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ORIGINAL ARTICLE

Phase I, randomized, double-blind, placebo-controlled, single-dose escalation study of the recombinant factor VIIa variant BAY 86-6150 in hemophilia

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Summary. *Background:* BAY 86-6150 is a new human recombinant factor VIIa variant developed for high procoagulant activity and longer action in people with hemophilia with inhibitors. *Objectives:* To investigate the safety, tolerability, pharmacodynamics, pharmacokinetics and immunogenicity of BAY 86-6150 in non-bleeding hemophilia subjects. *Methods:* The study included non-bleeding men (18–65 years of age) with moderate or severe hemophilia A or B with or without inhibitors. Sixteen subjects were randomized 3 : 1 to four cohorts of escalating doses of BAY 86-6150 (6.5, 20, 50 or 90 μg kg^{-1} [n = 3 per cohort]) or placebo (n = 1 per cohort); an independent data-monitoring committee reviewed previous cohort data before the next dose escalation. Blood sampling was performed predose and postdose; subjects were monitored for 50 days postdose. *Results:* At the tested doses, BAY 86-6150 was not associated with clinically significant adverse events or dose-limiting toxicities. BAY 86-6150 pharmacokinetics exhibited a linear dose response, with a half-life of 5–7 h. Subjects demonstrated consistent, dose-dependent thrombin generation ex vivo in platelet-poor plasma (PPP) (mean peak effect, 26–237 nM thrombin from 6.5 to 90 μg kg^{-1}). Peak thrombin levels over time paralleled BAY 86-6150, with thrombin kinetics appearing to be slightly shorter; thus, circulating BAY 86-6150 retained activity. There were corresponding decreases in activated partial thromboplastin and prothrombin times. No subject developed de novo anti-BAY 86-6150 neutralizing antibodies during the 50-day follow-up. *Conclusions:* In this first-in-human, multicenter, randomized, double-blind, placebo-controlled, single-dose escalation study, BAY 86-6150 was tolerated at the highest dose (90 μg kg^{-1}), with no safety concerns. Safety and efficacy will be further evaluated in phase II/III studies.

Keywords: hemophilia, inhibitors, pharmacokinetics/pharmacodynamics, recombinant factor VIIa variant, safety, tolerability.

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Introduction

The standard for treatment of bleeding episodes in people with hemophilia A or B with inhibitors to factor VIII or FIX is the use of bypassing agents such as recombinant activated FVII (rFVIIa) [1,2]. In this setting, rFVIIa has several important limitations; these include an inconsistent response of bleeding episodes to treatment with rFVIIa, its short half-life, and its relatively low procoagulant activity [2,3]. The reasons for inconsistent intrapatient and interpatient responses to treatment remain largely unknown; however, suboptimal thrombin generation may contribute to some treatment failures [4–6]. The short 2–3-h half-life of rFVIIa [2,3,7,8] necessitates repeated multiple dosing to control acute bleeding episodes, a practice that is both time-consuming and inconvenient. The relatively low procoagulant activity of exogenous rFVIIa could in part be attributable to competition with endogenous FVII for binding to tissue factor [9], as well as the intrinsically weak activity of the enzyme in the

absence of tissue factor and its inactivation by tissue factor pathway inhibitor [10].

The mechanism of action of rFVIIa includes the tissue factor-dependent and tissue factor-independent pathways. In the latter, rFVIIa binds to activated platelets, with consequent generation of activated FX on the surfaces of these activated platelets, whose downstream effect is thrombin generation and clot formation [11,12]. Therefore, there is an unmet need to develop a treatment for inhibitor subjects with a favorable pharmacokinetic profile and improved hemostatic properties.

Several approaches have been explored to create a new generation of rFVIIa products with improved pharmacokinetic and therapeutic properties. These include PEGylation and protein fusion technology, as well as rational and targeted protein design. In the development of rFVIIa variants, amino acid changes were made in the wild-type rFVIIa that resulted in conformational change without loss of hemostatic properties [13]. In vitro and preclinical studies indicate that these rFVIIa variants have improved thrombin generation potential, resulting in reduced blood loss in a mouse model [14,15]. BAY 86-6150 is a novel rFVIIa variant with four amino acid changes (P10Q, K32E, A34E, and R36E). These changes were introduced to increase binding to activated platelets, and they lead to increased tissue factor-independent activation of FX and consequently improved procoagulant activity [16]. BAY 86-6150 has two additional mutations (T106N and V253N) that introduce two more N-linked glycans, which may increase the circulating half-life of BAY 86-6150 as compared with wild-type rFVIIa [17]. In preclinical (mouse) studies and in vitro studies, BAY 86-6150 has been shown to be well tolerated, to have increased procoagulant activity and to have an increased half-life as compared with currently available rFVIIa products [16,18].

