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ORIGINAL ARTICLE

Predicting dosing advantages of factor VIIa variants with altered tissue factor-dependent and lipid-dependent activities

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Summary. *Background:* Recombinant factor VIIa (rFVIIa) is an FX-cleaving coagulation enzyme licensed for the treatment of bleeding episodes in hemophiliacs with inhibitory antibodies. Even though the optimal dosing and comparative dose efficacy of rFVIIa remain poorly understood, genetic or chemical modifications of rFVIIa have been proposed, with the goal of achieving faster and longer hemostatic action. No ongoing trial is currently comparing rFVIIa variants with each other. *Objectives and methods:* We used mathematical modeling to compare the pharmacokinetics, dose–response (pharmacodynamics) and dose-effect duration (pharmacokinetics/pharmacodynamics) of rFVIIa variants to predict their optimal doses. The pharmacodynamic (PD) model of FXa generation by FVIIa in complexes with tissue factor (TF) and procoagulant lipids (PLs) was validated against published *ex vivo* and *in vitro* thrombin generation (TG) experiments. To compare variants' safety profiles, the highest non-thrombogenic doses were estimated from the clinical evidence reported for the licensed rFVIIa product. *Results:* The PD model correctly described the biphasic TF-dependent and PL-dependent dose response observed in TG experiments *in vitro*. The pharmacokinetic/PD simulations agreed with published *ex vivo* TG data for rFVIIa and the BAY 86-6150 variant, and explained the similar efficacies of a single dose of 270 μg kg^{-1} (as reported in the literature) and repeated doses of 90 μg kg^{-1} of unmodified rFVIIa. The duration of the simulated hemostatic effect after a single optimal dose was prolonged for rFVIIa variants with increased TF affinity or extended half-lives, but not for those with modulated PL activity. *Conclusions:* Some modifications of the rFVIIa molecule may not translate into a prolonged hemostatic effect.

Keywords: factor VIIa; hemophilia; pharmacology; thrombin; thromboplastin.

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Introduction

Recombinant factor VIIa (rFVIIa) (NovoSeven; Novo Nordisk A/S, Plainsboro, NJ, USA) is an FX-cleaving blood coagulation enzyme whose proteolytic activity is revealed in complex with either tissue factor (TF) or procoagulant platelet lipids (PLs). In hemophilic patients with inhibitors, rFVIIa is administered at physiologically high doses between 90 μg kg^{-1} (labeled dose in the USA) and 270 μg kg^{-1} (off-label use in the USA), which result in rFVIIa plasma levels 2–8-fold greater than the endogenous level of FVII zymogen. The need for supraphysiologic dosing remains poorly understood, although the low PL-dependent proteolytic activity and the short half-life of rFVIIa (~ 2.5 h) have been proposed as explanations [1].

At least 10 genetically or chemically modified rFVIIa variants with improved FXa-generating activity [2–5] and circulatory half-lives [6–11] have recently been investigated in either preclinical or clinical trials in an attempt to improve treatment by either prolonging the interval between administrations or achieving stable hemostasis after fewer doses (Table 1). Unfortunately, the feasibility of performing the necessary robust clinical trials that might support the clinical superiority claims is limited by the small number of hemophilic patients with inhibitors, variability in individual responses between patients, complexity of bleeding episodes, and safety considerations regarding underdosing and overdosing (reviewed by Retzios [12]). The current prelicensing clinical trials may provide limited data on the relative effectiveness of rFVIIa variant doses, resulting in a dose selection that may not be optimal. Suboptimal dosing may lead to unfavorable conclusions about drug benefits during clinical trials if participants are exposed to uncontrolled bleeding because of

1. **. Results: The PD model correctly described the biphasic TF-dependent and PL-dependent dose respo...**
Anchor Name: dose response *[YYAS (Anja Stampar)]*

2. **Suboptimal dosing may lead to unfavorable conclusions about drug benefits during clinical trials i...**
Anchor Name: Dosing to ensure consistent efficacy
[NTWR (Nimisha Tiwari)]

Table 1 Characteristics of recombinant factor VIIa (rFVIIa) variants in preclinical and clinical development for treatment of bleeding in hemophilic patients with inhibitors

rFVIIa variant	Company	Changed parameters: fold change in comparison with wild-type FVIIa*	References
N7-GP†, glycoPEGylated rFVIIa	Novo Nordisk A/S	5 × half-life 0.5 × activity on TF Higher molecular weight	Ljung *et al.* [10] Sorensen *et al.* [11]
Vatreptacog alfa‡ (formerly NN1731), mutant rFVIIa	Novo Nordisk A/S	Shorter half-life Similar activity on TF 30 × activity on PLs Similar molecular weight	Persson *et al.* [4] Allen *et al.* [5]
7TF-M306D, mutant rFVIIa	Novo Nordisk A/S	2400 × activity on PLs	Nielsen *et al.* [3]
CB 813a, mutant rFVIIa	Pfizer (Cambridge, MA, USA)	5–10 × activity Similar molecular weight	Pittman *et al.* [2]
PF-05280602 (formerly CB 813d), mutant rFVIIa	Pfizer	3 × half-life 5–10 × activity Similar molecular weight	Pittman *et al.* [2]
BAY 86-6150‡ (formerly Bay7), mutant rFVIIa	Bayer (San Francisco, CA, USA)	5 × half-life 4–10 × activity on PLs Similar molecular weight	Mahlangu *et al.* [6]
rVIIa-FP, rFVIIa–albumin fusion	CSL Behring (Marburg, Germany)	6 × half-life 1.6 × affinity for TF 30–40% reduced activity Higher recovery Higher molecular weight	Metzner *et al.* [7]
FVIIa-CTP, rFVIIa–CTP-hCG fusion	OPKO Biologics (Nes Ziona, Israel)	5 × half-life Similar activity Higher recovery Higher molecular weight	Hart *et al.* [8]
FVIIa-XTEN, rFVIIa–scFv and rFVIIa–$XTEN_{288}$ double fusion	Biogen Idec (Cambridge, MA, USA)	8 × half-life Higher activity High affinity for platelets	Tan *et al.* [9]
PEGLip-FVIIa, rFVIIa formulated with PEGylated liposomes	Omri Laboratories (Nes Ziona, Israel)	Same half-life Similar activity Higher recovery Same molecular weight	Spira *et al.* [16]

CTP-hCG, C terminus peptide of human chorionic gonadotropin; PL, procoagulant lipid; TF, tissue factor. *Reported fold changes should be taken with caution. Various studies have utilized different wild-type FVIIa comparator molecules, e.g. plasma-derived FVIIa (NovoSeven) or rFVIIa produced in-house. Some studies did not specify the source of wild-type FVIIa. Although it is usually assumed that all wild-type rFVIIa preparations are the same, their functional properties may be different. Furthermore, differences in purity and functional properties between research-grade and commercial-scale preparations of rFVIIa variants used in various studies can be expected. †The company announced termination of the clinical development program, owing to insufficient efficacy at the tested doses (prophylaxis study). ‡The company announced termination of the clinical development programme, owing to immunogenicity concerns.

underdosing and excessive coagulation because of overdosing.

