

The New and Improved (?) Activated Factor VII Molecules

The Clinical Development Challenges of Recently Announced Modified and Biosimilar rFVIIas

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I. ABSTRACT

A number of new recombinant activated Factor VII (rFVIIa) molecules are currently in various phases of development. Some of these molecules have been structurally modified from the original rFVIIa (NovoSeven®), to increase their platelet surface-dependent activity or their half-life (or both) and some are biosimilars that possess no specific enhancements. These molecules are being developed at a time when rFVIIa efficacy in hemophilia has substantially improved by the introduction of higher dosing, and the hemostatic role of rFVIIa in non-hemophilia patients is seriously challenged by the results of various clinical studies.

In the past two decades, the original rFVIIa has been tested in hemophilia A and B complicated by inhibitors, in surgical situations with anticipated substantial blood loss, in acute intracerebral hemorrhage, in blunt and penetrating trauma and in a variety of other situations associated with excessive bleeding.

In hemophilia, the originally approved dosing of rFVIIa led to less-than-optimal hemostasis. Recent studies with substantially higher doses showed that these were both safe and highly effective, providing effective hemostasis with a single infusion in >90% of cases. If there is a scope for improvement in hemophilia care by these new rFVIIa analogs, it must be established with clinically meaningful endpoints and appropriate sample sizes. Certain possibilities are discussed in this review. In addition, safety must at least meet if not exceed that of rFVIIa. An extensive safety record with the original rFVIIa (NovoSeven®) is now available, a record that the newer molecules will require a substantial amount of time to challenge effectively.

In surgeries with anticipated substantial blood loss and high transfusion requirements, the issues are more complex. Various studies with rFVIIa failed to establish a clinical benefit. However, most of these studies have been exploratory in nature utilizing aggressive assumptions. With improvements in surgical techniques and extensive use of antifibrinolytic agents, it was not surprising that these aggressive assumptions were not been met in these clinical studies. Any new development effort in this indication (prophylactic use in major surgery) may have to assess these assumptions; carefully consider the enrollment criteria in order to include patients more likely to experience substantial blood loss; and utilize more sensitive and more statistically efficient endpoints. Certain suggestions towards these improvements are included in this review.

In acute intracerebral hemorrhage (ICH), there is likely little scope for further development. The proposition that hematoma growth after presentation is connected to poor outcome and higher mortality remains unproven and available opinion in support of this proposition is substantially skewed by bias. Furthermore, decrease in hematoma growth can be achieved more efficiently by other methodologies. In addition, there are a large number of technical issues in development in this indication that make further development very risky. These include insensitive endpoints and substantial difficulties in balancing randomized groups in anything else than very large studies. The development program by Novo Nordisk has failed to identify a clinical benefit due to a combination of the problems mentioned above.

In trauma, the situation is as ambivalent as in major surgery. Clinical trials have failed to establish a role for rFVIIa and the latest pivotal clinical trial in this indication (CONTROL) has been terminated early for futility. However, recent studies on trauma coagulopathy may point the way forward. These studies have indicated that trauma coagulopathy has an independent and substantial effect on mortality. Unfortunately, the rFVIIa studies in trauma performed thus far did not limit enrollment to patients with strong evidence of coagulopathy. Nor have the patients been evaluated for the potential requirement of massive transfusion. Future efforts may have to incorporate more stringent enrollment criteria in order to be successful.

In summary, the challenges remain substantial in establishing a role for rFVIIa in a variety of clinical situations in which adequate control of hemostasis is important.

Obviously, these challenges have not deterred the introduction of new rFVIIa molecules and biosimilars. It remains to be seen, however, if these challenges would be adequately addressed in future clinical studies.

II. INTRODUCTION

Hemophilia treatment is about to undergo substantial revolution with a number of longer acting Factor VIII and Factor IX molecules emerging from discovery labs and/or undergoing clinical development. Although these molecules have received the lion share of attention from the hemophilia community, a number of new recombinant Factor VIIa (recombinant activated Factor VII, rFVIIa) molecules are also in development. Some of these new rFVIIas are assumed to have “improved” properties (Novo’s NN1731 and N7-GP, Bayer/Maxygen’s Bay86-6150, Catalyst’s/Wyeth’s CB-813d, Recoly’s LongSeven) while others appear to be biosimilar (Baxter’s and Inspiration/Ipsen’s rFVIIa). These new rFVIIas may have not generated the same level of excitement, but they are worthy of an in-depth examination. In fact, because of the results of prior development effort with the original rFVIIa (NovoSeven®) both in hemophilia and other hemorrhagic clinical situations, the arrival of “new” or “new and improved” rFVIIa analogs is intriguing. These molecules would certainly face serious questions and challenges in clinical development and regulatory strategy.

Thus, in the context of these developments, this summary analysis investigates some of these molecules, their potential for improvements in hemophilia care and other hemostatic indications, as well as the challenges of the clinical development in this area. Although there are claims of increased efficacy and safety of the new rFVIIa analogs, there are substantial challenges in proving these in a clinical setting.

III. THE MECHANISM OF ACTION OF RECOMBINANT FACTOR VIIA

A. Hemophilia Treatment and Complications

Hemophilia is an inherited, sex-linked hemorrhagic disorder caused by the absence or defect of crucial constituents of the coagulation cascade, the process that leads to thrombin generation and fibrin clot formation.¹ There are a number of hemophilias, distinguished by the missing or defective element of the coagulation cascade (the coagulation cascade is discussed in summary in [Section III.B](#)). The most common, hemophilia A, is caused by defects in Factor VIII,

whereas a less common form, hemophilia B is caused by defects in Factor IX. There is also a very rare milder form, hemophilia C, limited to specific populations, which results from defects in the Factor XI gene. Patients with hemophilia experience spontaneous bleeding episodes mainly in their joints but also in deep muscle, and internal organs. They also bleed profusely following trauma. The frequent bleeding in joints leads to the development of severe deformity.

Since the 1960's, the typical treatment of hemophilia is by "substitution" therapy, in which the missing factor, produced either by plasma fractionation or by recombinant DNA technology, is infused in concentrated form restoring normal hemostasis following a spontaneous or traumatic bleeding episode. Currently, mostly in advanced countries, there is extensive use of prophylaxis, which consists of regular infusions of factor concentrates at doses targeted to maintain a certain minimum level of either Factor VIII or Factor IX in patient's blood. The use of prophylaxis avoids the development of joint deformity and incapacity and restores normal quality of life, although frequent intravenous infusions certainly impose a burden on patients.

A serious complication of hemophilia is the development of neutralizing antibodies (inhibitors) to the exogenous Factor VIII or IX. These inhibitors are mostly transient but they persist in about 20% of hemophilia A patients (but only in about 1% of hemophilia B ones) complicating treatment choices. Also, individuals without congenital impairment of the hemostatic system occasionally develop spontaneous inhibitors to their endogenous Factor VIII (acquired hemophilia).

When factor inhibitors are at high enough titer to render substitution therapy ineffective, there is a need for alternative treatments. Porcine Factor VIII can certainly be effective for a period of time in some of these patients. However, in most cases, a treatment agent is utilized that "bypasses" the normal flow of the coagulation cascade. Factor VIIa (activated Factor VII or FVIIa for short) is such a "bypass" agent.

B. The Coagulation Cascade and Factor VIIa

In order to better understand the action of the "bypass" agents and the effects of the modifications in the "improved" version of Factor VIIa, a short introduction to the process of blood coagulation is necessary.

Upon injury, stable blood clots are formed in normal individuals that contain and stop the hemorrhage. These blood clots consist of activated aggregated platelets reinforced by a mesh of a cross-linked polypeptide called fibrin. The activation and aggregation of platelets in the site of injury is the process of primary hemostasis. The reinforcement of the platelet clot by the fibrin mesh comprises the secondary hemostasis. In hemophilia, it is the secondary hemostasis process which fails to work adequately.

Fibrin is produced by the cleavage of fibrinogen, a molecule abundant in blood, by a serine protease called thrombin (Factor IIa). This conversion of fibrinogen to fibrin is the focal point of the “coagulation cascade.” The “cascade” consists of a series of sequential activations of typically inactive proenzymes to their active forms. The activations occur by proteolytic cleavages that bring inactive enzymes (proenzymes) and their cofactors into their catalytic or binding (for cofactors) configuration by re-orienting structural domains. These enzymes are all serine proteases (a serine residue is an essential component of the active site in these proteases). The coagulation cascade is shown diagrammatically in Figure 1.