The objectives of this phase I, first-in-human, randomized, placebo-controlled, double-blind dose escalation study were to evaluate the safety and tolerability of BAY 86-6150 in non-bleeding men with hemophilia A or B with or without inhibitors to FVIII or FIX. The pharmacokinetic and pharmacodynamic profiles, potential immunogenicity and effects of BAY 86-6150 on hemostasis markers and coagulation tests in men with hemophilia were also assessed.

Materials and methods

Subjects

The study protocol and all amendments were reviewed and approved by the independent ethics committee or institutional review board of each participating study site. Subjects (or their legally authorized representatives) were required to provide written informed consent and be willing to comply with the requirements of the study protocol.

This study included men aged 18–65 years with a history of moderate or severe congenital hemophilia A or B with or without inhibitors to FVIII or FIX. Eligible individuals were required to have adequate venous access, be able to discontinue factor replacement during the study (unless required for the treatment of an acute bleeding episode), and be willing to use effective contraception until day 30 postdose. Subjects were excluded if they received any procoagulant or antifibrinolytic therapy within 5 days of study drug administration or if they were planning to do so during the study. Other exclusion criteria were as follows: the presence of coagulation disorders other than hemophilia A or B; an acute bleeding episode or any ongoing bleeding episode at any time within 7 days of study drug administration; history of cancer, angina, disseminated intravascular coagulopathy, stage 2 hypertension, transient ischemic attack, stroke, myocardial infarction, coronary artery disease, congestive heart failure, or thromboembolic event; thrombocytopenia (platelets $< 100\,000$ mm^{-3}); levels of alanine aminotransferase, aspartate aminotransferase or serum creatinine that were more than two-fold higher than the limits of the local reference range; or human immunodeficiency virus infection with an absolute CD4 lymphocyte cell count of < 400 cells mm^{-3}.

Study design and procedures

This randomized, double-blind, placebo-controlled, single-dose escalation study in non-bleeding subjects was conducted between January and December 2009 at four hemophilia treatment centers (two in South Africa and one each in Poland and the UK). Within each of four cohorts ($n = 4$ per cohort; total study, $N = 16$), subjects were randomized 3 : 1 to receive BAY 86-6150 or placebo. An unblinded pharmacist (or authorized designee) dispensed BAY 86-6150 or placebo according to a computer-generated randomization scheme; syringes of study drug were labeled by the study pharmacist to maintain blinding. Each single-dose treatment was administered via slow intravenous infusion over 2–5 min. Subjects were assigned to one of four BAY 86-6150 single-dose cohorts (6.5, 20, 50 or 90 μg kg^{-1}), starting with the lowest dose. The planned sample size was 16 subjects; however, four additional subjects could be added at each dose level if required by the dose escalation guidance rules (as determined by an independent Data Safety Monitoring Board [DSMB] for a maximum sample size of 32). The DSMB, as well as an unblinded monitor, had access to unblinded data. Subjects were monitored for 50 days postdose.

Enrollment started with the recruitment of cohort 1 (6.5 μg kg^{-1} BAY 86-6150). For any given dose, escalation to the next higher dose proceeded if the DSMB did not identify safety and tolerability concerns after reviewing safety data, especially for dose-limiting toxicity (DLT), which was defined as any adverse event of grade 3 or more based on the Common Terminology Criteria for Adverse Events (CTCAE) version 3.0 (except for changes in activated partial thromboplastin time [APTT] or any confirmed thrombotic event [e.g. deep vein thrombosis or pulmonary embolism]). The following dose escalation rules guided the DSMB: (i) if none of three subjects receiving BAY 86-6150 at a given dose developed DLT, the dose was to be increased for the next cohort; and (ii) if one of

three subjects at a given dose developed DLT, four additional subjects were to be enrolled at the same dose (3 : 1; BAY 86-6150/placebo). If additional DLTs were not observed in this expanded cohort, the dose was to be increased; if two or more of six subjects developed DLT, dose escalation was halted; and (iii) if two or more of three subjects at a given dose developed DLT, dose escalation or expansion was halted. Dose escalation was also halted, pending review by the DSMB, if any patient developed deep vein thrombosis, pulmonary embolism, or any other confirmed thrombotic event (e.g. myocardial infarction or stroke).