We speculate that the 25 years of clinical and *in vitro* knowledge on the licensed rFVIIa product can be helpful to formulate expectations about optimal dosing of variants if we translate modified rFVIIa properties into dose-effect predictions. Mathematical modeling was used previously to overcome challenges related to pharmacokinetic (PK) evaluations in another hemophilia treatment, long-acting FIX [13]. Indeed, for FIX products, the duration of the dose effect can be plausibly derived from the PK data, because bleed treatment and prophylaxis have a well-established association with FIX activity in plasma [13]. The approach is not directly applicable to rFVIIa, because the relationship between rFVIIa's plasma concentration and therapeutic effect remains unknown. Therefore, we used the thrombin-generating capacity of rFVIIa as a potential surrogate pharmacodynamic (PD) response, because the thrombin generation (TG) test is routinely incorporated in PK and PD evaluations of rFVIIa [6,14–16] and has been used for experimental dose selection [17].

In this study, we developed and validated a mathematical PK/PD model of rFVIIa-dependent FXa generation in terms of mechanistic equations of protein complex formation, enzymatic activation, inhibition, and decay. Although this approach is not intended to replace animal and clinical investigations, the results obtained provide, for the first time, a translation of rFVIIa modifications into predictions about the duration of therapeutic effect that can be used as a supportive tool for dose evaluation during the design and analysis of clinical trials.

Materials and methods

In vitro *rFVIIa PD model component*

The previously published *in vitro* PD model of rFVIIa-dependent procoagulant activity [18] uses a simplified coagulation cascade defined by only the initial interactions between FVIIa, FVII (in some calculations), FX, FXa, TF, TF pathway inhibitor, antithrombin III, and procoagulant lipid surfaces, and provides the total FXa generation after 40 min. This output parameter was shown to correlate with thrombin peak heights from *in vitro* TG experiments, TG being a commonly accepted measure of the rFVIIa procoagulant effect. All simulations in this study used the model version of FVIII/FIX-deficient platelet-rich plasma, in which activated platelets are replaced with a functionally equivalent level of PL vesicles [19].

Ex vivo *rFVIIa PK model component*

The PK model is a two-compartment, linear PK equation derived from data in a recent experimental PK study of rFVIIa by Morfini *et al*. [20]. Briefly, the mean concentration–time data were extracted with DIGITIZEIT software (version 1.5; ShareIt Inc, Eden Prairie, MN, USA), and fitted to estimate PK constants and parameters with WINNONLIN (version 5.2.1; Pharsight Corporation, Sunnyvale, CA, USA), from the equation:

$$\mathrm{FVIIa}\,(t) = A \cdot e^{-at} + B \cdot e^{-bt}$$

The following parameters were obtained (mean ± standard error): A, 223 ± 25 IU mL^{-1}; B, 86 ± 24 IU mL^{-1}; a, 2.25 ± 0.57 h^{-1}; b, 0.32 ± 0.07 h^{-1}.

For the purpose of this study, the established linearity of the PK model for doses between 17.5 and 70 μg kg^{-1} [21] was extended to 5–350 μg kg^{-1}.

FVIIa PK/PD model: ex vivo *and* in vivo *variations*

The PK/PD model links the PD model [18] and PK model to describe the complete time course of the hemostatic response to a dosage regimen of rFVIIa. The PK component provides the rFVIIa concentration–time course, and the PD component relates the PK concentration to the hemostatic response, defined in the PD model. An *ex vivo* variation describes the experimental situation when the concentration of rFVIIa is maintained in a blood sample once it is removed from the circulatory system, and is therefore not subjected to decay during the 40-min interval necessary for TG assay data acquisition. The *in vivo* variation incorporates an additional equation to correct the PK/PD model output for the rFVIIa decay that continuously occurs *in vivo* during the 40-min acquisition period of the TG response.

To compare the hypothetical rFVIIa variants, the PK/PD model assumes that all variants follow the pharmacokinetics of rFVIIa: 50% recovery of the injected dose [21,22] and two-compartment, linear pharmacokinetics. The PK/PD model equations can be found in the Supporting information (see Section 'Mathematical Model Equations').

Results

Limits of agreement between the PD model and available in vitro *TG data*

We have previously demonstrated that the shape of rFVIIa's TG peak height dose–response curve in FVII-deficient plasma can be predicted from the mechanistic *in silico* PD model of the TF-dependent and PL-dependent FXa-generating activities of rFVIIa [18]. We now further compared this mechanistic *in silico* model with various other experimental studies of rFVIIa-dose dependent TG in hemophilic plasma [23–29]. The absolute values of dose responses, in terms of thrombin peak heights, were discordant between studies (Fig. S1), possibly because of the differences in experimental conditions (Table S1). Nevertheless, the shapes of the experimental dose–response relationships can be compared after normalization (Fig. 1A).

The normalized *in vitro* and *in silico* simulated dose–response curves were comparable in two characteristic features (Fig. 1B, dashed lines): (i) a rapid increase in responses with an rFVIIa concentration of > 25 nM in the presence and absence of TF, which, according to the model, is driven by rFVIIa activity on PL (data not shown); and (ii) a slow, nearly flat response with an rFVIIa concentration of < 25 nM, whose height was proportional to the concentration of TF, as a consequence of rFVIIa activity on TF. Additionally, the lack of dose response seen with an rFVIIa concentration of < 1 nM was masked by the presence of zymogen, because the removal of zymogen from *in silico* calculations revealed a dose–response in this range (Fig. 1B, solid lines).

Overall, the simple mechanistic model predicted the shape of experimental dose responses in hemophilia A plasma within the meaningful pharmacologic range of rFVIIa treatment dosing.

Evaluating rFVIIa modifications with the PD model

The potential effects of TF and PL pathway modifications in rFVIIa variants were simulated by increasing or decreasing the corresponding PD model parameters, e.g. k_{cat} or K_d. In order to fully reveal the effects of TF-dependent FVIIa modifications on the dose–response curve, zymogen was omitted from these calculations. Increases in TF affinity produced an earlier TG response to lower rFVIIa concentrations, with concurrent lengthening of the plateau period (Fig. 2A), whereas increases in the activity on TF above 10-fold produced only a minor

