octapharma

CLINICAL STUDY PROTOCOL

Immunogenicity, Efficacy and Safety of Treatment with *Human-cl rhFVIII* in Previously Untreated Patients with Severe Haemophilia A

Investigational Product	*Human-cl rhFVIII*
Indication	Severe Haemophilia A
Study Design	Prospective, multicentre, multinational, open-label, non-controlled
Sponsor	Octapharma AG Seidenstrasse 2, 8853 Lachen, Switzerland
Study Number	GENA-05
EudraCT and/or IND Number	2012-002554-23 BB-IND 13722
Development Phase	Phase III
Clinical Start	1st Quarter 2013
Clinical End	4th Quarter 2018 for all patients, except those continuing Immune Tolerance Induction Therapy
Date of 4th amended Protocol Date of 3rd amended Protocol Date of 2nd amended Protocol Date of 1st amended Protocol Date of Protocol	01-Feb-2018 11-Nov-2014 30-Sep-2013 17-Dec-2012 01-Aug-2012
Version	05
Co-ordinating Investigator	[redacted]

STUDY OUTLINE

<table>
<tr><td colspan="2">Name of Sponsor/Company:
Octapharma AG, 8853 Lachen, Switzerland</td></tr>
<tr><td>Name of Investigational Product:
Human-cl rhFVIII</td><td>Protocol Identification Code:
GENA-05</td></tr>
<tr><td>Name of Active Ingredient:
Coagulation FVIII</td><td>Date of Final Protocol:
01-Feb-2018</td></tr>
<tr><td colspan="2">Title of Study:
Immunogenicity, Efficacy and Safety of Treatment with Human-cl rhFVIII in Previously Untreated Patients with Severe Haemophilia A</td></tr>
<tr><td colspan="2">Indication:
Severe Haemophilia A (FVIII coagulation activity [FVIII:C] <1%)</td></tr>
<tr><td colspan="2">Number of Study Centre(s):
Around 40 centres worldwide are planned to participate in this study</td></tr>
<tr><td>Study Duration:
This study started in the 1st quarter 2013 and will be completed in the 4th quarter 2018 for all patients, except those continuing Immune Tolerance Induction Therapy</td><td>Development Phase: III</td></tr>
<tr><td colspan="2">Objectives:
Primary:
• To investigate the immunogenicity of Human-cl rhFVIII in 100 previously untreated patients (PUPs) suffering from severe Haemophilia A (FVIII:C < 1%)
Secondary:
• To assess the efficacy of Human-cl rhFVIII during prophylactic treatment (based on the frequency of spontaneous break-through bleeds)
• To assess the efficacy of Human-cl rhFVIII during treatment of bleeds
• To assess the efficacy of Human-cl rhFVIII in surgical prophylaxis
• To assess the safety and tolerability of Human-cl rhFVIII</td></tr>
<tr><td colspan="2">Study Design:
Prospective, multicentre, multinational, open-label, non-controlled</td></tr>
<tr><td colspan="2">Number of Patients:
100 evaluable PUPs will be enrolled in this study</td></tr>
<tr><td colspan="2">Patient Selection Criteria:
The patient population will mainly entail new-borns or infants; however, there is no limitation of age for study admission.
Inclusion criteria:
1. Male patients
2. Severe Haemophilia A (FVIII:C < 1%)
3. No previous treatment with FVIII concentrates or other blood products containing FVIII
4. Voluntarily given, fully informed written and signed consent obtained before any study-related procedures are conducted (obtained from the patient’s parent/legal guardian)</td></tr>
</table>

Name of Sponsor/Company: Octapharma AG, 8853 Lachen, Switzerland	
Name of Investigational Product: *Human-cl rhFVIII*	Protocol Identification Code: GENA-05
Name of Active Ingredient: Coagulation FVIII	Date of Final Protocol: 01-Feb-2018

Exclusion criteria:

1. Diagnosis with a coagulation disorder other than Haemophilia A
2. Severe liver or kidney disease (alanine amino transferase (ALT) or aspartate transaminase (AST) levels >5 times of upper limit of normal, creatinine >120 µmol/L)
3. Concomitant treatment with any systemic immunosuppressive drug
4. Participation in another interventional clinical study currently or during the past 4 weeks

Test Product, Dose, Mode of Administration, and Batch Number(s):

Human-cl rhFVIII is a purified B-domain deleted FVIII glycoprotein that is synthesised by a genetically engineered human embryonic kidney cell line (HEK 293F). *Human-cl rhFVIII* will be provided in single use vials containing a nominal potency of 250, 500, 1000 or 2000 International Units (IU) each of freeze-dried recombinant FVIII (rFVIII) concentrate to be reconstituted in 2.5 mL of water for injection. Full vials are to be injected, preferably - except for recovery investigations.

Human-cl rhFVIII should be used for intravenous injection only (maximally 4 mL/minute).

Dose:

Prophylactic treatment is recommended, but finally, it is the decision of the responsible treating physician whether patients will be treated prophylactically or on-demand. Patients may switch from on-demand to prophylactic treatment, or from prophylactic to on-demand treatment during the course of the study.

Prophylactic treatment:

Patients will be treated prophylactically with a recommended dose of 20-50 IU FVIII/kg body weight (BW).

Starting prophylaxis with the first bleeding episode (BE) is highly recommended.

The frequency of treatment will depend on the patient's clinical situation. For example, prophylaxis may be initiated with every other day injections (in order to keep the FVIII trough level >1%), or with once weekly injections, followed by twice and three times weekly, and every other day treatment. In cases of inadequate response, *Human-cl rhFVIII* administration frequency or dose adjustments can be considered at Investigator's discretion.

On-demand treatment:

In case of any bleed, the patients can be treated on-demand. The dosage and duration of treatment of spontaneous or traumatic bleeds depend on the location and the extent of bleeding as well as on the clinical situation of the patient. Dosage recommendations are given as follows:

- Minor haemorrhage: 20-30 IU FVIII/kg BW to achieve an intended target peak level of about 40% to 60%. Repeat dose every 8-24 hours until BE is resolved.
- Moderate to major haemorrhage: 30-40 IU FVIII/kg BW to achieve an intended target peak level of about 60% to 80%. Repeat dose every 6-24 hours until BE is resolved.

Name of Sponsor/Company: Octapharma AG, 8853 Lachen, Switzerland	
Name of Investigational Product: *Human-cl rhFVIII*	Protocol Identification Code: GENA-05
Name of Active Ingredient: Coagulation FVIII	Date of Final Protocol: 01-Feb-2018

- Major to life-threatening haemorrhage: initial dose of 40-60 IU FVIII/kg BW to achieve an intended target peak level of 100% to 120%. Repeat dose of 20-50 IU FVIII/kg BW every 6-12 hours until BE is resolved.

Surgical prophylaxis:

The dosage and duration of treatment with *Human-cl rhFVIII* will depend on the type of surgery and the patient's individual incremental recovery. Dosage recommendations are given as follows:

- Minor surgeries including tooth extractions: 25-30 IU FVIII/kg BW starting within 3 hours prior to surgery to achieve an intended target peak level of >30%. Repeat one dose every 12-24 hours if needed. Trough levels should be maintained at ≥ 30%.
- Major surgeries: 40-60 IU FVIII/kg BW within 3 hours prior to surgery to achieve an intended target peak level of approximately 100%. Repeat if necessary after 6-12 hours initially and for at least 6 to 14 days until healing is complete and recurrence to regular prophylactic treatment is possible. Trough levels should be maintained at > 50%.

Recovery investigation (optional):

Patients will receive 40 IU FVIII/kg BW for *in vivo* recovery evaluation. Blood samples are taken at baseline, 15 minutes and 1 hour after the IMP administration.

Immune tolerance induction (ITI) (if applicable):

Patients who develop a clinically significant and non-transient inhibitor will be offered to start ITI with the Investigational Medical Product (IMP).

The modified Bonn Protocol for inhibitor elimination is recommended:

- Low responders (<5 Bethesda Units [BU]) should receive 50-100 IU FVIII/kg BW daily or every second day. In case the inhibitor increases to 5 BU or more, the patient should switch to the high responder regimen.
- High responders (≥5 BU) should receive 100-150 IU FVIII/kg BW every 12 hours.

Once the inhibitor is eliminated (<0.6 BU), the FVIII recovery is ≥66% of normal, and the half-life of FVIII is at least 6 hours, a continuous reduction of about 10% of the initial ITI dosage should be initiated, until the patient has reached a prophylactic treatment regimen of 30-50 IU FVIII/kg BW every other day.

Any other ITI approach is possible.

All treatment details, including the needs of bypassing agents, and the further development of the inhibitor titre need to be documented.

Batch Number(s):

Several batches of *Human-cl rhFVIII* will be used.

Duration of Treatment:

The patients should stay in the study for 100 exposure days (EDs) and for a maximum period of 5 years from screening.

In patients who develop FVIII inhibitors and start ITI treatment, the maximum length of ITI will be 36 months.

Name of Sponsor/Company: Octapharma AG, 8853 Lachen, Switzerland	
Name of Investigational Product: *Human-cl rhFVIII*	Protocol Identification Code: GENA-05
Name of Active Ingredient: Coagulation FVIII	Date of Final Protocol: 01-Feb-2018
Reference Therapy, Dose, Mode of Administration, and Batch Number(s): N/A	

Study Outcome Parameters (Primary and Secondary Endpoints):

Primary endpoint

Immunogenicity:

Inhibitor activity will be determined by the modified Bethesda assay (Nijmegen modification), using congenital FVIII-deficient human plasma spiked with *Human-cl rhFVIII* at the following time points:

- At baseline (Screening Visit)
- Every 3-4 EDs until ED 20
- Every 10-12 EDs or every 3 months ± 2 weeks, (whichever comes first) from ED 20 to ED 100
- At study completion
- Any time in the case of a suspicion of inhibitor development.

In case of a positive inhibitor result, an inhibitor re-testing using a second separately drawn sample should be performed.

Secondary endpoints

Efficacy:

Efficacy of prophylactic treatment: The efficacy of *Human-cl rhFVIII* in the prophylactic treatment will be assessed based on the frequency of spontaneous break-through bleeds under prophylactic treatment. The dates and times of study drug infusions, details of dose(s) and product batch numbers used for the prophylactic treatment will be documented. Details of any BEs occurring under prophylactic treatment will be documented. Study drug consumption data (FVIII IU/kg per month, per year) per patient and in total will be evaluated.

Efficacy of treatment of bleeds: The efficacy of *Human-cl rhFVIII* in the treatment of bleeds will be assessed based on an objective haemostatic efficacy scale. Details of the bleed (type, site and severity of the bleed, and the start and end date and time of the bleed), the amount of *Human-cl rhFVIII* needed and the number of injections necessary to stop the bleed will be documented.

Efficacy of surgical prophylaxis: The efficacy of *Human-cl rhFVIII* in surgical prophylaxis will be assessed based on:

- Overall efficacy assessment (taking the intra- and post-operative assessment into account) after the end of the surgical prophylactic treatment phase, done together by the surgeon and the haematologist.
- Average and maximum expected estimated blood loss compared to the actual estimated blood loss.

In addition, the location, severity, and type of surgery will be documented. Expected and actual duration of surgical procedure and details of administered dose(s) of *Human-cl rhFVIII* (pre-, intra- and/or post-operatively) will be recorded. FVIII plasma levels (pre-,

Name of Sponsor/Company: Octapharma AG, 8853 Lachen, Switzerland	
Name of Investigational Product: *Human-cl rhFVIII*	Protocol Identification Code: GENA-05
Name of Active Ingredient: Coagulation FVIII	Date of Final Protocol: 01-Feb-2018

intra-, and post-operatively) will be measured. Details of concomitantly administered products (except standard anaesthesia) along with a brief narrative describing the outcome of the intervention will be recorded.

Safety:

Vital signs: Blood pressure, heart rate, respiratory rate and body temperature will be assessed at screening, 3-monthly and at study completion. In case IMP is injected at study site during these visits, one pre- and one post-treatment control is obligatory.

Safety laboratory parameters: The following routine safety laboratory parameters will be tested at screening, and at 3-monthly (± 2 weeks) control visits: haematological parameters (red blood cell count, white blood cell count, haemoglobin, haematocrit, platelet count) and clinical chemistry (ALT, AST, serum creatinine). Samples are analysed in the local laboratory.

Tolerability: The occurrence of any adverse event (AE) will be monitored throughout the study.

Additional analyses

Recovery investigation (optional): A recovery investigation is recommended to be performed. This can be done with the first *Human-cl rhFVIII* administration, or within the first three months after treatment start with *Human-cl rhFVIII*, in any case in a non-bleeding patient. A dose of 40 IU FVIII/kg BW will be administered and the *in vivo* recovery will be calculated from the FVIII levels before infusion and the peak level obtained from the 15 min and 1 hour post-infusion samples. Recovery investigations are recommended to be repeated approximately every 6 months.

FVIII gene mutation analysis (mandatory).

Immunogenotyping (optional): HLA-typing, immune response gene profiling and FVIII ethnic haplotype. Immunogenotyping will be performed in order to investigate genetic factors that might influence / predict development of FVIII inhibitors. Blood samples (EDTA) required for this analysis can be obtained at any time during the study.

RNA expression analysis (optional): RNA expression analysis will optionally be performed in order to provide an understanding of the transcript activity of the genes involved in immune responses that may be responsible for FVIII inhibitor formation in patients receiving exogenous FVIII. RNA expression analysis will also be optionally performed on patients undergoing ITI to provide information on the transcript activity of genes involved in immune tolerance. The analysis will be carried out using a customised PAXgene protocol in which RNA samples will be obtained by using commercially available PAXgene RNA blood collection tubes and centrally created customised microfuge tubes.

In vitro immunogenicity of FVIII products (optional): Immunogenicity of *Human-cl rhFVIII* will optionally be assessed by culturing peripheral blood monocyte cells (PBMC) (including a positive control) with *Human-cl rhFVIII*. The nature of T cell response will be assessed by analysing cytokine expression (measured in a multiplex format) and T cell proliferation.

Name of Sponsor/Company: Octapharma AG, 8853 Lachen, Switzerland	
Name of Investigational Product: *Human-cl rhFVIII*	Protocol Identification Code: GENA-05
Name of Active Ingredient: Coagulation FVIII	Date of Final Protocol: 01-Feb-2018

Epitope mapping (optional): Epitope mapping is optionally performed in patients who developed an inhibitor against FVIII and started an ITI.
In addition to these, samples available from patients who later on developed an inhibitor and started ITI, and from an inhibitor negative control group will be tested: already available plasma samples from ~20 PUPs included into the study in Ukraine and Moldova, which were drawn prior to the first exposure to FVIII are planned to be analysed in retrospect.
Additional health economic parameters: the resource use of patients treated with *Human-cl rhFVIII* is examined – and, if the size of the subgroups allows, the resource use of patients treated prophylactically or on-demand. In case patients develop an inhibitor, the resource use is examined in this subgroup. Parents will be asked to fill out a short "Additional Health Economic Evaluation" questionnaire on a quarterly basis starting from the Screening visit, which asks about their time commitments, and productivity loss.

Summary of Study Procedures and Statistical Analysis Plan:
Study Procedures:
All administrations of *Human-cl rhFVIII*, the occurrence of bleeds, the occurrence of AEs and the administration of concomitant medication during the entire study period will be thoroughly documented to assess the exposure to FVIII, the efficacy in the prevention of and the treatment of bleeds, and the overall safety and tolerability.
Screening Visit:
Patients' parent(s)/legal guardian(s) will be informed about the study details and will be required to give their written informed consent before any investigations and assessments can be performed. The following will be documented/assessed:

- Check of inclusion- and exclusion criteria
- Demographics, including patient's blood type and FVIII inhibitor risk factors
- Medical history
- Vital signs
- Height
- Body weight
- Physical examination
- Safety laboratory parameters
- FVIII:C (CHR and OS assay)
- FVIII inhibitor screen
- Additional Health Economic Evaluation
- RNA expression analysis (optional)
- FVIII gene mutation analysis (mandatory, can be performed later during the study)
- Immunogenotyping (optional, can be performed later during the study): HLA-typing, immune response gene profiling, FVIII ethnic haplotype determination
- *In vitro* immunogenicity of FVIII products (optional)
- Epitope mapping (optional)

Name of Sponsor/Company: Octapharma AG, 8853 Lachen, Switzerland	
Name of Investigational Product: *Human-cl rhFVIII*	Protocol Identification Code: GENA-05
Name of Active Ingredient: Coagulation FVIII	Date of Final Protocol: 01-Feb-2018

The Investigator will hand out the patient diary and instruct the patient/legal guardian how to document details of treatment with *Human-cl rhFVIII*, bleeds, AEs and concomitant medication. The patient/legal guardian will receive a sufficient amount of the study product for home treatment (in cooling boxes, if appropriate) and will be instructed on how to store and administer it. Details of home treatment (parents' home treatment training, the involvement of the general practitioner or a study nurse is documented).

Follow-Up Visits (every 3-4 EDs until ED 20):

The patients will visit the study site every 3-4 EDs until 20 EDs have been reached. At these visits, patients' body weight will be checked, and blood samples will be obtained for FVIII inhibitor screen and optionally RNA expression analysis (after a wash-out period of at least 2, preferably 3 days after the previous IMP administration). IMP injection is possible after blood sampling.