It is recognized that no specific hemostasis marker has been shown to correlate with efficacy or safety outcomes upon treatment with rFVIIa, and the mechanism of action of BAY 86-6150 is believed to be identical to that of rFVIIa. Hence, hemostasis markers could not be prespecified for decisions regarding dose escalation or cessation. However, quantitative changes in these markers in relation to predose status were carefully evaluated, and could be used by the DSMB to guide decisions regarding dose escalation.

Safety and immunogenicity assessments

The primary objective of the study was to evaluate the safety and tolerability of BAY 86-6150 in non-bleeding men with hemophilia. The safety evaluation included the frequency, duration and causality of adverse events recorded from the screening period through study day 30; serious adverse events were monitored through day 50. Adverse events were coded with the CTCAE, version 3.0 [19]. Further evaluation included clinical laboratory assessments consisting of complete blood count, urinalysis, clinical chemistry, markers of hemostasis, and immunogenicity. Blood samples for evaluating anti-BAY 86-6150 antibody level, BAY 86-6150 neutralizing activity and FVIIa inhibitor titer were collected on study day 1 (before dosing) and on days 30 and 50 (± 5 days). Samples were analyzed for neutralizing activity only if anti-BAY 86-6150 antibodies were detectable on study day 30 or 50.

Anti-BAY 86-6150 antibodies were evaluated by use of an ELISA, with a microtiter plate coated with BAY 86-6150 protein; cynomolgus polyclonal antibody was used as a surrogate positive control. During assay validation, the assay cutoff value was established to yield a 5% positive rate ($n = 50$ samples in four runs). Predose and postdose samples were analyzed on the same ELISA plate; postdose samples were compared with the sample-specific floating cutoff value if it was lower than the assay cutoff value. Positive samples were further evaluated for specificity to rFVIIa (eptacog alfa) and BAY 86-6150 by adding excess rFVIIa or BAY 86-6150 to the sample to deplete the antibody. Subsequent signal reduction would indicate specific antibodies; no signal change suggested a false-positive. A confirmed positive sample was titrated to estimate the antibody titer. Samples were analyzed at a 1 : 50 dilution, so titers of > 50 were reported.

To measure the neutralizing activity of the BAY 86-6150 antibody, serial dilutions of plasma samples into FVII-depleted plasma were made, and positive and negative controls in FVII-depleted plasma were prepared with or without rabbit anti-FVIIa polyclonal antibody, respectively. BAY 86-6150 and phospholipid vesicles were added to the sample, and this was followed by incubation for 30 min at 24 °C before recalcification. Clotting time was determined when the optical density at 340 nm increased by 0.1 above baseline. The clotting time results were calculated as a ratio between postdose and predose values. When this ratio was greater than the sample-specific cutoff, that dilution was reported as the neutralizing titer.

Pharmacokinetic and pharmacodynamic assessments

Blood samples for pharmacokinetic and pharmacodynamic evaluation were collected at study day 1 within 15 min before dosing and at 15 min (± 2 min), 1, 2, 4, 8 and 12 h (± 15 min) and 24 and 48 h (± 30 min) postdose. BAY 86-6150 plasma levels were plotted as a function of time. Pharmacokinetic parameters were calculated with the model-independent (compartment-free) method and WinNonLin 4.1.a software (Pharsight Corporation, Sunnyvale, CA, USA). BAY 86-6150 plasma levels were assessed with a quantitative ELISA that discriminated between FVII and FVIIa (only detecting FVIIa via the active site), and plotted as a function of time. Pharmacokinetic parameters included area under the concentration vs. time curve from time 0 to time t after administration ($AUC_{0–TN}$), elimination half-life ($t_{1/2}$), clearance (Cl), maximum observed plasma concentration (C_{max}), time to C_{max}, apparent volume of distribution (V_{ss}), and mean residence time (MRT). Pharmacodynamic assessments included ex vivo thrombin generation assay (TGA), prothrombin time (PT), and APTT, and ELISA measurement of fibrinogen, antithrombin III, thrombin–antithrombin (TAT) complex, D-dimer and prothrombin fragment 1 + 2 levels. The TGA was performed on a Calibrated Automated Thrombogram System (Thrombinoscope BV, Maastricht, the Netherlands) in citrated platelet-poor plasma with 50 μg mL^{-1} corn trypsin inhibitor, using synthetic phospholipids (phosphatidylserine/phosphatidylcholine/phosphatidylethanolamine 20 : 40 : 40 [Avanti Polar Lipids, Alabaster, AL, USA]) and calcium as initiator, S9625 (Sigma-Aldrich, St Louis, MO, USA) substrate, and thrombin calibrator (Diagnostica Stago, Asnières sur Seine, France) as calibrator, with peak thrombin values being reported.

