the populations studied by Guérois *et al.*[12], Bray *et al.*[5] and Lusher *et al*.[6] A more limited study by Lusher and Salzman with the pasteurized Factor VIII concentrate Monoclate P® showed an incidence of inhibitor formation of 18 percent.[14]

A study of the incidence of inhibitors in previously-treated patients (PTPs) carried out by McMillan et al. included 1306 hemophilia patients whose mean age was 18, and who were followed for up to four years. Thirty-one of these individuals (2.4 percent) developed inhibitors to Factor VIII; of these, 29 had been diagnosed with severe hemophilia A. The incidence of new inhibitors was eight per 1,000 patient-years of observation.

In a study of PTPs treated with recombinant Factor VIII (Kogenate®), by Schwartz *et al*, inhibitor formation was observed in only 1 of 86 (1.2%) patients following repeated exposure to the product.[16] The mean annual per capita consumption of Factor VIII in this subgroup was estimated to be approximately 78,000 units annually.

2 RATIONALE

Alphanate® is a high-purity plasma-derived preparation of Factor VIII (Antihemophilic Factor, AHF) in which Factor VIII:C is present in complexes of high-molecular weight multimers of von Willebrand Factor (VWF).

Alphanate® is prepared from plasma found to be nonreactive for hepatitis B surface antigen (HBsAg) and negative for antibodies to human immunodeficiency virus HIV-1 and HIV-2 and hepatitis C virus (HCV) using FDA approved assays. All units of plasma used in the manufacture of

Alphanate® are also tested for HIV and HCV genomic material using polymerase chain reaction (PCR) assays and using a three-stage sample pooling method approved by the US Food and Drug Administration (FDA). In addition, all units of plasma used in the preparation of Alphanate® have an alanine aminotransferase (ALT) level less than twice the upper limit of normal for the assay method used.

Two specific viral reduction processes are used in the manufacture of Alphanate®:

(1) treatment with an organic solvent/detergent mixture [tri(n-butyl)phosphate (TNBP)/polysorbate 80] to inactivate lipid-enveloped viruses such as HIV-1, HIV-2, hepatitis B (HBV) and HCV; and

(2) exposure of the lyophilized product in the final container to heating for 72 hours at 80ºC.

These processes have the theoretical potential to expose new antigenic sites in the Factor VIII molecule. If new antigenic entities are created, individuals with hemophilia A who have not developed antibody inhibitors in response to treatment with other plasma-derived Factor VIII concentrates could develop inhibitors to these new antigenic sites. The generation of antibody inhibitors to Factor VIII could result in treatment complications.

3 OBJECTIVE

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

4 STUDY DESIGN

This is a phase IV, non-randomized, multicenter study involving at least 50 evaluable subjects diagnosed with severe hemophilia A (levels of Factor VIII:C in plasma less than 0.01 IU/mL), who are male, at least six years of age and not more than 65 years of age, and with a Karnofsky Performance Score of at least 50. All subjects will have adequate immune system function (CD4 count must be greater than or equal to 400 cells/µL and subject must not have an immune system impairment or damage by disease or treatment).

Enrolled subjects will be treated at home and with in-clinic therapy exclusively with Alphanate® as their sole source of FVIII concentrate for prophylaxis and treatment of all bleeding episodes and surgical procedures for a period of at least two years and a minimum of 50 exposure days, or, if 50 exposure days are not reached, for a maximum of 30 months. Alphanate® will be administered in accordance with the subject's usual pre-study treatment regimen. An exposure day is defined as any day on which a subject receives one or more infusions of any Factor VIII containing product. However, only one exposure day will be accrued for each surgical episode, regardless of the number of days of treatment or the number of infusions of Factor VIII associated with that surgical procedure.

A subject will continue treatment, as specified above, unless

- The subject develops inhibitors to Factor VIII at a titer greater than or equal to 5 Bethesda units (BU/mL);
- treatment with Factor VIII becomes ineffective at providing hemostasis even for a subject who has developed inhibitors with a titer of less than 5 BU/mL; or
- the subject exhibits severe or serious adverse events that prevent completion of participation in the study.

In all instances, only the inhibitor titer obtained by the central reference laboratory will be used to determine the subject's eligibility to continue his participation in the trial.

The primary endpoint of this study is:

the incidence of Factor VIII inhibitor after exposure to Alphanate®.

The secondary endpoints are:

1. The frequency, nature, causality and severity of adverse events.
2. The occurrence of changes in biochemical parameters which would indicate alterations in renal or hepatic function.
3. Evidence for seroconversion to HIV-1/HIV-2, HAV, HBV, HCV and parvovirus B19 for subjects who are seronegative for these viruses upon enrollment.
4. The amount of product used per year as part of at-home prophylaxis and therapy for bleeding episodes.

5. A physician's qualitative assessment of hemostasis (evaluated as "None," "Moderate," "Good," or "Excellent") in response to therapy with Alphanate® received by the subject in a hospital or at another location under the physician's direct supervision.

The design of this study satisfies the recommendations of the European Commission Committee for Propriety Medicinal Products (CPMP) regarding eligibility criteria and end points for studies, in previously-treated patients, of concentrates with marketing authorization in which a manufacturing process has been added. In addition, this study conforms to the CPMP recommendation that all enrolled subjects be vaccinated against hepatitis A and B. Any candidate for this trial who has not yet been immunized against hepatitis A will be offered a course of hepatitis A vaccination.

5 SELECTION OF SUBJECTS

The following entrance criteria must be satisfied for a patient to be enrolled into this study:

5.1 Inclusion Criteria

1. The subject must be male, at least six years of age and not more than 65 years of age.

2. The subject must have signed and dated an Informed Consent Form and Patient Authorization for Release of Information approved by the appropriate Institutional Review Board (IRB) prior to screening and enrollment. If the subject is a minor (*i.e.*, less than 18 years of age) both he and his parent or legal guardian must sign and date the informed consent.

3. The subject must have been diagnosed with severe hemophilia A and must have levels of Factor VIII less than 0.01 IU/mL.

4. The subject must have received treatment with cryoprecipitate, Factor VIII concentrates, and/or whole blood, for at least 150 cumulative exposure days (CEDs) prior to enrollment.

5. The subject must not have received treatment with cryoprecipitate, Factor VIII concentrate, or any other blood product, for at least 72 hours prior to screening.

6. The subject must never have been diagnosed with inhibitors to Factor VIII at any detectable titer.

7. The subject must never have been diagnosed with nonspecific inhibitors of coagulation.

8. The subject must test negative for the presence of Factor VIII inhibitors at screening and enrollment.

9. The subject must have CD4 counts greater than or equal to 400 cells/ µL.

10. The subject must have been vaccinated against hepatitis A and hepatitis B, or have evidence of antibodies against hepatitis A and hepatitis B. However, a subject who has no prior immunity against hepatitis A will be offered a course of vaccination for hepatitis A.