At these visits, the patient diary will be reviewed (any IMP injection, any bleed) in order to transfer data into the Case Report Form (CRF). The occurrence of AEs and changes in concomitant medication will be checked and documented.

Follow-Up Visits (every 10-12 EDs):

After ED 20 has been reached and until 100 EDs, patients will visit the study site every 10-12 EDs. At these visits, patients' body weight will be checked, and blood samples will be obtained for FVIII inhibitor screen (after a wash-out period of at least 2, but preferably 3 days after the previous IMP administration). IMP injection is possible after blood sampling. The patient diary will be reviewed (any IMP injection, any bleed) in order to transfer data into the CRF. The occurrence of AEs and changes in concomitant medication will be checked and documented.

3-Monthly (± 2 weeks) Follow-Up Visits (calculated from Screening Visit):

If appropriate, the above described follow-up visits may be combined with the regular quarterly (every 3-months (± 2 weeks)) Follow-Up visits, where some additional investigations will be performed: the safety laboratory parameters will be assessed and – in case IMP is injected after blood sampling – vital signs will be measured pre- and 30-60 minutes post-injection.

At these visits, patients' body weight will be checked, and blood samples will be obtained for FVIII inhibitor screen (after a wash-out period of at least 2, but preferably 3 days after the previous IMP administration). The patient diary will be reviewed (any IMP injection, any bleed) in order to transfer data into the CRF. The occurrence of AEs and changes in concomitant medication will be checked and documented. The Additional Health Economic Evaluation will be completed by the parents/legal guardians.

Once every 6 months a physical examination is performed.

If the optional *in vivo* recovery investigation is done, it is recommended with the first *Human-cl rhFVIII* administration, or within the first three months after treatment start with *Human-cl rhFVIII*, in any case in a non-bleeding patient. Controls approximately once every 6 months are recommended. Recovery visits should be timed so that they coincide with other study visits whenever possible. The *in vivo* recovery will be calculated from the

Name of Sponsor/Company: Octapharma AG, 8853 Lachen, Switzerland	
Name of Investigational Product: *Human-cl rhFVIII*	Protocol Identification Code: GENA-05
Name of Active Ingredient: Coagulation FVIII	Date of Final Protocol: 01-Feb-2018

FVIII plasma levels before infusion and the peak level obtained from the 15 minutes and 1 hour post-infusion samples.
In case the patient did not require any IMP from screening until the 3-Monthly Follow-Up visits during the first year, the blood sampling (inhibitor screen, safely laboratory) after 3 and 9 months is optional. After 6 and 12 months a sample is mandatory.

ITI Patient Follow-Up:

Patients who develop an - non-transient - inhibitor and do not agree to start an ITI will be withdrawn from the study.
Patients developing an inhibitor and starting ITI will be closely monitored, once their parents / legal guardians have been informed about the ITI treatment details and gave their written consent before ITI initiation. Depending on the development of the inhibitor: a bi-weekly (later on monthly to 3-monthly) inhibitor titre testing is considered appropriate.
Optional epitope mapping can be performed once the inhibitor is detected.
Optional RNA expression analysis can be performed alongside the inhibitor testing at initiation of ITI, 2 and 4 weeks after ITI initiation, and then on a monthly basis until month 6. This may give information on motifs predicting success that should be evident in the first 6 months and give mechanistic information on immune-tolerance.
Once the inhibitor is eliminated, a normalised recovery and half-life are expected during the continued ITI. Patient observation including testing visits is recommended according to progress of the ITI. Samples to test the recovery are recommended to be drawn at baseline and 15 minutes post-injection. For the half-life evaluation, the following sampling time points are recommended: baseline, 15 minutes, 3, 6, 9, 12, (24) hours post-injection.
Patients will complete study participation after a complete success of the ITI (inhibitor-free, normalised recovery and half-life), or after a maximum ITI period of 36 months.

Surgical Visits:

Study subjects may undergo surgical interventions during the course of the study. Depending on the type of surgery, subjects may be hospitalised or not (in case of certain minor surgeries with low risk of post-operative bleeding) at the discretion of the Investigator. Details of surgery, *Human-cl rhFVIII* dosing, expected and actual blood loss, concomitant treatment (including need for additional blood transfusions), and lab investigations will be documented, as well as details of hospitalisation (start and end date, regular ward, intensive care unit stay).

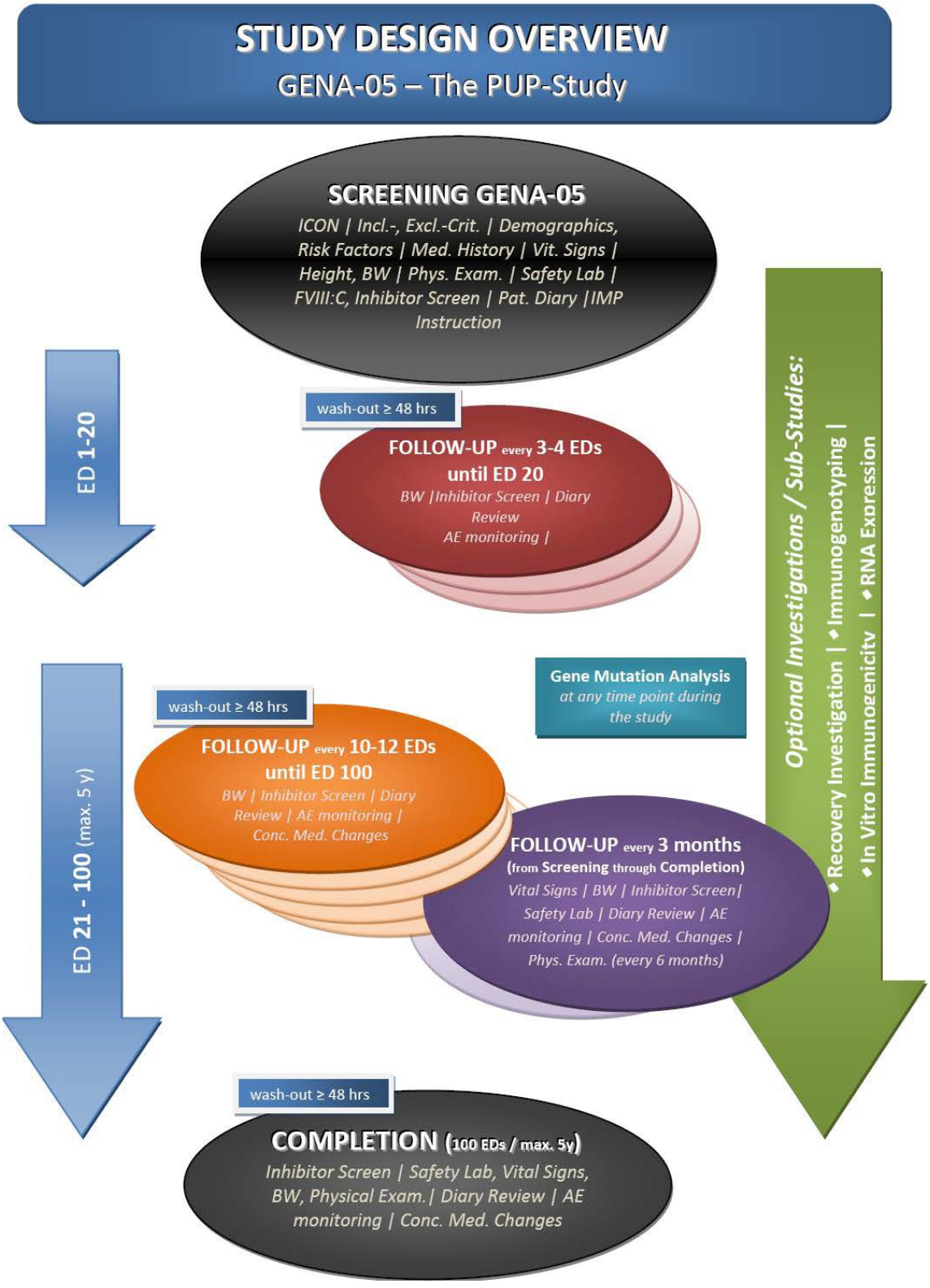
Completion Visit:

For each patient, the study is completed once 100 EDs have been reached, or after a maximum of 5 years' study participation - from screening.
Patients undergoing an ITI complete the study after a successful elimination of the inhibitor, but after a maximum ITI period of 36 months.
At these visits, patients' body weight will be checked, and blood samples will be obtained for FVIII inhibitor screen (after a wash-out period of at least 2, preferably 3 days after the previous IMP administration) and the safety panel. Vital signs will be checked and a physical examination will be performed. The occurrence of AEs and changes in concomitant medication will be checked and documented.

Name of Sponsor/Company: Octapharma AG, 8853 Lachen, Switzerland	
Name of Investigational Product: *Human-cl rhFVIII*	Protocol Identification Code: GENA-05
Name of Active Ingredient: Coagulation FVIII	Date of Final Protocol: 01-Feb-2018
At this visit, the patient diary will be reviewed (any IMP injection, any bleed) in order to transfer data into the CRF. **Statistical Analysis:** No inferential analysis involving formal testing is planned in this non-controlled trial. The sample size is determined by a CHMP guideline. Consequently, no formal sample size estimation is performed. The statistical analyses of the primary and secondary endpoints will be descriptive. An interim analysis is planned to be conducted after 50 patients have achieved at least 50 EDs.	

The study assessments and scheduled time points are summarised in the Flow Chart:

FLOW CHART



PROTOCOL SIGNATURES

Signature of the Sponsor's Representative

This study is intended to be conducted in compliance with the protocol, Good Clinical Practice and the applicable regulatory requirements.

On behalf of the Sponsor

Signature of the Author of the Protocol/ Clinical Project Manager

This study is intended to be conducted in compliance with the protocol, Good Clinical Practice and the applicable regulatory requirements.

Signature of the Biostatistician

This study is intended to be conducted in compliance with the protocol, Good Clinical Practice and the applicable regulatory requirements.

Signature of the Coordinating Investigator

This study is intended to be conducted in compliance with the protocol, Good Clinical Practice and the applicable regulatory requirements.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF ABBREVIATIONS

ADR	Adverse drug reaction
AE	Adverse event
ALT	Alanine aminotransferase
AST	Aspartate transaminase
BE	Bleeding episode
BLEED	Study population of BEs
BMI	Body mass index
BU	Bethesda unit
BW	Body weight
CHO	Chinese hamster ovary (cells)
CHR	Chromogenic
CHMP	Committee for Medicinal Products for Human Use
CRO	Contract Research Organisation
CVAD	Central Venous Access Device
ED	Exposure day
EMA	European Medicines Agency
FVIII	Coagulation factor VIII
FVIII:C	Factor VIII coagulation activity
GCP	Good Clinical Practice
GLP	Good Laboratory Practice
HEK	Human Embryonic Kidney
HLA	Human Leukocyte Antigen
ICU	Intensive care unit
IDMC	Independent Data Monitoring Committee
IEC	Independent Ethics Committee
IMP	Investigational Medicinal Product
IRB	Institutional Review Board
ITI	Immune tolerance induction
ITT	Intention to treat
IU	International Unit
IVR	In vivo recovery
LOCF	Last observation carried forward
MedDRA	Medical Dictionary for Regulatory Activities
OC	Observed cases
OS	One-stage
PBMC	Peripheral blood mononuclear cells
PP	Per protocol
PROPH	Study population of subjects with prophylaxis
PTP	Previously-treated patient
PUP	Previously-untreated patient
RBC	Red blood cell
rFVIII	Recombinant FVIII
SAEs	Serious adverse events
SAP	Statistical analysis plan
SOC	System organ class
SOP	Standard operating procedure
SURG	Study population of surgeries
ULN	Upper limit of normal
WBC	White blood cell
WFI	Water for injection

1 INTRODUCTION

Haemophilia A is an inherited gender-related coagulation disorder in which affected males do not produce functional coagulation factor VIII (FVIII) in sufficient quantities to achieve satisfactory haemostasis. Therefore, patients suffer from bleeding diathesis. Most bleeding episodes (BEs) occur in joints and muscles. Without adequate treatment these repeated haemarthroses and haematoma lead to long-term sequelae with severe disability. Other bleeding sites, although less frequent but more severe, are the central nervous system, the urinary or gastrointestinal tract, eyes and the retro-peritoneum. Hence, affected patients are at high risk to develop major and life-threatening bleeds after surgical procedures, even after minor ones such as tooth extraction.

The optimal effective treatment of the disorder is the replacement of FVIII by using FVIII concentrate either obtained by fractionation of human plasma or manufactured by recombinant DNA technology. However, the occurrence of inhibitory antibodies against the infused FVIII is a major clinical complication in the treatment of Haemophilia A. It is not known whether recombinant FVIII (rFVIII) products carry an enhanced immunogenic risk and whether there are differences between rFVIII products in their relative immunogenicity (1).

All commercially available recombinant rFVIII concentrates are exclusively produced in hamster cells, either in baby hamster kidney (BHK) cells (e.g. Kogenate FS) or in Chinese hamster ovary (CHO) cells (e.g. Advate, ReFacto). The intrinsic immunogenicity of current rFVIII products may be increased due to incorporation of non-human glycan structures during post-translational protein processing in mammalian cell lines.

Human-cl rhFVIII is a B-domain deleted rhFVIII expressed in genetically modified human embryonic kidney (HEK) 293F cells. Using a human cell line for the expression of rFVIII ensures that non-human immunogenic epitopes are absent, in contrast to rFVIII expressed in hamster cells. For example, N-glycolylneuraminic acid, which is reported to be antigenic in man (2) and present in recombinant glycoproteins expressed by CHO cells (3), was not detected in *Human-cl rhFVIII*. Furthermore, the antigenic carbohydrate epitope Galα1,3Gal, which has been reported to be present in recombinant proteins such as full-length FVIII from BHK cells (4), is not present in *Human-cl rhFVIII* either. The use of a human cell-line for the expression of rFVIII is expected to provide a more genuine human glycosylation pattern than achieved with murine cell-lines. This may result in an improved function and reduced immunogenicity of the rFVIII expressed from human cell-lines.

Clinical data on efficacy and safety of *Human-cl rhFVIII* in the targeted indications in previously treated patients (PTPs) are available. Since paediatric patients may respond differently to FVIII treatments than adults, European Medicines Agency (EMA) CHMP guidelines (5) specify that paediatric studies should be conducted within the clinical investigation program of FVIII products. Moreover, the clinical development strategy for FVIII products in paediatric patients should follow a stepwise approach, in order to gain experience in older patients before investigations are initiated in younger patients. Study GENA-03 was assessing efficacy, immunogenicity, pharmacokinetic profiles and safety of *Human-cl rhFVIII* in paediatric patients between 2 and 12 years. With the availability of 6 months' treatment data from more than 20 previously treated children, and with having finished all baseline pharmacokinetic assessments, the present study has been designed to investigate the immunogenicity, efficacy and safety of *Human-cl rhFVIII*, in previously untreated patients (PUPs) with inherited severe Haemophilia A.

A summary of findings from non-clinical as well as clinical investigations can be found in the Clinical Investigator's Brochure.

1.1 Rationale for Conducting the Study

The occurrence of an antibody against factor VIII, a so called inhibitor, is the most important complication in haemophilia treatment. Inhibitors occur in up to about 30% of PUPs with severe haemophilia, usually within the first 50 exposure days (EDs) to a FVIII concentrate. As specified in the CHMP Guideline on the Clinical Investigation of Recombinant and Human Plasma-Derived Factor VIII Products (CHMP/BPWP/144533/2009) (5), clinical trials in PUPs are required depending on the type of factor VIII product (e.g. novel modified proteins to extend half-life).

This clinical study is designed in compliance with the requirements set up in the CHMP guideline (CHMP/BPWP/144533/2009) (5). At the time when this study in children is planned to be started (in the course of the 4th quarter 2012), pharmacokinetic, efficacy, safety and immunogenicity data from further studies with *Human-cl rhFVIII* in PTPs will be available (including a children study, as required by the guideline).

The aim of this study is to investigate the immunogenicity of *Human-cl rhFVIII*, to assess the efficacy of *Human-cl rhFVIII* during prophylactic treatment, in the treatment of bleeds, and in surgical prophylaxis, and to assess the safety and tolerability of *Human-cl rhFVIII* in PUPs with severe Haemophilia A (FVIII coagulation activity [FVIII:C] <1%) over 100 EDs and for a maximum period of five years from screening.

Additionally health economics data are increasingly requested by decision makers in healthcare. This is because resources are scarce and there is a need to achieve the best outcomes possible within the resources available. Therefore, in addition to immunogenicity, efficacy and safety data of *Human-cl rhFVIII* resource use data will be collected. The resource use data that will be collected alongside this clinical trial could be linked to reference unit costs for a specific country of interest to obtain insight in the average cost of treatment of a Haemophilia A patient.