Statistical analysis

In accordance with the study protocol, four subjects were enrolled in each dose cohort, and the safety and tolerability analyses were performed on data from all subjects who received a dose of study treatment. Clinical pharmacology analyses were performed on data from all subjects who completed the study. Because of the low planned number of subjects in each cohort ($n = 4$), descriptive statistics were used to summarize all demographic, safety, tolerability, pharmacokinetic and pharmacodynamic outcomes by dose cohort. Changes in pharma-

cokinetic parameters across doses were assessed with an explorative analysis of variance.

Results

Patient characteristics

Of the 21 subjects with hemophilia who were screened, 16 met the eligibility criteria and were randomized into the four sequential dose cohorts, and five were screen failures. Each dose cohort included four subjects at a 3 : 1 randomization ratio of BAY 86-6150 to placebo. All 16 subjects completed the study and were included in the safety, pharmacokinetic and pharmacodynamic analyses.

Baseline demographic and hemophilia disease characteristics were generally similar across the four dose cohorts (Table 1). The majority of subjects (n = 11, 69%) were black (mean age, 30 years; range, 21–57 years), had hemophilia A (n = 14, 88%), and had severe disease (n = 14, 88%). On the basis of the blood samples obtained on study day 1 before dosing, four of the subjects (25%) had a history of inhibitors and had high-titer inhibitors at the time of study entry.

Safety and tolerability

Treatment-emergent adverse events were experienced by six (38%) subjects (BAY 86-6150, n = 4; placebo, n = 2) (Table 2); the most common was bleeding (BAY 86-6150, n = 3; placebo, n = 2). Of the three subjects receiving BAY 86-6150 who experienced a treatment-emergent bleeding incident as an adverse event, one receiving 50 μg kg^{-1} had an episode of right ankle joint bleeding (CTCAE grade 1) beginning 17 days after dosing, one receiving 90 μg kg^{-1} had an episode of hemarthrosis of the right knee (CTCAE grade 2) beginning 20 days after dosing, and one receiving 90 μg kg^{-1} had two episodes of right ankle joint bleeding (CTCAE grade 1) beginning 15 and 22 days after dosing, respectively. No other adverse event was recorded in more than one patient in any cohort, and the overall incidence of adverse events was similar across treatment cohorts. One patient receiving 90 μg kg^{-1} developed a swollen right arm (peripheral edema; CTCAE grade 1) unrelated to study drug occurring 37 days postdosing that resolved after 4 days. Of the treatment-emergent adverse events, two (both non-hemorrhagic) were considered to be possibly related to study drug: a single occurrence of trace of protein in the urine (CTCAE grade 1; no evidence of hemoglobin or red blood cells in the sample) that occurred 32 days postdose and resolved without any treatment seen as necessary after 14 days in a patient receiving 90 μg kg^{-1} BAY 86-6150 (this patient's predose urine sample also contained a trace amount of protein); and headache (CTCAE grade 2) in a patient randomized to placebo. No deaths or study withdrawals occurred, and there were no serious adverse events or CTCAE grade ≥ 3 adverse events. Ten subjects (BAY 86-6150, n = 7; placebo, n = 3) had clinical laboratory values above 1.5-fold the upper limit of normal. With placebo, one subject each had: elevated eosinophils, γ-glutamyl aminotransferase (GGT), and alanine aminotransferase (ALT) (all observed predose and postdose); elevated GGT (observed predose and postdose) and ALT (observed postdose); and elevated monocytes (observed postdose) and ALT (observed predose and postdose). With 6.5 μg kg^{-1} BAY 86-6150, one subject each had: elevated basophils, absolute basophil count, monocytes, and red cell distribution width (all observed postdose); elevated eosinophils, lymphocytes, and red cell distribution width (all observed postdose); and elevated GGT (observed predose and postdose) and monocytes (observed postdose). With 20 μg kg^{-1} BAY 86-6150, one subject had elevated GGT and monocytes (observed postdose) and one subject had elevated GGT and ALT (both observed predose and postdose). With 50 and 90 μg kg^{-1} BAY 86-6150, respectively, one subject had ele-