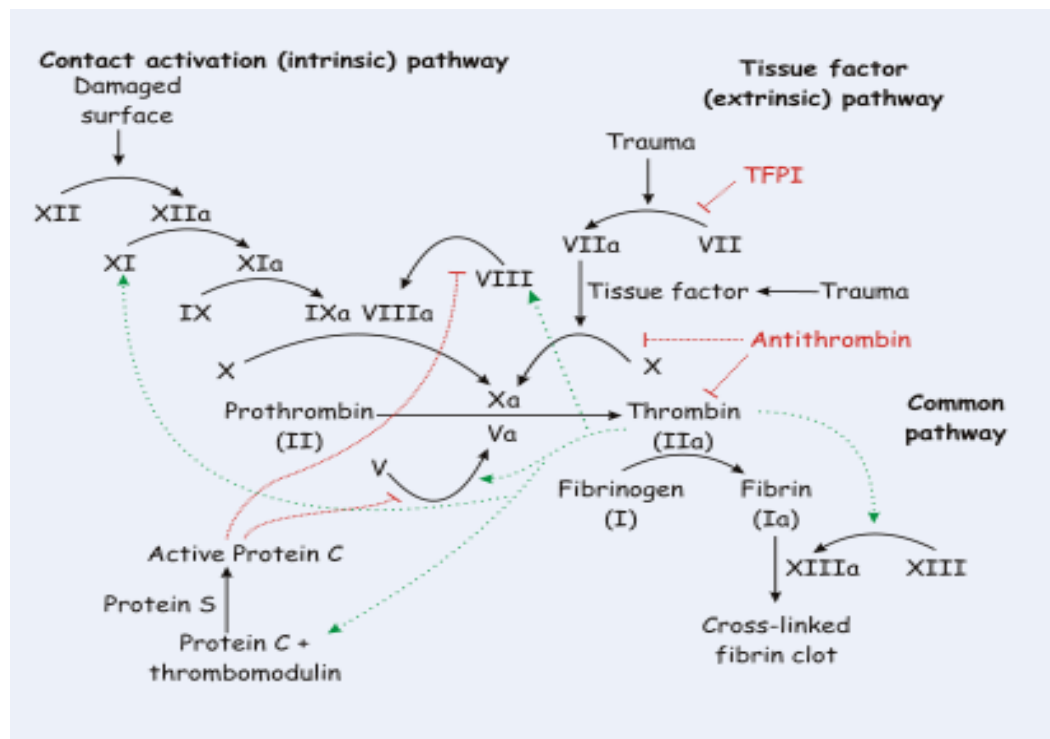


Figure 1: The Coagulation Cascade

There are two main consecutive phases in the coagulation cascade: (a) the initiation phase, which starts the process; and (b) the propagation/amplification phase which results in adequate amounts of fibrin been formed. In hemophilia, it is the propagation/amplification phase that fails to work adequately.

What is the place of Factor VIIa in this process? It plays a key role in the initiation phase. There are only small amounts of Factor VIIa present in plasma at any given time. Most of the molecule circulates in its inactive form (Factor VII). Upon injury, fibroblasts release tissue factor (TF) which readily binds Factor VIIa and Factor VII. The TF-FVIIa complex does activate Factor X to Factor Xa. Factor Xa binds to an activated cofactor, Factor Va; the resulting complex activates prothrombin (Factor II) to thrombin (Factor IIa). Thrombin then cleaves fibrinogen to fibrin. These steps comprise the extrinsic pathway of blood coagulation and constitute the initiation phase of blood coagulation. This phase results in too little fibrin being formed and it is quickly inhibited by the tissue factor pathway inhibitor (TFPI). For an effective clot, one needs substantially higher amounts of thrombin and thus, fibrin. These are provided by the activation of the intrinsic pathway and the commencement of the propagation/amplification phase of blood coagulation.

In the amplification/propagation phase, some of the thrombin formed activates Factor XI, one of the constituents of the intrinsic (or contact-phase) pathway of coagulation. Factor XIa then activates Factor IX (to Factor IXa) which subsequently in association with the activated form of a key cofactor, Factor VIIIa, activates Factor X (Factor Xa). As in the initiation phase, the FXa-cofactor Va complex activates substantial amounts of prothrombin to thrombin (Factor IIa). The presence of Factor VIII here is key, as it facilitates the creation of the catalytic complexes on the phospholipid membranes of activated platelets. This process generates substantially higher amounts of fibrin than the initiation phase and is essential in securing and reinforcing the platelet clot.

C. How Factor FVIIa Bypasses the Defects of the Propagation Phase of the Coagulation Cascade

From the above, it is clear that in hemophilia defects in Factor VIII or Factor IX (hemophilia B) result in the inability to activate adequate amounts of Factor X and thus thrombin. In the presence of neutralizing antibodies (inhibitors) to either Factor VIII or IX, even high exogenous amounts of these factors are

ineffective. Thus, bypassing the Factor X activation step becomes essential in creating a stable clot.

A number of bypass agents achieve this by providing a “cocktail” of inactive and active factors that include Factors VII, IX, X and II (prothrombin/thrombin).² These agents are usually called prothrombin complex concentrates (PCCs). There are “activated” versions of these PCCs (aPCCs) which contain higher amounts of the active factors. In the clinical setting, these concentrates have been found effective in providing hemostatic control to inhibitor patients. PCCs and aPCCs however are produced by blood fractionation and this process has the disadvantage of being open to the potential introduction of infectious agents (i.e., HIV, HCV) that are occasionally present in blood of donors. In addition, as they contain substantial amounts of Factor IX and trace amounts of Factor VIII, they may also increase the concentration of the inhibitors to these factors by an anamnestic response.

Thus, the disadvantages of the PCCs/aPCCs led investigators examine the capability of individual activated factors in bypassing the intrinsic pathway of coagulation. The high amounts of Factor VIIa in aPCCs as well as some early feasibility work in patients³ provided the major rationale for the development of recombinant Factor VIIa as an important agent in the treatment of hemophilia complicated with inhibitors.

There are various hypotheses as to how Factor VIIa effectively bypasses the the Factor IX-Factor VIII steps of the propagation phase to activate FX. Deducing the mechanism by which FVIIa generates adequate fibrin is crucial in designing “improved” versions of this molecule. One obvious explanation is that the additional exogenous amounts of FVIIa create more TF-FVIIa complexes, overwhelm TFPI and, thus, substantially amplify the initiation phase. However, it seems more likely that high amounts of FVIIa bind to activated platelet surfaces and activate there FX to FXa (which subsequently activates prothrombin to thrombin).^{4,5} Since this process is not inactivated by TFPI (the inhibitor that shuts down the initiation phase), it is more likely to create adequate amounts of fibrin to secure the clot. In such a putative mechanism, increasing platelet binding capacity may lead to increased efficacy for rFVIIa.

Although the original impetus for the development of FVIIa was to address the treatment of complications of hemophilia ([Section V.A](#)), this concentrate has been

evaluated in the treatment of trauma, hemorrhagic stroke and platelet defects, indications that substantially expand the range of its uses. The potential impact of the new and “improved” FVIIas in the investigation of new indications would be addressed in Sections [.V.B](#), [V.C](#) and [V.D](#).

IV. THE NEW FACTOR VIIA ANALOGS AND BIOSIMILARS

A. The New rFVIIa Molecules

Several pharmaceutical companies are actively developing new rFVIIas. Novo Nordisk is working on a variant called NN1731 and a pegylated form of the original rFVIIa, N7-GP. Bayer is concentrating on Bay86-6150 which it acquired from Maxygen in 2008. NN1731 and Bay 86-6150 have already commenced clinical development. Among others still in the preclinical phase, Wyeth (now Pfizer) is collaborating with Catalyst for the development of CB 813d, CSL Behring may be proceeding with its albumin-incorporating rFVIIa while Baxter is concentrating on a biosimilar FVIIa. A biosimilar rFVIIa is also being developed by Inspiration Biopharmaceuticals. Recoly is also developing a liposome-bound rFVIIa.

B. The Modifications of the New Factor VIIas and Early Clinical Studies

Novo Nordisk has published various studies on structural modifications that have been introduced to NN1731 and the effects of these changes on the properties of the enzyme. This variant incorporates three changes to the consensus primary sequence of rFVIIa: aspartic acid for valine in position 158, valine for glutamic acid in position 296 and glutamine for methionine in position 298. These changes have resulted in a rFVIIa variant with significantly higher activity on the surface of activated platelets.⁶

In a phase 1 single-dose escalation study in normal volunteers, a number of doses of NN1731 from 5 to 30 µg/kg were investigated.⁷ At each dose level, 8 subjects were enrolled, 2 infused with placebo and the remainder 6 with NN1731. The pharmacokinetic analysis revealed dose proportionality and a bi-phasic plasma activity/antigen decline profile, consistent with a two-compartment model. A fast, bi-exponential decline reduced activity by 73% in the first phase (with a $t_{1/2}$ of 20 min.), followed by a slower elimination phase with a $t_{1/2}$ of 3 hours. No subject showed evidence of inhibitors to NN1731 and

no thromboembolic events were noted. The pharmacokinetic profile of NN1731 does not appear to differ substantially from that of the original rFVIIa.⁸

N7-GP, also by Novo Nordisk, utilizes selective pegylation of a naturally-occurring N-glycan in rFVIIa.⁹ Thus, there are no alterations to the primary sequence of the protein and data indicate that the binding to TF and FX is largely unaffected.¹⁰ N7-GP shows a 6-fold increase in circulating half-life in hemophilic mice compared to the original FVIIa.¹¹

Bayer's Bay86-6150 incorporates more numerous changes to the original sequence of rFVIIa. Two of the changes, asparagine for threonine in position 106 and asparagine for valine in position 253, introduce additional N-linked glycans and extend the terminal half-life of the molecule. The remainder four changes are located near the N-terminus (γ -carboxyglutamic acid domain) of the molecule and increase binding to activated platelet surfaces without compromising enzymatic activity, as earlier studies have shown.¹² These are: glutamine for proline in position 10; glutamic acid for lysine in position 32; glutamic acid for alanine in position 34 and glutamic acid for arginine in position 36.