Collecting resource use data can provide insight into the differences in resource use of prophylactic administration of *Human-cl rhFVIII* against on-demand therapy. Also, data can be collected on resources (e.g. drug cost, monitoring cost) related to ITI for patients developing a clinically significant and non-transient inhibitor. As is known from literature ITI therapy is very expensive and cost may substantially vary depending on inhibitor status and choice of ITI strategy, further details on ITI therapy and its related cost are of considerable interest. This study can generate insight into resource use of patients developing inhibitors versus patients without inhibitors.

1.2 Dose Rationale

Human-cl rhFVIII will be provided in single use vials containing a nominal potency of 250, 500, 1000 or 2000 International Units (IU) each of freeze-dried rFVIII concentrate to be reconstituted in 2.5 mL of water for injection (WFI). Full vials are to be injected, preferably - except for recovery investigations. *Human-cl rhFVIII* should be used for intravenous injection only (maximum infusion speed: 4 mL/minute).

The dose and frequency of treatment of Haemophilia A depend on the individual patients' needs. As the current study will be conducted in PUPs, who will most likely be infants or

young children, and the recommendations in the published literature are controversial, no direct prophylactic dosing specifications are made, except that the starting dose should be 20-50 IU/kg BW. It is the Investigators' clinical judgement that will guide dose and frequency adjustments based on individual patients' needs.

1.3 Benefit-Risk Statement

On the basis of data available for adult and children (PTPs), the half-life and the recovery of *Human-cl rhFVIII* are similar to other already licensed rFVIII preparations. It can be concluded that *Human-cl rhFVIII* is efficacious in the prevention and in the treatment of BEs and during surgical prophylaxis in patients with inherited FVIII deficiency.

Comparable to other rFVIII preparations, the following adverse drug reactions (ADR) may occur by using *Human-cl rhFVIII*:

1. Allergic or anaphylactic types of reactions. Observed symptoms may include fever, chills, nausea, urticaria, pruritus, dizziness, chest tightness, shortness of breath, and – very rarely – anaphylactic shock.
2. Development of antibodies (inhibitors) against FVIII.

In conclusion, the hitherto existing clinical and pre-clinical data allow the conclusion that participating in this study does not represent any additional risk to the included patients in terms of immunogenicity and safety.

2 STUDY OBJECTIVES

2.1 Primary Objective

The primary objective of this clinical study is to investigate the immunogenicity of *Human-cl rhFVIII* in 100 PUPs suffering from severe Haemophilia A (FVIII:C < 1%).

2.2 Secondary Objective(s)

Secondary objectives of this trial are:

- To assess the efficacy of *Human-cl rhFVIII* during prophylactic treatment (based on the frequency of spontaneous break-through bleeds)
- To assess the efficacy of *Human-cl rhFVIII* during treatment of bleeds
- To assess the efficacy of *Human-cl rhFVIII* in surgical prophylaxis
- To assess the safety and tolerability of *Human-cl rhFVIII*

2.3 Further Objective(s)

Further objectives of this trial are:

- To examine the resource use of patients treated with *Human-cl rhFVIII*.
- And, if the size of the subgroups allow:
    - To examine the resource use of patients treated prophylactically and patients treated on-demand.
    - To examine the resource use of patients who developed inhibitors and those patients who did not develop inhibitors.

3 INVESTIGATIONAL PLAN

3.1 Study Endpoints

3.1.1 Primary Endpoint - Immunogenicity of Human-cl rhFVIII

Immunogenicity of *Human-cl rhFVIII* is the primary endpoint. Inhibitor activity will be determined by the modified Bethesda assay (Nijmegen modification), using congenital FVIII-deficient human plasma, spiked with *Human-cl rhFVIII,* at the following time points:

- At baseline (Screening Visit)
- Every 3-4 EDs until ED 20
- Every 10-12 EDs or every 3 months ± 2 weeks (whichever comes first) after ED 20 until 100 EDs are reached
- Any time in the case of suspicion of an inhibitor development

In case of a positive inhibitor result, an inhibitor retesting, using a second separately drawn sample, should be performed. A FVIII inhibitor is defined as "positive", if the retesting confirms the positive result, otherwise the result is considered as being "negative".

3.1.2 Secondary Endpoint(s)

3.1.2.1 Efficacy

Efficacy of prophylactic treatment

The efficacy of *Human-cl rhFVIII* in the prophylactic treatment will be investigated by calculating the frequency of spontaneous break-through bleeds under prophylactic treatment (see Section 7.2.2.1). Study drug consumption data (FVIII IU/kg per month, per year) per patient and in total will be evaluated. The dates and times of study drug infusions, the details of dose(s), and the product batch numbers used for the prophylactic treatment will be documented.

Efficacy of treatment of bleeds

The efficacy of *Human-cl rhFVIII* in the treatment of bleeds will be investigated by using a 4-point ordinal haemostatic efficacy scale (see Section 7.2.2.2). Details of the bleed, the amount of *Human-cl rhFVIII* needed and the number of injections necessary to stop the bleed will be documented.

Efficacy of surgical prophylaxis

In surgical procedures, the following parameters will be documented:

- Overall efficacy assessment (taking into account the intra- and post-operative assessment) after the end of surgical prophylactic treatment phase, done together by the surgeon and the haematologist (see Section 7.2.2.3)
- Average and maximum expected estimated blood loss, compared to the actual estimated blood loss
- Details on surgical procedure (see Section 7.2.2.3): location, severity, type, expected and actual duration
- Pre-, intra-, and post-operative FVIII plasma levels, if appropriate

- Details of administered dose(s) of *Human-cl rhFVIII* given pre-, intra- and/or post-operatively including dates, times and batch numbers
- Details on concomitantly administered drugs, including all blood and blood product transfusions, excluding standard anaesthetic drugs
- Details on all wound haematomas in terms of capturing, analysing, and reporting these, including any need for surgical evacuation
- Outcome of the intervention, described by means of a brief narrative.

3.1.2.2 Safety

Safety and tolerability

Safety and tolerability will be assessed by monitoring vital signs, standard laboratory parameters (red blood cells, white bleed cells, haemoglobin, hematocrit, platelets, ALT, AST, creatinine), and by monitoring adverse events (AEs).

3.1.3 Further Endpoint – Resource Use

Following a review of economic literature in haemophilia the most relevant parameters (in terms of volume and cost) were selected for inclusion in the current study. The resource use parameters that will be collected* and analysed are:

- FVIII concentrates
  Prophylaxis use or on-demand use, dose per kg body weight, the frequency of injection, duration of treatment, any change in treatment regimen, start date and stop date.
- Bleeding episodes: location, severity and duration.
- Inhibitor status, any change in FVIII use, use of other haemostatic agents, hospitalisation (including ward type) and its duration.
- ITI: ITI regimen details (regimen type, FVIII use, dose per kg body weight, the frequency of injection, start and stop date, other haemostatic agents, patients' inhibitor levels.
- CVAD: CVAD type, hospitalisation (including ward type) and duration, surgical procedures, complications due to CVAD use and management of the complication.
- Surgery prophylaxis: dosage and duration of treatment with FVIII for surgery prophylaxis including details on the type of surgery.

  * *The majority of the aforementioned resource parameters are collected as part of primary and secondary end points within the study. The same data will also be used to analyse resource use.*

- Non-medical resource use: The indirect costs brought about as a result of health care resource use should also be considered. Such costs include productivity losses of the parents (caregiver) and their opportunity costs arising through foregone time related to the child's disease.

The related resource use data will be evaluated by incorporating four questions (employment, household tasks and 'care' time) in the patient diary.

3.2 Overall Study Design and Plan

This study is designed as a prospective, multicentre, multinational, open-label, non-controlled phase III study in 100 male PUPs (i.e. patients without any previous treatment with FVIII concentrates or other blood products containing FVIII) who are suffering from severe Haemophilia A (FVIII:C < 1%). Around 40 centres worldwide are planned to participate in this study.

For each patient, the exposure to *Human-cl rhFVIII*, the efficacy of *Human-cl rhFVIII* in the prevention and the treatment of bleeds, the frequency of break-through bleeds in case of prophylactic treatment, the efficacy in surgical prophylaxis, and the overall safety and tolerability of *Human-cl rhFVIII* will be thoroughly assessed. In the course of the follow-up visits (i.e. every 3-4 EDs until ED 20, then every 10-12 EDs until ED 100), scheduled to be performed after the Screening Visit, FVIII inhibitor levels will be assessed for each patient. If appropriate, these visits may be combined with the additional regular 3-monthly (± 2 weeks) follow-up visits, stipulating some supplementary investigations are performed.

The occurrence of AEs and changes in concomitant medication will be checked and documented at each follow-up visit. A patient completes the study by reaching 100 EDs, or after a maximum study participation period of 5 years from screening. In patients who develop FVIII inhibitors and start ITI treatment, the maximum length of ITI will be 36 months. In patients undergoing surgical interventions, treatment details will be documented for the pre-, intra-, and post-operative phase, respectively.

The study is planned to start in the 4th quarter of 2012 and continues until the 4th quarter of 2018, except for those patients continuing Immune Tolerance Induction Therapy.

The tables below summarise the assessments to be performed at the individual visits:

Table 1 Screening Visit

Written informed consent for patients' parents / guardians	✓
Inclusion/exclusion criteria check	✓
Demographics, Medical History, risk factors	✓
FVIII gene mutation analysis*	✓
FVIII level	✓
FVIII inhibitor screen	✓
Safety lab tests	✓
Physical examination	✓
Vital signs	✓ (baseline and 30-60 minutes post-treatment, in case IMP is injected)
Body weight and height	✓
Immunogenotyping*† (HLA-typing, immune response gene profiling, FVIII ethnic haplotype determination)	✓
RNA expression analysis†¥	✓
Epitope mapping †¥ (analysed in all study patients who later on developed an inhibitor to FVIII and started ITI, and in a control group of ~20 patients who did not develop an inhibitor to FVIII during their 100 EDs with the IMP)	✓
In vitro immunogenicity testing†	✓
IMP injection after blood sampling (not mandatory)	✓
Diary & IMP instructions, plus completion of the "Additional Health Economic Evaluation" questionnaire	✓

* Suggested timing for these assessments; however, they can be performed at any point in the study. These analyses should be combined with scheduled study visits whenever possible. † Optional analyses. ¥ First blood sample must be drawn prior to the first *Human-cl rhFVIII* infusion.

Table 2 Follow-Up Visits

	Until ED 20	After ED 20*
FVIII inhibitor screen	Every 3-4 EDs	Every 10-12 EDs
IMP injection after blood sampling (not mandatory)	✓	✓
Review of diary	At every visit	At every visit
Body weight control	✓	✓
RNA expression analysis†	Every 3-4 EDs ‡	
AE monitoring	<throughout whole period>	<throughout whole period>

*These visits may be combined with follow-up visits as listed in Table 3. † Optional analysis. ‡ This analysis should be performed at the scheduled study visits whenever possible.

Table 3 Three-Monthly (± 2 weeks) Visits* (calculation from Screening)

	Before IMP injection	Time after end of IMP injection§
Body weight control	✓	
FVIII inhibitor screen	✓	
Safety lab tests (optional at 3 and 9 months if patient did not receive IMP)	✓	
IMP injection after blood sampling (not mandatory)	✓	
Vital signs	✓	30-60 minutes (in case IMP is injected)
Physical examination (once every 6 months)	✓	
Review of diary, plus completion of the "Additional Health Economic Evaluation" questionnaire	✓	
Recovery†‡	✓	baseline, 15 minutes, 1 hour
AE monitoring & changes in concomitant medication	<throughout whole period>	<throughout whole period>

§ Actual time points are to be documented. *These visits may be combined with follow-up visits as listed in Table 2.
† Optional analysis. ‡ Recommended to be performed within 3 months after treatment start and repeated approximately every 6 months thereafter.

Table 4 Completion Visit

	Before	Time after end of infusion§
Body weight	✓	
Physical examination	✓	
FVIII inhibitor screen	✓	
Safety lab tests	✓	
IMP injection after blood sampling (not mandatory)	✓	
Vital signs	✓	30-60 minutes (in case IMP is injected)
Review of diary, plus completion of the "Additional Health Economic Evaluation" questionnaire	✓	
Recovery†	✓	baseline, 15 minutes, 1 hour
AE monitoring & changes in concomitant medication	<throughout whole period>	<throughout whole period>

§ Actual time points are to be documented. † Optional analysis.

Table 5 Immune Tolerance Induction (ITI) Patient Follow-Up*

	Baseline	Time after end of infusion (in hours) §					
	0	0.25	3	6	9	12	24
Before ITI initiation, once ITI specific ICFs were signed							
FVIII inhibitor level	✓						
Epitope mapping†	✓						
RNA expression analysis†	✓						
During ITI (approximately every other week up to 3 months ITI, thereafter less frequently)							
FVIII inhibitor level	✓						
RNA expression analysis† (follow-ups week 2 & 4, monthly until month 6)	✓						
Epitope mapping (close after the peak inhibitor titer)†	✓						
Review of diary, plus completion of the "Additional Health Economic Evaluation" questionnaire	✓ every 3 months						
After inhibitor elimination (frequency of visits according to ITI progress)							
FVIII inhibitor screen	✓						
Epitope mapping†	✓						
Recovery	✓	✓					
Half-life evaluation	✓	✓	✓	✓	✓	✓	(✓)
Review of diary, plus completion of the "Additional Health Economic Evaluation" questionnaire	✓ every 3 months						

§ Actual time points are to be documented. * If applicable. † Optional analysis,

Table 6 Bleeding Episode (BE)

Treatment details	✓
Efficacy assessment at the end of the BE	✓
Type of bleeding (spontaneous, traumatic, post-operative, other)	✓
Site of bleeding	✓
Start date and time of occurrence/of noticing the bleed	✓
Severity of the bleed (minor, moderate to major, or major to life threatening)	✓
Date and time of end of BE	✓

Table 7 Surgery

	within 12 hours before start	within 3 hours before start	Surgery			POP Day 1	any POP Day	last POP Day
			start	intra-op	end			
Body weight	✓							
Estimation blood loss & Duration of surgery	✓							
Details on surgery (location, type, severity, duration)					✓			
Actual blood loss					✓			
Concomitant medications	<---------- throughout whole period ---------- >							
Details study drug injection(s)		🡻	(🡻)	(🡻)	(🡻)	(🡻)	(🡻)	(🡻)
Major surgery: FVIII level (mandatory for the first 3 postoperative infusions)*		✓		✓	✓	✓	✓	✓
Minor surgery: FVIII level*		✓		(✓)	(✓)	(✓)	(✓)	(✓)
Efficacy assessment					✓			✓
Safety laboratory tests	✓					✓	(✓)	(✓)
Vital signs	✓			✓		✓		
Wound haematomas						✓	✓	✓
Narrative of outcome								✓
AE monitoring	<---------- throughout whole period ---------->							

POP = post-operative
(🡻) = optional administration; (✓) optional investigation;
* <30 minutes prior to and after each study drug administration.

3.3 Discussion of Study Design

3.3.1 Study Design

The design of this study complies with the applicable European CHMP Guideline CHMP/BPWG/144533/2009 (5).

Accordingly, a PUP study needs to be conducted for all novel factor VIII products, such as novel genetic constructs or modifications of the factor VIII molecule, after availability of relevant PTP data, including data in pre-treated children.

Corresponding studies have been initiated by Octapharma since early 2009. With the presence of 6 months' treatment data from more than 20 children PTPs, and with having finished all baseline pharmacokinetic assessments, a clinical study in PUPs will now be initiated.

Human-cl rhFVIII is intended as replacement therapy in patients with Haemophilia A. Efficacy of treatment in this indication can be clearly distinguished from inactive therapy.

3.3.2 Control Group(s)

Not applicable.

3.3.3 Target Parameters

The following measurements and assessments are requested to be carried out, grouped by the different target parameters:

Immunogenicity

FVIII inhibitor screen

Prophylactic Treatment

Frequency of spontaneous breakthrough bleeds under three times weekly or every other day prophylactic treatment, and in case of surgical prophylaxis

Efficacy

Efficacy assessment at the end of each BE (based on the efficacy scale described in Section 7.2.2.2)

Recovery Investigation

In vivo recovery (calculated from the FVIII plasma levels before infusion and the peak level obtained from the 15 minutes and 1 hour post-infusion samples)

Safety

Vital signs (blood pressure, heart rate, respiratory rate and body temperature)

Safety laboratory parameters (red blood cell [RBC] count, white blood cell [WBC] count, haemoglobin, haematocrit, platelet count, alanine amino transferase [ALT], aspartate transaminase [AST], serum creatinine)

Tolerability (occurrence of any AE will be monitored throughout the study)

4 STUDY POPULATION

4.1 Population Base

Male patients (mainly new-borns or infants, although no age limitation is given) with severe Haemophilia A (FVIII:C < 1%), without any previous FVIII concentrate treatment or any previous treatment with other FVIII-containing blood products will be enrolled into the study.

In total, 100 PUPs will be enrolled by approximately 40 centres from around the world.