11. The subject must have a Kamofsky Perfomrance Score of at least 50 (Appendix 1).

5.2 Exclusion Criteria

1. The subject is receiving any immunosuppressive medications including intravenous immunoglobulins at the time of enrollment.

2. The subject has clinical signs or symptoms of an infection, such as fever, chills or nausea during screening or enrollment.

3. The subject has a history of frequent reactions to Factor VIII concentrates (e.g., chills or headaches).

4. The subject has received treatment with Alphanate® (Solvent-Detergent/ Heat-Treated).

5. The subject is immunocompromised (including HIV+ status or has an impaired immune system due to disease or treatment)**.**

6. TEST MATERIAL

Alphanate® is prepared from plasma found to be nonreactive for hepatitis B surface antigen (HBsAg) and negative for antibodies of HIV-1/HIV-2 and HCV using FDA approved assays. All units of plasma used in the manufacture of Alphanate® are also tested for HIV and HCV genomic material using polymerase chain reaction (PCR) assays and using a three-stage sample pooling method approved by the US Food and Drug Administration (FDA). In addition, all units of plasma used in the preparation of Alphanate® have an

alanine aminotransferase (ALT) level less than twice the upper limit of normal for the assay method used.

Alphanate® is manufactured by cryoprecipitation, polyethylene glycol (PEG) precipitation, heparin-agarose chromatography and glycine precipitation. Two specific viral reduction procedures are used in the manufacturing process:

- At an intermediate step in the manufacture of Alphanate®, the product is treated with an organic solvent/detergent mixture [tri(n-butyl)phosphate (TNBP)/polysorbate 80] to inactivate lipid-enveloped viruses such as HIV-1/HIV-2, hepatitis B and C (HBV, HCV); and

- The lyophilized product is heated at 80ºC for 72 hours in the final container and meets all internal specifications of Grifols Inc.

However, virus reduction methodologies and testing techniques cannot fully eliminate the risk of viral transmission.

Alphanate® is provided as a lyophilized powder in a vial containing at least 40 International Units Factor VIII:C/mL (200 IU/vial) (IU; all subsequent references to IU will be expressed as units) after reconstitution. A label indicating the manufacturer, lot number, number of Factor VIII:C units, and reconstitution volume is affixed to each vial. A vial containing the appropriate volume of sterile water for injection (SWI), USP, is provided with each vial of Alphanate®.

Alphanate® used in this clinical trial has met all internal product release specifications of Grifols Inc.

7 STUDY PROCEDURES (SEE APPENDIX 2)

7.1 Screening

All of the following procedures and laboratory procedures will be performed: a) **at least 72 hours after the most recent administration of cryoprecipitate, Factor VIII concentrate or any other blood product**, and b) before the first administration of Alphanate®.

See the table in Appendix 2 for the listing of laboratory tests to be performed.

1. Before any invasive screening tests are performed, the subject will be presented with an IRB-approved informed consent form and patient authorization form, which will be explained to him. The subject will not participate in any part of the clinical trial, including the initial screening until he has signed and dated the forms to indicate that he understands and

agrees to its contents (if he is less than 18 years of age, his parent or legal guardian must agree and sign).

2. The subject's medical and past treatment history will be reviewed to ascertain whether he meets all the entry requirements for the study.

3. Each subject will receive a physical examination that will include: vital signs (pulse, respiratory rate, oral temperature, blood pressure), height, weight, and a description of any physically observable abnormal conditions.

4. Hematologic status will be assessed by: complete blood count (CBC) with white blood cell (WBC) differential and platelet count.

5. Immune system function will be assessed, including a CD4 count.. Any patient with suspicion of a compromised immune system must be approved by the sponsor prior to enrollment.

6. Factor VIII coagulant activity (Factor VIII:C) will be assessed both by the local laboratory and the central reference laboratory **The value obtained by the central reference laboratory will determine the subject's eligibility.**

7. The Factor VIII inhibitor level (BU/mL) will be determined. Two sets of samples will be drawn: one for analysis at the local laboratory of the clinical site, and the other sample for analysis at the central reference laboratory. **The value obtained by the central reference laboratory will determine the subject's eligibility.**

8. Serum chemistry tests will be performed: alanine aminotransferase (ALT), aspartic aminotransferase (AST), alkaline phosphatase (AP), lactic acid dehydrogenase (LDH), blood urea nitrogen (BUN), total bilirubin and creatinine.

9. Viral and serological tests will be conducted: including testing for HBsAg, and for antibodies to HAV, HBcAb, HBsAb, HCV, HIV-1/HIV-2 and parvovirus B19 (by routine immunoassay).

10. A sample of venous blood will be drawn from which approximately 5 mL of serum will be prepared and aliquoted into three vials of at least 1.5 mL of sample each. The samples will be frozen and retained at -20°C in the event that they are required for later serological or virology tests.

7.2 Enrollment and Assignment of Subject Numbers

After the investigator has assured that the subject meets all of the enrollment criteria, the screening and enrollment case report forms will be completed. Note that with respect to Factor VIII coagulant activity (Factor VIII:C) and the Factor VIII inhibitor levels, the values obtained by the central reference laboratory will determine the subject's eligibility. The investigator may use the results from the local laboratory of the site to support clinical decisions in patient management.

After assuring that the subject meets all of the enrollment criteria, the following clinical and laboratory assessments will be performed:

1. Abbreviated physical examination including: interim medical history, vital signs (pulse, respiratory rate, oral temperature, blood pressure) and weight.

2. **If the subject has been treated with any of the products mentioned above in Section 7.1 (cryoprecipitate, Factor VIII concentrate or any other blood product) after the screening tests are performed**, blood will again be drawn no more than three days prior to the first treatment with Alphanate® for baseline testing of the following parameters:

   (a) Factor VIII coagulant activity (Factor VIII:C) for central lab testing,

   (b) Factor VIII inhibitor levels (BU/mL) for central testing,

   (c) Repeat viral and serological tests including anti-HIV-1/HIV-2, HBsAg, anti-HAV, anti-HBV, and anti-HCV, and anti-parvovirus B1.

3. A sample of venous blood will be drawn from which approximately 5 mL of serum will be prepared and aliquoted into three vials of at least 1.5 mL of sample each. The samples will be frozen and retained at -20°C in the event that they are required for later serological or virology tests.

In order to complete subject enrollment and receive the Subject Number, the participating study site will provide to the clinical monitor at Grifols Inc. a copy of the completed screening and enrollment CRFs, screening and enrollment tests and medical history. The results of the screening and enrollment tests and procedures will be reviewed and the clinical monitor will confirm the individual's eligibility and assign a subject number. Grifols Personnel assigned to the study can be contacted by telephone during working hours (8:00 a.m. to 5:00 p.m. Central Time) at [redacted] or fax [redacted].

7.3 At seven to fourteen days after the first infusion of Alphanate®

1. Parvovirus B19 PCR assay - only in subjects seronegative for parvovirus B19 at enrollment.
2. Collect a 2 mL sample of citrated plasma for inhibitor testing at the central reference laboratory.