Bayer recently completed a phase 1/2 clinical study with BAY 86-6150. This randomized, double-blind, single-dose escalation study investigated doses of BAY86-6150 from 6.5 to 90 $\mu\text{g}/\text{kg}$ and followed participating patients for 50 days after dosing. The pharmacokinetic analysis showed a linear profile over the dose range with a half-life of 5 – 7 hours. There was no dose-proportional increase in thrombogenicity markers such as TAT, prothrombin fragment 1+2 and D-dimer. There were no dose-limiting toxicities. On the other hand, one patient developed inhibitors to both BAY 86-6150 and rFVIIa with titers remaining stable over the 50-day observational period.

Pfizer and Catalyst have not provided a substantial amount of information on their FVIIa analogs (generally referred to as CB 813x) but the patent application indicates that modifications are targeted to same general domains as in NN1731 and Bay86-6150. In recent presentations, the companies presented information for certain of their molecules. A variant termed CB-813a achieved a 5-10-fold increase in catalytic activity whereas another, CB-813d, showed a 3-fold increase in duration of activity in hemophilic mice when compared to the original rFVIIa.¹³

CSL Behring has genetically modified rFVIIa by incorporating a human serum albumin sequence. The company presented data from preclinical work that show that this fusion protein extends the half-life of the rFVIIa activity 6 to 9-fold^{14,15} but this molecule has not as yet progressed to clinical development.

RecoLy is also developing a NecLip-rFVIIa. This form of rFVIIa is attached to the proprietary non-encapsulating, pegylated liposomes that RecoLy has developed.¹⁶ On the basis of communication of unpublished results by the company, a phase 1/2 clinical study in six patients in one Russian investigative site has shown what appears to be a doubling of rFVIIa half-life, although no specific numbers were given.

Other companies have decided to simply provide alternatives to the market that do not depart from the consensus sequence and biophysical properties of rFVIIa. Baxter is apparently completing the preclinical assessment of what appears to be a biosimilar rFVIIa. Inspiration Biopharmaceuticals, Inc. (recently acquired by Ipsen) is also developing a biosimilar rFVIIa, although details are sketchy.

V. THE CLINICAL DEVELOPMENT CHALLENGE

Recombinant Factor VIIa was originally approved in 1996 by the EMA and subsequently, in 1999, by the FDA under the trade name NovoSeven® (Novo Nordisk, Copenhagen, Denmark). In the remainder of the text, we would be referring to this molecule rFVIIa. This concentrate is indicated for the treatment of hemophilia A and B complicated with inhibitors, acquired hemophilia, Glasmann thrombocytopenia and Factor VII congenital deficiency. However, since approval by major regulatory bodies, rFVIIa has been extensively studied for a potential hemostatic prophylaxis role in surgeries associated with high blood loss, in hemorrhagic stroke (intracranial hemorrhage), and in the treatment of trauma. Its utilization in these clinical settings remains off label to this date and the arguments about its utility remain unsettled.

A. Hemophilia Complicated by Inhibitors

1. Clinical Studies Leading to Approval of rFVIIa

A number of studies have investigated the utility of rFVIIa in the treatment of bleeding events in hemophilia patients with high inhibitor titers. These

studies highlight the cautious approach followed in the investigation of this concentrate. Excellent reviews of the development in this field were published both by Puetz¹⁷ and Jonhansson and Ostrowski.¹⁸

One of the pivotal studies was conducted by Lusher *et al.*¹⁹ and investigated two doses of rFVIIa (35 µg/kg and 70 µg/kg) in a randomized, double-blind design. Participating patients were diagnosed with hemophilia A or B, with or without inhibitors. Dosing with rFVIIa was repeated every 2.5 hours until bleeding had stopped. The hemostatic control was rated as excellent, effective, partially effective or ineffective. Of the 116 subjects originally enrolled in this study, 84 were eventually treated for a total of 179 bleeding episodes in a hospital environment. There was no difference between the two doses in terms of hemostatic efficacy scoring. Both doses were rated excellent or effective for 71% of the treatments. However, patients who did not have inhibitors rated treatment with rFVIIa inferior to the treatment with FVIII or FIX concentrates. The mean number of doses was 3.1 for the 70 µg/kg dose and 2.7 for the 35 µg/kg dose. No safety concerns were noted and rFVIIa was tolerated well for both doses.

Key *et al.*²⁰ investigated the use of rFVIIa in the home environment in patient with hemophilia A or B complicated by inhibitors. This time, the dose was raised to 90 µg/kg. Dosing was to begin within 8 hours of the onset of bleeding and the patients could receive up to two additional infusions every three hours. If, at the end of this treatment hemostasis was rated as “effective”, an additional maintenance dose of rFVIIa was give. Sixty patients were enrolled in this study, 56 had a bleeding episode and 46 completed a year of treatment with rFVIIa. The treatment of 92% of bleeding episodes was rated as effective and an additional 5% as partially effective; a mean of 2.2 infusions were administered for each treatment. The only thromboembolic episode noted was of superficial thrombophlebitis. No subject showed evidence of inhibitors to FVIIa and none of them developed hypersensitivity reactions.

Surgery study Shapiro *et al.*²¹ investigated the efficacy and safety of two doses of rFVIIa in surgery. Patients were randomized to receive either 35 µg/kg or 90 µg/kg prior to the surgery and subsequent doses every two hours intra-operatively and every 2 -6 hours postoperatively for the following 3 days.

Hemostasis was evaluated over a period of 5 days. After this evaluation period, hemostasis was maintained by infusions with the higher dose (90 $\mu\text{g}/\text{kg}$). Twenty-nine patients underwent 11 major and 18 minor surgeries. Hemostasis was achieved intra-operatively for 28 of 29 patients. Twenty-three of the 29 patients successfully completed the study (did not require rescue treatment) with only one of the high dose group failing the hemostasis criteria. The investigators concluded that the 35 $\mu\text{g}/\text{mL}$ dose was suboptimal and surgery should utilize the higher dose of 90 $\mu\text{g}/\text{kg}$. Safety was acceptable, with one patient developing an internal jugular vein thrombosis at the site of the catheter.

These studies served as the basis of the approval of rFVIIa both in the US and EU. Following approval, a number of clinical trials investigated the efficacy and safety of higher bolus doses or dosing by continuous infusion in various clinical situations. Both of these efforts have now substantial implications in the development of the new rFVIIa analogs.

2. Clinical Studies Investigating Continuous Infusion

The clinical trials of the efficacy and safety of the continuous infusion method of administration* addresses the frequency of infusions required (every 2 to 3 hours) to maintain hemostasis post-operatively, especially following major surgeries in which the convalescence period is relatively long. Ludlam *et al.*²² explored the pharmacokinetics and efficacy of a continuous infusion dose of 50 $\mu\text{g}/\text{kg}/\text{hour}$ (until healing had occurred)[†] in 9 subjects undergoing major orthopedic surgery. According to the authors, this dose achieved adequate hemostatic control, although 6/9 subjects had breakthrough bleeding events treated with bolus infusions of rFVIIa. Lower doses did not appear to provide adequate hemostatic control. Smith *et al.*²³ utilized a dose of 16.5 $\mu\text{g}/\text{mL}/\text{hour}$ with rather disappointing results.

Pruthi *et al.*²⁴ compared the efficacy of bolus vs. continuous infusion in major surgery (most of the procedures were knee or hip replacements) in a randomized, open-label study. The participating patients (n =24) received a

* There are a number of technical issues regarding the aseptic reconstitution and maintenance of rFVIIa at room temperature in continuous infusion that are beyond the scope of this review.

