



Department of Health and Human Services  
Public Health Service  
United States Food and Drug Administration  
Center for Biologics Evaluation and Research



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**Pharmacology / Toxicology Primary Discipline Review**

**To:** File (Original BLA 125555/0)

**From:** La’Nissa A. Brown, PhD, Pharmacologist, Division of Hematology Clinical Review (DHCR)/ Office of Blood Research and Review (OBRR)

**Through:** Anne M. Pilaro, PhD, Supervisory Toxicologist, DHCR/OBRR

**Subject:** STN 125555/0 – Octapharma’s Original Biological License Application (BLA) for NuwIQ® antihemophilic factor, human recombinant beta domain deleted (codename rhFVIII-BDD)

**Indication:** Treatment for the control and prevention of bleeding episodes, including during and after surgery, in adults and children with Hemophilia A

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This memorandum is the final primary review of the nonclinical program based on pharmacology/toxicology data included in the Original Biological License Application (BLA) for Octapharma’s NuwIQ® antihemophilic factor, human recombinant beta domain deleted (codename rhFVIII-BDD). NuwIQ is indicated for the control and prevention of bleeding episodes, including during and after surgery, in adults and children with Hemophilia A. From the toxicology and pharmacology reviewer’s perspective, this original biological application STN 125555/0 is recommended for approval.

**I. Background**

NuwIQ®, Human Cell Line Recombinant Human Factor VIII beta domain deleted (codename rhFVIII-BDD; human-cl rhFVIII) is a fourth generation, recombinant Factor VIII product manufactured by Octapharma. The Applicant’s proposed indication is for prophylaxis for the control and prevention of bleeding episodes and as surgical adjunct in adults and children with Hemophilia A. Human-cl rhFVIII was derived from a B-domain deleted active form of human FVIII in human cell line, HEK 293F. The manufacturing includes a solvent/detergent treatment step for virus inactivation and removal, and nanofiltration for a second viral inactivation step. The Applicant claims that expression of rFVIII in a human cell line may reduce immunogenicity, leading to improved safety and improved functionality as compared to NuwIQ’s predecessors.

Hemophilia A is a recessive sex-linked hereditary disease characterized by congenital FVIII deficiency; historical data demonstrate that FVIII replacement therapy is the most widely utilized and effective therapy. However adverse events do occur from repeated FVIII use including thromboembolic events, antibody formation and increased inhibitor titers. Nevertheless, the longstanding use and efficacy of FVIII therapy substantiate its usefulness in the treatment of Hemophilia A.

**Related Files: (if any) IND and/or STNs:** IND 13722/0

## II. Proposed Use and Doses

Nuwiq® will be administered intravenously to adults and children with Hemophilia A for the treatment and prevention of bleeding episodes, including during and after surgery. The dose will be determined by the treating physician based on each patient's FVIII levels after initial dosing, the severity of bleeding, and the patient's clinical condition.

Nuwiq is supplied as lyophilized powder, together with its solvent for preparation of a solution for injection. The lyophilized powder is available in single-dose vials containing 250 IU, 500 IU, 1000 IU or 2000 IU recombinant Factor VIII per vial, and is reconstituted with a single-dose solvent pre-filled syringe containing 2.5mL of sterile water for injection (sWFI) before use. Each mL of reconstituted solution contains nominally 100, 200, 400 and 800 IU of Factor VIII activity, respectively.

### Prophylaxis

FVIII products may be administered on a regular schedule for prophylaxis of bleeding. For long-term prophylaxis against bleeding in patients with severe Hemophilia A, the recommended dose is 30 to 40 IU of rhFVIII-BDD/kg every other day. In children, the recommended dose for a regular prophylaxis schedule is 30 to 40 IU of rhFVIII-BDD/kg every other day or 3 times per week. Exact dosing is defined by the patient's clinical status and their response as determined by the treating physician.

Subjects treated for prophylaxis prior to major surgery will receive a pre-dose of 80 to 100 IU rhFVIII-BDD/kg body weight (BW) followed by repeated doses up to 3 times a day, for a duration of at least 7, until healing is resolved. On-demand (i.e., hemorrhage) subjects will receive human-cl rhFVIII-BDD therapy every 8 to 24 hours, with the dose and dosing interval to be prescribed by their treating physician, based on the type of bleeding episode.

Dose levels and dosing regimens for Nuwiq® are outlined in the table below, as excerpted from Octapharma's Proposed Package Insert, 2014.