Table 1 Patient demographics and baseline characteristics

		BAY 86-6150				
	Placebo (n = 4)	6.5 μg kg^{-1} (n = 3)	20 μg kg^{-1} (n = 3)	50 μg kg^{-1} (n = 3)	90 μg kg^{-1} (n = 3)	Total (N = 16)
Mean age (years)	29.5	30.3	31.0	38.7	22.0	30.3
Race, *n*						
Black	2	3	2	3	1	11
White	1	0	1	0	1	3
Asian	1	0	0	0	1	2
Hemophilia type, *n*						
A	4	2	2	3	3	14
B	0	1	1	0	0	2
Hemophilia severity, *n*						
Severe	4	3	2	3	2	14
Moderate	0	0	1	0	1	2
Inhibitor status, *n*						
Negative	4	1	2	2	3	12
Positive	0	2	1	1	0	4
BMI (kg m^{-2}), mean ± SD	25.3 ± 3.9	22.7 ± 6.1	23.1 ± 5.0	25.4 ± 4.1	23.9 ± 1.5	24.2 ± 3.9

BMI, body mass index; SD, standard deviation.

Table 2 Occurrence of adverse events (AEs)

	Subjects with any AE, *n* (%)*				
		BAY 86-6150			
	Placebo ($n = 4$)	6.5 μg kg^{-1} ($n = 3$)	20 μg kg^{-1} ($n = 3$)	50 μg kg^{-1} ($n = 3$)	90 μg kg^{-1} ($n = 3$)
Pretreatment	1 (25)	0 (0)	0 (0)	0 (0)	1 (33)
Treatment-emergent	2 (50)	0 (0)	0 (0)	1 (33)	3 (100)
Fever	0 (0)	0 (0)	0 (0)	0 (0)	1 (33)
Influenza-like illness	1 (25)	0 (0)	0 (0)	0 (0)	0 (0)
Hemorrhage	2 (50)	0 (0)	0 (0)	1 (33)	2 (67)
Upper airway infection	0 (0)	0 (0)	0 (0)	0 (0)	1 (33)
Peripheral edema	0 (0)	0 (0)	0 (0)	0 (0)	1 (33)
Proteinuria	0 (0)	0 (0)	0 (0)	0 (0)	1 (33)
Headache	1 (25)	0 (0)	0 (0)	0 (0)	0 (0)
Muscle pain	1 (25)	0 (0)	0 (0)	0 (0)	0 (0)
Treatment-related	1 (25)	0 (0)	0 (0)	0 (0)	1 (33)
Headache	1 (25)	0 (0)	0 (0)	0 (0)	0 (0)
Proteinuria	0 (0)	0 (0)	0 (0)	0 (0)	1 (33)

*Denominators for each AE occurrence are $n = 4$ for placebo (i.e. the total number of subjects receiving placebo) or $n = 3$ for BAY 86-6150 (i.e. the number of subjects receiving BAY 86-6150 per dose cohort).

vated ALT (observed predose) and one subject had elevated GGT (observed postdose). None of these elevated laboratory values was considered to be clinically significant by the investigator. No DLT precluded the progression to complete evaluation of the highest dose of BAY 86-6150 (90 μg kg^{-1}).

Immunogenicity

Evidence of apparent anti-FVIIa immunoreactivity was confirmed in one patient who had specific anti-FVIIa immunoreactivity that could be competed out with excess amounts of both BAY 86-6150 and rFVIIa (eptacog alfa). This anti-FVIIa antibody was present in this patient before and after dosing with BAY 86-6150, and the antibody titers remained unchanged after dosing. A test for neutralizing activity of these antibodies slightly exceeded the positive cutoff value in the two samples postdose, but the different detection limits for binding (3 μg mL^{-1}) and neutralizing (24 μg mL^{-1}) antibodies to FVIIa prevented determination of neutralizing activity in predose samples. Thus, in this study, there is no evidence of subjects developing de novo neutralizing antibodies in response to BAY 86-6150 exposure during the study or in the follow-up period (up to 50 days postdose).