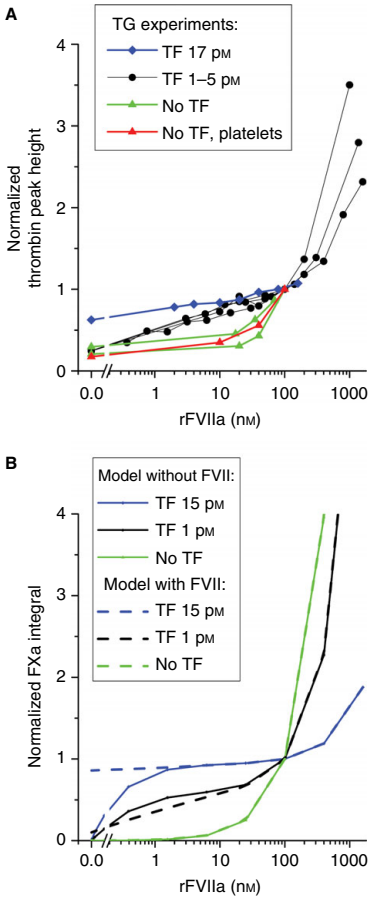

Fig. 1. The relationship between recombinant factor VIIa (rFVIIa) drug concentration and effect (i.e. pharmacodynamics) assessed experimentally and theoretically with various tissue factor (TF) concentrations. (A) Normalized thrombin peak heights observed in FVIII-deficient plasma supplemented with increasing concentrations of rFVIIa. Data were collected from seven publications [23–29] (as described in Table S1) and normalized to the thrombin generation (TG) response at 100 nM rFVIIa (raw data can be found in Fig. S1). (B) Normalized simulations of the *in vitro* pharmacodynamic model in the absence of FVIII and FIX and in the presence of procoagulant lipid (4 μM), TF (0, 1 and 15 pM), and rFVIIa. Solid lines denote simulations in the absence of endogenous FVII, and dashed lines denote simulations in the presence of 10 nM FVII and 0.1 nM FVIIa. Data from papers [23–29] is used with permission of respective publishers.

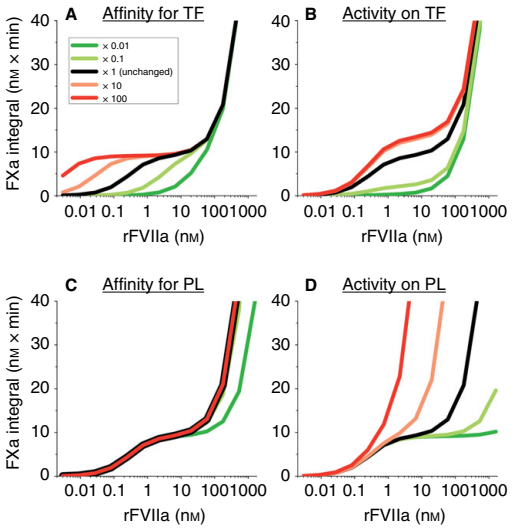

Fig. 2. *In vitro* pharmacodynamic model simulations of the dose–response curves produced by modifying FVIIa's proteolytic activity and affinity for tissue factor (TF) or procoagulant lipid (PL) in the absence of FVII. For each panel, the curves correspond to rFVIIa variants for which the respective constants for FVIIa binding affinity for TF (A), catalytic activity on TF (B), binding affinity for PL (C) and catalytic activity on PL (D) were changed to 0.01-fold (dark green), were changed to 0.1-fold (light green), were unchanged (black), were changed to 10-fold (light red), and were changed to 100-fold (dark red). Simulation conditions are as previously described in Fig. 1 (1 pM TF), but without FVII.

saturating 1.5-fold increase in plateau height (Fig. 2B). Decreases in both TF affinity and activity on TF abolished any TF contribution. In contrast, an increase in PL affinity produced no effect, but a decrease produced a slow rightward shift of the exponential growth portion of the dose–response curve (Fig. 2C). Changing the activity on PL was more effectual: a leftward shift in the exponential growth portion of the PD curve occurred for increased activity, whereas the opposite occurred for decreased activity (Fig. 2D).

Limits of agreement between the ex vivo *PK/PD modeling and* ex vivo *TG data*

To validate the PK/PD model, we simulated three *ex vivo* TG studies for the licensed rFVIIa [14–16] and one for mutant rFVIIa, BAY 86-6150 [6] (Fig. 3, black lines). The *ex vivo* variation of the PK/PD model was used for the simulations. PL and TF concentrations of the respective TG experiments were used for the corresponding simulations to allow the shapes (but not the absolute values) of the experimental and theoretical PK/PD curves to be compared (Fig. 3, red lines).

In the presence of TF, model and experimental TG responses of the licensed rFVIIa declined slowly between 0.5 h and 4–6 h after administration (Fig. 3A,B). In the absence of TF, model and experimental responses declined sharply during the first hour after administration, and thereafter only the experimental response showed an abrupt leveling off of the rate of decline to a

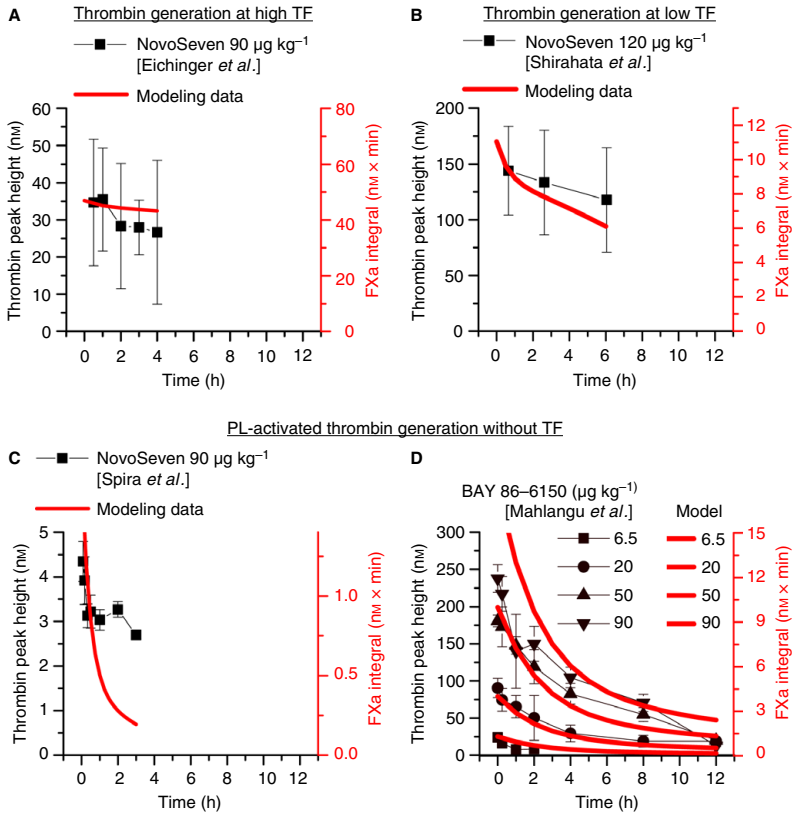

Fig. 3. Validation of the *ex vivo* pharmacokinetic (PK)/pharmacodynamic (PD) model with published experimental data. All *in silico* model lines in (A), (B) and (C) were normalized to the first experimental data point (separately for each panel). Dotted lines (black) show previously published results of thrombin generation test experiments performed on plasma samples collected at the indicated time points after administration of recombinant factor VIIa (rFVIIa) or the BAY 86-6150 variant. Solid lines (red) present FXa integrals obtained in mathematical model simulations in the absence of FVIII and FIX and the presence of FVII (10 nM). Thrombin generation was measured as follows [6,14–16]: (A) platelet-poor plasma (PPP) supplemented with 3.2 μM procoagulant lipid (PL) in the presence of 7.16 pM TF; (B) PPP supplemented with 4 μM PL in the presence of 1 pM tissue factor (TF); (C) PPP supplemented with 4 μM PL in the absence of TF; and (D) PPP supplemented with 4 μM PL in the absence of TF. Model concentrations of PL and TF were taken directly from the experimental data, and all simulations were performed with the *ex vivo* variation of the PK/PD model. Data from papers [6,14–16] is used with permission of respective publishers.

steady plateau (Fig. 3C). It should be noted that the thrombin values in the absence of TF were close to the lower limit of detection of the TG test (< 1% of prothrombin in plasma, ~ 2000 nM), and these experimental TG data may therefore be unreliable.