Degree of hemorrhage/ Type of surgical procedure	Required peak post- infusion FVIII activity (% of normal or IU/dL)	Frequency of doses (hours)/Duration of therapy (days)
<b>Hemorrhage</b>		
Early hemarthrosis, muscle bleeding or oral bleeding	20–40	Repeat infusion every 12 to 24 hours. At least 1 day, until the bleeding episode is resolved, as indicated by relief of pain, or healing is achieved.
More extensive hemarthrosis, muscle bleeding or hematoma	30–60	Repeat infusion every 12 to 24 hours for 3–4 days or more until bleeding episode is resolved (as indicated by relief of pain), or healing is achieved..
Life threatening hemorrhages	60–100	Repeat infusion every 8 to 24 hours until threat is resolved.
<b>Surgery</b>		
Minor (including tooth extraction)	30–60	Every 24 hours, at least 1 day, until healing is achieved.
Major	80–100 (pre- and post-operative)	Repeat infusion every 8 to 24 hours until adequate wound healing, then therapy for at least another 7 days to maintain a FVIII activity of 30% to 60% (IU/dL).

## III. Recommendations

Nonclinical studies to evaluate the general pharmacologic activity, pharmacokinetics, safety and toxicity of Nuwiq® for the proposed indication were included in the BLA submission. Based on review of the submitted pharmacology/toxicology data, this original biological application STN 125555/0 is recommended for approval. The clinical trials completed using Nuwiq further support the intended use of this product. There were no nonclinical deficiencies identified in this submission, and there are no

requests for any further nonclinical evaluations at this time. There are no outstanding issues from the nonclinical standpoint to prevent approval of this BLA.

#### **IV. Summary Basis for Regulatory Action (SBRA) for Nonclinical Nuwiq Data**

##### **Official Summary Basis for Regulatory Action (SBRA)**

#### **4. Non-clinical Pharmacology/Toxicology**

##### ***General Conclusions***

Nuwiq [Antihemophilic Factor (Recombinant), plasma/albumin free] was determined to be safe for its intended use as treatment for the control and prevention of bleeding episodes, including during and after surgery, in adults and children with Hemophilia A. This decision is based on nonclinical data demonstrating reasonable safety of rhFVIII-BDD from Good Laboratory Practices (GLP)-compliant and non-GLP studies, and on its clinical use both within and outside of the United States. The completed nonclinical program consisted of a series of studies to demonstrate the safety and effectiveness of rhFVIII-BDD in animals including hemophilic dogs, and wild-type FVIII expressing (b) (4) monkeys, rats, and rabbits.

##### ***Pharmacological/Toxicological Findings***

The Applicant (Octapharma Inc.) has completed an extensive nonclinical program to demonstrate the safety and effectiveness of Nuwiq [Antihemophilic factor, human (recombinant) beta domain deleted, or rhFVIII-BDD]. Based on data from GLP-compliant and non-GLP nonclinical studies, an adequate safety profile for rhFVIII-BDD has been established to support its intended use in the treatment of children and adult Hemophilia A patients for the control and prevention of bleeding episodes, including during and after surgery. Relevant animal models were employed to evaluate the pharmacologic activity and safety Nuwiq® including hemophilic dogs, and wild-type FVIII expressing (b) (4) monkeys, rats, and rabbits. To support the proposed clinical indications, the completed nonclinical program consisted of a series of studies to demonstrate the safety and effectiveness of rhFVIII-BDD in animals including: (a) safety pharmacology in hemophilia A dogs, (b) proof-of-principle in Hemophilia A dogs, (c) acute toxicity in rats and Hemophilia A dogs, (d) pharmacokinetics in monkeys and Hemophilia A dogs, (e) local tolerance in rabbits, (f) repeat dose toxicity, with toxicokinetics in rabbits and monkeys (g) and hemostatic activity of rhFVIII-BDD in Hemophilia A dogs.