7.4 Every 3 Months until Month 30 and/or Off-Study

Samples for quarterly testing will be drawn ± **10 days** of the scheduled visit date. At each of these visits, the following procedures will be performed:

1. Abbreviated physical examination including: interim medical history, vital signs (pulse, respiratory rate, oral temperature, blood pressure) and weight;
2. Factor VIII coagulant activity (Factor VIII:C);
3. Factor VIII inhibitor levels (BU/mL) in two sets of samples;
4. Hematologic status will be assessed by: complete blood count (CBC) with white blood cell (WBC) differential and platelet count.
5. Serum chemistry tests including: alanine aminotransferase (ALT), aspartic aminotransferase (AST), alkaline phosphatase (AP), lactic acid dehydrogenase (LDH), blood urea nitrogen (BUN), creatinine and total bilirubin;
6. Viral and serological tests including: testing for HBsAg, and for antibodies to HAV, HBcAb, HBsAb, HCV, HIV-1/HIV-2 (only if seronegative at baseline) and parvovirus B19 by routine immunoassay (only in subjects who are seronegative at baseline);
7. A sample of venous blood will be drawn from which approximately 5 mL of serum will be prepared and aliquoted into three vials of at least 1.5 mL of sample each. The samples will be frozen and retained at -20°C in the event that they are required for later serological or virology tests.

If at any quarterly visit, **the subject tests positive for the presence of Factor VIII inhibitors (inhibitor titer of greater than 0.6 modified BU/mL), based upon the value determined by the central reference laboratory,** the procedures specified in section 7.12 (Subjects Who Develop Inhibitors) will also be performed.

7.5 Unscheduled Visits

For each unscheduled visit, the following tests will be performed:

1. Abbreviated physical examination including: interim medical history, vital signs (pulse, respiratory rate, oral temperature, blood pressure) and weight;

2. Factor VIII coagulant activity (Factor VIII:C);

3. Factor VIII inhibitor levels (BU/mL) in two sets of samples **(to be performed only if: a) there is clinical indication that the normal dosage of Alphanate® is not providing adequate hemostasis, or b) surgery;**

4. Hematologic status will be assessed by: complete blood count (CBC) with white blood cell (WBC) differential and platelet count;

5. Serum chemistry tests including: alanine aminotransferase (ALT), aspartic aminotransferase (AST), alkaline phosphatase (AP), lactic acid dehydrogenase (LDH), blood urea nitrogen (BUN), creatinine and total bilirubin;

6. Viral and serological tests **(to be performed only if subject exhibits clinical or subjective symptoms indicating possible viral infection)** including: testing for HBsAg, and for antibodies to HAV, HBcAb, HBsAb, HCV, HIV-1/HIV-2 (only if seronegative at baseline) and parvovirus B19 by routine immunoassay (only in subjects who are seronegative for parvovirus B19 at baseline);

7. A sample of venous blood will be drawn from which approximately 5 mL of serum will be prepared and aliquoted into three vials of at least 1.5 mL of sample each. The samples will be frozen and retained at -20°C in the event that they are required for later serological or virology tests.

If at an unscheduled visit, a test for Factor VIII **is positive for the presence of Factor VIII inhibitors (inhibitor titer of greater than 0.6 modified BU/mL), based upon the value determined by the central reference laboratory,** the procedures specified in section 7.12 (Subjects Who Develop Inhibitors) will be performed.

7.6 In Hospital and In Clinic Treatments

A physician's qualitative assessment of hemostasis (evaluated as "None," "Moderate," "Good," or "Excellent") in response to therapy with Alphanate® received by the subject will be recorded for all treatments administered in the hospital, clinic, or at another location under the physician's direct supervision.

7.7 Home Treatment

Following enrollment, the subject will have the choice of obtaining Alphanate® treatment at the participating study site in response to bleeding episodes, or to receive treatment at home (See Section 7.9.1).

7.8 Miscellaneous

All enrollment tests should be performed prior to the first administration of Alphanate®. Samples drawn for quarterly testing will be drawn ± 10 days of the scheduled visit date. The presence of inhibitors will be determined by the Nijmegen modification of the Bethesda assay (see below); the Factor VIII source for this assay will be normal pooled plasma. **Inhibitor titers will be considered valid if the subject's Factor VIII level is at or below baseline (less than 0.01 IU/mL),** therefore, the subject should be scheduled for the quarterly visit at least three days after an infusion with Alphanate®.

If the subject's Factor VIII levels are higher than baseline at the time the plasma sample is drawn, then the subject will be rescheduled for testing within one week of the notification of the invalid result.

Assays for inhibitor to Factor VIII will be performed locally (i.e., at the coagulation testing laboratory for each clinical site) to support decisions regarding treatment. Duplicate plasma samples will be collected at each time point for confirmatory testing at a central reference laboratory. The central laboratory will utilize the Nijmegen modification of the Bethesda assay to improve reliability.[17] The modification involves the following changes in reagents: (1) buffering of the normal pooled plasma used in the assay and control with 0.1 M imidazole to pH 7.4 and (2) replacing the imidazole buffer in the control mixture with immunodepleted Factor VIII-deficient plasma. In addition, to further improve the accuracy of the assays, a standardized immunodepleted plasma will be used for the performance of the assay at all clinical sites, as well as at the central reference laboratory.

The local laboratories will not be required to test for Factor VIII inhibitors using the Nijmegen modification of the Bethesda assay, but are required to provide the sponsor with a detailed description of the procedure used.

Only the results of the assays from the central reference laboratory will be used in the determination and analysis of the primary endpoint for the clinical trial.

To determine the risk of transmission of parvovirus B19, subjects who are seronegative at enrollment will be tested approximately one week after receiving the first infusion with Alphanate® for the presence of parvovirus B19 by Polymerase Chain Reaction (PCR) testing.

7.9 Administration of Alphanate®

7.9.1 Product usage and subject diaries

Alphanate® will be administered as needed during the study. Indications for infusion include:

- bleeding episode or hemorrhage;
- routine prophylaxis;
- prophylactic treatment prior to physiotherapy or surgery.

Following enrollment, the subject will have the choice of obtaining Alphanate® treatment at the participating study site in response to bleeding episodes, or to receive treatment at home. If the Alphanate® is to be self-administered, then the subject must maintain an infusion diary, which will contain the following information:

- date of infusion;
- number of units infused;
- number of vials used;
- lot number(s);
- reason for infusion;
- any adverse events

The site should provide the subject with adequate drug supply for three months based on the subject's anticipated needs.

7.9.2 Alphanate® dosing guidelines

All subjects will be infused with the appropriate dose of Alphanate® (as determined by the investigator) for the prevention and/or control of bleeding, as needed. The dose and duration of therapy with Alphanate® will depend on the severity of the Factor VIII deficiency, the location and extent of the bleeding and the individual's physical condition. The amount of coagulation factor administered and the frequency of dosing will always take into account the clinical effectiveness of the product in each individual. However, the

following regimen should be used as a guide for dosage calculation in the treatment of adults with hemophilia A.