For the modified highly PL-active rFVIIa molecule BAY 86-6150, experimental and simulated curves showed similarly declining, dose-dependent PK/PD responses (Fig. 3D). Disagreements between the model and experiments were observed at higher doses only; for example, the model overestimated the response at 90 μg kg^{-1}. Furthermore, our PK model had limited agreement with the experimental PK data (Fig. S2), suggesting that BAY 86-6150 does not follow the dose-dependent PK linearity of the unmodified rFVIIa product [21], which was assumed for the model calculations of variants. In order to extend the *ex vivo* PK/PD model to *in vivo* predictions of the hemostatic effect, an additional equation was introduced into the PK/PD model to account for rFVIIa decay. TG responses were predictably slightly lower for the *in vivo* PK/PD model variation than for to the *ex vivo* variation (Fig. S3).

Modeling the comparability of current rFVIIa dosing schemes by ue of the in vivo *PK/PD model*

Multiple clinical studies have tried to compare the efficacy of a single dose of 270 μg kg^{-1} and a repeat dose of

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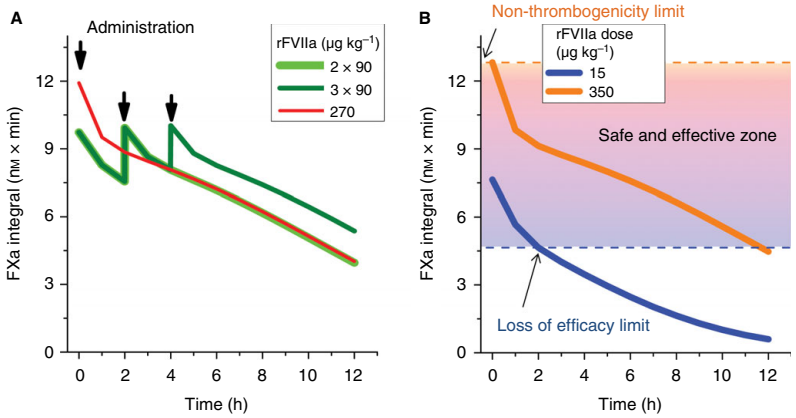

Fig. 4. Simulated *in vivo* pharmacokinetic (PK)/pharmacodynamic (PD) curves corresponding to single and repeated administrations of recombinant factor VIIa (rFVIIa) doses. (A) Comparison of the repeated licensed and single megadose schedules. *In vivo* PK/PD simulations of the licensed dosing corresponding to double or triple 90 μg kg^{-1} doses (every 2 h) are shown as light green and dark green lines, respectively; a 270 μg kg^{-1} megadose is shown in red. (B) Margins of the safe and effective zone were selected on the basis of data from [8–10]. The highest reportedly safe megadose of 350 μg kg^{-1} is shown in orange. The lowest reportedly effective dose of 15 μg kg^{-1} is shown in blue. The safe and effective zone low borderline was defined as the FXa response to an rFVIIa concentration at 2 h after administration of the lowest dose. The upper borderline corresponds to the maximal instantaneous FXa response at the highest rFVIIa dose. Simulations were conducted in the absence of FVIII and FIX, and in the presence of tissue factor (1 pM), procoagulant lipid (4 μM), and FVII (10 nM), with the *in vivo* variation of the PK/PD model. Calculations in the absence of zymogen gave similar results; see Fig. S4.

90 μg kg^{-1}. Our *in vivo* PK/PD modeling produced mostly overlapping dose–response time courses 1–4 h after the initial administration (Fig. 4A), which appears to be consistent with the comparable hemostatic efficacies of the two different administration schedules [30–34].

Mapping a safety–efficacy dosing scale of the licensed rFVIIa to evaluate rFVIIa variants

rFVIIa, characteristically of procoagulant drugs, is subject to a delicate balance between potential thrombotic conditions resulting from overdosing [35] and ineffective hemostatic efficacy resulting from underdosing [36]. Therefore, the dose-effect comparability of rFVIIa variants must be qualified against the maximum safe dose and the minimum efficacious dose of the licensed rFVIIa. In anecdotal or small case series of bleeding patients with hemophilia A, rFVIIa was shown to be hemostatically effective at low doses from 17.5 to 22.5 μg kg^{-1} [37,38], whereas administered doses as high as 346 μg kg^{-1} [39] were reported without thrombotic complications. Furthermore, anecdotal proof-of-concept studies with the plasma-derived FVIIa preparation demonstrated that doses from 9 to 20 μg kg^{-1} are hemostatically active in moderate to severe joint bleeds [40]. Therefore, *in vivo* PK/PD simulations of rFVIIa doses at 15 and 350 μg kg^{-1} were used to set a hypothetical safe and effective zone (Fig. 4B).

The selected upper and lower boundaries of this safe and effective zone do not account for individual variability of patients and bleeds, and should not be viewed as being equally safe and effective as the licensed dose schedule. Rather, these boundaries provide a formal account of the evidence that, in some patients, rFVIIa is effective at very low doses and can be non-thrombogenic at higher-than-licensed doses.

Evaluating the predicted pharmacokinetics/pharmacodynamics of rFVIIa variants

To assess the benefits of half-life, TF or PL affinity, and TF- or PL-dependent proteolytic activity modifications, we developed a set of eight model variants with functionally extreme and distinct characteristics (Table 2) that cover properties of known rFVIIa variants (Table 1). For simplicity, we assumed that doses of chemically or genetically fused multidomain variants are calculated per mass of the FVIIa domain; that is, we disregarded any change in the molecular mass of the variants.

In vivo PK/PD simulations of the hypothetical variants were performed at 90 and 270 μg kg^{-1}, which are common doses for wild-type rFVIIa (Fig. 5, black and red lines). The upper boundary of the safe and effective zone of wild-type rFVIIa was dramatically exceeded by the highly PL-active variants (Fig. 5A,D), regardless of half-life changes. Highly TF-active variants (Fig. 5B,E) and a simple variant with longer half-life (Fig. 5G) only slightly exceeded the simulated safety threshold. On the other hand, the dual TF low-active and PL low-active variant with a longer half-life (Fig. 5H) was below the efficacious boundary for both doses.