Pharmacokinetic and pharmacodynamic outcomes

Pharmacokinetic parameters for BAY 86-6150 are shown in Table 3. After dosing, the C_{max} of BAY 86-6150 increased in a dose-dependent manner, with similar rates of elimination across all doses (Fig. 1). BAY 86-6150 was detectable for up to 4 h postdose at 6.5 μg kg^{-1}, for up to 12 h at 20 μg kg^{-1}, and for up to 24 h at doses of 50 and 90 μg kg^{-1}. AUC_{0-TN} and C_{max} dose-dependently increased across the dose range. As expected, no clear dose dependence was seen for pharmacokinetic parameters related to elimination and distribution ($t_{1/2}$, Cl, V_{ss}, and MRT), owing to limited measurability of BAY 86-6150 following the two lowest dose levels. The $t_{1/2}$ of BAY 86-6150 determined from the two higher dose levels ranged from 5 to 7 h.

Table 3 BAY 86-6150 pharmacokinetic parameters*

	BAY 86-6150			
	6.5 μg kg^{-1} ($n = 3$)	20 μg kg^{-1} ($n = 3$)	50 μg kg^{-1} ($n = 3$)	90 μg kg^{-1} ($n = 3$)
AUC_{0-TN} (ng h mL^{-1})	246.5 (55.9)	1374 (14.8)	4689 (23.6)	8354 (25.2)
C_{max} (ng mL^{-1})	83.7 (14.5)	261.2 (29.7)	702.8 (12.4)	1412 (24.1)
$t_{1/2}$ (h)	3.5 (36.5)	5.5 (1.4)	6.7 (9.5)	5.8 (10.9)
Cl (mL h^{-1})	1231 (38.3)	826.1 (13.4)	735.0 (17.3)	764.7 (40.9)
V_{ss} (mL)	6355 (20.9)	6624 (12.1)	6606 (14.7)	5824 (24.4)
MRT (h)	5.2 (34.5)	8.0 (1.8)	9.0 (15.1)	7.6 (17.3)

AUC_{0-TN}, area under the curve for time 0 to time t after administration; Cl, clearance; C_{max}, maximum observed plasma concentration; MRT, mean residence time; $t_{1/2}$, elimination half-life; V_{ss}, apparent volume of distribution.
*Data are the mean (percentage coefficient of variation).

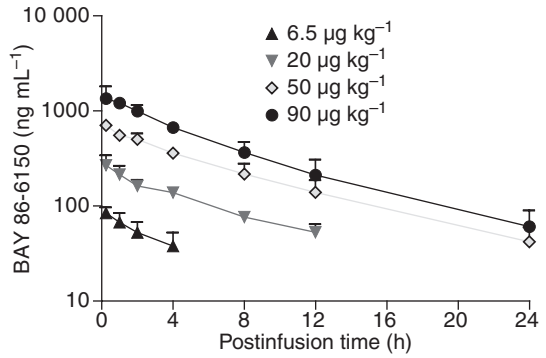

Fig. 1. Postinfusion BAY 86-6150 mean plasma concentrations (geometric mean ± standard deviation). $n = 3$ per treatment group.

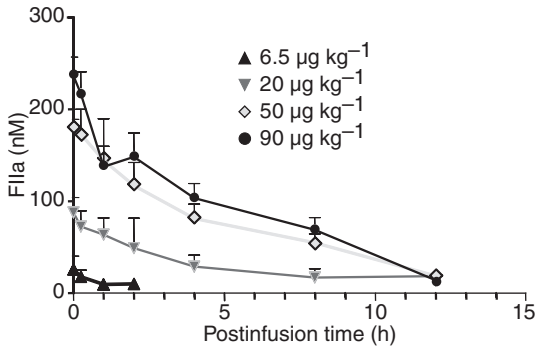

Fig. 2. Peak thrombin generation for BAY 86-6150 (geometric mean ± standard deviation). Sample size: $n = 3$ for all groups.