Antihemophilic factor (Factor VIII:C activity) potency is expressed in International Units (IU) on the product label, where one unit approximates the activity in one milliliter of normal plasma. Replacement therapy studies have shown a linear dose-response relationship, with a 2.0-2.5 % increase (0.020-0.025 IU/mL) in Factor VIII activity for each unit of Factor VIII:C per kg of body weight transfused, from which an approximate factor of 0.5 IU/kg can be calculated.[18, 19]

The following formula provides a guide for dosage calculation (the plasma Factor VIII may vary depending upon the age, weight, severity of hemorrhage, or surgical procedure of the subject):

Number of Factor VIII:C Required =
Body Weight (kg) x 0.50 (IU/kg) x Factor VIII:C Desired Increase (percent)

For example, in order to increase the Factor VIII:C concentration to 30 percent in a subject with a body weight of 50 kg, the formula is applied as follows:

50 kg x 0.50 IU/kg x 0.30 IU/mL $\times$ 100 = 750 IU Factor VIII:C

Mild to moderate hemorrhages may usually be treated with a single administration sufficient to raise the plasma Factor VIII level to 0.20 to 0.30 IU/mL. In more serious hemorrhages, the subject's plasma Factor VIII level should be raised to 0.3 to 0.5 IU/mL. Infusions are generally required at twice daily intervals over several days. [19]

Surgery requires that the Factor VIII level be raised to 0.50 to 0.80 IU/mL with the level maintained at or above 0.30 IU/mL for approximately two weeks postoperatively. For dental extractions, the Factor VIII level should be raised to 0.50 IU/mL immediately prior to the procedure; further Factor VIII may be given if bleeding recurs. [20]

In subjects who experience frequent hemorrhages, Antihemophilic Factor (Human), Alphanate®, may be administered prophylactically on a daily or every other day schedule to raise the Factor VIII level to approximately 0.15 IU/mL. [21]

In pediatric subjects, no clinical trials for safety and effectiveness have been conducted in 16 years of age or younger. Across well-controlled half-life and recovery clinical trials in patients previously treated with Factor VIII concentrates for Hemophilia A, the one pediatric subject receiving Alphanate® (Solvent/Detergent) responded similarly when compared with 12 adult subjects. [22]

Factor VIII levels should be monitored periodically to evaluate the response of individual patients to a given dosage regime.

7.10 Quarterly Clinic/Office Visits

Each participating subject will return to the study site at least quarterly for examination and collection of his infusion diary and for his quarterly drug supply. At each visit, the subject will bring all used and unused containers of Alphanate®, in addition to the diary. During the quarterly visits an abbreviated physical examination will be performed by the investigator or a staff member designated by the investigator. Blood samples will be collected for required testing, and the usage of the study drug, prescription medication and blood products will be determined. At each visit, the study nurse will discuss the information in the diary with the subject and will reconcile drug use. A new diary and a new supply of Alphanate® will be given to the subject.

Participating sites will:

- maintain these diaries in the subject files as part of the case report forms;
- verify, to the extent possible, their accuracy by comparing Alphanate® usage in these diaries against their own prescription information; and
- discuss with the subject any adverse events and their severity.

7.11 Subjects Who Develop Inhibitors

If at any quarterly visit, the subject tests positive for the presence of Factor VIII inhibitors, (inhibitor titer of 0.6 modified BU/mL or greater) based on the local lab test and confirmed by the central reference laboratory test result, the following steps should be taken:

- For an inhibitor titer of greater than or equal to 10 BU/mL,
  - Collect a 10 mL sample of citrated plasma and send to the central reference laboratory.
  - Withdraw the subject only after performing all required testing (see Section 7.4) and only after receiving confirmation of the inhibitor titer from the central reference laboratory.
- For an inhibitor titer less than 10 BU/mL,

- Collect a 50 mL sample of citrated plasma. This sample can be obtained in multiple draws within a two-week period and sent to the central reference laboratory.

- Repeat inhibitor titer assay at two-week intervals until:

1. the inhibitor titer decreases to < 0.6 modified BU/mL or
2. the subject's participation in the study is ended or
3. it becomes apparent that, after repeated assays at two-week intervals, the inhibitor titer will not decrease during the course of the study (the investigator should first consult with the Project Manager prior to making this determination).

- If the subject's inhibitor level is less than 5 BU/mL and treatment with Alphanate® at higher doses remains effective in achieving hemostasis, then the subject will continue the current treatment regimen and testing will be performed as described in Section 7.4 above.

- If Alphanate® treatment either becomes ineffective in achieving hemostasis, even if the inhibitor titer remains below 5 BU/mL, or if the inhibitor level rises to greater than or equal to 5 BU/mL, as confirmed by test results from the central reference laboratory, then the subject will be withdrawn from the study.

Subjects withdrawn from the study may either be treated with alternate concentrates or commence a treatment program with Alphanate® for the induction of immune tolerance. The treating physician, in consultation with Grifols Office of Medical Affairs, will determine the alternate treatment or product and the immune tolerance protocol.

7.12 Treatment with Other Blood Products

Subjects will be treated for hemophilia A exclusively with Alphanate® during the course of the study. If whole blood or other blood products are used during surgery or during the control of hemorrhagic episodes, the exact dose, date and duration of treatment and prescription etiology will be entered in the appropriate case report forms.

7.13 Concurrent Medication

All prescription and non-prescription medication administered to the subjects, including the name of the drug, dose, duration of treatment as well as the indication for use will be entered on the appropriate case report forms.

7.14 Discontinuation of Subjects

A subject will be discontinued from the study if any of the following occurs:

- A severe or serious adverse event, which, based on medical judgment, prevents completion of participation in the study (See Section 10 for classification of adverse events).
- Development of a Factor VIII inhibitor either
  - at a titer at or above 5 BU/mL **or**
  - at a titer lower than 5 BU/mL but that renders Alphanate® treatment ineffective in providing hemostasis.
- Treatment of the subject with another Factor VIII concentrate while in the study.
- Treatment of the subject with immunosuppressive drugs.
- Unwillingness and non-compliance of the subject with the provisions of the protocol.
- Withdrawal of the subject from the study on his/her own volition.
- Death of the subject during participation in the study.

If a subject withdraws after treatment, the clinical site will attempt to obtain all possible follow-up safety data from that subject.

8 CLINICAL ENDPOINTS

The safety of Alphanate® will be evaluated based on the following criteria:

8.1 Primary Endpoint

Incidence of antibody inhibitors to Factor VIII in at least 50 evaluable subjects will be determined following a period of at least two years and a minimum of 50 exposure days or if 50 exposure days are not reached, a maximum of 30 months. Only one exposure-day will be accrued for each surgical episode independent of the number of days of treatment or the number of infusions. For the purpose of this endpoint only, a subject is determined to be evaluable when his clinical experience after the date of enrollment includes at least 50 CEDs of treatment with Alphanate® or when he develops inhibitors with a titer greater than or equal to 5 BU/mL as determined by the central reference laboratory.