[†] At this dose, continuous infusion is not as cost-effective as bolus infusions of 90 $\mu\text{g}/\text{kg}$ administered every two to two and half hours.

pre-operative dose of 90 µg/kg and were then randomized to receive either bolus or continuous infusion. The bolus infusion group received infusions at 90 µg/kg every two hours for perioperative days 0 – 5, and every four hours for days 6 to 10. The continuous infusion patient group received 50 µg/kg/hour until Day 5, and then the dose was reduced to 25 mg/kg/h for days 6-10. Efficacy was comparable in both groups with approximately 75% of patients in each group displaying adequate hemostasis.

3. Clinical Studies Investigating Higher Dosing

The reason that 50 µg/kg/hour was borderline efficacious in orthopedic surgery and why the typical dose for the treatment of bleeding episodes had patients assess hemostasis with rFVIIa as of lower efficacy than that achieved by factor VIII or IX concentrates was actually explained by Kjalke *et al.*²⁵ These investigators observed that the typical dose of 90 µg/kg result in concentrations of rFVIIa in blood of approximately 50 nM. Their studies indicated that a three-fold increase in concentration, to 150 nM, is required in order to normalize the thrombin burst that occurs in the propagation/amplification stage of blood coagulation.

On the basis of this information, various studies explored rFVIIa doses substantially higher than those approved. Santagostino *et al.*²⁶ performed a prospective, randomized, crossover but open-label study comparing the safety and efficacy of the standard dose of 90 µg/kg repeated as necessary every three hours to that of a single dose of 270 µg/kg (if hemostasis was not achieved within 9 hours for the high dose, treatment continued with standard dosing). The patients assessed the progress of resolution of their symptoms in a visual analog scale (graded from 0 to 100) for 48 hours after the initial treatment and rated the treatment as effective, partially effective or ineffective. Eighteen patients treated 32 hemarthroses with the standard dose and 36 with the high dose. There was no significant difference in overall efficacy rating between the two doses (64% for high dose and 66% for standard dose at 48 hours); there was, however, a statistically significant difference the median number of infusions required to achieved effective hemostasis: three (3) infusions were required in the standard dose, but only 1 in the high-dose set of treatments. The amount of rFVIIa utilized was similar for the two dose schemes.

Similar observations were obtained in similar but blinded studies. Kavakli *et al.*²⁷ performed a prospective, randomized, crossover, double-blind study comparing 90 µg/kg x 3 administered every three hours after the onset of the bleeding episode to 270 µg/kg followed by two infusions of placebo every three hours. Twenty-two subjects treated in the study rated the high dose as effective in similar proportions to the standard dose (65% to 70%, respectively). The overall global assessment rates were very similar to those observed in the Santagostino *et al.*²⁶ study although the two studies utilized different assessment instruments and the Santagostino *et al.*²⁶ study was open label.

Young *et al.*²⁸ also compared the efficacy of the standard dose (90 µg/kg) given three times every three hours to that of the high dose (270 µg/kg) followed by two placebo infusions every three hours. The study included an open-label arm in which patients were treated with an activated PCC (aPCC) at a single dose of 75 U/kg. Efficacy was assessed at 9 hours after the original infusion using a response scale and the need for rescue treatment. The high and standard dose groups did not differ in the use of rescue medication (8.3% and 9.1%, respectively) or in their global assessment of efficacy.

Konkle *et al.*²⁹ examined the use of the high and standard dose in prophylaxis. In this study, after an observation period of 3 months to establish the baseline hemorrhage frequency, 22 patients were randomized to receive either the high (270 µg/kg) or the standard dose (90 µg/kg) daily for a period of 3 months. There was a substantial reduction of the bleeding frequency (by 59% for the high dose and 45% for the standard dose). The differences in response between the doses were not statistically significant.

None of the studies investigating the high dose identified any safety concerns with the high dose.

4. The Development Challenge for New and “Improved” rFVIIa Analogs in Hemophilia

Overall, the studies with the substantially increased dosing of rFVIIa showed that such dosing can provide effective hemostasis for the great majority of bleeding episodes with a single infusion without any substantial safety concerns. Since in both studies examining the high dose (270 µg/kg),

approximately 90% of the episodes were controlled with a single infusion, any potential improvement by the new rFVIIa analogs in this parameter of efficacy seem rather difficult to achieve.

Therefore, different primary endpoints must be sought. Most of these studies used a three-point scale for global efficacy assessment (effective, partially effective, and ineffective). Approximately 65% of the patients scored the treatment as effective with the rest scoring it as partially effective (presumably, symptoms did not recede very quickly after treatment). Only about 10% of the patients scored their treatment as ineffective (with either the standard or the high dose) in these studies. Despite the similarity in outcomes, it should be noted that all three studies used different sets of symptom assessment scales to render the final global efficacy score. In addition, although most of the studies necessitated treatment to begin within 8 hours of the onset of bleeding, the published data do not present a detailed analysis on how the time between bleeding onset and treatment affected the outcome.

It is just possible that the new rFVIIa analogs may perform better in the percentage of patients scoring the treatment as effective, when they are compared to the rFVIIa high dose (as they must, since the current dose is approved in the EU).[‡] Is the percent of patients scoring the treatment as effective clinically relevant when even the patients with partial effectiveness achieve hemostasis with a single infusion? Is the percent of patients that use of rescue medication more clinically relevant? What may be regarded as “clinically relevant” in the symptom assessment tools and global efficacy scores is open to discussion (and these discussions would certainly take place). The new endpoints would require larger studies than the ones performed thus far. Targeting an increase in the “effective” score of the global efficacy assessment from 65% to 85% (a relative 30% increase in efficacy) in a study with 80% power and level of significance at 0.05 (two-tailed) in a parallel, (at least) two-arm design would require as many as 70 patients per group. With the same design specifications, targeting a 50% decrease in the use of rescue medication (from approximately 10%, as noted

[‡] Mechanistically, there is no reason to assume that the “improved” Factor VIIa analogs will perform any better than the high dose (270 µg/kg) of the existing rFVIIa. If one assumes that the work of Kjalke *et al.*²⁵ is accurate, the high dose of rFVIIa fully normalizes the thrombin burst and creates a normal clot.

in two of the high dose studies,^{26,28} to 5%) would require a prohibitively high number of subjects per group (> 300) in a well-powered study.

Crossover designs, although complex to plan and difficult to regulate in a home-treatment environment, may lower the sample size requirements. But the design of crossover studies in a clinical setting in which one expects to treat a variety of joint bleeding episodes depends on rather difficult to justify assumptions on discordant pairs (or even definitions as to what constitutes a discordant pair). The problem with discordant pairs may be solved by specifying only specific “target” joints for treatment, but this creates issues of carry-over effects of treatment of joints not involved in the study with regular medication. Crossover designs, if they can be logically constructed, may also obviate the need for stratification, a requirement in relatively small studies utilizing parallel arms (randomization, on its own, may be inadequate in balancing the treatment groups in small studies). In any case, the rarity of patients may force studies with the new FVIIa analogs to adhere to the hemophilia clinical development “gestalt” of mostly underpowered studies following negotiations with regulatory agencies on both sides of the Atlantic.

5. The Competitive Landscape in Hemophilia

Even if studies with the “improved” rFVIIa show some advantages in terms of marginally increased “efficacy” scoring, the original rFVIIa, the new rFVIIa analogs and the biosimilars will, most likely, compete on equal footing in terms of efficacy for a share of the market. Safety is a different issue. The currently marketed rFVIIa has compiled a long and relatively good safety record. Abshire and Kenet³⁰ and Abshire³¹ calculated that the rate of thromboembolic events following the use of rFVIIa was approximately 4/100,000 doses and that this rate did not change when higher doses become prevalent after 2003.

Assuming safety would not become a pivotal issue, the major differentiator for the companies pursuing these new rFVIIa analogs/biosimilars may well be the price. Considering that rFVIIa is currently available from a single pharmaceutical company, Novo Nordisk, at a relatively high price, the possible arrival of a number of new rFVIIa molecules represents a substantial benefit to the patients and health authorities. It is difficult to see how the “enhanced” rFVIIa analogs can achieve higher pricing per treatment unit –at

least in hemophilia- than the current rFVIIa. Increased safety may achieve this goal, but a safety database that would challenge the lengthy experience with the original rFVIIa would take many years to compile. On top of this, biosimilars (such as the rFVIIa molecule by Baxter and/or Inspiration) will certainly put a downward pressure on prices. Novo Nordisk certainly has the advantage of an experienced manufacturing process and an extensive safety database. Additionally, Novo Nordisk may also manage to market at least one of the new analogs (depending on the results of the clinical studies) to protect its current advantage.

B. Hemostatic prophylaxis during major surgery

With hemophilia probably being a therapeutic area in which the new rFVIIa analogs will have an uphill struggle to establish an advantage over rFVIIa, the attention of the persons planning the development of these molecules must turn to a number of other indications. Prophylactic use of rFVIIa in major surgeries in which substantial blood loss may contribute to morbidity and mortality has been an area in which the original rFVIIa has compiled a record of mostly failed studies over the last decade; thus, one may assume that improved molecules may achieve better results. However, as we will discuss later, both the original rFVIIa and biosimilars may have a chance of greater success here if they modulate elements of study design to take advantage of the lessons learned.

