Preclinical Safety of Baxter's Recombinant Factor VIIa

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Introduction

Baxter is developing a recombinant FVIIa (rFVIIa) product for the potential treatment of patients with hemophilia A or B who have inhibitors. Baxter's new rFVIIa is produced by a genetically engineered Chinese hamster ovary (CHO) cell line in a cell culture medium free from any animal or human proteins. The objective of this preclinical study program was to evaluate the safety of Baxter's rFVIIa in different species.

Methods

All animal experiments accorded with either Austrian or UK laws governing animal experimentation and were additionally approved by the Institutional Animal Care and Use Committee (IACUC)

OVERVIEW PRECLINICAL SAFETY STUDIES

Dose : Baxter's rFVIIa mg/kg BW *	Animals/ Group	Study Duration	Endpoints
Hypotensive Anaphylactoid Reaction in Rats			
1.2	6 male	single administration	decrease in mean arterial blood pressure greater than 30% from baseline
Bronchospastic Anaphylactoid Reaction in Guinea Pigs			
1.2	6 male	single administration	increase in pulmonary inflation pressure ≥ 30% from baseline lasting for ≥ 1 minute
Whole Body Bias Flow Plethysmogram in Rats			
0.12, 0.6, 1.2	8 male	single administration	respiratory variables
Thrombogenic Potential (Wessler Test) in Normal Rabbits			
0.3, 0.6, 0.9, 1.2	3 male/ 3 female	single administration	thrombus formation in isolated jugular vein segments
Cardiovascular Effects (Telemetry) in Cynomolgus Monkeys			
0.12 ,1.2	4 male/ 4 female	single administration	arterial blood pressure (systolic, diastolic and mean), heart rate and lead II ECG (PR, QRS, QT and QTcR) intervals and morphology
Single Dose Toxicity in FVIII Knock Out Mice			
1.2, 2.7, 6.0,12	5 male/ 5 female	single administration, necropsy after 1 and 14 days	clinical condition, bodyweight, hematocrit, platelet count, macro- and histopathology
Single Dose Toxicity in Rats			
1.2, 2.7, 5.43	5 male/ 5 female	single administration, necropsy after 1 and 14 days	clinical condition, bodyweight, food consumption, hematology, blood chemistry, organ weight, macro- and histopathology
Single Dose Toxicity in Cynomolgus Monkeys			
1.2, 2.7, 6.5	2 male/ 2 female	single administration, observation period 14 days	clinical condition, bodyweight, food consumption, hematology, blood chemistry, toxicokinetics, organ weight, macro- and histopathology
Repeated Dose Toxicity including TK in Rats			
1.2, 2.7	10 male/ 10 female	14 daily doses, 2 week's recovery	clinical condition, bodyweight, food consumption, ophthalmic examination, hematology, blood chemistry, toxicokinetic, antibody assay, urinalysis, organ weight, sperm analysis, macro- and histopathology
Repeated Dose Toxicity Including TK in Cynomolgus Monkeys			
0.09, 2.7	3 male/ 3 female	one month (dosing every other day), 2 week's recovery	clinical condition, bodyweight, food consumption, ophthalmic examination, electrocardiography, hematology, blood chemistry, thrombogenic markers, toxicokinetics, binding and neutralizing antibodies, urinalysis , organ weight, macro- and histopathology
Local Tolerance in Rabbits			
5mL (IV or IA) or 0.5 mL (PV)	2 male / 2 female	single administration	macroscopic and histopathological examination of injection sites
Immunogenicity in E17 HLA-DR15 Mice			
40, 100, 400 µg/kg	10 male	8 weekly doses	development of antibodies against human rFVIIa, development of antibodies against CHO protein
Immunogenicity in Mice (immunologically tolerant to human FVII)			
400 µg/kg	5 male/ 5 female	8 weekly doses	development of antibodies against human rFVIIa
Immunogenicity in Mice (immunologically tolerant to human FVII)			
100, 400 µg/kg	5 male/ 5 female	8 weekly doses	development of antibodies against human rFVIIa
Immunogenicity in Wildtype Mice			
100, 400 µg/kg	10 male	8 weekly doses	development of antibodies against human rFVIIa

*** commercially available rFVIIa was used as the comparator product**

Summary and Conclusions

- Studies on safety pharmacology with Baxter's new rFVIIa revealed an excellent safety profile
- High doses of Baxter's new rFVIIa and licensed rFVIIa resulted in exaggerated pharmacological effects in rodents, which are well- known effects for this class of compound
- Repeated dose toxicity studies in cynomolgus monkeys did not reveal any adverse effect
- **Our studies on the safety profile of Baxter's new rFVIIa provide the evidence necessary for proceeding with human trials**

Results: Toxicology

Single Dose Toxicity Studies

Single application of Baxter's new rFVIIa did not result in any systemic toxicity in rats and cynomolgus monkeys

- **NOAEL for rat : 5.43 mg/kg**
- **NOAEL for cynomolgus monkey: 6.5 mg/kg**

Mice treated with high doses of either Baxter's new rFVIIa or licensed rFVIIa showed signs of exaggerated pharmacological effects including thrombus formation in the heart, lungs, and kidneys

- **NOAEL for FVIII knock-out mice: 2.7 mg/kg**

Repeated Dose Toxicity studies

Treatment of rats with normal hemostasis with either Baxter's new rFVIIa or licensed rFVIIa did result in the formation of some thrombi detected at microscopic pathology examination. Baxter's new rFVIIa caused no mortalities, the clinical condition of the animals remained good throughout the study, and thrombi formation did not cause damage to the affected tissues