1. **Our in vivo PK /PD modeling produced mostly overlapping dose–response time courses 1 – 4 h after th...**
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Table 2 Characteristics of hypothetical simulated recombinant factor VIIa (rFVIIa) variants A–H used for evaluation of rFVIIa effects on the treatment pharmacokinetic/pharmacodynamic (PD) parameters; the pharmacokinetics/pharmacodynamics of variants A–H are shown in Fig. 5A–H

Hypothetical rFVIIa variant	Estimated highest dose* of rFVIIa domain†, μg kg^{-1} (fold relative to wild type)	Duration of protection at the highest dose‡, h (fold relative to wild type)	Protection time per mg of rFVIIa§, h mg^{-1} kg)
Unchanged half-life			
Wild type	350 (× 1.0)	12 (× 1.0)	34
A: high activity on PL (× 20)	22.5 (× 0.1)	4 (× 0.3)	178
B: high activity on TF (× 10)	67.5 (× 0.2)	12 (× 1.0)	178
C: high affinity for TF (× 10)	350 (× 1.0)	18 (× 1.5)	51
Prolonged half-life			
D: long half-life (× 5); high activity on PL (× 10); low activity on TF (× 0.1)	60 (× 0.2)	5 (× 0.4)	83
E: long half-life (× 5); low activity on PL (× 0.1); high activity on TF (× 10)	210 (× 0.6)	77 (× 6.4)	367
F: long half-life (× 5); high affinity for TF (× 10)	240 (× 0.7)	89 (× 7.4)	371
G: long half-life (× 5)	250 (× 0.7)	54 (× 4.5)	216
H: long half-life (× 5); low activity on PL (× 0.2); low activity on TF (× 0.2)	2650 (× 7.6)	15 (× 1.3)	6

*The highest dose was defined as the highest dose that would maintain the PD effect in the safe and effective zone described in Fig. 4. A dose selected in this way will give the longest duration of the PD effect within the safe and effective zone. However, note that the highest dose may not be optimal from the safety point of view if the product is thrombogenic at this dose; †The dose is calculated per unit weight of the rFVIIa domain of fused or modified rFVIIa molecules. ‡Protection time was determined from PD simulations as the longest time interval where the PD curve for the highest dose stayed in the safe and effective zone. §Protection time per mg of rFVIIa domain was calculated as the ratio of the protection time to the highest single dose.

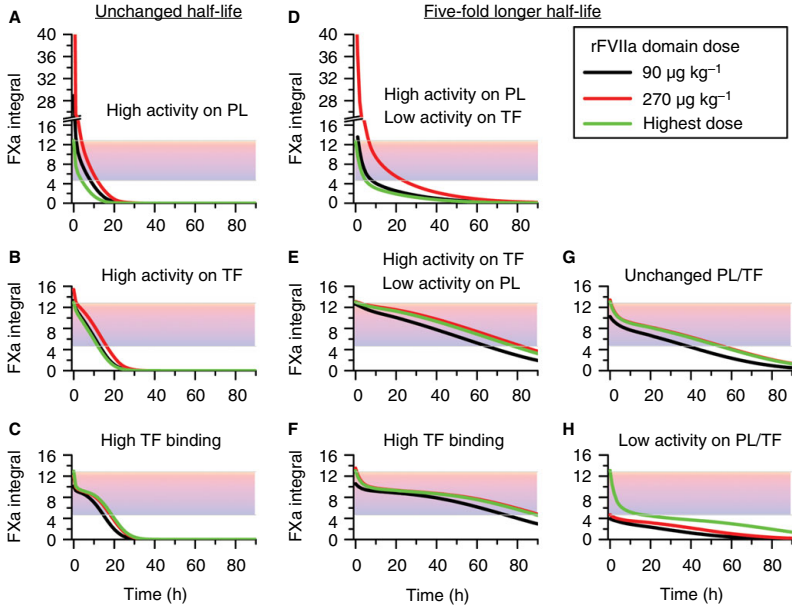

Fig. 5. *In vivo* pharmacokinetic (PK)/pharmacodynamic (PD) modeling of the dosing advantages provided by the hypothetical recombinant factor VIIa (rFVIIa) variants A–H (see Table 2). Simulated *in vivo* PK/PD curves for rFVIIa variants were obtained for three single doses: 90 μg kg^{-1}, the recommended dose for the licensed rFVIIa product (black lines); 270 μg kg^{-1}, an off-label megadose used for the licensed rFVIIa (red lines); the highest possible single dose for each variant that remained within the safe and effective zone, defined as the dose that produces a response that does not exceed the upper safety limit and maximizes the response duration above the lower therapeutic limit (green lines). Shaded areas show the safe and effective zone of the licensed rFVIIa as defined in Fig. 4B. Simulations were performed in the absence of FVIII and FIX, and in the presence of tissure factor (TF) (1 pM), procoagulant lipid (PL) (4 μM), and FVII (10 nM), with the *in vivo* variation of the PK/PD model. Calculations in the absence of zymogen gave similar results; see Fig. S5.

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An additional *in vivo* PK/PD simulation was performed for each hypothetical variant, to identify the highest possible dose whose peak effect would not exceed the safety threshold (Fig. 5, green lines). These doses would provide the safe effect for the longest period of time. In comparison with wild-type rFVIIa (Fig. 4A), increasing PL and TF activity (Fig. 5A,B) alone did not provide prolongation of the dose effect, whereas an increased half-life coupled with either unchanged TF activity (Fig. 5G), higher TF activity (Fig. 5E) or TF binding (Fig. 5F) was the most advantageous.

Discussion

In this study, a mathematical model of the dose–response (PD) and dose-effect duration (PK/PD) was used to compare the licensed rFVIIa product with the investigated rFVIIa variants. Our simulations identified, for each rFVIIa variant, the doses and dosing intervals that might match the safety and efficacy of the licensed rFVIIa drug. We found that variants with increased platelet-dependent activity achieved the same FXa-generating effect at lower doses than the licensed drug, but that the dose effect declined more rapidly after drug administration. In contrast, increased activity on TF or binding to TF can provide a longer duration of action, allowing for longer intervals between doses. These results could assist in planning the optimal dosing regimen for use in human and animal clinical evaluations of the safety and efficacy of new procoagulant rFVIIa-like molecules.

Our mathematical PK/PD model has important assumptions that may or may not limit the predictive value. First, we assume that the hemostatic effect of rFVIIa is determined solely by its effect on TG. Second, the model does not account for platelet activation, receptor exposure, or phospholipid content of phospholipid membranes, because these phenomena have not been shown to significantly modulate the rFVIIa effect on TG. Moreover, prior studies with platelets and other cells have not been performed in a manner that facilitates quantitative comparison with results obtained with PL vesicles. Third, we assume that TG is regulated by rFVIIa-dependent FX activation in complexes with the cofactors TF and PL. This TF-centric and PL-centric nature of our mathematical model does not contradict published evidence on rFVIIa action, and, simultaneously, provides straightforward mechanistic predictions of the dose effect of rFVIIa and its variants. For example, the modest dose–response dependence at pharmacologic rFVIIa concentrations (< 25 nM for a 90 μg kg^{-1} administration) in the presence of TF is explained in the model by high rFVIIa affinity for TF, which results in near saturation of picomolar TF concentrations by nanomolar rVIIa concentrations [41] (Figs 1A and 3A,B). The TF concentration apparently dictates the height and flatness of the dose–response plateau (Fig. 1), which is explained by high TF-dependent proteolytic activity of rFVIIa. Consequently, rFVIIa variants with improved TF binding and activity may provide the boost to low doses of rFVIIa (Fig. 2) and improve the duration of dose effect. These features of TF-dependent rFVIIa action explain the very slow decline in the TG response for up to 6 h after administration, as reported by Eichinger *et al*. [14] and Shirahata *et al*. [15] (Fig. 3A,B). On the other hand, modest rFVIIa activity on platelets in the absence of TF explains the lack of rFVIIa-induced TG with an rFVIIa concentration of < 25 nM *in vitro* (Fig. 1) and the rapid decline in response during the first hour after administration observed by Spira *et al*. [16] (Fig. 3C). Poor affinity prevents saturation of the platelet surface by increasing doses of rFVIIa, explaining the steep decline in the dose response demonstrated for unmodified rFVIIa *in vitro* (Fig. 1, > 25 nM) and by the highly lipid-active BAY 86-6150 variant *ex vivo* (Fig. 3D).