Overall, the nonclinical safety profile of Nuwiq did not identify any unexpected findings or significant concerns; toxicities that were observed were due to exaggerated pharmacological effect of excess amounts of Coagulation Factor VIII, which are expected for products in this class. The single dose toxicity of rhFVIII-BDD was tested in animals at doses up to 10,000 IU/kg, (i.e., approximately 100 to 125 times the intended starting clinical dose of 80 to 100 IU/kg BW rhFVIII-BDD for peri-operative management), without unexpected adverse events. Repeat-dose toxicity studies were completed with daily dosing of up to 500 U/kg for up to 4 weeks (i.e., approximately 14 times the median intended prophylactic clinical dose of 35 IU/kg BW rhFVIII-BDD) and the product was generally well-tolerated. Animal study results supported results from clinical trials investigating safety and efficacy of rhFVIII-BDD prophylaxis regimens. In animal studies, the exaggerated pharmacological effects of rhFVIII-BDD that were considered adverse included thrombogenic events and local reactions at the treatment site, as well as hypersensitivity. These effects were reported after repeat dosing with rhFVIII-BDD doses approximately 8 to 25-fold greater (i.e., 500 IU/kg rhFVIII-BDD) than the proposed clinical dose of 20 to 60 IU/kg for use in the repeat dose setting. These findings were predictive for human use of the product, as confirmed by the adverse events reported in the clinical trial.

Toxicokinetic profiles demonstrated a linear dose-dependent increase in the levels of Factor VIII, followed by a time-dependent decrease in product levels. This profile was maintained in the test animals until anti-product antibody formation occurred, resulting in decreased recombinant rhFVIII-BDD activity. Although immunologic responses may occur in patients following repeated product administration and are a potential safety concern, the formation of anti-product antibodies in animals is not unexpected and is not predictive of an immunogenic response to rhFVIII-BDD in humans. There were no reports of neutralizing anti-Factor VIII antibodies or anaphylaxis in clinical trials with rhFVIII-BDD.

Based on the intended use of rhFVIII-BDD, nonclinical reproductive or developmental toxicity studies, long-term animal studies to evaluate carcinogenic potential, and studies to determine genotoxicity and the effects of Nuwiq® on fertility were neither required according to ICH guidelines nor performed. A toxicological risk assessment was completed on potential extractable and leachable impurities associated with the Nuwiq® manufacturing process and container closure system. There were no concerns identified regarding these impurities, nor unexpected toxic effects that would require additional safety studies.

**Recommendation:** The results from the nonclinical program suggested that treatment of patients with Hemophilia A with Nuwiq will be reasonably safe for use for the labeled clinical indications. The Pharmacology/Toxicology Reviewer, La’Nissa A. Brown PhD, recommends that the Biological License Application (BLA) 125555/0 for Nuwiq be approved, based on the results from both the toxicological risk assessment, and the data from the nonclinical studies conducted by the Applicant.

#### **IV. Nonclinical Labeling for the Package Insert (PI) for STN 125555/0**

The label was revised to reflect current labeling guidelines and the relevant information for prescribing data based on nonclinical and clinical experience using Nuwiq™

##### **Clean Revised Version of Label for Nonclinical**

#### **8.1 Pregnancy**

Pregnancy Category C. Animal reproduction studies have not been conducted with Nuwiq. It is also not known whether Nuwiq can cause fetal harm when administered to a pregnant woman or can affect reproduction capacity. Nuwiq should be given to a pregnant woman only if clearly needed.

#### **8.3 Nursing Mothers**

It is not known whether Nuwiq is excreted in human milk. Because many drugs are excreted in human milk, caution should be exercised when Nuwiq is administered in a nursing woman.

#### **13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility**

Long-term studies in animals to evaluate the carcinogenic potential of Nuwiq or studies to determine the effects of Nuwiq on genotoxicity or fertility have not been performed.

##### **FDA Revisions to Applicant’s Label**

#### **Applicant’s Language (Section edited):**

#### **8.1 Pregnancy**

Animal reproduction studies have not been conducted with FVIII. Based on the rare occurrence of hemophilia A in women, experience regarding the use of FVIII during pregnancy and breast-feeding is not available. Therefore FVIII should be used during pregnancy and lactation only if clearly indicated.

**FDA Revision:** Section 8.1 was modified to reflect labeling guidelines as per 21 CFR 201.57.

### **8.1 Pregnancy**

Pregnancy Category C. Animal reproduction studies have not been conducted with Nuwiq. It is also not known whether Nuwiq can cause fetal harm when administered to a pregnant woman or can affect reproduction capacity. Nuwiq should be given to a pregnant woman only if clearly needed.

**Justification:** Revised the language to be consistent with that provided in the CFR to describe the Pregnancy Category C designation for Nuwiq.

