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

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Introduction

The current standard treatment for haemophilia A patients – prophylactic or on-demand to stop bleeds – consists of factor VIII (FVIII) concentrates which are either derived from human plasma or from recombinant technology produced in hamster cell lines.

Immunogenicity - inhibitor development is the most important complication in haemophilia treatment, occurring in up to 40% of previously untreated patients (PUPs) with severe haemophilia A usually within the first 50 exposure days (EDs) to a FVIII concentrate.[1]

Human-cl rhFVIII is the first recombinant factor VIII (rFVIII) concentrate derived from a genetically modified human cell-line (human embryonic kidney (HEK) 293F cells). Its glycosylation pattern is comparable to that of normal human plasma FVIII.[2] *Human-cl rhFVIII* is devoid of the antigenic Neu5Gc or α-Gal epitopes that are present in chinese hamster ovary- and baby hamster kidney-cell derived rFVIII products[3-5] and thus may be less immunogenic in humans.

The production process of *Human-cl rhFVIII* is completely free of added materials of human or animal origin, and any impurities are removed which might also reduce the risk of hypersensitivity reactions.[6]

Since 2009, 6 prospective GCP studies with *Human-cl rhFVIII* have been conducted in more than 130 previously treated adults and children with severe haemophilia A, with at least 50 EDs per patient, and an observational period of at least 6 months. None of the pre-treated patients (PTPs) treated exclusively with *Human-cl rhFVIII* developed an inhibitor.

According to the current European CHMP Guideline CHMP/ BPWG/ 144533/2009, a study in previously untreated patients (PUPs) needs to be conducted for all novel FVIII products, as soon as sufficient data of PTPs are available (including children).

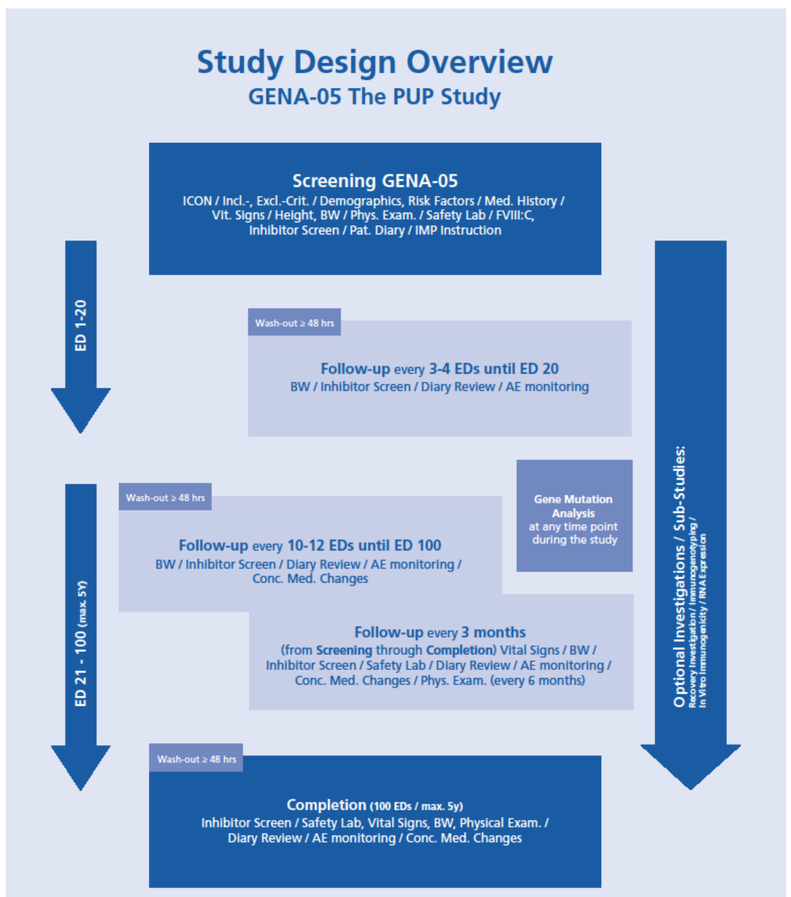
Consequently, a prospective, multicentre, multinational, open-label Phase III study including 100 PUPs was initiated early 2013. This study investigates the immunogenicity, efficacy and safety of *Human-cl rhFVIII* in PUPs.

Materials and Methods

Study design / Study procedures

This is a prospective, multicentre, multinational, open-label, non-controlled study in 100 PUPs with severe haemophilia A (FVIII coagulation activity [FVIII:C] < 1%). The study design is summarised as follows:

Figure 1. Study design



Study duration

- Entire study: early 2013 - end 2018
- Patients: 100 EDs, max 5 years (from screening to final follow-up)
- Patients with FVIII inhibitors: ITI treatment duration of max. 36 months

Figure 2. Study objectives

Primary objective	■ Immunogenicity of *Human-cl rhFVIII* in PUPs suffering from severe haemophilia A
Secondary objectives	■ Efficacy of *Human-cl rhFVIII* during prophylactic treatment ■ Efficacy of *Human-cl rhFVIII* during treatment of bleeds ■ Efficacy of *Human-cl rhFVIII* in surgical prophylaxis ■ Safety and tolerability of *Human-cl rhFVIII*

Study endpoints

Primary endpoint: Immunogenicity
Inhibitor activity will be determined by the modified Bethesda assay (Nijmegen modification):

- 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

Secondary endpoints

Efficacy:

Efficacy of prophylactic treatment

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

Efficacy of treatment of bleeding episodes, as well as **efficacy during and post surgical prophylaxis** is assessed by using 4-point haemostatic efficacy scales with objective criteria.