Interestingly, rFVIIa at any concentration *in vitro* provides a continuous dose response (at least, in the absence of the inhibitory effect of FVII zymogen), suggesting that rFVIIa may have a hemostatic effect at any concentration; this *in vitro* dose–response thus provides no evidence about *in vivo* dose success or failure. Therefore, we modeled the clinical evidence collected before and after the licensing of the first rFVIIa drug. Although the rFVIIa recommended license dose is 70–90 μg kg^{-1}, early studies in bleeding hemophiliacs showed hemostatic effects at much lower doses, e.g. 17.5 μg kg^{-1} [38], which is consistent with earlier reports of plasma-derived FVIIa effectiveness at 3–17.5 μg kg^{-1} [40]. However, evidence from hemophilic patients with inhibitors [42] and off-label use in non-hemophilic patients [43] suggests that higher-than-licensed doses can carry an additional risk of thrombosis. As no robust clinical study has evaluated doses above 270 μg kg^{-1}, we decided to use anecdotal evidence of non-thrombogenicity at 345 μg kg^{-1} as the upper margin of the highest effective but not excessively thrombogenic dose. Although arbitrary, the upper and lower limits of the safe and effective zone (Fig. 4B) provide optimistic depictions of the licensed drug's safety and efficacy.

The PK/PD model provides a novel explanation for the clinically observed similar hemostatic effectiveness of the single dose (megadose) at 270 μg kg^{-1} and the repeated dose at 90 μg kg^{-1} [31–34,44]. Simulations showed that the pharmacokinetics/pharmacodynamics for the megadose and two consecutive standard doses mostly overlap, maintaining the effect within the safe and effective zone for 12 h. In addition, the megadose provided a modest increase in simulated effect, suggesting that little gain in efficacy was achieved, which is consistent with the lack of the much desired rapid or more efficacious response in megadose trials (reviewed by Shapiro [45]).

PK/PD simulations of the highly lipid-active variants presented in Fig. 5 and Table 2 suggest little dose-effect advantage and dose-effect prolongation advantage, which

seems to agree with the development history for the NN1731 variant: despite having 10–100-fold higher activity on platelets *in vitro* [4,5], NN1731 had only three-fold to four-fold higher efficacy in a mouse bleeding model [46], and comparable efficacy to NovoSeven at NovoSeven-like doses and administration intervals in phase 2 safety and dose-finding studies [47]. On the other side of the variant spectrum, simulated low-active rFVIIa with a prolonged half-life was less effective than rFVIIa (Fig. 5H), consistent with recent disappointing results [10] for the prophylactic 90 μg kg^{-1} dose of a characteristically similar rFVIIa variant, a glycoPEGylated variant of rFVIIa with a prolonged half-life [10] and reduced proteolytic activity [11] (see Fig. S6 for N7-GP variant simulations). Unfortunately, our theoretical PK/PD predictions for NN1731 and N7-GP cannot be directly verified, because there are no published studies on TG after infusion of these two molecules.

An estimate of the model's predictive value can be potentially derived from studies of hemostatic efficacy in animals. For example, we compared *in vivo* PK/PD simulations with the results of a mouse tail bleeding model of Feng *et al*. [48]. Simulated FXa integrals and the clotting time data [48] demonstrated comparable hemostatic effect for the unmodified and chimeric rFVIIa variant that lacks TF-dependent activity (Fig. S7). These results agree with our previous observations of PL-dependent mechanism dominance at very high rFVIIa concentrations (100–1600 nM) [18], as were used by Feng *et al*., that are achieved immediately after megadose injections. Our *in silico* model predicts a shortened dose-effect duration for megadoses and a reduced efficacy of smaller doses for the TF-inactive variant, because the TF mechanism dominates at low rFVIIa concentrations.

The only modification not reported in the literature, but predicted to provide the highest duration of protection, was the increase in FVIIa affinity for TF. Different affinities for TF were reported for FVII zymogen (three-fold lower [49]) and rFVIIa with an inhibited active site (five-fold higher [50]), suggesting a feasible future for such a modification.

In conclusion, our work provides a tool that can be used for predicting various dosing regimens for the evaluation of rFVIIa variant efficacy. Despite general agreement with available clinical data, the model has important limitations; for example, it is not suitable for the prediction of treatment efficacy for individual patients. In addition, the underlying PK/PD model hypothesis that TG *in vitro* predicts efficacy *in vivo* requires confirmation via clinical trials.

Addendum

A. M. Shibeko developed the mathematical model and conducted theoretical simulations. I. Mahmood developed the PK model. N. Jain contributed to the analysis of clinical relevance. A. M. Shibeko and M. V. Ovanesov wrote the manuscript with assistance from S. A. Woodle and N. Jain. M. V. Ovanesov supervised the project and the preparation of the manuscript.

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Disclosure of Conflict of Interests

The authors state that they have no conflict of interest.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Fig. S1. Experimental PD dose–response curves under static conditions.
Fig. S2. Experimental and simulated pharmacokinetics of BAY 86-6150 variant.
Fig. S3. Comparison of *in vivo* and *ex vivo* PD models describing experimental data presented in Fig. 3 of the main text.
Fig. S4. The effect of FVII zymogen on the simulated PK/PD curves after a single administration or repeated administrations of rFVIIa.
Fig. S5. Comparison of the action of FVIIa variants in the presence or absence of FVII zymogen.
Fig. S6. Predicting PK/PD responses of rFVIIa-GP under treatment conditions described in Ljung *et al*.
Fig. S7. *In vivo* PK/PD model predictions for wild-type rFVIIa and the TF-independent rFVIIa chimeric variant described by Feng *et al*.
Table S1. Description of experimental studies presented in Fig. S1.
Table S2. Description of mathematical model variants used for calculation of figures.
Section 2. Mathematical Model Equations.

References

1 Hoffman M, Monroe DM III. The action of high-dose factor VIIa (FVIIa) in a cell-based model of hemostasis. *Semin Hematol* 2001; **38**: 6–9.

[no notes on this page]

2 Pittman D, Weston S, Shields K, Parng C, Arkin S, Madison E, Nichols TC, Fruebis J. A novel FVIIa variant with increased potency and duration of effect compared to wildtype FVIIa. A study in a dog model of hemophilia A. *Blood* 2011; **118**: 2252.