4.1.1 Inclusion Criteria

In order to qualify for study enrolment, the following criteria must be fulfilled before study entry:

1. Male patients
2. Severe Haemophilia A (FVIII:C < 1%)
3. No previous treatment with FVIII concentrates or other blood products containing FVIII
4. Voluntarily given, fully informed written and signed consent given before any study-related procedures are conducted (obtained from the patient's parent(s)/legal guardian(s))

4.1.2 Exclusion Criteria

Patients will not be included if any of the following exclusion criteria are met:

1. Diagnosis of a coagulation disorder other than Haemophilia A
2. Severe liver or kidney disease (ALT or AST levels >5 times of upper limit of normal (ULN), creatinine >120 μmol/L)
3. Concomitant treatment with any systemic immunosuppressive drug
4. Participation in another interventional clinical study currently or during the past 4 weeks

4.2 Prior and Concomitant Therapy

As this study is conducted in PUPs, patients should not have received prior treatment with FVIII concentrates as per exclusion criterion.

4.2.1 Permitted Concomitant Therapy

Concomitant administration of therapies not interfering with the objectives of the study is permitted. Details of any concomitant therapies must be recorded in the Case Report Form (CRF).

Vaccinations are permitted prior and during the study, and immunisations should be given as recommended by the country standards. However – once treatment with IMP has started – vaccinations are recommended to be done after the 20th ED. Additionally, a subcutaneous administration on a day without FVIII administration is recommended.

Patients may only receive anti-fibrinolytics if this is medically indicated / standard of care, e.g. in the course of tooth extractions or other surgeries.

4.2.2 Forbidden Concomitant Therapy

No FVIII concentrates other than *Human-cl rhFVIII* must be administered (except for emergency situations).

Patients permanently switching to another FVIII product within the study participation period will be assessed as treatment failures in the efficacy analyses. However, there are exceptions to this rule. Patients will hence not be considered treatment failures in the efficacy analyses, if:

- the use of another FVIII concentrate was due to an emergency case (example: accident requiring treatment with FVIII without patient or intensive care unit [ICU] personnel) having access to Investigational Medicinal Product [IMP])
- the IMP was not available for the patient in time (example: patient experiences a severe bleed but does not have enough product available).

The reason for a patient switching to another FVIII product should be clearly documented in the CRF (and patient diary, if appropriate).

Patients may not receive immuno-modulating drugs (other than anti-retroviral chemotherapy), such as alpha-interferon, prednisone (equivalent to >10 mg/day), or similar drugs.

For patients who developed an inhibitor and undergo an immune tolerance induction it is highly recommended not to be vaccinated during the ITI.

4.3 Withdrawal and Replacement of Patients

4.3.1 Premature Patient Withdrawal

Subjects have the right to withdraw from the study at any time for any reason, without the need to justify. The Investigator also has the right to withdraw subjects in case of AEs, protocol violations, or for administrative reasons. Since an excessive rate of withdrawals can render the study non-interpretable, the unnecessary withdrawal of subjects must be avoided.

The Investigator will obtain all the required withdrawal details and document the reason(s) for discontinuation in the CRF. Should the reason for removal of a subject be an AE, the main specific event or laboratory test will be recorded in the CRF, and the Investigator will make thorough efforts to clearly document the outcome.

In case of a positive FVIII inhibitor but no ITI initiation, the subject has to be withdrawn from the study.

4.3.2 Patient Replacement Policy

Patients withdrawn from the study after having started treatment with the IMP will not be replaced, if they have received more than 50 exposures with the IMP. Patients with less than 50 EDs need to be replaced.

4.4 Assignment of Patients to Treatment Groups

As this is a single-arm study, assignment of patients to treatment groups is not necessary.

4.5 Relevant Protocol Deviations

In case of any major protocol deviation or violation, the Investigator and Octapharma will decide on the further participation of the patient in this study after having discussed all relevant aspects.

4.6 Subsequent Therapy

Not applicable.

5 INVESTIGATIONAL MEDICINAL PRODUCT(S)

5.1 Characterisation of Investigational Product(s)

Human-cl rhFVIII is a B-domain deleted, human cell-line derived recombinant FVIII concentrate for intravenous use and will be provided in single use vials containing a nominal potency of 250, 500, 1000 or 2000 IU each of freeze-dried rFVIII concentrate to be reconstituted in 2.5 mL of WFI.

Several batches of the product will be used in the study.

The final product will be released by the responsible Octapharma Quality Control Department, in accordance with a defined final product specification.

5.2 Packaging and Labelling

The open-label study design does not necessitate the blinding of study participants or study site personnel with respect to treatment information. Thus, the IMP will be packed and labelled according to local regulations, together with a pre-filled syringe containing 2.5 mL WFI (which will be transferred to the product vial), 1 disposable syringe (10 mL), 1 vial adapter, 1 butterfly needle and 2 alcohol swabs.

Details regarding master labelling can be found in Appendix 1.

5.3 Conditions for Storage and Use

The product has to be stored at 2-8°C protected from light. The product must not be frozen.

The Investigators must ensure that the investigational product is stored under appropriate conditions with restricted access. The Investigators will inform the patients' parent(s)/legal guardian(s) of necessary storage conditions once the patients start home treatment.

5.4 Dose and Dosing Schedule

Prophylactic treatment is recommended, but finally, it is the decision of the responsible treating physician whether patients will be treated prophylactically or on-demand. Patients may switch from on-demand to prophylactic treatment, or from prophylactic to on-demand treatment during the course of the study.

Prophylactic Treatment

1 IU factor VIII per kg body weight raises the plasma factor VIII activity by 1.5 % to 2 %.

In case prophylactic treatment is chosen, the patients will be treated with a recommended dose of 20-50 IU FVIII/kg body weight (BW).

Starting the prophylaxis with the first BE is highly recommended.

The frequency of treatment will mainly depend on the patient's clinical situation. For example, prophylaxis may be initiated with every-other-day injections (in order to keep the FVIII trough level >1%) or with once weekly injections, followed by twice and three times weekly, and every other day treatment. In cases of inadequate response, *Human-cl rhFVIII* administration frequency or dose adjustments can be considered at Investigator's discretion.

On-demand Treatment

In case of bleeds, the patients can be treated on-demand. The dosage and duration of treatment of spontaneous or traumatic bleeds depend on the location and the extent of bleeding as well as on the clinical situation of the patient. Dosage recommendations are given as follows:

- Minor haemorrhage (superficial muscle or soft tissue and oral bleeds): 20-30 IU FVIII/kg BW to achieve an intended target peak level of about 40% to 60%. Repeat dose every 8-24 hours until BE is resolved.
- Moderate to major haemorrhage (haemorrhage into muscles, into oral cavity; haemarthrosis, known trauma): 30-40 IU FVIII/kg BW to achieve an intended target peak level of about 60% to 80%. Repeat dose every 6-24 hours until BE is resolved.
- Major to life-threatening haemorrhage (intracranial, intra-abdominal, gastro-intestinal or intrathoracic bleeds, central nervous system bleeds, bleeding in retropharyngeal spaces or iliopsoas sheath, eyes/retina, fractures or head trauma): initial dose of 40-60 IU FVIII/kg BW to achieve an intended target peak level of 100% to 120%. Repeat dose of 20-50 IU FVIII/kg BW every 6-12 hours until BE is resolved.

Surgical Prophylaxis

The dosage and duration of treatment with *Human-cl rhFVIII* will depend on the type of surgery and the patient's individual incremental recovery. Dosage recommendations are given as follows:

- Minor surgeries including tooth extractions: 25-30 IU FVIII/kg BW starting within 3 hours prior to surgery to achieve an intended target peak level of >30%. Repeat one dose every 12-24 hours if needed. Trough levels should be maintained at ≥30%.
- Major surgeries: 40-60 IU FVIII/kg BW within 3 hours prior to surgery to achieve an intended target peak level of approximately 100%. Repeat if necessary after 6-12 hours initially and for at least 6 to 14 days until healing is complete and recurrence to regular prophylactic treatment is possible. Trough levels should be maintained at >50%.

Recovery Investigation (Optional)

A dose of 40 IU FVIII/kg BW (labelled potency) will be administered on the occasion of the recovery investigations. For the calculation of recovery, the actual potency (measured using both chromogenic (CHR) and one-stage (OS) assays) of *Human-cl rhFVIII* will be used. *In vivo* recovery will be calculated from the FVIII plasma level before infusion and the peak level obtained from the 15 minutes and 1 hour post-infusion samples. Recovery investigations can be done with the first *Human-cl rhFVIII* administration, or within the first three months after treatment start with *Human-cl rhFVIII*, in any case in a non-bleeding patient. They are recommended being controlled every 6 months thereafter.

Immune Tolerance Induction (ITI) (if applicable)

Patients who develop a clinically significant and non-transient FVIII inhibitor will be offered to start ITI with the IMP. Inhibitors that `disappear´ without any clinical signs or symptoms, where no FVIII dosing increase was required, and that turn to <0.6 BU within a period of 6 months after first detection are regarded as "transient".

The modified Bonn Protocol for inhibitor elimination is recommended:

- Low responders (<5 Bethesda Units [BU]) should receive 50-100 IU FVIII/kg BW daily or every second day. In case the inhibitor increases to 5 BU or more, the patient should switch to the high responder regimen.
- High responders (≥5 BU) should receive 100-150 IU FVIII/kg BW every 12 hours.

Once the inhibitor is eliminated (<0.6 BU), the FVIII recovery is ≥66% of normal (defined as 1 IU FVIII/kg BW raises the plasma FVIII activity by 1.5%), and the half-life of FVIII is at least 6 hours, a continuous reduction of about 10% of the initial ITI dosage should be initiated, until the patient has reached a prophylactic treatment regimen of 30-50 IU FVIII/kg BW every other day.

Any other ITI approach using *Human-cl rhFVIII* as the selected FVIII concentrate is possible.

All treatment details and the further development of the inhibitor titre need to be documented.

Patients who do not start ITI within one year after inhibitor detection are withdrawn from the study.

5.5 Preparation and Method of Administration

Human-cl rhFVIII will be provided in single use vials containing a nominal potency of 250, 500, 1000 or 2000 IU each of freeze-dried rFVIII concentrate to be reconstituted in 2.5 mL of WFI.

Prior to injection, the solution must have reached room temperature, without taking any specific warming-up measures. The preparation should be administered within 3 hours after reconstitution at one single occasion. The solution is a clear or slightly opalescent colourless solution. Solutions that are cloudy or have deposits must not be used. *Human-cl rhFVIII* should be injected intravenously by bolus injection (maximally 4 mL/minute) by using aseptic technique.

5.6 Blinding, Emergency Envelopes and Breaking the Study Blind

Not applicable.

5.7 Treatment Compliance

5.7.1 Drug Dispensing and Accountability

A drug dispensing log will be kept up to date by the Investigator, detailing the dates, batch numbers, and quantities of investigational product dispensed to each patient. The inventory will be available to the Study Monitor in order to verify drug accountability in the course of the study. Patients' parent(s)/legal guardian(s) will be advised to return any empty vials and unused investigational product on the occasion of the study visits.

Any unused investigational product not dispensed, or returned by the patient, will be counted and returned to the Sponsor. In certain cases, unused supplies might have to be destroyed at the study site. However, this is only applicable after drug accountability has been verified and fully re-conciliated, and after written approval from the Sponsor has been obtained.

The Investigator will ensure that such a disposition does not expose patients to risks linked to the investigational product. The Investigator and the Sponsor will maintain records of any such alternate disposition to permit accurate drug accountability.

5.7.2 Assessment of Treatment Compliance

Patients will usually undergo at-home treatment (treatment will likely be administered by parents/guardians due to the expected young age of the patients or by general practitioners or nurses). At the first visit, the Investigator will provide them with a sufficient amount of trial medication (in cooling boxes, if appropriate) and a diary. The Investigator will advise the parents/guardians on how to fill in the diary. The diary will include detailed written instructions for all assessments to be made. Furthermore, the efficacy rating criteria and the criteria for assessing the severity of a bleed will be explained. The Investigator will emphasise the necessity of a careful documentation of all treatment details. The recording of at-home injections will include the date and time, dose, batch number, and reason for injection (i.e. prophylaxis, BE treatment). IMP is provided with tear-off labels that must be placed in the diary.

In case of treatment of BEs, the patient's parents/guardians will document the site and severity of the bleed, the start and end dates and time, and carry out an efficacy assessment according to the explanation provided in the diary.

Study product administrations that are overseen by the Investigator (e.g. treatment in relation to the recovery investigations or in case of severe BEs treated at the study site), are to be documented in the patient's journal (by the Investigator), as well as in the patient's diary (by the parents/guardians).

In addition, the patient's parents/guardians will record any concomitant medications taken during the study period in their diaries.

For each follow-up visit at the study site, the parents/guardians must bring all diaries to be reviewed and validated by site personnel.

6 STUDY CONDUCT

6.1 Observations by Visit

6.1.1 Screening (Visit 1)

Parents/legal guardians have to be informed about the study details and have to give their written informed consent before any investigations and assessments can be performed. The following will be documented/assessed:

- Check of inclusion- and exclusion criteria
- Demographics, including patient's blood type and FVIII inhibitor risk factors
- Medical history
- Vital signs
- Height
- Body weight
- Physical examination
- Safety laboratory parameters
- FVIII:C (CHR and OS assay)
- FVIII inhibitor screen
- FVIII gene mutation analysis (mandatory, can be performed later during the study)
- Immunogenotyping (HLA-typing, immune response gene expression), (optional)
- RNA expression analysis (optional)

- *In vitro* immunogenicity of FVIII products (optional)
- Epitope mapping: optional, available pre-treatment samples will be analysed from study patients who later on developed an inhibitor to FVIII and started ITI. In addition, samples available from an inhibitor negative control group will be tested: already available plasma samples from ~20 PUPs included into the study in Ukraine and Moldova, which were drawn prior to the first exposure to FVIII are planned to be analysed in retrospect.

The Investigator will hand out the patient diary and instruct the parent(s)/legal guardian(s) on how to document details of treatment with *Human-cl rhFVIII*, bleeds, AEs and concomitant medication. The parent(s)/legal guardian(s) will receive a sufficient amount of study product for home treatment (in cooling boxes, if appropriate) and will be instructed on how to store and administer it.

Immunogenotyping is suggested to be performed at screening; however, it can be performed at any point during the study. It is suggested that the sampling coincides with regular study visits whenever possible.

6.1.2 Procedures During the Follow-Up Part of the Study

Follow-Up Visits (until ED 20):
The patients will visit the study site every 3-4 EDs until 20 EDs have been reached. At these visits, blood samples will be obtained for FVIII inhibitor screen and optionally RNA expression analysis (after a wash-out period of at least 2, preferably 3 days after the previous IMP administration). Samples are analysed in the central laboratories.

At these visits, the patient diary will be reviewed (any IMP injection, any bleed) in order to transfer data into the CRF. The occurrence of AEs and changes in weight and concomitant medication will be checked and documented.

Follow-Up Visits (after ED 20 until ED 100):
After ED 20 has been reached, patients will visit the study site every 10-12 EDs. At these visits, blood samples will be obtained for FVIII inhibitor screen (after a wash-out period of at least 2, preferably 3 days after the previous IMP administration).

At these visits, the patient diary will be reviewed (any IMP injection, any bleed) in order to transfer data into the CRF. The occurrence of AEs and changes in concomitant medication will be checked and documented. Samples are analysed in the central laboratory.

Follow-Up Visits (every 3 months ± 2 weeks):
If appropriate, the above-described follow-up visits may be combined with regular 3-monthly follow-up visits (± 2 weeks), stipulating some supplementary investigations are performed: the safety laboratory parameters will be assessed (samples are analysed locally) and vital signs will be measured pre- and – in case IMP was injected – 30-60 minutes post-injection and the patient's BW will be measured.
Once every 6 months a physical examination is performed.

If the optional recovery investigation is done, it is recommended that it will be performed with the first *Human-cl rhFVIII* administration, or within the first three months after treatment start and every 6 months thereafter, in any case in a non-bleeding patient.

Recovery visits should be timed so that they coincide with other study visits whenever possible. The *in vivo* recovery will be calculated from the FVIII plasma levels before infusion and the

peak level obtained from the 15 minutes and 1 hour post-infusion samples. Samples are analysed in the central laboratory.

In case the patient did not require any IMP from screening until the 3-Monthly Follow-Up visits during the first year, the blood sampling (inhibitor screen, safely laboratory) after 3 and 9 months is optional. After 6 and 12 months a sample is mandatory.

Additional Health Economic Evaluation:

Parents / Caregivers will be asked to fill out a short questionnaire as part of the patient diary that asks about their time commitments, and productivity loss.

These questions should be answered by both parents (if applicable) on a quarterly basis, finally covering the entire study period.

- Do you currently hold a paid job?
- Did your child's Haemophilia A oblige you to be off work at any time in the past three months?
- Did other people take over and perform your usual household tasks in the past three months in connection with your child's Haemophilia A?
- How much of the time you cared for your child in the past 3 months was specifically related to Haemophilia A? *Consider time related to product administration, receiving education on home treatment, time related to hospital visit, physicians visits etc.*

ITI Patient Follow-Up:

Patients developing an inhibitor and not starting ITI will be withdrawn from the study, once it can be ruled out that the inhibitor is transient / not requiring ITI. Inhibitors that `disappear´ without any clinical signs or symptoms, where no FVIII dosing increase was required, and that turn to <0.6 BU within a period of 6 months after first detection are regarded as "transient".

