Consistent clinical factor VIII equivalency is unlikely for non-factor therapies in hemophilic mice

Thibaud Sefiane,[1*] Geneviève McCluskey,[1*] Marie Clavel,[2] Hortense Maynadié,[1,3] Ivan Peyron,[1] Tovo David,[4] Camille Brochier,[5] François Saller,[1] Mariem Khamari,[1] Cécile V. Denis,[1,6] Olivier D. Christophe,[1] Peter J. Lenting,[1] Vincent Muczynski[1,7#] and Caterina Casari[1#]

[1]Université Paris-Saclay, Institut National de la Santé et de la Recherche Médicale, Hémostase Inflammation Thrombose U1176, 94276, Le Kremlin-Bicêtre, France; [2]Inovarion, Paris, France; [3]Centre de Référence Hémophilie, Hôpital Bicetre, AP-HP, Université Paris-Saclay, Le Kremlin-Bicetre, France; [4]F. Hoffmann-La Roche Ltd., Basel, Basel-Stadt, Switzerland; [5]Institut Roche, Boulogne-Billancourt, France; [6]Centre Hospitalier Régional Universitaire Nancy, Nancy, France and [7]University College London – Cancer Institute, London, UK

TS and GM contributed equally as first authors.
#VM and CC contributed equally as senior authors.

Correspondence: P. J. Lenting
peter.lenting@inserm.fr

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Abstract

Non-factor therapies are changing the treatment paradigm in hemophilia A, which was previously dominated by replacement-therapy using factor VIII (FVIII) concentrates. However, the FVIII equivalence of these new therapies has remained unclear, since *in vitro* assays generate variable responses. Here we used four different *in vivo* bleeding models to compare FVIII to emicizumab and to a sequence-identical analog of the tissue factor pathway inhibitor-targeting antibody marstacimab (SIA-marstacimab). The severity of these models was variable, each requiring different doses of FVIII to reduce blood loss to levels of wild-type mice. For example, a dose of 2.5 IU/kg FVIII was needed for full correction in the tail vein transection (TVT) model, whereas 25 IU/kg was needed in the saphenous vein puncture (SVP) model. Intermediate doses were required in the tail artery transection (TAT) model (5 IU/kg) and tail clip model (7.5 IU/kg). Importantly, FVIII treatment produced stable clots, without spontaneous rebleeds being observed. Both emicizumab and SIA-marstacimab (used at therapeutic doses of 55 μg/mL and 16 μg/mL, respectively) displayed a variable, model-dependent FVIII equivalence. For example, emicizumab proved equivalent to 5 IU/kg FVIII in the tail clip model, and to 10 IU/kg in the SVP model. Strikingly, both emicizumab and SIA-marstacimab treatment resulted in spontaneous rebleeds in the TVT, TAT and tail clip models, further distinguishing them from FVIII treatment. Our data suggest that there is unlikely to be a single FVIII equivalence for emicizumab, SIA-marstacimab, and similar molecules, because their activity is dependent on local conditions and severity of the injury.

Introduction

The clinical management of hemophilia A has experienced major advances over the past decade.[1] In particular, the development of non-factor therapies has changed the treatment paradigm, which was previously dominated by factor VIII (FVIII)-replacement therapy. One example is emicizumab, a bispecific antibody that binds both the enzyme FIXa and its substrate FX, thereby mimicking part of the FVIIIa function.[2-4] Emicizumab allows, therefore, the generation of a certain amount of thrombin without completely correcting coagulation. Clinical studies have shown marked therapeutic benefit of emicizumab, with average annual bleeding rates for treated bleeds being below 2 among patients with and without inhibitors treated with emicizumab prophylactically.[5] Because of its efficacy and its subcutaneous mode of application, increasing numbers of hemophilia A patients with and without inhibitors are using emicizumab as prophylactic therapy.[6] A second example concerns the strategy to restore hemostasis by interfering with anticoagulant molecules, such as tissue factor pathway inhibitor (TFPI). Several anti-TFPI monoclonal antibodies are currently at an advanced stage of clinical evaluation, including concizumab and marstacimab, and promising data have been reported with distinctly reduced bleeding complications in both inhibitor and non-inhibitor

hemophilia A and B patients.[7-9]

One issue that is much debated concerning non-factor therapies is how they compare to FVIII in terms of mode of action and, perhaps more importantly, activity (referred to as FVIII equivalence). One way to compare these non-factor agents to FVIII is to test them in FVIII-activity assays, like one-stage clotting assays, chromogenic FVIII-activity assays, and global hemostasis assays (thrombin generation assays, rotational thrombo-elastometry). However, a different FVIII equivalence has been found for emicizumab when using these different activity assays.[10-12] Indeed, as its mode of action differs from that of FVIII, it is unlikely that *in vitro* assays (which are optimized to measure FVIII activity) could provide an absolute FVIII equivalence for emicizumab and, by extension, to other non-factor therapies.

Here, we explored *in vivo* testing of non-factor molecules emicizumab and a sequence-identical analog of marstacimab (SIA-marstacimab) to evaluate whether or not an absolute FVIII equivalence exists for these agents in this setting. To this end, we used several different bleeding models in which both molecules at their therapeutic concentration (55 μg/mL for emicizumab and 16 μg/mL for SIA-marstacimab) were compared to FVIII for their capacity to correct bleeding in FVIII-deficient mice. We noticed that depending on the bleeding model used, emicizumab and SIA-marstacimab displayed a different FVIII equivalence. These observations indicate that the existence of a single FVIII equivalence for individual non-factor molecules is unlikely. Conceivably, their activity is guided by local conditions and severity of the injury.