8.2 Secondary Endpoints

All subjects will be considered to be evaluable for the secondary endpoints.

1. The frequency, nature, causality and severity of adverse events.

2. The occurrence of variations in biochemical parameters which would indicate renal or hepatic impairment.

3. Evidence for seroconversion to HIV-1/HIV-2, HAV, HBV, HCV and parvovirus B19 for subjects who are seronegative for these viruses upon enrollment.

4. The amount of product used per year as part of at-home prophylaxis and therapy for bleeding episodes.

5. A physician's qualitative assessment of hemostasis (evaluated as "None," "Moderate," "Good," or "Excellent") in response to therapy with Alphanate® received by the subject in a hospital or at another location under the physician's direct supervision.

With this section, the design of this clinical trial satisfies the recommendations of the CPMP for trials in previously-treated patients of concentrates with marketing authorization in which a manufacturing process has been added.

9 STATISTICAL PLAN

9.1 Sample Size

The sample size of 50 to 80 evaluable patients followed for at least two years for renal or hepatic function and evidence of seroconversion to HIV-1/HIV-2, HAV, HBV, HCV and parvovirus B19 is in accordance with the recommendations of the CPMP regarding post-authorization surveillance of previously-treated patients infused with a coagulation factor concentrate:

- for which a marketing authorization has already been granted and
- in the production of which a manufacturing process has been added.

9.2 Planned Method of Analysis

9.2.1 Primary Endpoint

Incidence of Antibody Inhibitors to Factor VIII in Individuals Treated with Alphanate®

Assuming a maximum biannual rate of inhibitor incidence of three percent in multi-transfused hemophilia patients, the 95% one-sided confidence interval for this incidence rate in a sample of 50 subjects is up to two percent. Thus,

with a sample of 50 evaluable subjects monitored for two years, if one subject develops an antibody inhibitor to Factor VIII with a titer higher than 0.6 modified BU/mL, that persists for at least one month, the incidence of inhibitors to Factor VIII will be judged to be higher than that for patients with hemophilia A who are treated with currently marketed Factor VIII concentrates. If the sample size is increased to 80 subjects, no more than 1 subject may develop an antibody inhibitor to Factor VIII.

9.2.2 Secondary Endpoints

1. Adverse Events

   The percentage of subjects experiencing one or more adverse events will be calculated. The frequency of adverse events will be reported as a proportion of the total number of infusions with Alphanate®. Causality [the proportions of adverse events judged to be definitely, probably, possibly, probably not and definitely not related to treatments with Alphanate®] and severity will be reported as percentages of the total number of adverse events occurring during the course of the clinical trial.

2. Assessment of Biochemical Tests on Serum

   Descriptive statistics will be presented, with a focus on parameters which when elevated may indicate alterations in hepatic or renal function.

3. Viral Safety

   Viral safety will be determined by a lack of seroconversion to reactivity against the specific blood-borne viruses for which subjects will have been tested and found to be nonreactive at the time of enrollment.

4. Consumption Analysis

   Descriptive statistics will be employed to evaluate the use of Alphanate® by subjects, both for self-administration and for treatment by the investigator at the clinical site or other places of treatment.

5. Hemostasis

   Descriptive statistics will be employed to evaluate the physicians' qualitative assessments of the ability of Alphanate® to control bleeding episodes when administered at the clinical site.

10 ADVERSE EVENTS

Any adverse events observed by the investigator or study staff or reported by the subject and which occur during the study will be recorded on the appropriate case report form. These events will be reported regardless of the perceived relation of the event to study treatment.

10.1 Adverse Event Monitoring

At each visit of the subject to his treatment center an assessment will be performed of any adverse events occurring since the previous visit. Any adverse event, which is observed by the investigator or study staff or reported by the subject and which occurs during the study, will be reported on the appropriate CRF. Any substantive or unexpected new abnormality will be recorded and monitored until the abnormal finding returns to normal for that subject.

Each new event that appears to be independent of any prior event will be reported separately. The description of the event will include:

- its nature,
- the date of onset,
- the duration and severity of each sign or symptom,
- the investigator's opinion regarding the likelihood of causal relationship to the study drug,
- the course of action taken and
- the resolution of the event.

10.2 Serious Adverse Events

Any serious adverse event, including death, that occurs while the subject is receiving an investigational drug, irrespective of the investigator's opinion regarding drug relationship, will be reported by telephone **within 24 hours of its occurrence** to the Grifols' Personnel assigned to the study, [redacted], who can be contacted during working hours (8:00 a.m. to 5:00 p.m. Pacific Time) at [redacted] or [redacted] during non-working hours.

A **serious** adverse event is defined by FDA regulations in the Code of Federal Regulations (21 CFR 312.32) as any adverse event that suggests a significant hazard, contraindication, side effect or precaution. With respect to human clinical events, a serious event includes any event that results in the following outcomes: Death, a life-threatening adverse drug event, inpatient hospitalization or prolongation of existing hospitalization, a persistent or significant disability/incapacity, or a congenital anomaly/birth defect. Important medical events that may not result in death, be life-threatening, or require

hospitalization may be considered serious adverse drug events when, based upon appropriate medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in the definition.

If a serious adverse event occurs, based on the investigator's judgment, appropriate therapy should be administered. Subjects will then be monitored closely as appropriate.

Oral reports of serious adverse events must be confirmed in writing within five (5) calendar days of the occurrence to Grifols Inc. and to the Institutional Review Board within their reporting guidelines.

10.3 Grading Scale

An adverse event may occur during or following the administration of the investigational compound or control agent. Each event will be graded according to severity using the following criteria as appropriate:

- **Mild:** the event was noted but the administration of the compound was not interrupted; the event resolved spontaneously or no treatment was required beyond administration of nonprescription analgesics.
- **Moderate:** the administration of the compound was not necessarily interrupted; the event required momentary treatment with prescription drugs and produced no sequelae.
- **Severe:** the event caused interruption of the administration of the compound, produced sequelae and required prolonged treatment. Infusion of the compound will not be resumed until all symptoms are resolved.

10.4 Causality

The investigator must attempt to explain each adverse event and its relationship to drug treatment (definite, probable, possible, probably not, definitely not). Criteria for determining the relationship of clinical adverse reactions to test drug administration are as follows:

- **Definite:** an event that follows a reasonable temporal sequence from administration of the drug or in which the drug level has been established in body fluids or tissues; that follows a known response pattern to the suspected drug; and that is confirmed by improvement on stopping the drug (dechallenge), and reappearance of the reaction on repeated exposure (rechallenge).
- **Probable:** an event that follows a reasonable temporal sequence from administration of the drug; that follows a known response pattern to the suspected drug; 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 drug; that follows a known response pattern to the suspected drug; but that could have been produced by the subject's clinical state or other modes of therapy administered to the subject.
- **Probably not:** an event that follows a reasonable temporal sequence from administration of the drug; that does not follow a known response pattern to the suspected drug; and that could be reasonably explained by the known characteristics of the subject's clinical state.
- **Definitely not:** an event that occurs prior to the administration of study drug, is proven to be caused by the subject's disease or condition or another drug through rechallenge, or does not recur following rechallenge.