- **NOAEL for rat following 14 days of treatment: 2.7 mg/kg/day**

Treatment of cynomolgus monkeys with Baxter's new rFVIIa for 4 weeks did not result in any evidence of adverse effects. As expected, the pharmacological action of both Baxter's new rFVIIa and licensed rFVIIa resulted in changes to clotting function. The magnitude of these effects was similar for both rFVIIa products and was not of a level that was considered adverse

- **NOAEL for cynomolgus monkey after 1 month of treatment: 2.7 mg/kg**

Local Tolerance

- Baxter's new rFVIIa was well tolerated

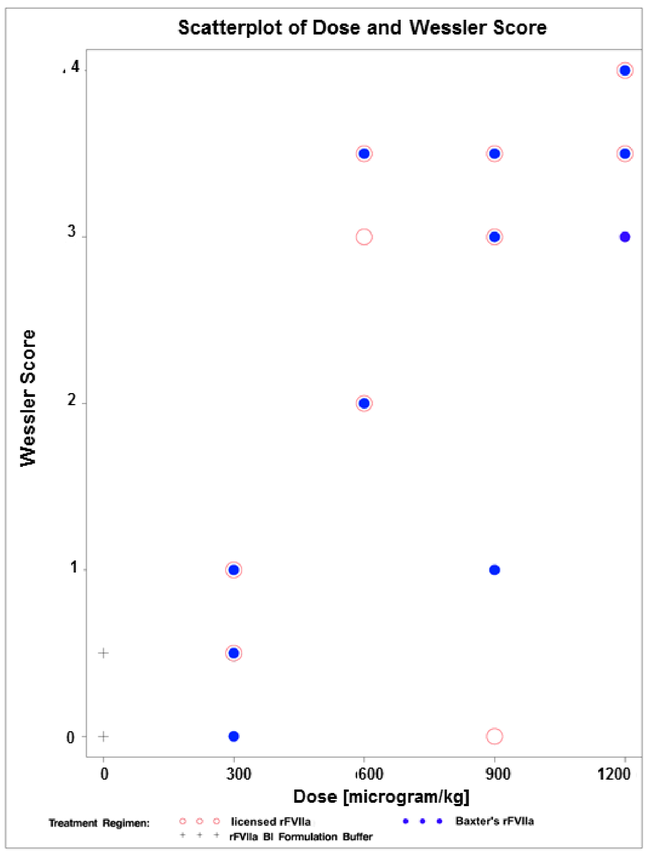
Immunogenicity

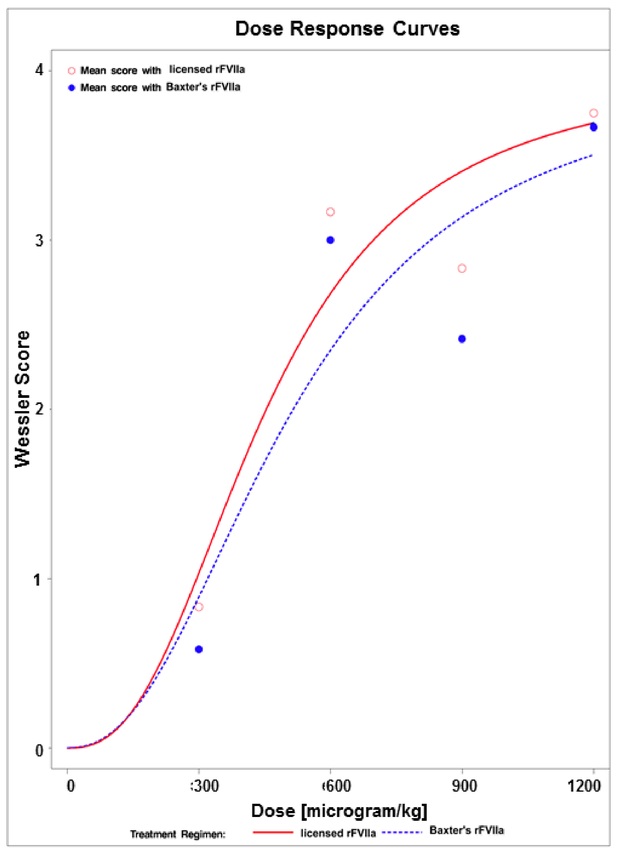
- Comparative immunogenicity studies demonstrate that Baxter's new rFVIIa and licensed rFVIIa have a similar immunogenicity profile

Results: Safety Pharmacology

- None of the animals had a positive hypotensive anaphylactoid reaction
- None of the animals had a positive bronchospastic anaphylactoid reaction
- No physiologically relevant effect on any of the respiratory variables
- No adverse effect on any of the cardiovascular variables measured
- **Thrombogenicity of Baxter's new rFVIIa in Wessler stasis model in rabbits with normal hemostasis is similar to that of licensed rFVIIa**

Comparative evaluation of thrombogenicity of Baxter's new rFVIIa in a Wessler stasis model with licensed rFVIIa





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Barbara Dietrich, Susan Kubik, Wilfried Auer, Birgit Reipert, Frank, Horling, Hartmut Ehrlich, Friedrich Scheiflinger, Hans Peter Schwarz and Eva-Maria Muchitsch are full-time employees of Baxter Innovations GmbH, Vienna, Austria.

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