1. Clinical Studies with rFVIIa in this Indication

The potential of rFVIIa to limit blood loss during major surgery and diagnostic procedures was investigated in a number of studies in various types of operations known to be accompanied by high blood loss and transfusion requirements, elements that contribute to the increased morbidity of these clinical situations. Such operations include:

- Laparoscopic liver biopsy in liver disease patients³²
- Retropubic prostatectomy³³
- Pelvic reconstruction³⁴
- Complex non-coronary heart surgery^{35,36}
- Partial hepatectomy^{37,38}
- Liver transplantation^{39,40}
- Skin excision and grafting⁴¹

- Spinal fusion surgery⁴²

Overall, most of these studies have failed to show convincingly that rFVIIa is capable of providing effective hemostatic prophylaxis in high-blood loss operations. In the majority of cases, rFVIIa did not perform any better than placebo. Certain studies, such as the one by Friederich *et al.*³³ in retropubic prostatectomy, had a positive outcome (lowered or eliminated RBC transfusions), but the sample size was very small ($n = 36$). The summary of the experience evaluated in a number of meta-analyses did not support the use of rFVIIa in hemostatic prophylaxis in surgery.^{43,44}

Despite the notable lack of success, the new and “improved” rFVIIa analogs may want to revisit these clinical situations. As noted, the studies have been mostly small and even those with more robust sample sizes were powered on the basis of rather aggressive treatment effects. However, there are various problems in utilizing these studies as templates for further development. The dosing of rFVIIa was not standardized; doses in various studies were as low as $5 \mu\text{g}/\text{kg}$ ³² and as high as $400 \mu\text{g}/\text{kg}$.³⁷ The endpoints also differed encompassing either the percentage of patients requiring transfusion, blood loss, or number of red blood cell (RBC) units transfused. The challenge for new rFVIIa analogs is now substantial. Even assuming that in some of the new analogs that the platelet-dependent activity has quadrupled, defining the intervention dose still remains challenging on the basis of the information at hand. In addition, the current rFVIIa (Novoseven[®]) and biosimilars may be able to obtain similar outcomes (to those of the analogs) by increasing doses. As mentioned earlier, increasing the dose of rFVIIa in patients with hemophilia increases efficacy without compromising safety.

A detailed discussion on each one of these surgical indications is beyond the scope of this paper. However, examining carefully some of the studies highlights the problems in the existing data and the complications that may be encountered in future development. Therefore, the discussion here is limited to the studies in liver transplantation and resection. Both of these surgical interventions constitute an excellent challenge in determining the potential of any agent to limit blood loss. It is also the clinical situations in which the most robust studies investigating the efficacy of rFVIIa in major surgery have been performed. Lodge *et al.* performed studies in partial

hepatectomy³⁸ and liver transplantation³⁹. These studies enrolled a relatively robust number of patients, although the powering considerations were based on rather aggressive assumptions. A smaller study in liver transplantation was performed by Planinsic *et al.*⁴⁰

In the partial hepatectomy study,³⁸ 204 non-cirrhotic patients were randomized to receive either placebo, 20 µg/kg or 80 µg/kg rFVIIa (dosing could have been repeated 5 hours after the original dose if the operation lasted for more than 5 hours). There were two primary endpoints to this study, the percentage of patients receiving transfusions and the number of RBC units transfused. Prothrombin Time (PT) was monitored every hour during the surgery. Both rFVIIa groups had significantly lower PT for most of the period of the surgery. Despite the improvement in PT, the study did not show any significant differences in the primary endpoints: 37% of the placebo group, 41% of the 20 µg/kg group and 25% of the 80 µg/kg group patients required transfusions perioperatively; as mentioned, these differences were not statistically significant and the same was true for any differences noted in total blood loss and volume of red blood cells infused. There were 9 thromboembolic adverse events in the study (3 in each of the groups), there was no dose-response relationship and there were attributed to patient risk factors, not to rFVIIa.

The Planinsic *et al.*⁴⁰ study in liver transplantation was small in sample size and definitely exploratory in its design. It was a multicenter, randomized, double blind trial for which the primary endpoint was the number of RBC units infused during the operation and for 24 hours following the operation. The assumptions for the study design were aggressive and consistent with its exploratory nature. According to the investigators, the study had 80% power to detect a 60% decrease in mean RBC units transfused when comparing the two highest rFVIIa dose groups to placebo. Eighty-nine subjects were randomized equally into four groups: placebo, 20, 40 and 80 µg/kg rFVIIa. Only a single administration of placebo/rFVIIa was given (in contrast to the Lodge *et al.*³⁹ study, discussed below, that allowed multiple infusions every

two hours).[§] The safety results did not indicate any specific issues with rFVIIa.

In the 2nd study in liver transplantation, conducted by Lodge *et al.*,³⁹ dosing was more aggressive; In this multicenter, randomized, double-blind trial, the patients were randomized to receive either placebo, 60 µg/kg or 120 µg/kg rFVIIa; the dose was repeated every 2 hours until the end of the operation. The primary endpoint was the same as in the Planinsic *et al.*⁴⁰ study: the mean RBC units transfused within 24 hours of the operation. According to the investigators, the study was designed for an 80% power to discern a decrease of 40% in mean RBC units transfused. One hundred and seventy-nine patients were randomized and treated in this study. Although a reduction of 15% and 23% was seen for the low and high dose of rFVIIa, respectively, these reductions were not statistically significant. The investigators noted that there was a significant difference between the groups in the number of patients that avoided transfusions altogether, but it is important to note that the study was not powered to discern such differences and there were no adjustments for multiplicity. Despite the fact that the dosing was substantially increased when compared to the Planinsic *et al.*⁴⁰ effort, there were no specific safety concerns for rFVIIa and there was no dose-dependent increase in thromboembolic complications. Interestingly, the investigators tried to define the levels of rFVIIa clotting activity in the plasma of treated patients. Recombinant FVIIa activity was in line with dosing.

Neither of the studies in liver transplantation, nor the Lodge *et al.*³⁸ study in liver resection showed that rFVIIa performed significantly better than placebo. Despite the substantial increase in dosing in liver transplantation by Lodge *et al.*,³⁹ there was no significant increase or noted trend in the rate of thromboembolic complications.

2. Development Challenges and Opportunities in Hemostatic Prophylaxis during Major Surgery

It would be interesting to see if the new rFVIIa analogs intend to revisit research in this indication. Antifibrinolytics have shown conclusively the ability to lower blood loss and reduce transfusion requirements.^{45,46} rFVIIa

[§] It is important to note that in this study the mean duration of the operation was 7.3 hours. As such, the dosing given was substantially less than in the Lodge *et al.* study.

used in combination with antifibrinolytics may simply provide limited benefits, below the threshold of clinical relevance. Certainly, most of the studies so far indicate that this is the case, although most have been rather small and underpowered. Although current techniques have led to substantial improvement in blood loss in liver transplantation, there may still be room for improvement and rFVIIa analogs are likely to take up the challenge. In this case, they would be facing various challenges. One of those is the continuous improvement in the surgical technique and graft preservation, as well as the utilization of antifibrinolytic drugs, all of which have reduced blood loss and the need for blood cell transfusions. De Boer *et al.*⁴⁷ reported that such improvements have resulted in several centers reporting no requirement for transfusions in about 30% of the patients.

Thus, this is an uphill task which will require careful definition of a number of parameters that do affect these studies. Enrollment criteria should be revisited. Both studies in liver transplantation enrolled patients in a Child-Pugh⁴⁸ category of B and C. Categorizing patients on the Child-Pugh score is helpful in deciding who is requiring transplantation, but may be inadequate for the purposes of a study. The Child-Pugh score is an aggregate number, consisting of individual scores on the severity of certain laboratory and clinical findings. The Child-Pugh score is also not predictive of substantial bleeding during surgery. Of its components, only INR has been found most sensitive to the need of transfusion of blood products,⁴⁹ although its specificity is not high. Thus, instead of concentrating on the total score, it may be best to enroll patients in the study with INR values ≥ 2.0 , if possible. Alternatively, patients may be entered in these studies according to the risk index for massive blood transfusion in liver transplantation developed by McCluskey *et al.*⁵⁰ Such approaches may yield much better results than the current enrollment criteria. In addition, these studies utilized specific “triggers” for infusion that can be re-assessed in newer studies, as they can certainly affect the power of the studies.

Dosing would also be a challenge as no dose-response curve has been established as yet. Taking together the Planinsic *et al.*⁴⁰ and Lodge *et al.*³⁹ studies, it seems that infusion of 120 $\mu\text{g}/\text{kg}$ repeated every two hours (with infusions also given at some time postoperatively), should be utilized as the starting dose level in future studies.