Patients developing an inhibitor and starting ITI will be closely monitored. Depending on the development of the inhibitor level: a bi-weekly (later on monthly to 3-monthly) inhibitor titre testing is considered appropriate. Optional epitope mapping can be performed once the inhibitor is detected (pre-ITI start), as close as possible after the peak inhibitor level, and after inhibitor eradication. These patients' epitope mapping samples optionally taken at screening / prior to the first exposure to FVIII will also be analysed. Optional RNA expression analysis can be performed alongside the inhibitor testing at initiation of ITI, 2 and 4 weeks after ITI initiation, and then on a monthly basis until Month 6. This may give information on motifs that predict success that should be evident in the first 6 months and give mechanistic information on immune-tolerance.

Once the inhibitor is eliminated, a normalised recovery and half-life are expected during the continued ITI. Patient observation visits including inhibitor screen and FVIII measurements are recommended according to progress of the ITI. Samples to test the recovery are recommended to be drawn at baseline and 15 minutes post-injection. For the half-life evaluation, the following sampling time points are recommended: baseline, 15 minutes, 3, 6, 9, 12, 24 hours post-injection. No wash-out period needs to be respected prior to blood sampling; no ITI injection should be skipped prior to or during the half-life evaluation (last blood sampling should be performed before the next regular ITI dose is injected).

Patients will complete study participation after a complete success of the ITI (inhibitor-free, normalised recovery and half-life), and the start of regular prophylactic treatment, or after a maximum ITI period of 36 months.

Surgical Visits:

Study subjects may undergo surgical interventions during the course of the study. Depending on the type of surgery, subjects may be hospitalised or not (in case of certain minor surgeries with low risk of post-operative bleeding) at the discretion of the Investigator. Patient's BW will be recorded within 12 hours before the start of surgery. Estimations of blood loss and duration of surgery will be recorded. Details of *Human-cl rhFVIII* dosing, concomitant treatment (including need for additional blood transfusions, excluding anaesthetic drugs), AEs (if any) and vital signs will be documented throughout the surgery. Details of surgery, actual blood loss, efficacy assessment, presence of wound haematomas and narratives of outcomes will be recorded after the surgery.

Completion Visit:

For each patient, the study is completed once 100 EDs have been reached, or after a maximum of 5 years study participation - from screening.

Patients undergoing an ITI complete the study after a successful elimination of the inhibitor ("complete success" criteria see above), but after a maximum ITI period of 36 months.

At this visit, blood samples will be obtained for FVIII inhibitor screen (after a wash-out period of at least 2, preferably 3 days after the previous IMP administration) and the safety panel. Samples for FVIII inhibitor screen are analysed in the central laboratory.

The vital signs and a physical examination will be performed. The patient's BW will be measured. The occurrence of AEs and changes in concomitant medication are checked and documented.

At this visit, the patient diary will be reviewed (any IMP injection, any bleed) in order to transfer data into the CRF.

6.2 Duration of Study

6.2.1 Planned Duration for an Individual Patient

The patients should stay in the study for 100 EDs and for a maximum study participation period of 5 years from screening.

In patients who develop FVIII inhibitors and start ITI treatment, the study is complete after a successful ITI, but the maximum length of ITI will be 36 months.

6.2.2 Planned Duration for the Study as a Whole

The study will be considered completed when all patients have terminated the planned observation period/Completion Visit. The total study period is scheduled to comprise 6 years. The study started in the 1st quarter 2013 and is planned to continue until the 4th quarter of 2018.

6.2.3 Premature Termination of the Study

Both the Investigator and the Sponsor reserve the right to terminate the study at any time. Should this be necessary, the required procedures will be arranged on an individual basis after review and consultation by both parties. In terminating the study, the Sponsor and the

Investigator will ensure that adequate consideration is given to the protection of the patients' interests.

7 ASSESSMENTS AND METHODS

7.1 Background / Baseline Information

The following information will be captured upon enrolment:

Demographics: sex, age, weight and height (calculated body mass index [BMI]), and ethnic origin, family history of haemophilia and family history of FVIII inhibitor development

Medical history: obtained from existing medical charts and by interviewing the parent(s)/legal guardian(s) and by performing a physical examination

Previous and concomitant medication: obtained from existing medical charts and by interviewing the parent(s)/legal guardian(s)

Analysis of FVIII gene mutations: blood samples collected by the Investigator and analysis performed by a central laboratory

7.2 Efficacy/Immunogenicity Assessments

7.2.1 Assessments for Primary Endpoint

The primary objective is to investigate the immunogenicity of *Human-cl rhFVIII*.

Inhibitor activity will be determined centrally by the modified Bethesda assay (Nijmegen modification), using congenital FVIII-deficient human plasma spiked with *Human-cl rhFVIII* at the following time points:

- At baseline (Screening Visit)
- Every 3-4 EDs until ED 20
- Every 10-12 EDs or every 3 months ± 2 weeks (whichever comes first) after ED 20
- At study completion
- Any time in the case of a suspicion of inhibitor development.

In case of a positive inhibitor result, an inhibitor re-testing using a second separately drawn sample should be performed centrally. The definitions for thresholds are ≥ 0.6 to < 5 BU for a "low titre" inhibitor and ≥5 BU for a "high-titre" inhibitor.

Patients who develop a non-transient inhibitor will have an option to start on ITI treatment. Patients developing inhibitors and not starting ITI will be withdrawn from the study. Inhibitors that `disappear´ without any clinical signs or symptoms, where no FVIII dosing increase was required, and that turn to <0.6 BU within a period of 6 months after first detection are regarded as “transient”, and continue regular treatment within the study until they have reached ED 100 or a maximum period of 5 years..

7.2.2 Assessments for Secondary Endpoints

7.2.2.1 Prophylactic treatment

The following parameters will be documented:

- Dates and times of study product injections
- Details of dose(s) (in IU) and product batch numbers
- Details of BEs (if any) occurring under prophylactic treatment

Study drug consumption data (FVIII IU/kg per month, per year) per patient and in total (for patients on prophylactic treatment) will be evaluated.

Efficacy Assessment of Prophylactic Treatment

The efficacy of *Human-cl rhFVIII* in prophylactic treatment will be evaluated based on the frequency of spontaneous breakthrough bleeds per months under three times weekly or every other day prophylactic treatment and will be calculated and assessed as excellent, good, moderate or poor:

Excellent: Less than 0.75 spontaneous BEs per month

Good: Between 0.75 and 1 spontaneous BEs per month

Moderate: Between more than 1 and 1.5 spontaneous BEs per month

Poor: More than 1.5 spontaneous BEs per month

The time period for prophylactic treatment will comprise the time periods between first prophylactic treatment with *Human-cl rhFVIII* until the administration of the last prophylactic treatment + 2 days or completion visit whatever comes first) minus time periods from start of a surgery until final assessment of the surgery, and minus time period of on demand treatment, if any.

The frequency of treatment will mainly depend on the patient's clinical situation and may vary from every other day to once weekly injections. In cases of inadequate response, *Human-cl rhFVIII* administration frequency or dose adjustments can be considered at Investigator's discretion.

The number of BEs counted for prophylactic treatment efficacy assessment will comprise all BEs starting during the time periods for prophylactic treatment defined above, i.e. BEs occurring between start of surgery until final assessment of the surgery will not be included in the prophylactic treatment assessment.

7.2.2.2 Bleeding episodes

For all BEs occurring within the study period, the following data will be documented:

- Type of bleeding (spontaneous, traumatic, post-operative, other)
- Site of bleeding
- Start date and time of occurrence/of noticing the bleed
- Severity of the bleed (minor, moderate, major or life threatening, see Section 5.4)

- Date and time of end of BE*
- Efficacy assessment at the end of the BE (definitions see below)
- Details of dose(s) and batch number used to treat BE (in IU)
- Dates and times of study product injections

* If the treatment of a BE in one bleeding site is interrupted for more than 48 hours, two separate BEs will have to be recorded; if another – in addition to the original – bleeding site is affected, the events will be recorded as separate BEs at any time.

All above listed parameters will be documented by the parents (together with the Investigator in case of on-site treatments) in the patient's diary. Patients experiencing a major or life-threatening BE should preferably be treated at the study site.

After the end of a BE the patient is intended to return to his regular prophylactic treatment regimen.

Efficacy Assessment of Bleeding Episodes

At the end of a BE, the following efficacy assessment will be made by the patient's parent(s)/legal guardian(s) (together with the Investigator in case of on-site treatment):

Excellent: Abrupt pain relief and/or unequivocal improvement in objective signs of bleeding within approximately 8 hours after a single infusion

Good: Definite pain relief and/or improvement in signs of bleeding within approximately 8 – 12 hours after an infusion requiring up to 2 infusions for complete resolution

Moderate: Probable or slight beneficial effect within approximately 12 hours after the first infusion requiring more than two infusions for complete resolution

None: No improvement within 12 hours, or worsening of symptoms, requiring more than 2 infusions for complete resolution

The assessment will be made at the end of a BE.

7.2.2.3 Surgical Prophylaxis

In case surgical procedures are performed, the following data will be documented:

- Location and type (planned or emergency) of surgery
- Severity of surgery (minor or major, definitions see below)
- Expected duration of surgical procedure
- Actual BW prior to surgery (kg)
- Actual duration of surgical procedure (start and end times, i.e. skin to skin)
- Pre-, intra-, and post-operative FVIII plasma levels (time-points see below)
- Expected and actual blood loss (see below)
- All wound haematomas, incl. capture, analysis, and reporting, noting whether surgical evacuation is required

- Laboratory tests (haematology, chemistry: before and 24 hours after end of surgery)
- Details on concomitantly administered products including any blood/blood product transfusions but *excluding* drugs given for routine anaesthesia
- Details of administered dose(s) of *Human-cl rhFVIII* given pre-, intra- and/or post-operatively (definitions see below) including dates, times, and batch number
- A brief narrative describing the outcome and efficacy of the intervention
- Overall efficacy assessment at the end of surgical prophylaxis done together by the surgeon and the haematologist (definitions see below)

Classification of Surgeries

Surgeries are defined as major if any of the following criteria are met:

- Requiring general or spinal anaesthesia
- Requiring opening into the great body cavities
- In the course of which hazards of severe haemorrhage is possible
- Requiring haemostatic therapy for at least 6 days
- Orthopaedic interventions involving joints (ankle, knee, hip, wrist, elbow, shoulder)
- 3rd molar extraction or extraction of ≥3 teeth
- Surgeries/conditions in which the patient's life is at stake

The classification is made prospectively. All other surgeries are classified as minor.

FVIII Plasma Level

FVIII plasma level (both assays, local and central lab) will be documented at the following time-points:

- Immediately (≤30 minutes) before and after pre-operative injection of study drug
- Immediately (≤30 minutes) before and after each intra-operative bolus dose (if any); not mandatory for minor BEs
- Immediately (≤30 minutes) before and after each post-operative dose (if any); in case of major surgery: mandatory for the first 3 post-operative doses

Continuous infusion is not allowed in this study, only bolus injections are permitted.

Estimation of Blood-Loss

Prior to surgery, the surgeon will provide written estimates of the following:

Volume (mL) of *average* expected blood loss for the planned surgical procedure, as it would be expected for the same procedure in a patient with normal haemostasis, of the same sex, age, and stature.

Volume (mL) of *maximal* expected blood loss for the planned surgical procedure as it would be expected for the same procedure in a patient with normal haemostasis, of the same sex, age, and stature.

Following the surgery, the **actual** blood loss will be estimated by the surgeon.

Definitions of Pre-, Intra-, and Post-Operative Doses

A pre-operative administration is defined as any dose of *Human-cl rhFVIII* applied within 3 hours prior to surgery start.

An intra-operative administration is defined as any injection of *Human-cl rhFVIII* applied during surgery.

A post-operative administration is defined as any dose of *Human-cl rhFVIII* applied after the end of the surgery ("end of surgery" is defined as "last suture"), for at least 2 days (minor surgeries), or for at least 6 to 14 days (major surgeries), respectively, until healing is achieved and recurrence to the regular prophylactic treatment regimen.

Efficacy Assessment of Surgical Prophylaxis

Efficacy will be assessed at the end of surgery by the surgeon and post-operatively by the haematologist using the following scales:

Intra-operatively (at the end of the surgery [= after last suture]):

- Excellent: Intra-operative blood loss was lower than or equal to the average expected blood loss compared with the same type of procedure performed in a patient with normal haemostasis and of the same sex, age, and stature.
- Good: Intra-operative blood loss was higher than average expected blood loss but lower or equal to the maximal expected blood loss compared with the same type of procedure in a patient with normal haemostasis.
- Moderate: Intra-operative blood loss was higher than maximal expected blood loss compared with the same type of procedure performed in a patient with normal haemostasis, but haemostasis was controlled.
- None: Haemostasis was uncontrolled necessitating a change in clotting factor replacement regimen.

Post-operatively:

- *Excellent*: No post-operative bleeding or oozing that was not due to complications of surgery. All post-operative bleeding (due to complications of surgery) was controlled with *Human-cl rhFVIII*, as anticipated for the type of procedure.
- *Good*: No post-operative bleeding or oozing that was not due to complications of surgery. Control of post-operative bleeding due to complications of surgery required increased dosing with *Human-cl rhFVIII* or additional infusions, not originally anticipated for the type of procedure.
- *Moderate*: Some post-operative bleeding and oozing that was not due to complications of surgery; control of post-operative bleeding required increased dosing with *Human-cl rhFVIII* or additional infusions, not originally anticipated for the type of procedure.
- *None*: Extensive uncontrolled post-operative bleeding and oozing. Control of post-operative bleeding required use of an alternate FVIII concentrate.

An overall efficacy assessment taking both the intra- and post-operative assessment into account will be done together by the surgeon and the haematologist.

The conclusion of the post-operative phase of a major surgery is defined as follows: date of

discharge, or at least post-operative Day 6, whichever occurs later.

In the end of a surgical prophylactic treatment period the patient is intended to return to his regular prophylactic treatment regimen.

7.3 Safety Assessments

7.3.1 Adverse Events

All AEs and SAEs will be monitored and recorded throughout the study.

7.3.1.1 Definitions

Adverse event (AE): An AE is any untoward medical occurrence in a study patient receiving an IMP and which does not necessarily have a causal relationship with this treatment. An AE can therefore be any unfavourable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of an IMP, whether or not related to the IMP.

Adverse drug reaction (ADR): An ADR is any noxious and unintended response to an IMP related to any dose. The phrase "response to an IMP" means that a causal relationship between the IMP and an AE carries at least a reasonable possibility, i.e., the relationship cannot be ruled out.

Other significant AEs: Any marked laboratory abnormalities or any AEs that lead to an intervention, including withdrawal of drug treatment, dose reduction or significant additional concomitant therapy.

Withdrawal due to AE/ADR: Is a patient whose treatment with IMP is discontinued because of an AE or ADR. Any such events will be followed up by the Investigator until the event is resolved or until the medical condition of the patient is stable. All follow-up information collected will be made available to the Sponsor.

7.3.1.2 Collection

The condition of the patient will be monitored throughout the study. At each visit, whether scheduled or unscheduled, AEs will be elicited using a standard non-leading question such as "How have you been since the last visit / during the previous study period?" In addition, the patient diaries (if applicable) will be checked by the Investigator for any documented event.

Any AE or ADR which occurs during the study will be noted in detail on the appropriate pages of the CRF. If the patient reports several signs or symptoms, which represent a single syndrome or diagnosis, the latter should be recorded in the CRF. The Investigator responsible will grade the severity of all AEs or ADRs (mild, moderate or severe), the seriousness (non-serious or serious) and causality, as defined below (Sections 7.3.1.3, 7.3.1.4 and 7.3.2). The Sponsor is responsible to assess the expectedness of each ADR (expected or unexpected), as defined below (Section 7.3.1.4).

In the event of clinically significant abnormal laboratory findings, the tests will be repeated and followed-up until they have returned to normal and/or an adequate explanation is available.

Diseases, signs and symptoms and/or laboratory abnormalities already existing before the first administration of IMP are not considered as AEs when observed at a later stage unless they represent an exacerbation in intensity or frequency (worsening).

The Investigator responsible should always provide detailed information concerning any abnormalities and the nature of, and reasons for any necessary action(s), as well as any other observations or comments, which are useful for the interpretation and understanding of the patients' AEs or ADRs.

7.3.1.3 Severity

The intensity/severity of all AEs will be graded as follows:

- mild: an AE, usually transient, which causes discomfort but does not interfere with the patient's routine activities;
- moderate: an AE which is sufficiently discomforting to interfere with the patient's routine activities;
- severe: an AE which is incapacitating and prevents the pursuit of the patient's routine activities.

Grading of an AE is up to the medical judgement of the Investigator and will be decided on a case by case basis.