11 SUBJECT DEATHS

All deaths of subjects, regardless of cause, occurring within 24 months of the subject's entry into the protocol and which are known to the investigator will be reported in the case report on the appropriate CRF. Documentation of the subject's cause of death and a copy of the autopsy report, if any, will also be provided.

Grifols Inc. must be notified within 24 hours by telephone of all subject deaths; written follow-up must be received within 5 calendar days of initial notification.

12 INFORMED CONSENT FORM AND PATIENT AUTHORIZATION FORM

The investigator will be responsible for obtaining from every subject prior to his participation in the study a written Informed Consent signed and dated by the subject in accordance with US Federal regulations. Any study enrolling subjects less than 18 years of age must allow for the signing of informed consents by the subject's legally authorized representative. A written Informed Consent will be obtained from the subject after the investigator has provided a full explanation, both verbally and in writing, of the purpose, risks and discomforts involved, and potential benefits of the study. The original signed copy of the Informed Consent must be maintained in the institution's records, and is subject to inspection by a representative of Grifols Inc. The Informed Consent document must be approved by the Institutional Review Board and Grifols Inc. prior to study initiation and must conform to federal regulations. The document must include the following:

- A statement that the study involves research, an explanation of purposes of the research and the expected duration of the subject's participation, a

description of the procedures to be followed, and identification of any procedures that are experimental.

- A description of any reasonably foreseeable risks or discomforts to the subject.
- A description of any benefits to the subject or to others which may reasonably be expected from the research.
- A disclosure of appropriate alternative procedures or courses of treatment, if any, that might be advantageous to the subject.
- A statement describing the extent, if any, to which confidentiality of records identifying the subject will be maintained and that notes the possibility that representatives from the Food and Drug Administration and/or other regulatory agencies and Grifols Inc. may inspect and obtain copies of the records, including medical records.
- For research involving more than minimal risk, an explanation as to whether any compensation is provided and an explanation as to whether any medical treatments are available if injury occurs and if so, what they consist of, or where further information may be obtained.
- An explanation of whom to contact for answers to pertinent questions about the research and subject's rights, and whom to contact in the event of a research-related injury to the subject.
- A statement that participation is voluntary, that refusal to participate will involve no penalty or loss of benefits to which the subject is otherwise entitled, and that the subject may discontinue participation at any time without penalty or loss of benefits to which the subject is otherwise entitled. The subject must be assured that refusal to participate in the study or withdrawal from the study will not affect the quality of the medical care which he/she will receive.
- A statement that the particular treatment or procedure may involve risks to the subject (or to the embryo or fetus, if the subject is or may become pregnant) which are currently unforeseeable.
- Anticipated circumstances under which the subject's participation may be terminated by the investigator without regard to the subject's consent.
- Any additional costs to the subject that may result from participation in the research.
- The consequences of a subject's decision to withdraw from the research and procedures for orderly termination of participation by the subject. The subject must be assured that withdrawal will not affect the level of medical care which he/she will receive.

- A statement that significant new findings developed during the course of the research which may relate to the subject's willingness to continue participation will be provided to the subject.
- The approximate number of subjects involved in the study.

In conjunction with the Informed Consent, the investigator will also be responsible for obtaining the Patient Authorization Form for Release of Information, in accordance with the Health Insurance Portability and Accountability Act (HIPAA), effective 14 April 2003, prior to the subject's participation in the study.

The Patient Authorization Form will allow the investigator and his support staff to use or disclose identifiable health information to Grifols Inc., its employees and agents, members of, and auditors hired by, the independent board that approved this clinical trial; and members of government agencies in the US and other countries that grant approvals for drugs to be sold and used to treat patients. The authorization will apply to subjects enrolled in the clinical sites in the United States and other participating countries.

If the subject chooses not to sign the authorization form, he cannot participate in the trial. However, treatment not related to the study will not be withheld from him.

13 ADMINISTRATIVE PROCEDURES AND DOCUMENTS

13.1 Institutional Review Board and Privacy Board

US Federal regulations require that an Institutional Review Board (IRB) oversee all investigational drug studies (21 CFR 56). This board or committee, the makeup of which must conform to federal regulations, and any state and local guidelines regarding such, will approve all aspects of the study, including said protocol and written Informed Consent to be used, prior to initiation of the study. The investigator will provide Grifols Inc. with a copy of the communication from the committee to the investigator indicating approval of the protocol and consent form. All amendments to the protocol must be reviewed and approved prior to implementation, except where necessary to eliminate apparent immediate hazards to human subjects.

THE INVESTIGATOR WILL BE RESPONSIBLE FOR OBTAINING ANNUAL IRB RENEWAL FOR THE DURATION OF THE STUDY. COPIES OF THE INVESTIGATOR'S REPORT AND/OR COPIES OF THE IRB EXTENSION APPROVAL MUST BE SENT TO GRIFOLS INC.

The IRB or a duly established Privacy Board will review and approve the Patient Authorization Form to allow the release of the subject's protected health information to Grifols Inc. and other entities as required to fulfill the

obligations of this protocol. Any requested changes to the Authorization Form by the Board must be submitted to Grifols Inc.

13.2 Documents Required Before a Study Is Initiated

Prior to study implementation and drug shipment, the following documents must be submitted to Grifols Inc. for the purpose of FDA submission and/or procedural compliance:

- Executed Form FDA 1572, listing all subinvestigators and signed by the principal investigator.
- Investigator's Agreement from the final protocol signed by the principal investigator.
- Current Curricula Vitae for the principal investigator and all subinvestigators.
- Copy of the correspondence from the IRB indicating approval of the protocol and written Informed Consent, signed by the IRB chairperson or designee and containing the name and address of the IRB.
- A sample of the written Informed Consent that was reviewed and approved by the IRB, or a revised document if changes were requested by the committee.
- A sample Patient Authorization Form that was reviewed and approved by the IRB or Privacy Board, or a revised document if changes were requested by the Board.

13.3 Documents Required Within 30 Days of Study Initiation

- A membership roster of the IRB, listing names and occupations. In order to avoid a conflict of interest, investigators, subinvestigators and others directly participating in the study, should indicate in writing their abstention from voting on the protocol if they are also IRB members.
- Clinical laboratory ranges for all tests required for the protocol and documentation of laboratory licensure.

14 REPORTING AND MONITORING OBLIGATIONS OF INVESTIGATORS

14.1 Case Reports

A case report, comprised of individual case report forms, will be completed for every subject who signed a written Informed Consent form for participation in the study.