The endpoints of these studies bear some examination too. As mentioned before, the primary endpoints differed among the studies, including either number of patients requiring transfusion, blood loss, or number of RBC units transfused. These endpoints may not be sensitive enough to the benefits of rFVIIa in this indication, and they may lack the statistical efficiency required. Unfortunately, the publications of these studies do not include detailed information of various parameters and/or extensive follow-up of patients to allow for a careful evaluation of alternate endpoints.

Lodge *et al.*³⁹ in investigating the effects of rFVIIa in liver transplantation based the primary endpoint on the number of RBC units transfused. These investigators determined, (on the basis on assumptions that are partially obscured in the publication^{**}) that they required 60 subjects per group in order to prove that at least the higher dose group was superior to placebo by 40%. This was certainly aggressive, as were the variance assumptions considering the effects and experience of the antifibrinolytics in major surgery. However, the authors did not provide adequate data to calculate how close their assumptions (partially listed in the publication) were to the actual observations.

Assuming that the true treatment effect is near the range discussed by Lodge *et al.*³⁹ (an approximate mean reduction of 2 units of RBCs transfused) and using a realistic assumption on variance (unfortunately the observed variance is not available in the data published by Lodge *et al.*³⁹), a study that would discern this effect at 80% power would require at least 200 subjects per arm (or more, depending on higher estimates of variance). These are high numbers for studies in liver transplantation, especially if more than one dose level is going to be investigated.

Therefore, new endpoints may have to be investigated, possibly composite ones in order to provide a certain degree of statistical efficiency. Composite endpoints can increase statistical efficiency and they can be clinically meaningful assuming that they are properly constructed. These endpoints may not only include parameters related to transfusion requirements, but may also incorporate typical morbidities associated with excessive blood loss

^{**} Although the authors provided certain aspects of their sample size calculation, they did not provide the starting assumption regarding RBC unit transfusions

(death, longer than anticipated recovery, pulmonary complications, infections and re-operations).⁴⁷ These variables are clinically relevant and thus in accordance with the ICH-E9 guidance. Combining a variety of these variables into a single endpoint certainly avoids the complications of multiplicity testing (the required adjustments for the Type I error). In addition, individual parameters may be differently weighted for severity in a predefined algorithm. It is just possible that such composite endpoints may better define the benefits of rFVIIa in and avoid the substantial variance of single primary endpoints such as “RBC units infused” or the insensitivity of “the percentage of patients transfused.” Of course, any new composite endpoints may require validation and a certain regulatory acceptance, which a series of phase 2 studies should be able to provide.

In any case, since the current experience has not included any dose limiting events in these studies,^{††} the original rFVIIa and biosimilar molecules certainly have the capability of increasing doses to match the performance of rFVIIa analogs, assuming that positive results are obtained with these molecules in new studies..

C. Acute Intracerebral Hemorrhage

A number of studies investigated the use of rFVIIa in the treatment of spontaneous intracerebral hemorrhage (ICH). Intracerebral hemorrhage accounts of approximately 12 - 15% of all strokes⁵¹ and it is associated with higher mortality rate than the more frequent ischemic stroke.^{52,53} One third of ICHs are associated with intraventricular hemorrhage.^{##} The causes of ICH are various and involve high blood pressure, diabetes, infections, tumors, and arteriovenous malformations. In fact, the heterogeneity of causes of this condition is one of the many challenges in development in this indication, as these causes certainly have an influence on the outcome.

1. Recombinant FVIIa Studies in this Indication

The studies in this indication are of better quality (although still aggressive on their statistical assumptions) than the studies in major surgeries. The studies

^{††} Lodge *et al.*³⁸ remarked that in hepatectomy, the thromboembolic episodes noted tended to be more severe in the rFVIIa groups but they numbers of these episodes were just too small for any definitive statement.

^{##} The brain ventricles contain the cerebrospinal fluid of the brain and these ventricles are the continuation of the central canal of the spinal cord.

are well summarized in the review of their designs, issues and outcomes by Salman.⁵⁴

A number of phase 2 studies were performed in various geographies (Asia, US and Europe) and were followed by a phase 3 study. A study in Japan was never published.⁵⁴ thus data are not available for this summary. A phase 2a study was performed in the US.⁵⁵ In this randomized, double-blind, placebo-controlled, dose-escalation trial 40 subjects diagnosed with acute ICH within 3 hours of symptom onset were treated with either placebo or 5, 20, 40, or 80 $\mu\text{g}/\text{kg}$ rFVIIa (n = 8 patients per group). There were 10 thromboembolic complications in this study, 3 occurring in the placebo group, 3 in the 20 $\mu\text{g}/\text{kg}$ rFVIIa group, 2 in the 40 $\mu\text{g}/\text{kg}$ and 2 in the 80 $\mu\text{g}/\text{kg}$ rFVIIa group. The investigators regarded the safety profile as acceptable for proceeding to further studies.

Another early phase 2 study (phase 2a), was performed in Europe and various countries in Asia.⁵⁶ It was a multicenter, placebo controlled, dose-escalation, study that investigated a variety of doses of rFVIIa (10, 20, 40, 80, 120 and 160 $\mu\text{g}/\text{kg}$) in patients with symptoms of ICH. Patients that presented within 3 hours of onset of symptoms of ICH, were given a baseline computed tomography (CT) scan and were then treated with either placebo or the assigned dose of rFVIIa. Overall, 48 patients were treated: 12 were assigned to placebo and 6 to each of the rFVIIa dose groups. The main goal of the study was to determine the safety of rFVIIa in this clinical situation. There was no evidence in the study of thromboembolic complications and coagulopathies attributed to rFVIIa

Following these early studies, an expanded phase 2 (phase 2b) study was performed in the US.⁵⁷ It was a multicenter, placebo control, randomized, parallel and double-blind study. In this trial, 399 patients were randomized to receive either placebo or 40, 80 or 160 $\mu\text{g}/\text{kg}$ rFVIIa. As in the Europe/Asia phase 2 study, the patients were randomized if they presented to treatment centers within 3 hours of onset of symptoms; they were treated within an hour of the baseline computed tomography (CT) scan. In this study, as in the previous phase 2 studies, the primary endpoint was the expansion of the volume of hemorrhage. Hematoma volume increased by 29% in the placebo group 16%, 14% and 11% in the groups given 40, 80, and 160 $\mu\text{g}/\text{kg}$ of rFVIIa,

respectively. The difference in increase in hematoma volume for the three rFVIIa groups was statistically significant when compared with the placebo group ($p = 0.01$). Ninety (90)-day mortality was also significantly higher in the placebo group (29%) when compared to the combined rFVIIa groups (18%) ($p=0.02$).

These studies set the stage for the Phase 3 study.⁵⁸ In this multicenter, randomized and double-blind study, 841 patients were randomized to treatment by either placebo, 20 μg or 80 $\mu\text{g}/\text{kg}$ rFVIIa. The patients should have been admitted within 3 hours of onset of symptoms. Treatment was applied within 1 hour after the baseline CT scan and no later than 4 hours after the onset of symptoms. The main endpoint of the study was death or poor outcome as defined by the modified Rankin scale⁵⁸ (score 5 or 6) at 90 days post-treatment. Hematoma volume growth was followed by additional CT scans at 24 and 72 hours. The study was powered on the assumption that poor outcome would be noted in 45% of the placebo-treated patients and in 30% of the patients receiving rFVIIa. The power was set at 90% and the alpha was 0.025. This was an adaptive study with a planned (and executed) blinded analysis of poor outcomes at 50% of enrollment. Apparently, at the interim analysis, the poor outcomes were lower than expected, so the sample size was adjusted higher by 123 patients at that point. At the conclusion of the study, the effects of treatment on the growth of hematoma volume were similar to those noted in the phase 2 studies: the estimated increase was 26% in the placebo group, 18% in the 20 $\mu\text{g}/\text{kg}$ rFVIIa group and 11% in the 80 $\mu\text{g}/\text{kg}$ group ($p < 0.001$). However, this decrease in the volume of hematoma growth had no effect on outcome. Poor outcome was noted in 24% of patients in the placebo group, in 26% of patients of the 20 $\mu\text{g}/\text{kg}$ rFVIIa group and in 29% of patients treated with 80 $\mu\text{g}/\text{kg}$ rFVIIa. It is interesting to note that the 90- day mortality of the placebo group of the phase 3 study was 19%,

⁵⁸ The modified Rankin scale (mRS) is a 7-point scale (0 – 6) that is typically used to assess the degree of disability in patients who suffered a stroke. Other scales and or scores used include (a) the National Institutes of Health Stroke Scale (NIHSS), an 11-category assessment of neurological deficit with each category scored on 3 to 4-point scale (0-2 or 0-3); (b) the Barthel Index, a 10-category tool that assesses performance in activities of daily living; (c) Glasgow Outcome Scale (GOS), a 5-point scale assessing outcome. All these scales, their advantages, disadvantages, sensitivity and consistency are discussed in Section D,2.b. of [Why Do So Many Phase 3 Clinical Trials Fail? Part 1: The Effect of Deficient Phase 2 Trials in Therapeutic Areas with High Failure Rates in Phase 3 Studies](#) by Retzios AD.

substantially lower than the mortality of the placebo group of the phase 2b study (29%).