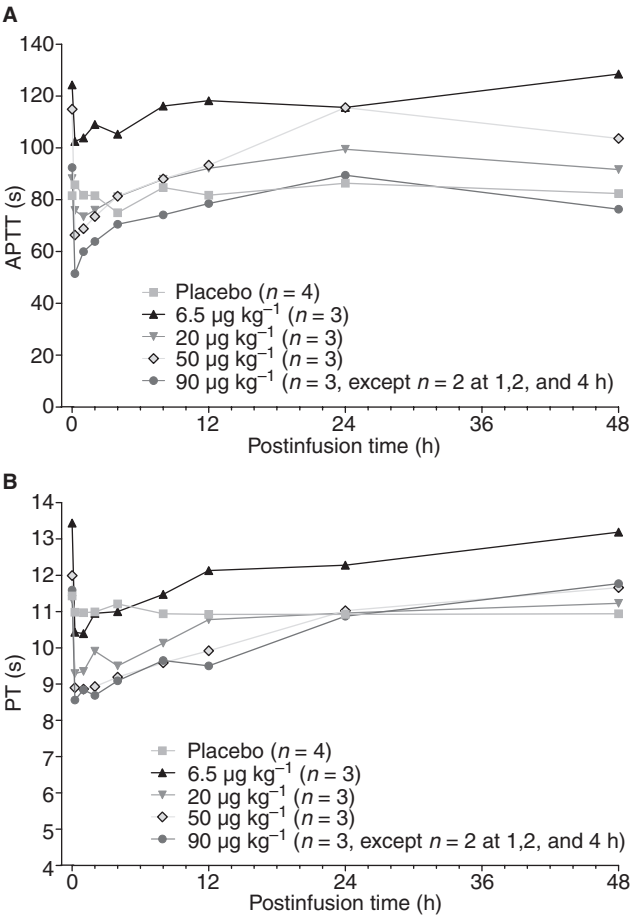

Fig. 3. Mean activated partial thromboplastin time (APTT) (A) and mean prothrombin time (PT) (B) for BAY 86-6150. Sample size: placebo, $n = 4$; 6.5–50 µg kg^{-1}, $n = 3$; 90 µg kg^{-1}, $n = 3$ at all points except 1, 2 and 4 h, where $n = 2$.

There was dose-dependent thrombin generation ex vivo as measured by the TGA in PPP (Fig. 2). Peak thrombin (FIIa) levels over time (mean peak effect, 26–237 nM from 6.5 to 90 µg kg^{-1}) paralleled the presence of BAY 86-6150, as determined by pharmacokinetic analysis (Figs 1 and 2). A decrease in clotting time in both the APTT (Fig. 3A) and PT (Fig. 3B) assays, peaking at 15 min postinfusion, was also observed. No effect was seen on fibrinogen levels. Although several abnormal hemostasis marker levels were observed, there was no relationship with dose or even with whether BAY 86-6150 or placebo was administered; subjects with abnormal levels were observed for D-dimer (above the upper limit of normal [232 ng mL^{-1}] before and/or after dosing: placebo, $n = 2$ subjects; BAY 86-6150, $n = 6$ subjects), prothrombin fragment 1 + 2 (above the upper limit of the reference range [325 pM] after dosing: placebo, $n = 1$; BAY 86-6150, $n = 4$), antithrombin III (below the lower limit of normal [83%]: placebo, $n = 1$ after dosing; BAY 86-6150, $n = 5$ before and/or after dosing), and TAT complex (above the upper limit of normal [4.1 µg L^{-1}] before and/or after dosing: placebo, $n = 4$; BAY 86-6150, $n = 12$).

One of the three subjects treated with a dose of 90 µg kg^{-1} had increases in TAT complex (predose, 3.8 µg L^{-1}; postdose peak, 25.4 µg L^{-1} at 2 h postdose), prothrombin fragment 1 + 2 (predose, 111 pM; postdose peak, 245 pM at 2 h postdose), and D-dimer (predose, 172 ng mL^{-1}; postdose peak, 370 ng mL^{-1} at 1 day postdose). However, although antithrombin III levels were initially below the lower limit of normal, there was no corresponding decrease after dosing (predose, 60.9%; postdose range, 61.5–83.8%).

Post hoc analyses showed significant correlations between BAY 86-6150 and thrombin generation levels ($r = 0.89$; $P < 0.0001$) and APTT and thrombin generation levels ($r = -0.7348$; $P < 0.0001$).

Discussion

This first-in-human, phase I, double-blind, placebo-controlled, dose escalation study investigated the safety, tolerability and pharmacokinetic and pharmacodynamic parameters of a novel rFVIIa variant, BAY 86-6150, in a cohort of 16 non-bleeding subjects with hemophilia A or B with or without inhibitors. No enrolled and/or dosed subjects withdrew from the study; therefore, this report includes all data collected in the study.

BAY 86-6150 was well tolerated in all dose cohorts, with no serious adverse events being reported. Only one subject exposed to BAY 86-6150 developed an adverse event (trace of protein in urine) that was considered to be possibly related to the study drug. However, as this was also seen in the same subject at the screening visit, it is not possible to confirm the causal relationship between BAY 86-6150 and the proteinuria. Importantly, the proteinuria was not associated with persistent renal or other organ dysfunction, and it resolved within 14 days. Treatment-emergent bleeding events, which were deemed to be unrelated to exposure to BAY 86-6150, were recorded in three subjects receiving BAY 86-6150 at between 15 and 22 days after dosing, and in two subjects given placebo. No DLTs were detected with up to 90 μg kg^{-1} BAY 86-6150. None of the subjects developed clinically evident thrombosis up to 50 days after exposure to BAY 86-6150. The significance of the results in the one subject with a decrease in antithrombin III and an increase in TAT complex requires further exploration.