**Applicant's Language (Section edited):**

### **8.3 Nursing Mothers**

Nuwiq<sup>®</sup> has not been studied in lactating women. It is not known whether this drug is excreted into human milk. Because many drugs are excreted into human milk, caution should be exercised when Nuwiq<sup>®</sup> is administered to a nursing mother. Prescribe Nuwiq<sup>®</sup> only if clinically needed.

**FDA Revision: Section 8.3**

### **8.3 Nursing Mothers**

It is not known whether Nuwiq is excreted in human milk. Because many drugs are excreted in human milk, caution should be exercised when Nuwiq is administered in a nursing woman.

**Justification:** Revised the language to be consistent with that provided in the CFR to describe the risks of use of Nuwiq in nursing mothers.

**Applicant's Language (Removed the entire Section 13, below):**

## **13 NON-CLINICAL TOXICOLOGY**

Preclinical studies evaluating Nuwiq<sup>®</sup> with rats, dogs, and non-human primates demonstrated efficacy and safety of the product.

**FDA Revision: Section 13 removed**

**FDA Revision:** Language immediately related to nonclinical data under the header for Section 13 was removed.

**Justification:** Removed nonclinical data in Section 13 due to redundancy with the clinical findings. The pharmacodynamic findings in animals are not essential for describing the clinical pharmacology; the Nuwiq product was evaluated for pharmacodynamic activity in clinical trials, and the results and safety profile are appropriately described in the clinical sections of the label.

**Applicant's Language (Section edited):**

## **13. Carcinogenesis, Mutagenesis, Impairment of Fertility**

Genotoxicity studies and carcinogenicity studies are not applicable for recombinant products. Long-term investigations cannot be performed due to the immune response to heterologous proteins in all non-human mammalian species. Therefore, studies to determine the effects of Nuwiq<sup>®</sup> on genotoxicity, carcinogenicity or fertility have not been performed.

**FDA Revision: Section 13.1****13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility**

Long-term studies in animals to evaluate the carcinogenic potential of Nuwiq or studies to determine the effects of Nuwiq on genotoxicity or fertility have not been performed.

**Justification:** Section 13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility section was edited to convey important information that was omitted by the Applicant (i.e., an assessment of carcinogenic risk was performed, although *in vivo* animal carcinogenicity testing was not conducted), and needed to be added to the label.

**V. General Comments**Report Findings and Conclusions

- Studies presented compared human-cl-rhFVIII versus ReFacto® or Amofil® (marketed plasma-derived FVIII products) for efficacy, pharmacokinetics, safety pharmacology, and repeat dose toxicity for *in vivo* studies. The remaining studies were for human-cl rhFVIII acute toxicity, dose-range finding, and local tolerance. Based on comparative analysis, human-cl rhFVIII displays similar pharmacokinetics, local tolerance, hemostatic efficacy, immunological, toxicological and safety pharmacological properties as its comparators, Amofil® and ReFacto®.
- No genotoxicity or carcinogenicity studies were completed as product is a recombinant derivative of human FVIII.
- No separate safety pharmacology studies were complete for AEs for human-cl rhFVIII analysis.
- Human-cl rhFVIII was well tolerated and exhibited similar efficacy as ReFacto in cross-over study in hemophilia A canine model. No statistical analysis were complete (n=2).
- A preliminary dose response finding study was done in (b) (4) monkeys while acute toxicity was simultaneously assessed. It appears that the proposed human dose of 50 IU/kg human-cl rhFVIII did not elicit pharmacological response or have any major effects in monkeys; while a 10-fold increase of maximal dose of 500 IU/kg human-cl rhFVIII did not cause significant acute toxicity. Immunological changes were observed including increased aPTT, FVIII:C inhibition, anti-FVIII inhibitors, clotting time with decreased FVIII activity.
- A 28-day repeat dose toxicity study with 2-week recovery phase in (b) (4) monkeys revealed that 500 IU/kg BW human-cl rhFVIII does cause alterations associated with FVIII treatment including increased FVIII antibodies formation, inhibitors, and clotting time comparable to 500 IU/kg BW Amofil. There were also significant alterations in clinical conditions and hematology for 500 IU/kg BW human-cl rhFVIII also similar to comparator. At dose of 50 IU/kg BW human-cl rhFVIII, the same afore mentioned observable changes occurred although to a lesser extent and not significantly.
- Human-cl rhFVIII appears to be well tolerated in acute toxicity study in rats which was indicated considerably above proposed human dose (200x higher). Even at the maximum dose of 10,000 IU/kg, few adverse effects occurred in parameters examined. It appears that human-cl rhFVIII will be safe in humans as prophylaxis based on results.
- Human-cl rhFVIII was well tolerated in rabbits (n=4) in local tolerance model at single dose of 200 IU/kg vs. vehicle (formulation buffer) over four days. There were no unfavorable clinical conditions observed.
- The proposed human dose of 50 IU/kg human-cl rhFVIII appears to be well tolerated, and not did elicit toxic responses in tested parameters for all animal models.
- A 10-fold increase of the maximal dose of 500 IU/kg human-cl rhFVIII demonstrated minimal toxicity in all parameters examined and was comparable to Amofil, a marketed FVIII product, in all animal models tested.
- Human-cl rhFVIII appears to be safe for repeat use in humans based on all animal models tested.