2. Issues with Clinical Development in Acute ICH

Two main questions must be asked prior to the beginning of any pharmaceutical development program: (a) is there any evidence for the utility of the test agent in the clinical condition investigated?; and (b) do we have the appropriate tools to define the effects of the drug on outcome of the treatment of the clinical condition?

On both these questions, the answers for rFVIIa in acute intracerebral hemorrhage are, at least, ambiguous. Regarding the utility of hemostatic treatment in acute intracerebral hemorrhage in which the hemorrhage has occurred upon presentation, one needs to define if hemostatic intervention leading to the decrease in the growth of hematoma volume affects the outcome of this type of stroke. The answer may not need to rise to the “proof of concept” level, but it must be convincing.

The case for a clinical benefit of restricting the growth of hematoma with early and aggressive hemostatic treatment was made by Mayer,⁵⁹ the same principal investigator who conducted all the rFVIIa studies in ICH. Mayer argued that a number of studies supported the notion that the increase of hematoma volume after presentation was connected to poor outcome.⁵⁹ Examining the argument today, with the knowledge of the outcome of the rFVIIa studies, it is easy to remark that the evidence for this proposition was ambiguous and depended mostly on retrospective studies. Of the studies that Mayer presented in defense of his argument, only the study by Brott *et al.*⁶⁰ was prospective; it examined hematoma growth during the first 20 hours after presentation. Patients were eligible for this study if they presented to the investigative sites within 3 hours of onset of symptoms. The patients had an immediate baseline CT scan, which was repeated at 1 hour and 20 hours after the baseline scan. The investigators determined that an expansion of hematoma was observed in approximately 38% of patients.^{***} It is important to note here, however, that most of these patients (26%) exhibited the hematoma volume increase at the 1-hour CT scan. The patients that exhibited

^{***} The investigators noted that the numbers may have been deceptively low because the 24-hour scan was available in approximately 75% of studied patients.

an increase in hematoma growth at the 1-hour scan, were more likely to display increased neurological deficit as defined by the NIHSS score.^{§§} However, no definitive relationship was found between hematoma increase and 30-day death rates, the modified Rankin scale^{§§} or Barthel Index^{§§} at 4 – 6 weeks.

The rest of the data bolstering Mayer's argument were derived from Japanese retrospective studies;^{61,62,63} these studies also showed that most of the increase in hematoma growth occurred relatively early after the baseline CT scan. In fact, as Kazui *et al.*⁶⁴ determined in an separate study that hematoma volume increase occurs relatively early after symptom onset (<6 hours); after 6 hours, it is unlikely that hematoma volume increase can be detected. Overall, the Brott *et al.*⁶⁰ data and the Japanese studies mentioned above set the stage for the requirement in the rFVIIa trials of delivering treatment within one hour of the baseline CT scan.

What appeared as a more definitive evidence that hematoma expansion in acute ICH is related to negative outcome was developed by Davis *et al.*⁶⁵ These investigators aggregated data of various studies that utilized CT scans over a period of 24 hours after presentation to assess hematoma growth and relate this growth to outcome parameters. The patients in these studies presented within 3 hours of symptom onset and had various several neurological assessments (Glasgow Coma Score, modified Ranking scale, and Barthel Index) during their period of treatment. It should be emphasized that substantial part of the data analyzed by the authors was derived from the placebo group of the rFVIIa phase 2 study (115 out 218 patients), with the remainder of the data mostly derived from the Brott *et al.*⁶⁰ study discussed above. Examining this combined-dataset analysis, one can discern the investigator bias that created the "positive" conclusion. Brott *et al.*⁶⁰ had provided (a) a strong rationale as to what was regarded definitive evidence of hematoma volume increase⁺⁺⁺ and, (b) excluded patients from analysis who

⁺⁺⁺ Hematoma growth was defined as an increase in the volume of intraparenchymal hemorrhage of >33% as measured by image analysis on the 1- or 20-hour CT compared with the baseline CT scan. The conservative number of 33% was chosen prospectively for two reasons. The investigators explained this by stating that a 33% change in the volume of a sphere corresponds to a 10% increase in diameter, a difference that can be seen by the person assessing serial CT scans. More importantly, however, the 33% limit was adopted because of observations that "increases" and "decrease" at below that amount may have been due to different positioning and angles of the CT slice images in the baseline and 1-hour CTs rather than to an actual decrease or increase in hemorrhage volume.

had surgical evacuation of the hematoma; these approaches were not followed by Davis *et al.*⁶⁵ Thus, in a more “relaxed” approach, this group of investigators determined that 72.9% of patients in these combined datasets now showed “some evidence of increase of hemorrhage volume” following admission. If one combines this conceptual increase in patients with hematoma expansion with the fact that the placebo group of the rFVIIa phase 2b study exhibited exceptionally high death rate and poor outcome, the results of the Davis *et al.*⁶⁵ analysis – a definitive link between hematoma volume increase and poor outcome (assessed by death rates, modified Rankin scale and Barthel Index) – was inevitable.

Thus, the evidence for a clinical benefit of an early hemostatic intervention was substantially influenced by the biases of the proponents of this type of intervention. A less biased approach may have found a much weaker relationship, if any at all, similar to the analysis results of Brott *et al.*⁶⁰

Even if one assumes that the decrease in hematoma growth in acute ICH is connected to poor outcome, it is highly questionable if the current tools for assessing outcome, neurological deficit and function (NIHSS, mRS, BI, GOS and others) are capable of discerning the effects of treatment. A review of these endpoints⁶⁶ shows that these tools do not have the sensitivity to discern effects of treatment in most therapeutic interventions and, as they stand, are likely responsible for the continued failure of stroke-directed treatments. Furthermore, it is apparent that the causes of acute ICH have substantial impact on the outcome and balancing groups for these causes is very important in discerning an effect. For example, acute ICH resulting from arteriovenous malformations has higher mortality than other causes as populations studies reveal.⁶⁷ It would be a substantial challenge to diagnose causes and stratify patients accordingly in future studies within the rather tight time-to-treatment requirement of these studies (no more than 4 hours after onset of symptoms).

Overall, pursuing major studies in ICH with the new rFVIIa analogs should be seriously questioned. The whole premise for intervention seems suspect and likely due to certain operating biases; in addition, the current methodology does not appear to allow appropriate balancing of treatment groups by appropriate stratification (mainly on the basis of the conditions

that caused the ICH) and the tools for assessing outcome need further development to become effective in discerning effects of interventions.

One additional question that needs to be asked is that if hemostatic intervention was the best way of limiting the increase of hematoma growth. The INTERACT study is now demonstrating that early control of hypertension is very effective in limiting hematoma growth.⁶⁸

D. Trauma

A number of studies were performed in trauma. These studies are well summarized by Lin *et al.*⁴⁴ although this particular review of rFVIIa studies did not include the design and outcome of the latest CONTROL Phase 3 study in blunt trauma.

1. A Brief Summary of rFVIIa Studies in this Indication

Boffard *et al.*⁶⁹ conducted two studies with rFVIIa in trauma. These multicenter, randomized, double-blind and controlled studies addressed the efficacy and safety of rFVIIa in both blunt and penetrating trauma. The study in blunt trauma accessed 158 patients; 143 patients were enrolled in the penetrating trauma trial. Eligible patients must have sustained the injury <12 hours prior to hospitalization, had a Glasgow Coma Scale score of ≥ 8 , had received < 8 units of packed red blood cells (RPCs) prior to the arrival to the hospital and required a transfusion of at least 6 units of RPCs. In both studies, after consent the patients were randomized to either placebo or rFVIIa treatment. The treatment consisted of an initial infusion of rFVIIa (or placebo) at 200 $\mu\text{g}/\text{kg}$, followed by infusions of 100 $\mu\text{g}/\text{kg}$ at 1 and 3 hours after the initial treatment. As these were regarded mostly exploratory studies, the primary endpoint utilized was the number of RPC units transfused within 48 hours of the initial treatment. Additional endpoints included 30-day mortality, other blood products used, length of hospital stay and adverse events during the 30 days post-treatment.