The plasma concentrations of BAY 86-6150 were dose-dependent in the dose range 6.5–90 μg kg^{-1}. The $t_{1/2}$ of BAY 86-6150 was 5–7 h, which is approximately two to three times longer than that of the currently available rFVIIa product [7]. The longer $t_{1/2}$ of BAY 86-6150 has the potential to improve care by decreasing the frequency of infusion and making prophylaxis a practical possibility.

There was also a correlation between BAY 86-6150 pharmacokinetics and TGA results, in that the generation of thrombin was dose-dependent and paralleled the BAY 86-6150 pharmacokinetic levels. This result indicates that, in the bleeding setting, BAY 86-6150 is expected to initiate clot formation and stop bleeding when used as a bypassing agent. Correspondingly, all subjects randomized to BAY 86-6150 demonstrated a consistent, dose-dependent decrease in APTT and PT, beginning at 15 min after dosing. Although the kinetics of thrombin generation seem to be shorter than those of BAY 86-6150, it should be noted that sampling for the TGA was not possible in some subjects at various time points, owing to the paucity of leftover plasma specimens, potentially complicating interpretation of these data. Specifically, one subject receiving 20 μg kg^{-1} BAY 86-6150 had no ex vivo TGA samples for all time points, one subject receiving 90 μg kg^{-1} had no TGA samples for the 1-h and 2-h time points, a second subject receiving 90 μg kg^{-1} did not have a sample for the 24-h time point, and the third subject receiving 90 μg kg^{-1} did not have a sample for the 15-min and 24-h time points. Decreases in clotting time were greater and more persistent in subjects receiving 50 and 90 μg kg^{-1} than in those receiving lower doses. The effect of BAY 86-6150 on APTT indicates that BAY 86-6150 may act, in part, through a phospholipid-dependent pathway.

None of the subjects in the current study developed neutralizing inhibitors upon exposure to BAY 86-6150. This finding is consistent with the known profile of the currently available rFVIIa product [20,21], as well as the profile of BAY 86-6150 observed in preclinical studies [16]. It is encouraging to note that, in the single subject with pre-existing anti-FVIIa antibodies, the profile remained unchanged after exposure to BAY 86-6150. The clinical significance of this observation remains uncertain, and will be monitored closely in the subsequent phases of the BAY 86-6150 development program.

Conclusions

The favorable safety, pharmacokinetic and pharmacodynamic profiles of BAY 86-6150 in this phase I first-in-human study indicates that it has the potential to improve the treatment of bleeding episodes in people with hemophilia. Furthermore, no potential safety concerns were identified with BAY 86-6150 in the studied treatment groups. There is no evidence for the development of de novo neutralizing antibodies in response to BAY 86-6150 exposure. The clinical significance of pre-existing anti-BAY 86-6150 antibody remains uncertain. The longer $t_{1/2}$ of BAY 86-6150 than of wild-type rFVIIa could reduce the dosing frequency and permit the use of prophylaxis regimens. Future clinical trials will compare the efficacy, tolerability and safety of BAY 86-6150 in acutely bleeding subjects with hemophilia with inhibitors.

Addendum

J. N. Mahlangu: lead study investigator and enrolled a majority of the study subjects – he was involved in the oversight of the trial, provision of subjects and data acquisition during the trial, in the analysis and interpretation of all analyses, and in the preparation and finalization of the manuscript; M. J. Coetzee, M. Laffan, J. Windyga, and T. T. Yee: oversight of the trial, provision of subjects and data acquisition during the trial, analysis and interpretation of all analyses, and preparation and finalization of the manuscript; J. Schroeder: design and oversight of the trial, analysis and interpretation of all analyses, and preparation and finalization of the manuscript; J. Haaning: design of the trial, analysis and interpretation of all analyses, and preparation and finalization of the manuscript; J. E. Siegel: analysis and interpretation of all analyses, and preparation and finalization of the manuscript; G. Lemm: design and oversight of the trial, analysis and interpretation of all analyses, preparation and finalization of the manuscript. All authors approved submission of the final version of the manuscript.

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Disclosure of Conflict of Interests

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