Safety

- Vital signs measurements
- Safety laboratory parameters
- Tolerability
- Continuous documentation of AEs

Additional analyses

- Recovery investigation (optional)
- FVIII gene mutation analysis (mandatory)
- Immunogenotyping (optional)
- **RNA expression analysis (optional)**
  For understanding of the transcript activity of the genes involved in immune responses to FVIII inhibitor
- **In vitro immunogenicity of *Human-cl rhFVIII* (optional)**
  Assessed by culturing PBMC (including positive control) with *Human-cl rhFVIII*
- **Epitope mapping (optional)**
  In patients who developed FVIII inhibitor

Health economic parameters

- Resource use of patients:
  1. Treated with *Human-cl rhFVIII*
  2. Treated prophylactically or on-demand (if possible)
  3. With/without inhibitors (if possible)
- Parent questionnaire asking about time commitments and productivity loss.

Patient selection criteria

Due to the fact that PUPs are included, the patient population will mainly consist of new-borns or infants, but there is no general age limitation for study inclusion.

Figure 3. Inclusion / Exclusion criteria

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. Fully informed written and signed consent preceding any study-related procedures (from the patient's parent/legal guardian)
Exclusion criteria	1. Coagulation disorder other than Haemophilia A 2. Severe liver or kidney disease 3. Concomitant treatment with systemic immunosuppressive drug 4. Participation in another interventional clinical study

Statistical analysis

- The statistical analyses of the primary and secondary endpoints will be descriptive
- Two interim analyses:
  1. When 30 patients have started treatment
  2. After 50 patients have documented at least 50 EDs

Test product

Human-cl rhFVIII

- Freeze-dried concentrate to be reconstituted in 2.5 mL of water for injection
- Single-use vials containing a nominal potency of 250, 500, 1000 or 2000 IU, each
- Intravenous injection only (max. 4 mL/minute)

This novel *Human-cl rhFVIII*, may have a low immunogenic potential, as it is produced in human embryonic kidney (HEK) 293 F cells and consequently does not have any immunogenic epitopes compared to currently available recombinant products produced in hamster cells. In addition, the production process of *Human-cl rhFVIII* is completely free of added materials of human or animal origin, and any impurities are removed, which may further reduce the risk of hypersensitivity reactions.

Dose and mode of administration

- Prophylactic treatment is recommended
- The responsible physician determines the type of treatment for each patient
- Patients may switch between on-demand and prophylactic treatment during the study

Figure 4. Treatment options

Prophylactic treatment	■ Recommended dose: > 20 IU FVIII/kg body weight (BW) ■ Start prophylaxis with the first BE ■ Frequency of treatment depends on the patient's clinical situation ■ Frequency or dose adjustments at Investigator's discretion
Recovery investigation (optional):	■ 40 IU FVIII/kg BW for in vivo recovery evaluation ■ Blood samples taken at baseline, 15 min and 1 h post-dose
On-demand treatment	■ Minor haemorrhage: 20-30 IU FVIII/kg BW; repeat dose every 8-24 h until BE is resolved ■ Moderate to major haemorrhage: 30-40 IU FVIII/kg BW; repeat dose every 6-24 h until BE is resolved ■ Major to life-threatening haemorrhage: 50-80 IU FVIII/kg BW; repeat dose > 20 IU/kg BW every 6-12 hours until BE is resolved
Surgical prophylaxis	■ Minor surgeries including tooth extractions: 25-30 IU FVIII/kg BW within 3 h prior to surgery. Repeat every 12-24 h if needed. Trough levels to be maintained at ≥ 30% ■ Major surgeries: > 50 IU FVIII/kg BW within 3 h prior to surgery. Repeat if necessary after 6-12 h, for at least 6 to 14 days until healing is complete. Trough levels to be maintained at > 50%
Immune tolerance induction (ITI)	■ Patients with clinically significant, non-transient inhibitor may start ITI ■ Recommended: modified Bonn Protocol ■ Low responders (< 5 Bethesda Units [BU]): 50-100 IU FVIII/kg BW every 1-2 days ■ High responders (≥ 5 BU): 100-150 IU FVIII/kg BW every 12 h Once the inhibitor is eliminated (< 0.6 BU) and recovery and half-life are back to normal, the dose is continuously reduced to prophylactic level.

Results

- By April 2014, 29 PUPs were enrolled
- 21 PUPs have started treatment
- Out of ~45 selected study centres in 16 countries, 31 centres have been initiated

Summary

Human-cl rhFVIII is the first recombinant FVIII concentrate from a human cell-line, with a glycosylation pattern comparable to human plasma-derived FVIII and without immunogenic epitopes present in rFVIII produced in hamster cell lines.

The newly developed product may be less immunogenic in humans.

- After having completed five prospective GCP studies with *Human-cl rhFVIII* in more than 130 pre-treated children and adults with severe haemophilia A, the PUP study was initiated early 2013.
- No inhibitors and no related serious adverse events were detected in PTPs with severe haemophilia A in finalized clinical studies.
- A first interim analysis of the study will be conducted after 30 PUPs have started treatment.

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