3 Nielsen AL, Ruf W, Holmberg HL, Buchardt J, Sorensen B, Olsen OH, Ostergaard H. Design of a potent phospholipid membrane-dependent factor VIIa variant. *J Thromb Haemost* 2013; **11**: 237.

4 Persson E, Kjalke M, Olsen OH. Rational design of coagulation factor VIIa variants with substantially increased intrinsic activity. *Proc Natl Acad Sci USA* 2001; **98**: 13583–8.

5 Allen GA, Persson E, Campbell RA, Ezban M, Hedner U, Wolberg AS. A variant of recombinant factor VIIa with enhanced procoagulant and antifibrinolytic activities in an *in vitro* model of hemophilia. *Arterioscler Thromb Vasc Biol* 2007; **27**: 683–9.

6 Mahlangu JN, Coetzee MJ, Laffan M, Windyga J, Yee TT, Schroeder J, Haaning J, Siegel JE, Lemm G. Phase I randomized, double-blind, placebo-controlled, single-dose escalation study of the recombinant factor VIIa variant BAY 86-6150 in hemophilia. *J Thromb Haemost* 2012; **10**: 773–80.

7 Metzner HJ, Weimer T, Schulte S. Half-life extension by fusion to recombinant albumin. *Therapeutic Proteins: Strategies to Modulate Their Plasma Half-Lives*. Weinheim: Wiley-VCH Verlag GmbH & Co. KGaA, 2012; 223–47.

8 Hart G, Hershkovitz O, Bar-Ilan A, Seligsohn U, Fima E. Factor VIIa-CTP, a novel long-acting coagulation factor, displays a prolonged hemostatic effect and augmented pharmacokinetics and pharmacodynamics following IV and SC administration in hemophilic animal models. *Blood* 2012; **120**: 1114.

9 Tan S, Salas J, Chen K, Kistanova E, Ashworth T, Acosta M, Johnson B, Pape B, Pierce GF, Light D, Mei B, Schellenberger V, Peters RT, Jiang H. A platelet-targeted factor VIIa.XTEN fusion protein with increased circulating half-life and improved clotting activity. *J Thromb Haemost* 2013; **11**: 586.

10 Ljung R, Karim FA, Saxena K, Suzuki T, Arkhammar P, Rosholm A, Giangrande P. 40K glycoPEGylated, recombinant FVIIa: 3-month, double-blind, randomized trial of safety, pharmacokinetics, and preliminary efficacy in hemophilia patients with inhibitors. *J Thromb Haemost* 2013; **11**: 1260–8.

11 Sorensen BB, Kjalke M, Zopf D, Bjorn SE, Stennicke HR. Platelet-dependent activity of GlycoPEGylated rFVIIa. *Blood (ASH Annual Meeting Abstracts)* 2007; **110**: 3140.

12 Retzios AD. The New and Improved (?) Activated Factor VII Molecules. http://adrclinresearch.com/Issues_in_Clinical_Research_links/The%20New%20Factor%20VIIas_18May2011.pdf. Accessed 18 June 2014.

13 Collins PW, Moss J, Knobe K, Groth A, Colberg T, Watson E. Population pharmacokinetic modeling for dose setting of nonacog beta pegol (N9-GP), a glycoPEGylated recombinant factor IX. *J Thromb Haemost* 2012; **10**: 2305–12.

14 Eichinger S, Lubsczyk B, Kollars M, Traby L, Zwiauer K, Gleiss A, Quehenberger P, Kyrle PA. Thrombin generation in haemophilia A patients with factor VIII inhibitors after infusion of recombinant factor VIIa. *Eur J Clin Invest* 2009; **39**: 707–13.

15 Shirahata A, Fukutake K, Mimaya J, Takamatsu J, Shima M, Hanabusa H, Takedani H, Takashima Y, Matsushita T, Tawa A, Higasa S, Takata N, Sakai M, Kawakami K, Ohashi Y, Saito H. Results of clot waveform analysis and thrombin generation test for a plasma-derived factor VIIa and X mixture (MC710) in haemophilia patients with inhibitors – phase I trial: 2nd report. *Haemophilia* 2013; **19**: 330–7.

16 Spira J, Plyushch O, Zozulya N, Yatuv R, Dayan I, Bleicher A, Robinson M, Baru M. Safety, pharmacokinetics and efficacy of factor VIIa formulated with PEGylated liposomes in haemophilia A patients with inhibitors to factor VIII – an open label, exploratory, cross-over, phase I/II study. *Haemophilia* 2010; **16**: 910–18.

17 Dargaud Y, Lienhart A, Negrier C. Prospective assessment of thrombin generation test for dose monitoring of bypassing therapy in hemophilia patients with inhibitors undergoing elective surgery. *Blood* 2010; **116**: 5734–7.

18 Shibeko AM, Woodle SA, Lee TK, Ovanesov MV. Unifying the mechanism of recombinant FVIIa action: dose dependence is regulated differently by tissue factor and phospholipids. *Blood* 2012; **120**: 891–9.

19 Butenas S, Branda RF, van't Veer C, Cawthern KM, Mann KG. Platelets and phospholipids in tissue factor-initiated thrombin generation. *Thromb Haemost* 2001; **86**: 660–7.

20 Morfini M, Jimenez-Yuste V, Eichler H, Fischer R, Kirchmaier CM, Scharling B, Bjerre J. Pharmacokinetic properties of two different recombinant activated factor VII formulations. *Haemophilia* 2012; **18**: 431–6.

21 Lindley CM, Sawyer WT, Macik BG, Lusher J, Harrison JF, Baird-Cox K, Birch K, Glazer S, Roberts HR. Pharmacokinetics and pharmacodynamics of recombinant factor VIIa. *Clin Pharmacol Ther* 1994; **55**: 638–48.

22 Agerso H, Tranholm M. Pharmacokinetics and pharmacodynamics of rFVIIa and new improved bypassing agents for the treatment of haemophilia. *Haemophilia* 2012; **18**: 6–10.

23 Turecek PL, Varadi K, Keil B, Negrier C, Berntorp E, Astermark J, Bordet JC, Morfini M, Linari S, Schwarz HP. Factor VIII inhibitor-bypassing agents act by inducing thrombin generation and can be monitored by a thrombin generation assay. *Pathophysiol Haemost Thromb* 2003; **33**: 16–22.

24 Ovanesov MV, Panteleev MA, Sinauridze EI, Kireev DA, Plyushch OP, Kopylov KG, Lopatina EG, Saenko EL, Ataullakhanov FI. Mechanisms of action of recombinant activated factor VII in the context of tissue factor concentration and distribution. *Blood Coagul Fibrinolysis* 2008; **19**: 743–55.

25 Klintman J, Astermark J, Berntorp E. Combination of FVIII and by-passing agent potentiates *in vitro* thrombin production in haemophilia A inhibitor plasma. *Br J Haematol* 2010; **151**: 381–6.