7.3.1.4 Causality

The relationship of AEs to the administered IMP will be assessed by the Investigator responsible:

- probable: reports including good reasons and sufficient documentation to assume a causal relationship, in the sense of plausible, conceivable, likely, but not necessarily highly probable. A reaction that follows a reasonable temporal sequence from administration of the IMP; or that follows a known or expected response pattern to the suspected medicine; or that is confirmed by stopping or reducing the dosage of the medicine and that could not reasonably be explained by known characteristics of the patient's clinical state.
- possible: reports containing sufficient information to accept the possibility of a causal relationship, in the sense of not impossible and not unlikely, although the connection is uncertain or doubtful, for example because of missing data or insufficient evidence. A reaction that follows a reasonable temporal sequence from administration of the IMP; that follows a known or expected response pattern to the suspected medicine; but that could readily have been produced by a number of other factors.
- unlikely: reports not following a reasonable temporal sequence from IMP administration. An event which may have been produced by the patient's clinical state or by environmental factors or other therapies administered.
- not related (unrelated): events for which sufficient information exists to conclude that the aetiology is unrelated to the IMP.
- unclassified: reports which for one reason or another are not yet assessable, e.g. because of outstanding information (can only be a temporary assessment).

Classification of ADRs:

ADRs will be classified by the Sponsor as either expected or unexpected:

- expected: an AE that is listed in the "Reference Safety Information" of the current edition of the Investigator's Brochure.
- unexpected: an AE that is not listed in the current edition of the Investigator's Brochure, or that differs because of greater severity or greater specificity.

7.3.1.5 Outcome

The outcome of all reported AEs has to be documented as follows:

1. recovered, resolved
2. recovering, resolving
3. not recovered, not resolved
4. recovered, resolved with sequelae
5. fatal
6. unknown

NOTE: A patient's **death** *per se* is not an event, but an outcome. The event which resulted into patient's death must be fully documented and reported, even in case the death occurs within 4 weeks after IMP treatment end, and without respect of being considered treatment-related or not.

7.3.1.6 Action(s) taken

AEs requiring action or therapy must be treated with recognised standards of medical care to protect the health and well-being of the patient. Appropriate resuscitation equipment and medicines must be available to ensure the best possible treatment of an emergency situation.

The action taken by the Investigator must be documented:

In general

- none
- medication (other than IMP) or other (e.g., physical) therapy started
- test performed
- other (to be specified)

On IMP

- none
- product withdrawn
- dose reduced
- dose increased

The responsible Investigator will follow-up each AE until it is resolved or until the medical condition of the patient is stable, and all relevant follow-up information will be reported to the Sponsor.

7.3.2 Serious Adverse Events

An SAE is any untoward medical occurrence that at any dose:

- results in death,
- is life-threatening,
- requires hospitalisation or prolongation of existing hospitalisation,
- results in persistent or significant disability/incapacity,
- is a congenital anomaly/birth defect,
- is another important medical event.

In addition, each FVIII inhibitor development is regarded as an SAE.

NOTE: The term "life-threatening" refers to an event in which the patient was — in the view of the reporting Investigator — at immediate 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.

Medical judgment should be exercised in deciding whether an AE/reaction is serious in other situations: Important AEs/ADRs that are not immediately life-threatening or do not result in death or hospitalisation but may jeopardise the patient or may require intervention to prevent one of the other outcomes listed in the definitions above, should also be considered serious.

In addition, although not classified under the seriousness criteria, all suspected transmissions of an infectious agent should be reported as SAE. A suspected virus transmission means that virus antigen has been detected in the patient. A passive transmission of antibodies alone does not constitute a suspected virus transmission.

SAE reporting timelines

All SAEs, whether suspected to be related to study treatment or not, are to be reported by telephone, fax or e-mail immediately to the Clinical Project Manager or designee.

Contact details will be communicated at the study initiation visit.

An Octapharma "Serious Adverse Event Report" must be completed and submitted within 24 hours after recognition of the event.

All SAEs should additionally be reported to:

Waiver from SAE reporting requirement:

The following SAEs do not require reporting in expedited manner:

- Hospitalisation for the treatment of a (disease-related) BE assessed as unrelated to IMP treatment.

These exceptions/waivers include surgeries that are elective or planned, and prolongation of the existing hospitalisations due to economic or social reasons, but not medical reasons. These should not be considered as SAEs.

7.3.3 Laboratory Safety Tests

Blood Sampling

The *actual* date and time of any blood sampling must be recorded in the CRF, on the label of the laboratory tubes and on the corresponding laboratory shipment forms.

If several blood samples have to be taken at one time point, the blood sampling will be done in the following sequence:

1. Haematology: RBC count, WBC count, haemoglobin, haematocrit, platelet count (EDTA blood)
2. Coagulation: FVIII plasma level and FVIII inhibitor control (citrated plasma)
3. Biochemistry: ALT, AST, serum creatinine (serum)
4. RNA expression analysis (optional) (whole blood, RNAse-free conditions)
5. Immunogenotyping: gene mutation analysis (mandatory), HLA-typing, immune response gene profiling, FVIII ethnic haplotype determination (optional), (EDTA blood)
6. *In vitro* immunogenicity of FVIII products (optional) (citrated blood)

It is essential that blood sampling after the injection of *Human-cl rhFVIII* is done from the other arm.

Blood samples taken for citrate plasma will be centrifuged after collection for 15 to 20 minutes at 1500 to 2000 g (about 3000 rpm). Aliquots of the supernatant are subsequently transferred into the tubes provided by the central laboratory (pre-labelled with date and time point of sampling, type of sample, patient identification and study number) and stored, respectively shipped under adequate conditions.

EDTA Blood

A 1 mL sample (or less, as required by the local laboratory) of EDTA blood will be collected for the measurement of haematology parameters (RBC count, WBC count, haemoglobin, haematocrit, platelet count). All haematology tests are to be done at the local laboratory.

A further 5 mL sample will be collected for the FVIII gene mutation analysis and the optional further immunogenotyping (HLA-typing, immune response gene profiling and FVIII ethnic haplotype analysis). The immunogenotyping will be done at the University laboratory of Prof. Johannes Oldenburg in Bonn, Germany. The blood samples will be stored at ≤–70°C and shipped to the central laboratory on dry-ice.

Citrated Plasma

For the analysis of coagulation factor and inhibitor screen, both performed in the central laboratory in the US, a maximum of around 2 mL of citrated blood will be collected. After collection and centrifugation, the plasma will be aliquoted into cryo-resistant tubes. Samples will be stored at ≤-70°C and shipped to the central laboratory on dry-ice.

For analysis performed locally, e.g. at screening and pre-, intra, and post-surgery, citrated blood as required by the local laboratory will be collected and processed in accordance with local requirements.

From patients developing an inhibitor to FVIII, and agree to participate in the epitope mapping sub-study, a further pre-ITI initiation 2 mL citrated blood sample (2 x 0,5 mL citrated plasma) is needed. After ITI initiation two further blood sampling time points are recommended: as close as possible after the peak inhibitor level, as well as after inhibitor eradication.

Serum

For the determination of clinical chemistry (ALT, AST, serum creatinine) a blood sample of about 0.5 mL (or less, as required by the local laboratory) will be collected. All tests are to be done at the local laboratory.

Citrated blood

If the (optional) analysis of immunogenicity of FVIII products (*in vitro* T cell assay) is to be performed, a blood sample of exactly 5 mL needs to be collected. Samples will be kept at room temperature and are immediately shipped to Antitope Ltd. at ambient temperature. The samples must arrive at the central laboratory within 24 hours after collection.

Whole blood (RNAse-free conditions)

If the RNA expression analysis is performed, about 1.0 mL of blood will be collected into an sterile (RNAase & DNase-free) syringe and directly transferred into a customised PAXGene tube (provided by the central laboratory and pre-labelled with date and time point of sampling, type of sample, patient identification and study number). Sample should be kept upright for a minimum of 2 hours at room temperature (18 – 25°C) following blood sampling (maximum time 72 h). Samples can be stored at -20°C or –70°C and shipped to the central laboratory on dry ice. See Appendix 2 for a detailed sampling protocol.

The “Ethical Considerations for Clinical Trials on Medicinal Products conducted with the Paediatric Population” are followed during the conduct of GENA-05. The Committee recommends the below blood volume limits for sampling in neonates:

Per individual, the trial-related blood loss should not exceed 3 % of the total blood volume during a period of four weeks, and should not exceed 1% at any single time. The total volume of blood is estimated at 80 to 90 ml/kg body weight; 3% is 2.4 ml blood per kg body weight.

Time-points and required blood volume needs for laboratory parameters are as follows (all optional blood samplings are greyed out):

Table 8 Time-Points and Blood Volume for Laboratory Parameters

	Screening	**Follow-Up**			**Surgery**	**ITI**
	(Baseline)	Every 3-4 EDs (until ED 20)	Every 10-12 EDs (after ED 20) / 3-Months Visits	Completion Visit	(Pre-, intra- and post-surgery)	Recovery / PK Visits
FVIII inhibitor screen (citrated blood/plasma) -central laboratory-US	2 mL/0.7 mL	2 mL/0.7 mL (5-6 x)	2 mL/0.7 mL	2 mL/0.7 mL		2 mL/0.7 mL
Haematology (local laboratory)	~1 mL EDTA blood		~1 mL EDTA blood	~1 mL EDTA blood	~1 mL EDTA blood	
Clinical chemistry (local laboratory)	~0.5 mL serum		~0.5 mL serum	~0.5 mL serum	~0.5 mL serum	
FVIII:C (3 samples if recovery determination is done) (citrated blood/plasma) -central laboratory-US	1.4 mL/0.7 mL		1.4 mL/0.7 mL		1.4 mL/0.7 mL	1.4 mL/0.7 mL
FVIII gene mutation analysis Optional: Immunogeno-typing: HLA-typing, immune response gene profiling, FVIII ethnic haplotype determination -central laboratory-Germany	5.0 mL (2 x 2.5 mL) EDTA blood					
RNA expression analysis* ([§]ITI baseline, 2 and 4 weeks after ITI start, monthly until month 6) -central laboratory-UK	1.0 mL whole blood	1.0 mL[#] whole blood				1.0 mL[#§]
In vitro immunogenicity* -central laboratory-UK	5.0 mL[†] citrated blood or Li heparin					
Epitope mapping in case of inhibitor development[‡] --central laboratory-Germany						2.0 mL citrated blood (preferred at 3 time-points)

* Optional analyses. [‡] If *in vivo* recovery analysis is to be performed. [†] Suggested timing of analyses. [#] This analysis should be performed at the scheduled study visits whenever possible.

All treatment-emergent rise in AST or ALT to >3 x ULN will be followed by assessing liver function tests until their return to normal; or (in case of *a priori* increased values) until their return to the individual's baseline; or until a definite diagnosis is determined.

If the abnormal values persist for more than 1 week, viral serology (hepatitis B virus, hepatitis C virus) and PCR testing will be performed by the local laboratory to rule out hepatitis B and C. In addition, PCR testing of the corresponding batches will be performed by the central lab.

The Investigator must assess the clinical significance of abnormal laboratory values outside the normal range as specified by the reference laboratory. Any clinically significant abnormalities

should be fully investigated.

Only laboratory abnormalities that have been rated as being clinically significant will be documented as AEs/ADRs. Clinically significant is defined as any laboratory abnormality that the Investigator feels is of clinical concern, and/or requires medical intervention and/or follow-up. Additional tests and other evaluations required to establish the significance, or aetiology of an abnormal result or to monitor the course of an AE should be obtained if clinically indicated. Any abnormal laboratory value that persists should be followed until resolution or for 14 days after the final study visit, whichever occurs first. Preferably, clinically significant lab abnormalities should be medically diagnosed and entered as a diagnosis into the AE form, if not already present at baseline.

The following table summarises all test parameters and the laboratory responsible for analysis:

Table 9 Test Parameters and Laboratories

Test	Material needed	Responsible laboratory
FVIII:C* (one-stage and chromogenic assay)	Citrated plasma	LabCorp Clinical Trials Laboratory Services 8490 Upland Dr, Ste. 100 Englewood, CO 80112, USA
Inhibitors to FVIII (modified Bethesda assay - Nijmegen modification)	Citrated plasma	
Haematology	EDTA blood	Local laboratory
Clinical chemistry	Serum	Local laboratory
Immunogenotyping (FVIII gene mutation analysis, HLA-typing‡, immune response gene profiling‡ and FVIII ethnic haplotype‡)	EDTA blood	Prof. Johannes Oldenburg Institute of Experimental Haematology and Transfusion Medicine Sigmund-Freud Strasse 25D 53127 Bonn, Germany
RNA expression analysis‡	Whole blood (RNAse-free conditions)	Dr Dan Hart and Dr Paul Batty Dept of Haematology Laboratory 4th Floor, Pathology and Pharmacy building Royal London Hospital Barts and The London School of Medicine and Dentistry 80 Newark Street. London E1 2ES, United Kingdom
In vitro immunogenicity of FVIII products‡	Blood (citrated or heparinised)	Antitope Ltd Business Development Group Babraham Research Campus Babraham Cambridge CB22 3AT, United Kingdom
Epitope mapping ‡	Citrated plasma	Prof. Johannes Oldenburg Institute of Experimental Haematology and Transfusion Medicine Sigmund-Freud Strasse 25D 53127 Bonn, Germany
PCR testing of *Human-cl rhFVIII* batches†	Human-cl rhFVIII	Octapharma AB Elersvägen 40 11275 Stockholm, Sweden

* Performed locally at screening and when required, e.g. pre-, intra- and post-surgery.
‡ Optional investigations.

† Performed if abnormal AST or ALT values persist for more than 1 week.

All remaining serum and plasma volumes will be labelled and stored as retention samples at the central laboratory for at least 2 years after the completion of the study and until Octapharma's written authorisation to destroy these samples.

7.3.4 Vital Signs and Physical Examination

Vital signs (blood pressure, heart rate, respiratory rate, body temperature) will be assessed at the following time-points: at screening, prior and – in case IMP was injected – 30-60 minutes after the end of the injection at 3-monthly (± 2 weeks) visits, and prior and – in case IMP was injected – 30-60 minutes after the end of the injection at the completion visit. During surgical procedures vital signs will be assessed before, during and at the first post-operative day.

A physical examination needs to be performed at screening, once every 6 months and at study completion.

7.3.5 Other Relevant Safety Information

Post study related safety reports:

Any ADR (i.e., any AE with a suspected causal relationship to the IMP) that occurs after the completion of the study should be reported by the Investigator. The usual procedure for reporting post-marketing safety information should be followed, but relation to the clinical study should be stated on the report.

If a patient dies within 4 weeks after the last IMP administration, this should be reported as well, without being considered treatment-related or not.

Overdose, interaction, abuse, misuse, medication error and lack of efficacy:

The following safety relevant information should be reported as an AE or, if the reaction fulfils one of the criteria for seriousness, as a SAE.

Drug overdose:

An overdose is a deliberate or inadvertent administration of a treatment at a dose higher than that specified in the protocol, and higher than the known therapeutic dose and of clinical relevance. The reaction must be clearly identified as an overdose.

Interaction:

A drug interaction is a situation in which a substance/medicinal product affects the activity of an IMP, i.e., the effects are increased or decreased, or they produce an effect that none of the products exhibits on its own. The reaction must be clearly identified as drug interaction.

Misuse:

Misuse is the deliberate administration or use of the medicinal product outside its described indication or outside the current state of the art medical practice (off-label-use). The reaction must be clearly identified as misuse.

Medication error:

Medication error involves the inadvertent administration or unintended use of a medicinal product which may be caused by the naming, presentation of pharmaceutical form/packaging, instructions for use/labelling. The reaction must be clearly identified as a medication error.

7.4 Other Assessments

7.4.1.1 Recovery investigation (optional)

A recovery investigation is recommended to be performed with the first *Human-cl rhFVIII* administration, or within the first three months after treatment start with *Human-cl rhFVIII*, in any case in a non-bleeding patient. A dose of 40 IU FVIII/kg BW will be administered and the *in vivo* recovery will be calculated from the FVIII levels before infusion and the peak level obtained from the 15 min and 1 hour post-infusion samples. Recovery investigations are recommended to be repeated approximately every 6 months.

7.4.1.2 RNA Expression Analysis (Optional)

RNA expression analysis will optionally be performed in order to provide an understanding of the transcript activity of the genes involved in immune responses that may be responsible for FVIII inhibitor formation in patients receiving exogenous FVIII. RNA expression analysis will also be optionally performed on patients undergoing ITI to provide information on the transcript activity of genes involved in immune tolerance. The analysis will be carried out using a customised PAXgene protocol in which RNA samples will be obtained by using commercially available PAXgene RNA blood collection tubes and customised microfuge tubes. This customised approach has previously been used in both a paediatric and animal study where samples volumes are limited (6),(7). The full protocol for this sub-study can be found in Appendix 2.

7.4.1.3 Immunogenotyping (Partly Optional)

Immunogenotyping will be performed in order to investigate genetic factors that might influence development of FVIII inhibitors. It will include analysis of FVIII gene mutations, HLA-typing, immune response gene profiling and FVIII ethnic haplotype analysis. FVIII mutation analysis is a required assessment, whereas HLA-typing, immune response gene profiling and FVIII ethnic haplotype are optional. Blood samples required for this analysis can be obtained at any time during the study, but it is recommended that they be obtained at the same time as the samples for scheduled study visits.