## VI. List of Nonclinical Studies in STN BLA 125555/0

- Study Report OCT102007- Hemophilia Dog Study - Crossover Study of the Efficacy and Pharmacokinetics of a Novel B-domain-deleted Recombinant Coagulation Factor VIII Concentrate in a Canine Model of Hemophilia A
- Study Report OC11-0200 Pharmacokinetics of Human cl-rhFVIII in Hemophilia Dogs
- Study Report DWL 0001/063743 – Recombinant Human Factor VIII (rhFVIII) Preliminary Toxicity Study by Intravenous Administration to (b) (4) s Monkeys
- Study Report DWL 0002/064067 - Recombinant Human Factor VIII (rhFVIII) Toxicity Study by Intravenous Administration to (b) (4) Monkeys for 4 Weeks Followed by a 2 Weeks Recovery Period
- Study Report DWL 0003/063496 – Recombinant Human Factor VIII (rhFVIII) Toxicity Study by Intravenous Administration to CD Rats
- Study Report DWL 004/073723 – Recombinant Human Factor VIII (rhFVIII) Local Tolerance Study in the Rabbit Following Perivenous Injection

## VII. Summary of Nonclinical Studies in STN BLA 125555/0

In summary:

PEL (pharmacologically effective level) = 25 IU/kg

tSF (tentative safety factor) = approximately 10-fold over the NOAEL for the prophylaxis regimen proposed, using the clinical dose of 50 IU/kg Nuwiq

NOAEL = 500 IU/kg for repeat dose regimen and acute dosing regimen

### *Safety Pharmacology/Primary Pharmacodynamics*

**Study Report OCT102007 and Study Report OC11-0200 - Hemophilia Dog Study - Crossover Study of the Efficacy and Pharmacokinetics of a Novel B-domain-deleted Recombinant Coagulation Factor VIII Concentrate in a Canine Model of Hemophilia A (Pharmacokinetics of Human cl-rhFVIII in Hemophilia Dogs)**

*These studies were reviewed together. Both Study reports were of the same original study to include separate data sets.*

The **aim** of study was to evaluate pharmacokinetics and hemostatic efficacy to compare safety of human-cl rhFVIII versus ReFacto (comparable recombinant FVIII marketed product) after i.v. (125 IU FVIII:C/kg) injection in Hemophilia A canine cross-over model (n=2). Parameters were cuticle bleeding tests, whole blood clotting times, aPTT measurements, clinical observations (daily), hematology (complete blood count) and biochemistry panel (serum chemistries), and functional FVIII levels (1-stage clotting assay and chromogenic assay). **Results:** There were no pronounced differences in human-cl rhFVIII as compared to ReFacto in all of the parameters tested including hematologic or biochemical toxicities. Bleeding times were reduced and plasma FVIII levels were increased with decreased aPTT and whole blot clot times for both human-cl rhFVIII and ReFacto, although changes were not significant. The  $t_{1/2}$  was ~7.7 hrs for human-cl rhFVIII vs. ~ 9 hrs for ReFacto, and clearance at 5.65 mg/kg/L for human-cl rhFVIII vs. 4.65 mg/kg/L for ReFacto showing all data within an acceptable range. Based on these

data, the hemostatic efficacy and safety of human-cl rhFVIII is comparable to ReFacto in the study. It is important to note that one test subject; “Noah” had significant weight change, a single positive anti-human FVIII inhibitor test, and incurred reversible unfavorable reactions (adverse cardiovascular reactions) in crossover use associated with post-ReFacto treatment. **Conclusion:** Overall, human-cl rhFVIII showed favorable safety pharmacology, pharmacokinetics, and efficacy is comparable to ReFacto in hemophilia A canine model. This study was completed June 2007 at Queen’s University Kingston, Ontario, Canada non-GLP compliant. The study was not conducted under GLP compliance due to study model feasibility.