26 van Veen JJ, Gatt A, Bowyer AE, Cooper PC, Kitchen S, Makris M. The effect of tissue factor concentration on calibrated automated thrombography in the presence of inhibitor bypass agents. *Int J Lab Hematol* 2009; **31**: 189–98.

27 Nakatomi Y, Nakashima T, Gokudan S, Miyazaki H, Tsuji M, Hanada-Dateki T, Araki T, Tomokiyo K, Hamamoto T, Ogata Y. Combining FVIIa and FX into a mixture which imparts a unique thrombin generation potential to hemophilic plasma: an *in vitro* assessment of FVIIa/FX mixture as an alternative bypassing agent. *Thromb Res* 2010; **125**: 457–63.

28 Livnat T, Martinowitz U, Zivelin A, Rima D, Kenet G. A highly sensitive thrombin generation assay for assessment of recombinant activated factor VII therapy in haemophilia patients with an inhibitor. *Thromb Haemost* 2011; **105**: 688–95.

29 Martinowitz U, Livnat T, Zivelin A, Kenet G. Concomitant infusion of low doses of rFVIIa and FEIBA in haemophilia patients with inhibitors. *Haemophilia* 2009; **15**: 904–10.

30 Chambost H, Santagostino E, Laffan M, Kavakli K. Real-world outcomes with recombinant factor VIIa treatment of acute bleeds in haemophilia patients with inhibitors: results from the international ONE registry. *Haemophilia* 2013; **19**: 571–7.

31 Kavakli K, Makris M, Zulfikar B, Erhardtsen E, Abrams ZS, Kenet G. Home treatment of haemarthroses using a single dose regimen of recombinant activated factor VII in patients with haemophilia and inhibitors. A multi-centre, randomised, double-blind, cross-over trial. *Thromb Haemost* 2006; **95**: 600–5.

32 Santagostino E, Mancuso ME, Rocino A, Mancuso G, Scaraggi F, Mannucci PM. A prospective randomized trial of high and standard dosages of recombinant factor VIIa for treatment of

[no notes on this page]

hemarthroses in hemophiliacs with inhibitors. *J Thromb Haemost* 2006; **4**: 367–71.
33 Pan-Petesch B, Laguna P, Mital A, Stanley J, Torchet MF, Salek SZ, Salaj P. Single-dose (270 microg kg^{-1}) recombinant activated factor VII for the treatment and prevention of bleeds in haemophilia A patients with inhibitors: experience from seven European haemophilia centres. *Haemophilia* 2009; **15**: 760–5.
34 Young G, Shafer FE, Rojas P, Seremetis S. Single 270 microg kg^{-1}-dose rFVIIa vs. standard 90 microg kg^{-1}-dose rFVIIa and APCC for home treatment of joint bleeds in haemophilia patients with inhibitors: a randomized comparison. *Haemophilia* 2008; **14**: 287–94.
35 Lin Y, Stanworth S, Birchall J, Doree C, Hyde C. Use of recombinant factor VIIa for the prevention and treatment of bleeding in patients without hemophilia: a systematic review and meta-analysis. *CMAJ* 2011; **183**: E9–19.
36 Shapiro AD, Gilchrist GS, Hoots WK, Cooper HA, Gastineau DA. Prospective, randomised trial of two doses of rFVIIa (NovoSeven) in haemophilia patients with inhibitors undergoing surgery. *Thromb Haemost* 1998; **80**: 773–8.
37 Harrison JF, Fegan C, Dasani H, Bloom AL. Clinical effect of recombinant human activated factor VII (rVIIa NOVO). *Br J Haematol* 1991; **77**: 3 V008.
38 Macik BG, Lindley CM, Lusher J, Sawyer WT, Bloom AL, Harrison JF, Baird-Cox K, Birch K, Glazer S, Roberts HR. Safety and initial clinical efficacy of three dose levels of recombinant activated factor VII (rFVIIa): results of a phase I study. *Blood Coagul Fibrinolysis* 1993; **4**: 521–7.
39 Parameswaran R, Shapiro AD, Gill JC, Kessler CM. Dose effect and efficacy of rFVIIa in the treatment of haemophilia patients with inhibitors: analysis from the Hemophilia and Thrombosis Research Society Registry. *Haemophilia* 2005; **11**: 100–6.
40 Hedner U, Bjoern S, Bernvil SS, Tengborn L, Stigendahl L. Clinical experience with human plasma-derived factor VIIa in patients with hemophilia A and high titer inhibitors. *Haemostasis* 1989; **19**: 335–43.
41 Butenas S, Brummel KE, Bouchard BA, Mann KG. How factor VIIa works in hemophilia. *J Thromb Haemost* 2003; **1**: 1158–60.
42 Baudo F, Collins P, Huth-Kuhne A, Levesque H, Marco P, Nemes L, Pellegrini F, Tengborn L, Knoebl P. Management of bleeding in acquired hemophilia A: results from the European Acquired Haemophilia (EACH2) Registry. *Blood* 2012; **120**: 39–46.
43 Logan AC, Yank V, Stafford RS. Off-label use of recombinant factor VIIa in US hospitals: analysis of hospital records. *Ann Intern Med* 2011; **154**: 516–22.
44 Auerswald G, Muntean W, Kemkes-Matthes B, Klamroth R, Krause M, Kurnik K, Oldenburg J, Pabinger-Fasching I, Schramm W, Zimmermann R, Zotz RB. [Relevance of a single dose of 270 microg/kg recombinant factor VIIa for the treatment of patients with haemophilia and inhibitors – recommendations from the GTH experts]. *Hamostaseologie* 2009; **29**: 197–203.
45 Shapiro AD. Single-dose recombinant activated factor VII for the treatment of joint bleeds in hemophilia patients with inhibitors. *Clin Adv Hematol Oncol* 2008; **6**: 579–86.
46 Tranholm M, Kristensen K, Kristensen AT, Pyke C, Rojkjaer R, Persson E. Improved hemostasis with superactive analogs of factor VIIa in a mouse model of hemophilia A. *Blood* 2003; **102**: 3615–20.
47 de Paula EV, Kavakli K, Mahlangu J, Ayob Y, Lentz SR, Morfini M, Nemes L, Salek SZ, Shima M, Windyga J, Ehrenforth S, Chuansumrit A. Recombinant factor VIIa analog (vatreptacog alfa [activated]) for treatment of joint bleeds in hemophilia patients with inhibitors: a randomized controlled trial. *J Thromb Haemost* 2012; **10**: 81–9.
48 Feng D, Whinna H, Monroe D, Stafford DW. FVIIa as used pharmacologically is not TF dependent in hemophilia B mice. *Blood* 2014; **123**: 1764–6.
49 Bach R, Gentry R, Nemerson Y. Factor VII binding to tissue factor in reconstituted phospholipid vesicles: induction of cooperativity by phosphatidylserine. *Biochemistry* 1986; **25**: 4007–20.
50 Sorensen BB, Persson E, Freskgard PO, Kjalke M, Ezban M, Williams T, Rao LV. Incorporation of an active site inhibitor in factor VIIa alters the affinity for tissue factor. *J Biol Chem* 1997; **272**: 11863–8.

[no notes on this page]