7.4.1.4 In vitro immunogenicity of FVIII products (optional)

Immunogenicity of *Human-cl rhFVIII* will be assessed by culturing peripheral blood monocyte cells (PBMC) from the patients (including a positive control) with *Human-cl rhFVIII*. The nature of T cell response will be assessed by analysing cytokine expression (measured in a multiplex format) and T cell proliferation. Cytokines analysed will include IL-2, TNFα, IFNγ, IL-5, IL-6, IL-10, and IL-17 (TGFβ may also be included in an ELISA format).

7.5 Appropriateness of Measurements

All measurements used for the assessment of the immunogenicity, safety, and efficacy of *Human-cl rhFVIII* are in compliance with the requirements set up in the CHMP “Guideline on the clinical investigation of recombinant and human plasma-derived factor VIII products” (EMA/CHMP/BPWP/144533/2009) (5).

All FVIII inhibitor and FVIII plasma level samples obtained in the course of this study will be evaluated by an accredited central laboratory using validated methods and assays.

If clinically indicated, FVIII and inhibitor testing can additionally be performed in the local laboratory. The results obtained locally will be recorded in the CRFs.

8 DATA HANDLING AND RECORD KEEPING

8.1 Documentation of Data

8.1.1 Source Data and Records

Source data are defined as all the information related to clinical findings, observations, or other activities in the study, written down in original records or certified copies of original records allowing reconstruction and evaluation of the clinical study.

The Investigator will maintain adequate source records (e.g., case histories or patient files for each patient enrolled). Source records should be preserved for the maximum period of time required by local regulations.

For each patient enrolled, the Investigator will indicate in the source record(s) that the patient participates in this study.

All data entered in the CRF must be supported by source data in the patient records with the exceptions listed in Section 8.1.2.

The Investigator will permit study-related monitoring, audit(s), Independent Ethics Committee [IEC]/ Institutional Review Board [IRB] review(s) and regulatory inspection(s), by providing direct access to source data/records.

The Investigator may authorise site staff (e.g., sub-Investigators, nurses) to enter study data into the CRF. This must be documented in the "Delegation of Authority Log", filled in and signed by the Investigator responsible.

8.1.2 Case Report Forms

For each patient enrolled, a CRF will be completed and signed by the Investigator or an authorised Co-Investigator. All forms will be filled out using an indelible pen, and must be legible.

8.1.3 Changes to Case Report Form Data

Errors occurring in CRFs will be crossed out without obscuring the original entry, the correction will be written alongside the initial entry, and the change will be initialled and dated by the Investigator or authorised study site personnel. When changes to CRF data are necessary following removal of the original CRF from the study site, any such changes will be documented on data clarification / resolution forms, which will be submitted to the Investigator for signature.

If reason for the change is not obvious, then a reason should be given. The Principal Investigator must, as a minimum, sign the final CRF page to attest the accuracy and completeness of all the data. Once the data have been entered onto the database, they will be checked and any discrepancies will be raised and returned to the Investigator for resolution. Data will be monitored and tabulated in accordance with the Data Management Plan.

8.2 Information of Investigators

An Investigator's Brochure will be handed out to the Investigator before the start of the study. This Brochure contains all information in the Sponsor's possession necessary for the Investigator to be fully and accurately informed about the safety of the IMP under evaluation and the respective benefit-risk ratio.

The Investigator's Brochure will be updated by the Sponsor at regular intervals and in case new information concerning the IMP becomes available.

The Investigators will be informed about the methods for rating relevant study outcomes and for completing CRFs in order to reduce discrepancies between participating Investigators and study sites.

The Investigator will be kept informed of important data that relate to the safe use of the IMP as the study proceeds.

8.3 Responsibilities

The Co-ordinating Investigator of this study is Dr Raina J Liesner, Great Ormond Street Hospital for Children, NHS Trust, Haemophilia Centre, Great Ormond Street, London WC1N 3JH, United Kingdom.

The central laboratory for all coagulation parameters and inhibitor testing is Covance (former LabCorp Clinical Trials, Laboratory Services), 8490 Upland Dr, Ste. 100, Englewood, CO 80112, USA.

The central laboratory for immunogenotyping (FVIII mutation, HLA-typing, immune response gene profiling and FVIII ethnic haplotype) is Prof. Johannes Oldenburg, Institute of Experimental Haematology and Transfusion Medicine, Sigmund-Freud Strasse 25D, 53127 Bonn, Germany.

The central laboratory for the RNA expression analysis is Dr Dan Hart and Dr Paul Batty, Dept of Haematology Laboratory, 4th Floor, Pathology and Pharmacy building, Royal London Hospital, Barts and The London School of Medicine and Dentistry, 80 Newark Street, London E1 2ES, United Kingdom.

The central laboratory for the determination of immunogenicity of FVIII products (*in vitro* T cell assay) is Antitope Ltd, Business Development Group, Babraham Research Campus, Babraham, Cambridge CB22 3AT, United Kingdom.

The central laboratory for epitope mapping in patients developing an inhibitor is Prof. Johannes Oldenburg, Institute of Experimental Haematology and Transfusion Medicine, Sigmund-Freud Strasse 25D, 53127 Bonn, Germany.

Statistical advice during the planning phase of the study was given by Accovion GmbH – Clinipace Worldwide, Softwarecenter 3, 35037 Marburg, Germany.

Study data management and statistics will be delegated under an agreement of transfer of responsibilities to Accovion GmbH – Clinipace Worldwide , Softwarecenter 3, 35037 Marburg, Germany.

All Octapharma procedures and policies have to be met by external parties (Contract Research Organisations [CROs] and central laboratories), discrepancies or exceptions are to be approved by Octapharma.

All parties involved in the study are responsible to comply with local and international obligations, regulatory requirements and duties in accordance with local laws, using the principles of good clinical practice (GCP) and good laboratory practice (GLP) guidelines, SOPs and complying with all other applicable regulations.

8.4 Investigator's Site File

At each study site, the Investigator is responsible for maintaining all records to enable the conduct of the study to be fully documented. Essential documents as required by GCP guidelines and regulations (e.g., copies of the protocol, study approval letters, all original informed consent forms, site copies of all CRFs, drug dispensing and accountability logs, correspondence pertaining to the study, etc.) should be filed accurately and kept by the Investigator for the maximum period of time required by local regulations.

The Investigator is responsible for maintaining a confidential patient identification code list, which provides the unique link between named source records and CRF data for the Sponsor. The Investigator must arrange for the retention of this confidential list for the maximum period of time required by local regulations.

No study document should be destroyed without prior written agreement between the Investigator and the Sponsor. Should the Investigator elect to assign the study documents to another party, or move them to another location, the Sponsor must be notified in writing.

8.5 Provision of Additional Information

On request, the Investigator will supply the Sponsor with additional data relating to the study, or copies of relevant source records, ensuring that the patient's confidentiality is maintained. This is particularly important when CRFs are illegible or when errors in data transcription are encountered. In case of particular issues or governmental queries, it is also necessary to have access to the complete study records, provided that the patient's confidentiality is protected in accordance with applicable regulations.

8.6 Independent Data Monitoring Committee

Inhibitor and safety data this study will be monitored at specified intervals by an Independent Data Monitoring Committee (IDMC). The IDMC will be composed of recognised experts in the field of haemophilia who are not actively recruiting patients. The study Sponsor will formally convene the IDMC at least once per year until completion of the trial program. Ad-hoc meetings will be held, if needed.

9 STATISTICAL METHODS AND SAMPLE SIZE

The statistical analysis will be delegated under an agreement of transfer of responsibilities to an external CRO. All Octapharma procedures and policies have to be met by this CRO. Discrepancies or exceptions are to be approved by the Sponsor's Manager of Biometrics.

9.1 Determination of Sample Size

No inferential analysis involving formal testing is planned in this non-controlled study. Consequently, no formal sample size estimation was performed, but the sample size was chosen to satisfy current CHMP recommendations.

According to the CHMP "Guideline on the Clinical Investigation of Recombinant and Human Plasma-Derived Factor VIII Products" (EMA/CHMP/BPWP/144533/2009) (5), a minimum of 50 PUPs evaluated for efficacy and safety during at least 50 ED, connected with a post-approval commitment to follow up at least 100 PUPs (50 from efficacy/safety trial and 50 new) for a minimum of 100 ED, is necessary.

9.2 Statistical Analysis

A formal statistical analysis plan (SAP) describing all details of the analyses to be performed will be prepared by the study statistician and approved by the Sponsor prior to the start of the study.

The statistical analysis of the endpoints is to be understood in the exploratory sense. No confirmatory hypothesis testing is planned.

9.2.1 Population for Analysis

For the analysis of this study, the following populations will be considered:

- Safety analysis population: All subjects who received at least one dose of *Human-cl rhFVIII*;
- Intent to Treat (ITT) analysis population: All subjects in the safety analysis population for whom any data was collected post treatment with *Human-cl rhFVIII*;
- Efficacy: Per Protocol (PP) analysis population: All subjects in the ITT analysis population who completed the trial without significantly violating the inclusion/exclusion criteria or other aspects of the protocol considered to potentially affect the efficacy results.
  Especially the following subjects will be excluded from this population:
  - Subjects who violate the following inclusion criteria:
    - severe Haemophilia A (FVIII:C <1%; historical value as documented in subject records),
    - No previous treatment with FVIII concentrates or other blood products containing FVIII
  - Subjects who fulfil the following exclusion criteria:
    - other coagulation disorder than Haemophilia A
    - severe liver or kidney disease (ALT and AST levels >5 times of ULN, creatinine >120 µmol/L),
    - concomitant treatment with any systemic immunosuppressive drug
    - subjects who use concomitant medication that may confound study results, like e.g. alpha-interferon or prednisone
  - Subjects with significant non-compliances with the protocol such as non-compliance to complete the diary in a proper manner or more than 30% of haemostatic efficacy assessments missing.
  - A minimum of inhibitor tests (baseline, after 1-4 EDs, 10-15 EDs and 50 EDs) is available
  - Subjects with dosing or treatment errors like e.g. the use of other FVIII products (except for emergencies as mentioned above) or several *unexplained* and significant deviations from the recommended dose regimen.
  - Patients with less than 100 days of exposure to *Human-cl rhFVIII*.

- Subpopulations of the ITT populations will be
  - Subjects of each ethnic group
  - Subjects with ITI treatment
  - Subjects with breakthrough bleedings, if appropriate
  - Subjects with surgical interventions, if appropriate
  - Subpopulations especially for inhibitor incidence analysis:
    - Subjects with different FVIII gene mutations
    - Subjects with/without family history of inhibitors
    - Subjects by classes of EDs prior to inhibitor generation
    - Subjects by reason for treatment prior to inhibitor generation
    - Subjects by intensity of treatment with/without peak treatment moments before inhibitor appearance (peak treatment moments defined as ≥3 subsequent days with FVIII dosing)
    - Subjects by classes of prophylactic treatment (amount and frequency)
- Population of subjects on prophylactic treatment schedule (PROPH): All subjects in the ITT population who have at least one prophylactic treatment
- Per-protocol population of subjects on prophylactic treatment schedules (PROPH-PP): All subjects in the PP population who have at least 100 EDs of treatment and who have no significant dosing or treatment errors, like e.g. unexplained interruptions of the prophylaxis with *Human-cl rhFVIII*
- Population of bleedings (BLEED): All documented bleeds - except those occurring during and after surgery - of subjects in the ITT population for which
  - any amount of treatment with *Human-cl rhFVIII* is documented and which
  - start between first BE treated with *Human-cl rhFVIII* and the completion visit.
- Population of bleedings per protocol (BLEED-PP): All documented bleeds in the BLEED population of subjects in the PP population.
- Surgery population (SURG): All documented surgical interventions of subjects in the ITT population for which
  - any amount of *Human-cl rhFVIII* prior to, during or after the surgery is documented and
  - no other FVIII concentrate is documented within 24 hours prior to surgery.
- Surgery per-protocol population (SURG-PP): All documented surgical interventions of subjects in the PP population for which
  - any amount of *Human-cl rhFVIII* prior to, during or after the surgery is documented and
  - no other FVIII concentrate is documented within 72 hours prior to, during or after the surgery (until resuming regular prophylactic treatment or until discharge from hospital in case of a subject with on-demand treatment).

The subject disposition, i.e. the identification of significant violations to consider for the PP populations and the assignment of each subject, bleeding and surgery to these analysis populations, will be the joined decision of the trial statistician and the responsible medical expert prior to database lock.

The ITT population is considered to be the most relevant for analysis of immunogenicity data; the PROPH population is considered to be the most relevant for analysis of efficacy data on prophylaxis; the BLEED population is considered to be the most relevant for analysis of efficacy data on bleedings and the SURG population is considered to be the most relevant for analysis of efficacy data on surgeries. To evaluate the robustness of the study results, efficacy analyses will also be done on basis of the respective PP population.

9.2.2 Immunogenicity Analysis Plan

All recorded determinations of inhibitors against FVIII will be listed. The occurrence and cumulative incidence of inhibitors (inhibitor titre ≥0.6 and ≥5 BU respectively) will be presented in total, and as percentage of the analysis population with a 95% confidence interval. If justified by the number of events, the time period until the first inhibitor activity will be tabulated and displayed graphically by means of a Kaplan-Meier plot of survival without inhibitor versus cumulative EDs, and a logistic regression analysis of incidence rates of FVIII inhibitor development will be performed (prognostic factors: family history, weight, mean dose/ED, age, gene mutation defect, number of EDs before inhibitor development, age at first treatment, intensity of treatment). Number of EDs before inhibitor development will be tabulated for each patient who develops FVIII inhibitors. Incidence of FVIII inhibitors will also be presented grouped by ethnicity and FVIII gene mutations, incl. results of the immunogenotyping sub-study, if data are available.

Date from patients participating in sub-studies – RNA-expression analysis and the *In vitro* immunogenicity of FVIII products - will be evaluated by descriptive statistics.

9.2.3 Efficacy Analysis Plan

Efficacy will be evaluated by descriptive statistics.

- On bleeding rates (efficacy of prophylaxis) overall and by intensity of prophylaxis
- On efficacy assessments per bleed, basic bleed characteristics including severity, site and type
- On efficacy assessments per surgery
- Recovery investigation (optional): a recovery investigation is recommended to be performed. This can be done with the first *Human-cl rhFVIII* administration, or within the first three months after treatment start with *Human-cl rhFVIII*, in any case in a non-bleeding patient.

The frequency of bleeds, the number of infusions needed to treat a BE, the number of EDs, and study drug consumption data (FVIII IU/kg per infusion, per BE, per month, per year) per patient and in total will be evaluated. Furthermore, increased and decreased doses of *Human-cl rhFVIII* used to treat individual BEs (frequency and relative magnitude of dose changes) will be evaluated, as well as changes in the doses per infusion and changes in the total dose used to treat subsequent BEs of the same type (e.g. elbow, knee, etc.) in the same patient (frequency and relative magnitude of dose changes).

9.2.4 Safety Analysis Plan

All AEs occurring after initiation of study treatments (including events likely to be related to the underlying disease, or a concomitant illness or medication or clinical significant abnormalities in laboratory parameters or vital signs) will be displayed in summary tables, listings and figures.

Incidences of AEs will be given as numbers and percentages of patients and infusions with:

- Any AE.
- Any serious AE.
- Any AE probably or possibly related to the trial drug.
- Any AE that begins within 24 hours of the end of an infusion.
- Any severe AE.
- Any withdrawal due to AE.
- Any AE by MedDRA preferred term.
- Any AE by MedDRA System Organ Class (SOC).

Summary tables for AEs will be given by SOC and preferred term. Additionally, AEs will be summarised by severity and relationship to study treatment. These summary tables will feature total counts and counts by age group, sex, prior EDs to *Human-cl rhFVIII* and total amount of *Human-cl rhFVIII* used prior to the AE to evaluate the need of further investigation of any apparent pattern or trend in AE rates.

The MedDRA coded terms and the corresponding original (verbatim) terms used by the Investigator will be listed.

Vital signs

Blood pressure (systolic/diastolic), heart rate, respiratory rate and body temperature will be tabulated, and the sample characteristics will be presented by time point.

Routine laboratory data

Routine laboratory parameters (haematology, clinical chemistry) will be listed for all patients, using indicators for values outside the associated reference ranges. Changes from baseline (screening) will be provided where appropriate.

9.2.5 Resource Use Analysis Plan

The statistical analysis of resource use parameters will be descriptive.

Continuous characteristics (i.e. drug usage, duration of hospitalisation, days lost at work, etc.) will be described in terms of mean/median/ standard deviation and range). Dichotomous or categorical variables (i.e. number of hospitalisations, CVAD use, etc.) will be described by frequency tables and percentages.

If applicable, results will also be presented per subgroup.

Health economic modelling analysis with resource use parameters is planned at study closure.

9.2.6 Handling of Missing Data

In general, missing data will not be imputed: calculations pertaining to person-year computations will be based on observed values only. Only in case of missing BW, the last available weight measurement will be used for calculating the dose per kg bodyweight (last observation carried forward).

9.3 Interim Analysis

One interim analysis is planned after 50 patients have achieved at least 50 EDs.

The interim analysis will comprise the complete analysis planned for the final analysis, except for the Health economic modelling analysis. The analysis is based on available data at that time.