- Case report pages will be filled out in indelible dark ink or will be typewritten.
- Any corrections will be made by a single line stroke through the entry to be corrected, and the correct entry will be made above or near the deleted entry, initial and dated. Correction fluid must not be used.
- The Investigator must sign and date the certification of the case report form completion and return the originals, not copies, to Grifols, Inc. This signature will indicate that thorough inspection of the data has been made and will thereby certify the contents of the case report form.

All original source documentation (laboratory results, treatment records, audit queries, etc.) will be retained by the investigator or institution unless specified otherwise by the protocol. The results as they become available will be entered on the appropriate case report forms. Legible reproductions of the original laboratory reports for selected tests or variables may be included with the completed case report forms. The research manager will indicate which reports, if any, need to be reproduced. The investigator will retain a copy of the completed case report forms and necessary supporting laboratory data, and documentation before entrusting the original(s) to the mail.

Case report forms will be reviewed at the study site by a clinical monitor of Grifols Inc. who will make a decision as to their acceptability in regard to completeness and accuracy of the data. Query letters will be generated for omissions, corrections and clarifications. Data may also be reviewed in-house by a clinical assistant or data management personnel.

14.2 Clinical Monitoring

A representative of Grifols Inc. will visit the institution prior to initiating the study and periodically thereafter (at least once a year) to monitor acceptability of facilities, the agreement between CRF entries and original source documentation, adherence to the protocol and to applicable FDA and other regulatory agencies regulations and the maintenance of adequate clinical records. The monitor will have access to subject records, medication sheets, laboratory data and other source documentation.

14.3 Investigator's Final Report

A summary report must be submitted to the Institutional Review Board and a copy to Grifols Inc. within eight weeks after the study's completion or termination.

14.4 Regulatory Correspondence

The investigator will notify Grifols Inc. within three days following FDA or other regulatory agency contact with the investigative site. The investigator will provide Grifols in a timely fashion, with copies of all correspondence with the

FDA or other regulatory agency which may affect the review of the current study (e.g., Form 483, Inspection Observations, compliance responses) or their qualification as an investigator in studies conducted by Grifols Inc. (e.g., warning letters).

15 RECORDS

The Food and Drug Administration requires that an investigator will retain records for a period of two years following the date of the New Drug Application or Product License Application is approved for the drug for the indication for which is being investigated; or, if no application or license is to be filed or, if the application or license is not approved for such indication, until two years after the investigation is discontinued (21 CFR 312.62). In compliance with CPMP requirements, however, all records must be retained for a period of 15 years following the date of approval for the New Drug Application or Product License Application.

Investigator should ensure that the following records are maintained for the appropriate time period:

- Patient files containing copies of completed case reports and supporting documentation and the signed Informed Consent form.
- Investigator files containing copies of the documents required for the initiation of the study (executed Form FDA 1572, signed Investigator's Agreement, current *Curricula Vitae* for the principal and all subinvestigators, copy of the IRB approval of the protocol and Informed Consent form), copies of correspondence received from and sent to Grifols Inc.. In addition to these records required by regulations, Grifols Inc. requests that the investigator keep a copy of the Financial Agreement between Grifols Inc. and the investigator.
- Pharmacy files containing copies of the Investigational Drug Utilization Records (IDUR) or an equivalent form approved by Grifols Inc., instructions for the use of the IDUR and package inserts and/or the Investigator's Brochure.

16 HANDLING OF INVESTIGATIONAL DRUG

16.1 Investigational Drug Utilization Record Instructions

In accordance with federal regulations (21 CFR 312.62), all investigators are required to keep accurate records showing final disposition of all investigational drugs. The pharmacist in charge of the investigational drug may keep the records and sign the IDUR or the equivalent form approved by Grifols Inc. These records must be transmitted to Grifols Inc. on an ongoing

basis, showing accurate reconciliation of each and every shipment of investigational drug or control agent. A separate/new IDUR (or the equivalent) must be made for each new lot of drug received.

THE INVESTIGATOR MUST NOT USE MATERIAL PROVIDED FOR THIS PARTICULAR STUDY IN ANOTHER STUDY WITHOUT PRIOR WRITTEN APPROVAL FROM GRIFOLS INC.

Investigator or his/her designee will record:

- Person Responsible: The investigator or designee should sign the first report in full. On the Distribution Log, if the same individual signs the forms from day to day, his/her title need not be recorded after the first time.
- Lot Number: The lot number is indicated on the label applied to each container of the product.
- Expiration Date: The expiration date will be listed on the label of the product.
- Date Used: Date administered or dispensed to the subject.
- Disposition of Material: Indicate if administered, destroyed, damaged in transit and destroyed, or other final disposition of material. Material cannot be transferred for preclinical or other use without prior written approval from Grifols Inc.
- Unused Containers: Indicate any unopened units returned, as well as opened and partially used containers.
- Date Returned to Grifols Inc. or Destroyed: At the termination of the study, unused and opened and partially used containers will be returned to the clinical project manager or destroyed. However, the investigator will not destroy the supplies without prior consultation with the [redacted]. The [redacted], PhD, may be contacted at [redacted].
- Disposition of Investigational Drug Utilization Records: When an IDUR (or the approved equivalent form) is complete for any given lot, it is to be signed by the principal investigator or pharmacist in charge of the investigational drug. The white original (if Grifols' form) is to be mailed to Grifols Inc., the yellow copy is to remain in the pharmacy file and the pink copy is to be filed in the on-site investigator's file. Appropriate copies of an equivalent form will be filed at the pharmacy, the investigator's file and Grifols Inc.

16.2 Disposition of Clinical Supplies

Ultimate accountability for the receiving, dispensing and inventory of the test material lies with the investigator. US federal regulation requires that storage of the substance be in a secure enclosure, access to which is limited, to prevent theft or diversion. Neither the pharmacist nor investigator may supply the test material to any person outside of this protocol. (See previous section for details of the record-keeping for the investigational material.)

The investigational drug provided by Grifols Inc. for use in this study is intended for use only in the clinical trial outlined in this protocol and will be administered only to patients appropriately enrolled in this trial. The use of the drug for other clinical or preclinical situations is strictly prohibited. The use of the investigational drug by the investigator, subinvestigators or any third party, outside of the provisions stated in this protocol, without the express written permission of Grifols Inc., is strictly prohibited.

Alphanate® is an investigational drug for use in Clinical Trial GBI 04-01. The possession and use of Alphanate® by investigators must be closely controlled and monitored. All Alphanate® that is not used in the course of this clinical trial will be returned to Grifols Inc. or disposed of according to the written direction of the Clinical Research Manager.

On the written approval of the Project Manager, outdated Alphanate® will be destroyed and the containers and/or labels returned to Grifols Inc.

Material that is not outdated at the completion of the study will be returned by the investigator to an address provided by Grifols Inc. or will be handled otherwise according to written instructions from Grifols Inc.