**Reviewer Comment:** The sample size may be too small and treatment time course administration varied cancelling true comparison of products for safety for human-cl rhFVIII.

### ***Pharmacokinetics***

#### **Study Report DWL 0001/063743 – Recombinant Human Factor VIII (rhFVIII) Preliminary Toxicity Study by Intravenous Administration to (b) (4) Monkeys**

The **purpose** of the first portion of this study was to assess the pharmacokinetics and *in vivo* exposure of human-cl rhFVIII following bolus i.v. administration in (b) (4) monkeys (n= 4, 2 groups of 1M/1F per group). These data were analyzed upon performance of preliminary dose-range finding toxicity study by measuring several endpoints. The parameters measured were for FVIII: C by chromogenic assay and Coatest SP parallel-line assay and ADME for pharmacokinetics.

**Dose:** The dose administered i.v were 50 IU/kg for group one; or 500 IU/kg, then 1500 IU/kg group two in 3mL/day of (vehicle) formulation buffer, respectively.

**Results and conclusion:** The results of pharmacokinetic parameters tested stated human-cl rhFVIII were similar to those of ReFacto including distribution, metabolism, and absorption (ADME) rates. The values for FVIII:C were similar for human-cl rhFVIII and ReFacto. Overall, this study claimed the pharmacokinetics were similar for ReFacto and rhFVIII-BDD with no significant changes noted. This study was completed May 2008 at (b) (4) under Good Laboratory Practices (GLP) compliance and (b) (4) GLP guidelines.

### ***Toxicology***

#### **Study Report DWL 0003/063496 – Recombinant Human Factor VIII (rhFVIII) Toxicity Study by Intravenous Administration to CD Rats**

The **objective** of this study was to assess acute toxicity and establish lethal and non-lethal doses of human-cl rhFVIII (preliminary 2,500 IU/kg BW for n=2 with no toxicity; thereafter 10,000 IU/kg BW in main study) after i.v. administration in randomized rats (n=10, 5M/5F). Endpoints for toxicity were body weight (days 0, 8, and 15), death, unusual behavior or physiological changes (clinical conditions) and macroscopic pathology by necropsy (post mortem) daily for 14 days post-dose.

**Results:** There were no significant differences in body weight, macroscopic alterations, deaths or other toxicity endpoints examined. To note, two treatment animals displayed abnormal necropsies of a pale liver (1 male) and congestion of spleen and pale kidneys (1 female).

**Conclusion:** The study dose for human-cl rhFVIII appears to be well tolerated considerably above proposed dose range in humans. This study was completed September 2006 at (b) (4) under Good Laboratory Practices (GLP) compliance and (b) (4) GLP guidelines.

**Reviewer Comments:** Based on the tests completed, a lethal dose was not determined and a dose range finding was not achieved or a rationale for doses selected in study given. This study does not constitute acute toxicity findings, although it does support safety of proposed dose of human-cl rhFVIII in humans (500x proposed dose).

#### **Study Report DWL 0001/063743 – Recombinant Human Factor VIII (rhFVIII) Preliminary Toxicity Study by Intravenous Administration to (b) (4) Monkeys**



The **aim** of this study was to determine dose range findings for human-cl rhFVIII following bolus intravenous administration in (b) (4) monkeys (n=4, 2 groups of 1M/1F). This preliminary study used several measured parameters for toxicity testing including:

- Clinical observations or clinical conditions (daily; and immediate pre-and post-dosing)
- Mortality (twice daily)
- Anti-FVIII antibodies (b) (4)
- Body weight (BW) (days 0, 8, and 15)
- Clotting Function by activated thromboplastin time [aPTT] (days 1, 18 and 25; group 2 additional days 35, 49 and 56)
- Blood chemistry (hematology panel)
- FVIII activity (chromogenic assay)
- FVIII:C inhibition (modified Bethesda Assay)
- Necropsy (Macroscopic pathology, tissue fixation, etc.)
- Organ weight (post-mortem)

**Dose:** Administered i.v bolus, the dosage of human-cl rhFVIII was for the maximal clinical dose of 50 IU/kg BW for group one; or 10-fold increase of the maximal dose of 500 IU/kg BW, then additionally 1500 IU/kg BW for group two. There were 10 doses on days 1-7, 11, 14, and 21; 7 additional doses for group 2 on days 29, 31, 33, 35, 37, 39 and 41.