The results of these studies were uneven. Data were available for analysis in 143/158 patients in blunt trauma and 134/143 in penetrating trauma. There was a statistically significant difference in RBCs infused in the blunt trauma study (a reduction of 2.6 units), but not in penetrating trauma. The same trend was seen for the need for massive infusions (> 20 RBC units). In blunt

trauma, there was a statistically significance difference in that endpoint (14% of rFVIIa versus 33% of placebo patients; $p = 0.03$). No statistical significant difference in the need for massive transfusion was seen in penetrating trauma. Although the investigators claimed that a trend in improvement in 48-hour and 30-day mortality was seen, the data does not really bear this out. For blunt trauma, 48-hour mortality was 18% for placebo and 19% for rFVIIa; 30-day mortality was 30% for placebo and 25% for rFVIIa. For penetrating trauma, 48-hour mortality was 16% for placebo and 17% for rFVIIa; 30-day mortality was 28% in placebo and 24% in rFVIIa. Of the adverse events, the only observed statistically significant difference was in acute respiratory distress syndrome (ARDS) occurrence in blunt trauma (16% in placebo versus 4% in rFVIIa). However, these were exploratory analyses and were not adjusted for multiplicity.

Following these two studies, a phase 3 clinical study (the CONTROL trial) was initiated.⁷⁰ The CONTROL trial, that started in 2005 and terminated in 2008, was a multicenter, randomized, placebo-control, double-blind study. The study utilized two primary endpoints: 30-day mortality and 30-day durable morbidity defined as pulmonary and/or renal dysfunction at day 30. The analysis of these two endpoints was tiered: if superiority could not be established at first for 30-day mortality, the data were tested for non-inferiority in 30-day mortality and superiority in durable morbidity. There were a number of secondary endpoints that included RBC units transfused, other blood products infused (platelets, fresh frozen plasma, cryoprecipitate, fibrinogen concentrate), need for massive transfusion (≥ 10 units of RBCs), days free of organ failure, days in ICU, days of hospitalization and others.

The study was targeted to enroll 1502 subjects with $GCS \geq 5$ in order to detect a 16.7% reduction in mortality with rFVIIa assuming a 30% mortality in the placebo arm for blunt trauma (a lower estimate was utilized for mortality of penetrating trauma bringing the a total assumed placebo mortality to 27.5%). Subject treatment followed the same algorithm as the Boffard *et al.*⁶⁹ exploratory clinical studies.

Although the authors do not clearly state so, two interim blinded analyses of mortality were planned. In the first interim analysis based on data from 447 subjects, it was determined that the mortality was running substantially

lower than assumed (10.8% versus the assumed 27.5%). Thus, the clinical study was terminated for futility. Data from 573 treated patients were available for analysis at the time of discontinuation.

Of the 573 patients analyzed, 468 were admitted with blunt and 86 for penetrating trauma. Examining specifically the data for blunt trauma, 218 placebo and 242 rFVIIa-treated patients were available for analysis. There were no differences in mortality (10.7% for placebo versus 11% for rFVIIa) or durable morbidity (9.5% for placebo versus 8.7% for rFVIIa). Statistically significant differences were seen in allogeneic blood, RBC units and FFP transfusions. No statistically significant differences were seen in the much smaller penetrating trauma population. In adverse events, very much as in the phase 2 study, a post-hoc analysis showed that the ARDS incidence was higher in the placebo group.

2. Development Challenges in Trauma

The problems with the CONTROL study demonstrate the difficulties of performing pivotal studies in this indication. Even with rather aggressive assumptions, the sample sizes are large and enrollment is slow and fraught with issues of informed consent. The CONTROL study enrolled 573 patients in 150 hospitals in 26 different countries over a period of 3 years. Even assuming that at least 30% of this period was consumed by concluding agreements with the participating institutions and obtaining permissions from ethics committees, the targeted enrollment would have required at least 5 years to be reached.

The CONTROL study was terminated early because of much lower than expected mortality. However, the much lower rate of mortality (just over a third of what was projected) should not have been unexpected. According to the investigators of CONTROL, the previous trials by Boffard *et al.*⁶⁹ were utilized for the prediction of the placebo arm 30-day mortality. However, the enrollment criteria were substantially relaxed in the CONTROL study. For example, although eligibility was set at ≥ 8 GCS in the earlier studies, this criterion was lowered to ≥ 5 GCS in CONTROL. Other inclusion/exclusion criteria were also relaxed when compared to those utilized in the exploratory studies. It is peculiar that, after these changes, the investigators expected the same rate of 30-day mortality as in the Boffard *et al.*⁶⁹ studies.

If the exclusion criteria are somewhat tightened to increase mortality in future studies to about 20% in the placebo arm and if the same or slightly higher benefit is assumed (a 16.7 - 20% reduction in mortality), a study with such assumptions, a power of 80% and a two-tailed alpha at 0.05 would require approximately 2000 patients per group. This is a substantial commitment by any organization and it would be based on rather sparse evidence of any benefit in mortality.

Aside from the specific issues with the studies discussed thus far, the major conceptual problem of utilizing rFVIIa in the treatment of trauma is the effect of trauma-related coagulopathy on mortality. Trauma coagulopathy appears to be caused by a variety of endogenous or exogenous factors that include hemodilution, hypothermia, hypoperfusion, acidosis, and disseminated intravascular coagulation.^{71,72} The coagulopathy is mostly defined by the occurrence of abnormal Prothrombin Time (PT), Partial Thromboplastin Time (PTT) or International Normalized Ratio (INR) values. Utilizing these parameters, coagulopathy affects approximately 25% - 35% of trauma victims depending on the threshold values utilized.⁷³ Mortality is greatly elevated in patients with evidence of coagulopathy. Brohi *et al.*^{74,75} showed that patients with abnormally long PT or PTT (PT > 18 sec or PTT > 60 sec) had at very high mortality rate (46 - 62%) when compared to trauma patients with normal PT or PTT values (24- 10%, respectively). McLeod *et al.*,⁷⁶ in large prospective registry study in a Level 1 trauma center, determined that abnormal PTT (>35 sec) affected 28% of trauma patients; this defect increased the risk of mortality by 326%, independent of other conditions. On the other hand, abnormal PT (>14 sec) was noted in only 8% of trauma victims and its effects were very moderate, increasing mortality risk by 35%. Similar findings were published recently by Dirks *et al.*⁷⁷

The studies examining the frequency of coagulopathy in trauma and its implication in increased mortality point the way forward for rFVIIa. If only one in four patients presents acute coagulopathy when admitted, it is reasonable to direct treatment with rFVIIa to these patients. The Boffard *et al.*⁶⁹ studies and the investigators in the phase 3 CONTROL study⁷⁰ have failed to utilize such enrollment criteria. Thus, any further exploration of the role of rFVIIa and the newer rFVIIa analogs in trauma should be limited to patients experiencing substantial coagulopathy and utilize tools such as the

TASH score (Trauma Associated Severe Hemorrhage) which can serve to limit entry to those patients more likely to require massive transfusion.⁷⁸

Therefore, a study that goes beyond a mere tightening up of the entry criteria of the CONTROL study, becomes a serious possibility. By setting eligibility to at least ≥ 8 GCS (as in the Boffard *et al.*⁶⁹ studies) and selecting only patients with demonstrated coagulopathy (PTT > 60 sec) and TASH score >18, it is possible to elevate mortality for the placebo population to at least 40%. In this case, targeting a treatment benefit of a 16.7 - 20% reduction in mortality (the target of the CONTROL study), only 500 - 700 subjects per arm would be required. Such a study may incorporate certain adaptive methodologies⁷⁹ in order to attain higher efficiency.

VI. DISCUSSION AND SUMMARY

In conclusion, the discussion above has shown that the benefits of new and “improved” rFVIIas in hemophilia maybe marginal and difficult to prove in a clinical setting. In addition, defining the effects of coagulopathy in outcomes in various clinical situations and the scope of intervention with rFVIIa remains quite challenging. Although rFVIIa may be able to correct the underlying coagulopathy, it would have limited utility unless the coagulopathy per se contributes significantly to mortality or any lasting/permanent morbidity. This may be so in trauma but not in intracerebral hemorrhage. If the coagulopathy contributes independently to increase mortality or durable morbidity (as seems to be the case in trauma), then, indices for the coagulopathy need to be introduced into the enrollment criteria of the planned studies, a practice that has not been followed thus far in the development of rFVIIa. Studies with better focus on patients likely to profit from treatment may be better able to define the role of rFVIIa in a variety of indications.

Overall, the challenges for the new rFVIIa molecules, especially the modified ones, are substantial. The clinical development process would need to provide a strong and direct evidence that these molecules are safe and can obtain better patient outcomes than the original rFVIIa (NovoSeven).[®] Biosimilars may be able to match the efficacy of these molecules in certain indications by increasing dosing within the appropriate safety margin. If the improved molecules fail to strongly differentiate themselves, it is unlikely that premium pricing can be obtained and the original

rFVIIa and biosimilar molecules should be able to compete effectively based on pricing and availability.

VII. REFERENCES

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