17 MISCELLANEOUS PROVISIONS

17.1 Use of Information and Publication Rights

All information and data, including the terms of this protocol, and all data, clinical results and research conducted hereunder concerning Grifols Inc.'s products and operations including Grifols' patent applications, formulas, manufacturing processes, basic scientific data and formulation information that has been supplied by Grifols Inc. and not previously published are considered confidential by Grifols and will remain the sole property of Grifols Inc. The investigator understands and agrees that said proprietary and/or confidential information disclosed to or produced by him/her thereunder is highly valuable to Grifols Inc. and will be used EXCLUSIVELY by the investigator in accomplishing this study and will not use it for any other purposes without Grifols' prior written consent. The investigator agrees that he/she will not use

any such proprietary and/or confidential information for any other purpose. The investigator also understands and agrees that such disclosure will not be deemed to grant to the investigator a license for use of said proprietary and/or confidential information, except as expressly provided herein.

It is understood by the investigator that the information developed in the clinical study will be used by Grifols Inc. in connection with the development of Alphanate®, therefore, may be disclosed and used solely by Grifols as required to such third parties and agencies as Grifols, in its sole discretion, warrants. In order to allow for the use of the information derived from the clinical studies, it is understood that there is an obligation to provide to Grifols complete test results and all data developed in this study. The investigator agrees to promptly answer all inquires from the clinical research manager regarding completion, legibility or accuracy of trial data in the case report.

The investigator may publish the results of this study. In this regard, however, at least 30 days before submitting a manuscript to a publisher and 7 days before submitting an abstract for presentation at a scientific meeting, a copy will be provided by the investigator to Grifols for review. Grifols will have the right to modify or amend such a manuscript to ensure that no confidential or proprietary information of Grifols is disclosed and to insure that reported data are factually correct. Grifols agrees that before it publishes any results of this study in a refereed journal, it will provide the investigator, for review, a prepublication manuscript at least 30 days prior to the submission of the manuscript to the publisher.

18 REFERENCES

1. Goldsmith JC: Introduction: The challenges of inhibitor patient care. Sem Hematol. 30, Suppl 1:1-2, 1993.

2. Aledort L: Inhibitors in hemophilia patients: Current status and management. Am. J. Hematol. 47:208-217, 1994.

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21. Data on file at Grifols Inc.

19 INVESTIGATOR'S AGREEMENT

Investigator's Name: _____
Institution: _____

I attest that I have read Clinical Protocol GBI 04-01 (formerly ATC 02-01 of Alpha Therapeutic Corporation) entitled: "A Study of Immunologic Safety for Alphanate® in Previously Treated Patients Diagnosed with Hemophilia A", dated 10 January 2007 - incorporating protocol amendment six.

I understand the contents of Clinical Protocol GBI 04-01 and agree to adhere to the design, conduct and reporting requirements of the study as stated in the clinical protocol and to my obligations to Grifols Inc. as described in the protocol and executed contracts between myself, my institution and Grifols Inc.

I understand that if any questions about the design or conduct of the trial or about my responsibilities to Grifols Inc. (or those of Grifols Inc. to me) arise before or during the trial, I will promptly contact the clinical project manager at Grifols Inc. for clarification.

I understand that only I and the subinvestigators named on Form FDA-1572, completed for participation in Clinical Protocol GBI 04-01 are authorized to review and sign the case reports relating to this trial. I understand that I and my subinvestigator(s) are fully responsible for ensuring the completeness, correctness and timely entry of data on all case report forms relating to Clinical Protocol GBI 04-01.

I understand that the Alphanate® provided for this study is designated for use in Clinical Protocol GBI 04-01 only. I will not use this material for the performance of any other preclinical or clinical studies unless so requested and approved in writing by Grifols Inc. nor will I make this material available to any other person for any reason whatsoever.

I understand that for the purpose of this clinical protocol the Alphanate® consigned to me will become outdated on the expiration date listed on the product label or Investigator's Brochure. Any outdated material or material remaining at the conclusion of the trial will be returned to Grifols Inc. or disposed of according to their directions.

I agree that the data acquired in the performance of Clinical Protocol GBI 04-01 will not be used by me or any of the named subinvestigators in any publication except that which reports the results of this study alone and that all manuscripts and/or abstracts will be submitted to Grifols Inc. prior to submission for publication as specified in the protocol.

I furthermore agree to adhere to applicable Food and Drug Administration regulations regarding the conduct of clinical trials and the responsibilities of Clinical Investigators.

_____	_____	_____
Signature of Principal Investigator	Type or Print Name as Signed	Date
_____	_____	_____
Signature of Project Manager Grifols Inc.	Type or Print Name as Signed	Date

20 APPENDICES

APPENDIX 1: KARNOFSKY PERFORMANCE SCORE

100	Normal; no complaints, no evidence of disease
90	Able to carry on normal activity; minor signs of symptoms of disease
80	Normal activity with effort; some signs of symptoms of disease
70	Cares for self, unable to carry on normal activity or do active work
60	Requires occasional assistance, but is able to care for most personal needs
50	Requires considerable assistance and frequent medical care
40	Disabled; requires special care and assistance
30	Severely disabled; hospitalization is indicated, although death is not imminent
20	Very sick; hospitalization necessary; active support treatment is necessary
10	Moribund; fatal process progressing rapidly
0	Dead

APPENDIX 2: SCHEDULE OF EVENTS

Test	Screening[1]	Enrollment[2]	Seven to 14 days Post Infusion 1	In Hospital and In Clinic Treatments	Every Three Months until Month 30 and/or Off-Study[3]	Unsched
Sign Informed Consent Form	X					
Physical Examination medical & past treatment history, vital signs, height & weight	X					
Abbreviated Physical Exam interim medical history, vital signs, & weight		X			X	X
Hematology Panel CBC with WBC differential and platelet count	X				X	X
Biochemistry Panel ALT, AST, AP, LDH, BUN, total bilirubin, and creatinine	X				X	X
Viral Serology HBsAg, and antibodies to HAV, HBcAb, HBsAb, HCV, HIV1/HIV-2, and parvovirus B19 (by routine immunoassay)	X	X[2]			X	X[5]
Parvovirus B19 PCR Assay performed only if seronegative for parvo B19 at enrollment			X			
CD4 Count	X					
Factor VIII:C	X	X[2]			X	X
Factor VIII:C Inhibitor (two sets of samples)	X	X[2]			X[4]	X[6,7]
Collect 2 mL citrated plasma for inhibitor testing			X			
Plasma for inhibitor characterization[7] performed only if subject develops an inhibitor					X[7]	
Serum for storage Draw approx 5 mL of serum and aliquot into three vials of at least 1.5 mL of sample each. Freeze and retain the sample at -20°C for later serological & virological tests.	X	X			X	X
Patient Diary if the Alphanate® is to be self-administered			X	X	X	X
Physician's Assessment of Hemostasis evaluated as “None,” “Moderate,” “Good,” or “Excellent”				X		

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