10 ETHICAL / REGULATORY, LEGAL AND ADMINISTRATIVE ASPECTS

10.1 Ethical / Regulatory Framework

This study will be conducted in accordance with the ethical principles laid down in the Declaration of Helsinki. The study protocol and any subsequent amendment(s) will be submitted to an IEC/IRB and to the Regulatory Authority. The study will be conducted in compliance with the protocol, GCP regulations and applicable regulatory requirements.

The regulatory application or submission for regulatory approval will be made by the Sponsor or designated third party (e.g. CRO) as required by national law.

10.2 Approval of Study Documents

The study protocol, a sample of the patient information and informed consent form, any other materials provided to the patients, and further requested information will be submitted by the Sponsor or the Investigator to the appropriate IEC/IRB and the Regulatory Authority. The study approval letter must be available before any patient is exposed to a study-related procedure.

The Sponsor, the Investigator and any third party (e.g., CRO) involved in obtaining approval, must inform each other in writing that all ethical and legal requirements have been met before the first patient is enrolled in the study.

10.3 Patient Information and Informed Consent

The Investigator will obtain a freely given written consent from each patient after an appropriate explanation of the aims, methods, anticipated benefits, potential hazards and any other aspect of the study which is relevant to the patient's decision to participate. The informed consent form must be signed, with name and date and time noted by the patient, before the patient is exposed to any study-related procedure, including screening tests for eligibility.

For patients not qualified to give legal consent, written consent must be obtained from the legal parent(s)/guardian(s). If children are old enough to understand the risks and benefits of the study, they should also be informed and provide their written consent.

The Investigator will explain that the patients are completely free to refuse to enter the study or to withdraw from it at any time, without any consequences for their further care and without the need to justify. The Investigator will complete the informed consent section of the CRF for each patient enrolled.

Each patient will be informed that his/her medical (source) records may be reviewed by the study monitor, a quality assurance auditor or a health authority inspector, in accordance with applicable regulations, and that these persons are bound by confidentiality obligations.

10.4 Protocol Amendments

Any prospective change to the protocol will be agreed between the Investigator (co-ordinating Investigator in multi-centre studies) and the Sponsor prior to its implementation. Any such

amendments will be submitted to the IEC(s)/IRB) and/or competent authority responsible as required by applicable regulations. IEC(s)/IRB approval will at a minimum be requested for any change to this protocol which could affect the safety of the patients, the objective/design of the study, any increase in dosage or duration of exposure to the IMP an increase in the number of patients treated, the addition of a new test or procedure, or the dropping of a test intended to monitor safety.

10.5 Confidentiality of Patients´ Data

The Investigator will ensure that the patient's confidentiality is preserved. On CRFs or any other documents submitted to the Sponsor, the patients will not be identified by their names, but by a unique patient number. Documents not for submission to the Sponsor, i.e., the confidential patient identification code list, original consent forms and source records will be maintained by the Investigator in strict confidence.

11 QUALITY CONTROL AND QUALITY ASSURANCE

11.1 Periodic Monitoring

The monitor will contact and visit the Investigator periodically to review all study-related source data/records, verify the adherence to the protocol and the completeness, correctness and accuracy of all CRF entries compared to source data. The Investigator will co-operate with the monitor to ensure that any discrepancies identified are resolved.

For this study, the first monitoring visit shall take place shortly after the inclusion of the first patient. Thereafter, monitoring frequency will depend on study progress.

The monitor must be given direct access to source documents (original documents, data and records). Direct access includes permission to examine, analyse, verify and reproduce any records and reports that are important to the evaluation of the clinical study. Source data will be available for all data in the CRFs, including all laboratory results.

11.2 Audit and Inspection

The Investigator will make all study-related source data and records available to a qualified quality assurance auditor mandated by the Sponsor, or to IEC/IRB/regulatory inspectors, after reasonable notice. The main purposes of an audit or inspection are to confirm that the rights and welfare of the patients have been adequately protected, and that all data relevant for the assessment of safety and effectiveness of the IMP have been reported to the Sponsor.

12 REPORTING AND PUBLICATION

12.1 Clinical Study Report

A clinical study report (in accordance with relevant guidelines and Sponsor’s SOPs) will be prepared by the Sponsor after the completion of the study. The co-ordinating Investigator will approve the final study report after review.

12.2 Publication Policy

The results of this study may be published or presented at scientific meetings. If this is envisaged by an Investigator, the Investigator agrees to inform the Sponsor and to submit all manuscripts or abstracts to the Sponsor prior to submission to an editorial board or scientific review committee. This will allow the Sponsor to protect proprietary information and to provide comments based on information that may not yet be available to the Investigator.

In accordance with standard editorial and ethical practice, the Sponsor will support publication of multi-centre studies only in their entirety and not as individual centre data. Authorship will be determined by mutual agreement.

13 LIABILITIES AND INSURANCE

In order to cover any potential damage or injury occurring to a patient in association with the IMP or the participation in the study, Octapharma AG will contract insurance in accordance with local regulations.

The Investigator is responsible for dispensing the IMP according to this protocol, and for its secure storage and safe handling throughout the study.

14 REFERENCES

(1) Report of Expert Meeting on Factor VIII Products and Inhibitor Development. EMEA/CHMP/BPWP/123835/2006; London, 22 February 2007]

(2) Kobayashi T, and Ezzelarab M. Glycobiology relating to xenotransplantation. Curr Opin Organ Transplant 2006;11:1541-59.

(3) Hokke CH, Bergwerff AA, van Dedem GWK, van Oostrum J, Kamerling JP, and Vliegenthart JFG. Sialylated carbohydrate chains of recombinant human glycoproteins expressed in Chinese hamster ovary cells contain traces of N-glycolylneuraminic acid. FEBS Lett 1990;275:9-14.

(4) Hironaka T, Furukawa K, Esmon PC, Fournel MA, Sawada S, Kato M, et al. Comparative study of the sugar chains of factor VIII purified from human plasma and from the culture media of recombinant baby hamster kidney cells. J Biol Chem 1992;267:8012-20.

(5) Guideline on the clinical Investigation of Recombinant and Human Plasma-Derived Factor VIII Products (EMEA/CHMP/BPWP/144533/2009), London, 21 July 2011

(6) Carrol ED, Salway F, Pepper SD, Saunders E, Mankhambo LA, Ollier WE, et al. Successful downstream application of the Paxgene Blood RNA system from small blood samples in paediatric patients for quantitative PCR analysis. BMC Immunol 2007;8:20.

(7) Krawiec JA, Chen H, Alom-Ruiz S, Jaye M. Modified PAXgene method allows for isolation of high-integrity total RNA from microliter volumes of mouse whole blood. Lab Anim 2009;43:394-8.

15 APPENDICES

Appendix 1: IMP Master Label for GENA-05 (500 IU vial example)

PRODUCT VIAL & PRODUCT VIAL BOX LABELS

Product Vial Cover:

500 IU Human-cl rhFVIII **GENA-05** **EudraCT No.: 2012-002554-23**
See attached booklet for local languages
(I):
(II):
(III):
(IV):

Tear-off label for Product Vial Cover:

Study: GENA-05 500 IU *Human-cl rhFVIII*
(I):
(II):
(III):

Product Vial Page 2:

Sponsor: Octapharma AG, Seidenstrasse 2, 8853 Lachen, Switzerland, Tel.: +41-55-4512121

Product Vial Page 3:

US, GB, CA…4-5
DE…6-7
FR, CA…8-9
PL…10-11
ES, CO, VE…12-13
RU, UA, GE…14-15
MD…16-17
BR…18-19

Product Vial Content Pages:

500 IU Human-cl rhFVIII **Clinical Study GENA-05 EudraCT No.: 2012-002554-23**
Freeze dried powder rhFVIII for reconstitution: 500 IU FVIII.
For intravenous injection after reconstitution with supplied solvent (2.5 ml Water for Injection).
Use immediately after reconstitution.
Do not use turbid or incompletely dissolved solutions.
FOR CLINICAL TRIAL USE ONLY
Caution: New Drug – Limited by Federal (or United States) Law to Investigational Use
Do not freeze, store at +2°C to +8°C, protected from light.
Keep out of reach of children
Batch no.:…….(I) Expiry date:…….(II) Patient no.:…….(III)
Investigator: ……(IV)

Product Vial Last Pages:

500 IU Human-cl rhFVIII **Clinical Study GENA-05 EudraCT No.: 2012-002554-23**
Sponsor: Octapharma AG, Seidenstrasse 2, 8853 Lachen, Switzerland, Tel.: +41-55-4512121

Appendix 1: IMP Master Label for GENA-05 (500 IU vial example) - continued

EQUIPMENT BOX LABELS

Equipment Box Cover:

500 IU Human-cl rhFVIII **Clinical Study GENA-05 EudraCT No.: 2012-002554-23**
See attached booklet for local languages
Sponsor: Octapharma AG, Seidenstrasse 2, 8853 Lachen, Switzerland, Tel: +41-55-4512121
(I):
(II):
(III):
(IV):

Equipment Box Page 3:

US, GB, CA…4-5
DE…6-7
FR, CA…8-9
PL…10-11
ES, CO, VE…12-13
RU, UA, GE…14-15
MD…16-17
BR…18-19

Equipment Box Content Page 4-5 (US, GB, CA, IN):

500 IU Human-cl rhFVIII **Clinical Study GENA-05 EudraCT No.: 2012-002554-23**
Equipment pack for reconstitution and injection.
This package contains:
1 vial of *Human-cl rhFVIII* concentrate (500 IU)
1 pre-filled syringe containing 2.5 ml Water for Injection Ph. Eur.
1 Vial adapter
1 Disposable syringe (10 ml)
1 Infusion set (butterfly needle)
2 Tissues
Do not freeze. Store at +2°C to +8°C (+36°F to + 46°F). If refrigeration space is limited, store only the concentrate vial in the refrigerator; the equipment pack can be stored at room temperature.

For clinical trial use only
Caution: New Drug – Limited by Federal (or United States) Law to Investigational Use

Keep out of reach of children

Batch no.:.....(I) Expiry date:.....(II) Patient no.: ……(III)

Investigator:.....(IV)

Equipment Box Last Page:

500 IU Human-cl rhFVIII **Clinical Study GENA-05 EudraCT No.: 2012-002554-23**
See attached booklet for local languages
Sponsor: Octapharma AG, Seidenstrasse 2, 8853 Lachen, Switzerland, Tel.: +41-55-4512121

PRE-FILLED SYRINGE:

Pre-filled syringe:

Pre-filled syringe containing **Clinical Study GENA-05**
2.5 mL Water for Injection for reconstitution of one (1) vial *Human-cl rhFVIII*
Store at +2 °C to +25 °C
Batch no.: _____
Expiry date: _____

Appendix 2: RNA Expression Sub-Study

Inhibitory antibody formation remains the greatest challenge in the management of severe haemophilia A. The focus of genetics studies of inhibitor risk has been on variations / mutations in the inherited DNA sequences of the factor VIII (FVIII) gene (causative haemophilia mutation or FVIII wild type haplotype) and genes involved in the immune response (e.g. IL-10, TNF-α and CTLA-4). However, such DNA based technology only gives the baseline gene profile of the individual without taking into account the dynamic changes that will occur in actual gene expression around the time of exposure to a novel immunogenic stimulus. Quantitative assessment of transcribed messenger Ribonucleic Acid (mRNA) offers a way of measuring differential changes in gene activity in these circumstances. Although technology for assessment of mRNA expression has been available for some years, the arrival of next generation sequencing (RNA-Seq) offers the ability to perform high throughput analysis of gene transcript activity on small volumes of venous blood in the setting of an international clinical study. This technology offers the ability to sequence the gene transcript products of millions of genes from each clinical sample. This enables an unbiased assessment of the genes involved in the immune response as well as other potential novel biological pathways that may prove to be of key importance in the process of inhibitor formation.

This is the first clinical study to prospectively evaluate changes in gene expression around the time of exposure to exogenous FVIII and during immune tolerance induction. Measurement of real-time changes in mRNA expression may give further insight into the complex dynamic processes and pathways involved with the aim of identifying “bio-signatures” that can predict those at greatest risk of inhibitor formation or failure of immune tolerance induction. Sampling of mRNA is feasible in a paediatric study as it only requires small volumes of venous whole blood. Due to instability in mRNA and changes in gene expression following phlebotomy, stabilisation is required at the point of collection (1,2,3). Modifications of commercially available stabilisation systems have been successfully trialled in both paediatric and animal studies demonstrating satisfactory mRNA yields whilst minimising required blood volumes (4,5).

RNA Expression Analysis Sampling Schedule

Formal written consent should be obtained at study entry for storage of whole blood (mRNA) samples for research purposes to investigate the mechanisms of inhibitor formation / immune tolerance.

For a patient enrolled on this optional sub-study, peripheral venous blood samples will be collected on commencement of Human-cl rhFVIII treatment at the time points shown in table 1.

A screening sample (prior to first FVIII exposure) is necessary to proceed with follow up samples. If the screening sample is missed, do not collect subsequent follow up samples. **However, these patients could still be enrolled in the ITI RNA-Seq study (see Table 2), with a new pre-ITI baseline sample and subsequent follow up samples.**

Table 1: RNA expression analysis sampling schedule: First 20 Exposure Days (20ED)

	Pre-1st exposure (Screening)	Every 3 – 4 ED (with routine inhibitor screen)	20 ED or at inhibitor formation
RNA expression analysis	1mL	1mL	1mL

For patients diagnosed with an inhibitor (commencing on ITI), enrolled on this optional ITI RNA expression analysis sub-study, peripheral venous blood samples will be taken at the time points shown in table 2.

Table 2: RNA expression analysis sampling schedule: Immune Tolerance Induction (ITI)

	Pre-ITI Initiation	Week 2	Week 4	Week 8	Week 12	Week 16	Week 20	Week 24
RNA expression analysis	1mL	1mL	1mL	1mL	1mL	1mL	1mL	1mL

RNA Expression Study Materials

Investigation centres will be provided with centrally prepared customized PAXgene mRNA sampling tubes. The materials required for this study are as follows:

1) Customized PAXgene mRNA sampling tube: sterile(RNAse / DNAse free) cryovial (4.5 mL volume) containing 2.76mL PAXgene reagent (ratio 1mL blood : 2.76mL reagent) (provided)
2) Sterile butterfly / winged infusion kit (not provided)
3) Sterile 1mL syringe (not provided)
4) Sterile gloves (not provided)
5) Skin surface wipe (not provided)

RNA Expression Sampling Protocol

1) Keep RNA expression sample tube at room temperature (18 – 25°C) prior to sampling. **Do not re-use once opened.**
2) Collect 1mL venous blood into a 1mL sterile syringe using the local centre's protocol for venepuncture, wearing sterile gloves.
3) Open PAXgene sample tube and directly transfer 1mL of venous blood following venepuncture into the PAXgene tube.
4) Close sample tube.
5) Gently invert the customized PAXgene tube 8 to 10 times.
6) Label sample with patient identifiable information / study number / and study time-point.

RNA Expression Transportation and Storage Protocol

1) Keep sample upright for **4-6 hours at room temperature (18 – 25°C)** following blood sampling, prior to freezing. Early freezing of samples will substantially decrease the RNA yield.
2) Freeze sample at **–70°C** in a wire rack (not a stryofoam tray as this may lead to sample tubes cracking).

3) Transport frozen PAXgene stabilised RNA study samples to LabCorp (LabCorp Clinical Trials, Laboratory Services, 8490 Upland Dr, Ste. 100, Englewood, CO 80112, USA) on dry ice.
4) RNA Samples kept at -70°C will be stable for a period of up to 60 months (6).

RNA Extraction and Transcriptosome Analysis

All extraction, purification of RNA and next generation sequencing (RNA-Seq) will be performed at The Genome Centre, John Vane Science Centre, United Kingdom. Interpretation of results will be performed by Dr Paul Batty / Dr Daniel Hart in collaboration with the Bioinformatics Department at The Genome Centre, John Vane Science Centre, United Kingdom.

References

(1) Pahl A, Brune K. Gene expression changes in blood after phlebotomy: implications for gene expression profiling. Blood 2002 Aug 1;100(3):1094-5.

(2) Rainen L, Oelmueller U, Jurgensen S, Wyrich R, Ballas C, Schram J, et al. Stabilization of mRNA expression in whole blood samples. Clin Chem 2002 Nov;48(11):1883-90.

(3) Pahl A, Brune K. Stabilization of gene expression profiles in blood after phlebotomy. Clin Chem 2002 Dec;48(12):2251-3.

(4) Carrol ED, Salway F, Pepper SD, Saunders E, Mankhambo LA, Ollier WE, et al. Successful downstream application of the Paxgene Blood RNA system from small blood samples in paediatric patients for quantitative PCR analysis. BMC Immunol 2007;8:20

(5) Krawiec JA, Chen H, Alom-Ruiz S, Jaye M. Modified PAXgene method allows for isolation of high-integrity total RNA from microlitre volumes of mouse whole blood. Lab Anim 2009 Oct;43(4):394-8.

(6) Weber DG, Casjens S, Rozynek P, Lehnert M, Zilch-Schoneweis S, Bryk O, et al. Assessment of mRNA and microRNA Stabilization in Peripheral Human Blood for Multicenter Studies and Biobanks. Biomark Insights 2010;5:95-102.

Contacts

[redacted]