**Results and Conclusion:** Human-cl rhFVIII was well tolerated with no mortalities; and no pronounced immunological responses, any necropsy parameters, or treatment-related effects were recorded. The 500/1500 IU/kg BW (group 2) human-cl rhFVIII group exhibited prolonged clotting time, slightly low neutrophils and slightly high lymphocyte, increased aPTT, FVIII:C inhibition, anti-FVIII inhibitors with decreased FVIII activity. Similar results were observed in the 50 IU/kg IU/kg BW human-cl rhFVIII (low dose) group for FVIII:C inhibitor levels, FVIII levels, and anti-FVIII antibodies to a lesser extent.

**Outcome:** These observed responses following human-cl rhFVIII administration were dose-related and expected because of product species cross reactivity, and observations were comparable to approved rFVIII product responses. This study was completed May 2008 at (b) (4) under Good Laboratory Practices (GLP) compliance and (b) (4) GLP guidelines.

#### **Study Report DWL 0002/064067- Recombinant Human Factor VIII (rhFVIII) Toxicity Study by Intravenous Administration to (b) (4) Monkeys for 4 Weeks Followed by a 2 Weeks Recovery Period**

The **purpose** of these studies was to determine repeat dose toxicity for 28 days after dose range finding study completion following bolus i.v. administration of human-cl rhFVIII in monkeys (n=36, 7 groups in two cohorts; see tables below). Monkeys were also observed for a 2-week recovery phase after 4 week treatment.

**Dose:** Administered bolus i.v daily, the dosage and groups are specified on table below:

Group	Treatment	Dose group (mg/kg/day)	Days of use	Male	Female
1	Control (vehicle)	0	28	3	3
2	Human-cl rhFVIII	50	28	3	3
3	Human-cl rhFVIII	500	28	3	3
4	Amofil	500	28	3	3

An additional cohort of animals (n=12 in 3 groups; 2F/2M per group) was utilized to determine recovery of immunologic functions. Animals were examined for recovery phase two weeks after 28-day treatment regimen as follows:

Group	Treatment	Dose group (IU/kg/day)	Male	Female
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1	Control (vehicle)	0	2	2
2	Human-cl rhFVIII	500	2	2
3	Amofil	500	2	2

Numerous endpoints and parameters were examined including:

- Clinical observations (daily and immediate pre-and post-dosing)

-Anti-FVIII antibodies (b) (4)

- Body weight

-Organ weight

-Hematology (peripheral blood)

Hematocrit (Hct)

Hemoglobin concentration (Hb)

Erythrocyte count (RBC)

Reticulocyte count (Retic)

Mean cell hemoglobin (MCH)

Mean cell haemoglobin concentration (MCHC)

Mean cell volume (MCV)

Total white cell count (WBC)

Differential WBC count

Neutrophils (N)

Lymphocytes (L)

Eosinophils (E)

Basophils (B)

Monocytes (M)

Large unstained cells (LUC)

Platelet count (Plt)

- Blood Chemistry

Alkaline phosphatase (ALP)

Alanine aminotransferase (ALT)

Aspartate aminotransferase (AST)

Total bilirubin (Bili)

Urea

Creatinine (Creat)

Glucose (Gluc)

Total cholesterol (Chol)

Total protein (Total Prot.)

Albumin (Alb)

Sodium (Na)

Potassium (K)

Chloride (Cl)

Calcium (Ca)

Inorganic phosphorus (Phos)

Albumin/Globulin ratio (A/G ratio)

-Electrocardiography

-Ophthalmoscopy

-Urinalysis

-Clotting Function by activated thromboplastin time [aPTT] (days 1, 18 and 25; group 2 additional days 35, 49 and 56)

- FVIII activity (chromogenic assay)

- FVIII inhibition (Nijmegen Assay)
- Necropsy (Macroscopic pathology, histology, organ weight)
- Cardiovascular parameters
- Immunogenicity (anaphylactoid reactions) versus Amofil (comparator)
- Histopathology Analysis

### Results:

- There were no significant effects in body weight, clinical pathology, ophthalmoscopy, electrocardiography, or organ weight associated with human-cl rhFVIII treatment.
- Human-cl rhFVIII does exhibit significant differences compared to its comparators and dose-related responses were minimal in all parameters examined.
- The 500 IU/kg BW human-cl rhFVIII group exhibited prolonged clotting time, one euthanized mortality from internal blood loss (1F/Day 30), lower urine pH, bruising, and increased aPTT, anti FVIII antibodies and anti-FVIII inhibitors compared to controls (Amofil and vehicle). There were also changes in 500 IU/kg BW human-cl rhFVIII compared to comparator or control in blood chemistry and hematologic parameters including increased reticulocytes and bilirubin and decreased hematocrit, hemoglobin, and RBCs.
- The 500 IU/kg BW human-cl rhFVIII group had incidences of subcutaneous hemorrhage (n=1/F), pallor livers (n=2/F), skeletal muscle hemorrhage (n=2, 1F/1M), and subcutaneous swelling (n=1/M). These adverse effects were closely correlated with highest antibody concentrations exhibited in animals.
- Human-cl rhFVIII 500 IU/kg BW induces higher detectable levels of anti-FVIII inhibitors than Amofil 500 IU/kg BW, but with less incidences (n= 1/4 vs. 2/4) of induction suggesting human-cl rhFVIII may be better tolerated immunologically than its comparator, Amofil.
- Intravenous administration of recombinant human Factor VIII (human-cl rhFVIII) to (b) (4) monkeys was clinically well tolerated, but resulted in decreased factor VIII activity and inhibition against human-cl rhFVIII and endogenous factor VIII due to the generation of anti-factor VIII antibodies.
- Human-cl rhFVIII appears to be a more potent FVIII product than previously approved FVIII comparator products including Amofil based on levels of FVIII activity; anti-FVIII antibody titres; inhibitor antibody levels (see table below); longer recovery period required; lower RBCs, hemoglobin and hemocrit; and higher bilirubin.

*Inhibitor antibody levels (mean BU/ml in affected animal, frequency of affected animals and range of BU/ml in affected animals) in monkey plasma at pre-treatment during 28-day treatment and after the 14-days recovery period.*

Product and Dose FVIII:C	Pre-treatment	Day 1	Day 13	Day 21	Day 29	Recovery Day 14
Human-cl rhFVIII 50 IU/kg/day	No inhibitors	No tested	Not tested	No tested	No inhibitors	N/A
Human-cl rhFVIII 500 IU/kg/day	No inhibitors	Not tested	9.75  (4 of 10, 2-23)	34.75  (4 of 10, 23-52)	78.5  (6 of 10, 2- 157)	17.75  (4 of 4, 2-54)

Amofil 500 IU/kg/day	No inhibitors	Not tested	4.5 (8 of 10, 2-9)	10 (6 of 10, 2-21)	19.6 (8 of 10, 3- 55)	12.7 (3 of 4, 4-29)
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**Conclusion:** Based on immunological and clinical results along with high tolerance at all levels, both low and high dose groups were acceptable for repeat dose toxicity study to assess safety of human-cl rhFVIII as compared Amofil. This study was completed May 2008 at (b) (4) under Good Laboratory Practices (GLP) compliance and (b) (4) GLP guidelines.

**Study Report DWL 004/073723 – Recombinant Human Factor VIII (rhFVIII) Local Tolerance Study in the Rabbit Following Perivenous Injection**

The **purpose** of these studies is to evaluate the extent of local tolerance reactions in (b) (4) rabbits (n=4) by perivenous injection acute administration of human-cl rhFVIII. Endpoints for local tolerance included body weight (days 1 and 5), macroscopic pathology and histopathology (necropsy), dermal irritations (local reactions), death/moribund (twice daily), unusual behavior or physiological changes (clinical conditions) or toxicity daily. Injections sites were assessed and scored based on oedema, erythema or eschar formation.

**Dose:** Each rabbit (n=4) received single perivenous injection 200 IU/mL to lateral vein of left ear at dose 0.2 mL in clinical formulation buffer or only clinical formulation buffer (vehicle) at dose of 0.2 to right lateral ear vein.

**Results and conclusion:** There appeared to be good tolerance for single administration of human-cl rhFVIII with no dermal irritation, body weight changes, no mortality, or adverse clinical signs from treatment. There were no macroscopic or microscopic abnormalities or treatment related reactions observed associated with human-cl rhFVIII administration. To note, a small number changes seen were considered related to route of administration. This study was completed February 2008 at (b) (4) under Good Laboratory Practices (GLP) compliance and (b) (4) GLP guidelines.