Methods

Ethics statement

All experiments were performed using FVIII-deficient mice[13] which were backcrossed (>10 times) on a C57Bl/6J background and their wild-type (wt) littermates. Male and female mice were used throughout the study (8-12 weeks old, 20-25 g). Animal housing and experiments were performed in accordance with French regulations and the experimental guidelines of the European Community. This project was approved by the local ethical committee of Université Paris-Saclay (Comité d'Ethique en Experimentation Animale n. 26, protocols APAFIS#26333-2020070214599234-v1, APAFIS#26510-202007061525281-v2, APAFIS#38228-2022 08021331789-v2 and APAFIS #37499-2022052712138879-v3).

Proteins

Recombinant FVIII (Advate) was obtained from Takeda France (Paris, France). Emicizumab (marketed as Hemlibra) was obtained from F. Hoffmann-La Roche (Basel, Switzerland). Sequence identical analog (SIA)-marstacimab was produced via transient expression in HEK293F cells, and purified to homogeneity via protein A-affinity chromatography. This antibody has similar affinity for human and murine TFPI.[14]

Tail vein transection model

For the tail vein transection (TVT) procedure, mice were anesthetized with isoflurane and a precise cut of a lateral caudal vein with the support of a template was performed as described.[15] After vein transection, the tail was immediately immersed in physiological saline, prewarmed at 37°C. Where indicated, occlusive clots were dislodged twice during the observation period (at 15 and 30 minutes [min] after transection) using a humidified compress to reinitiate bleeding. Clot removal has been introduced in this model to mimic a potential physical challenge to which the tail of the mice can be exposed when these mice can move freely.[15] Animals were sacrificed by cervical dislocation at the end of the assay (at 45 min).

Tail artery transection model

For the tail artery transection (TAT) model, a similar approach was used as that for the TVT procedure, except that the template was used to transect the tail artery. In addition, the observation time was limited to 30 min. At 15 min after transection, occlusive clots were dislodged using a humidified compress to reinitiate bleeding. Animals were sacrificed by cervical dislocation at the end of the assay.

Tail clip model

Mice were anesthetized using ketamine (100 mg/kg)/xylazine (10 mg/kg). The terminal 3 mm of the tail was severed, and the tail was immersed in pre-warmed physiological saline (37°C). Blood was collected for 30 min. Animals were sacrificed by cervical dislocation at the end of the assay.

Saphenous vein puncture model

The saphenous vein puncture (SVP) model was performed as described elsewhere with mice being under ketamine (100 mg/kg)/xylazine (10 mg/kg) anesthesia.[16] Under observation with an operating microscope, the saphenous vein was exposed and the initial injury was inflicted by puncturing the saphenous vein with a 23-gauge needle. When hemostasis occurred, the injury was enlarged to 1 mm using micro-scissors. Subsequently, clots were dislodged using a humidified compress to reinitiate bleeding. The total number of clots formed (hemostatic events) within a 30 min period was recorded. In this model, higher numbers of reforming clots correspond to better hemostatic capacity.

Measuring blood loss

The mixture of collected blood and physiological saline was centrifuged at 1,500 g for 20 min. The red blood cell pellet was then lysed in H_2O and the amount of hemoglobin was determined by reading the absorbance at 416 nm. The volume of blood loss in each sample was calculated from

a standard curve, which was obtained by lysing defined volumes of mouse blood (20, 40, 60, 80, and 100 μL) in H_2O to extract hemoglobin.

Bleeding profile

For the TVT, TAT, and tail clip models, periods of bleeding and non-bleeding were noted in order to visualize the bleeding pattern.

Treatments

Factor VIII was given via retro-orbital injection 5 min before injury. Dosages were as follows: TVT model 0, 2.5, and 5.0 IU/kg; TAT model: 0, 2.5, 5.0, and 10 IU/kg; tail clip model: 0, 2.5, 5.0, 7.5, and 10 IU/kg; SVP model: 0, 2.0, 10, and 25 IU/kg. These doses result in FVIII plasma levels being (2 x IU/kg)/dL (e.g., 10 IU/kg results in 20 IU/dL), within a 10% margin. SIA-marstacimab was given as a single intravenous bolus (retro-orbital) at a dose of 1 mg/kg 5 min before injury, allowing for plasma levels at the start of the procedures to be approximately 16 μg/mL.[17] Emicizumab was given as a single intravenous bolus (retro-orbital) at a dose of 5 mg/kg 24 hours before injury, allowing for plasma levels at the start of the procedures to be approximately 55 μg/mL.[18] Since emicizumab does not react with murine factors IX and X, human factors IX and X were given intravenously 5 min before injury at a dose of 100 U/kg each.[18] Plasma levels of SIA-marstacimab (16 μg/mL) and emicizumab (55 μg/mL) correspond to the target steady-state plasma levels of patients that receive these molecules subcutaneously during prophylactic treatment.

Statistical analysis

Analysis of blood loss between groups was performed using one-way ANOVA with Tukey's correction for multiple comparisons (all *vs*. all).

Results

Hemostatic response in the tail vein transection model without clot removal

The amount of FVIII needed to arrest bleeding in FVIII-deficient mice similar to that of littermate wt mice was explored in five different bleeding models (Figure 1). In the TVT model without the clot being dislodged, vehicle-treated mice had an initial bleeding arrest, but started bleeding spontaneously several minutes later (Figure 2). This resulted in a blood loss of 800±89 μL (N=7). All other treatments (FVIII at 2.5 IU/kg, emicizumab or SIA-marstacimab) resulted in the formation of an occlusive clot, and no spontaneous rebleeds were observed under these conditions (Figure 2). As such, these mice were similar to wt mice, with blood loss being less than 100 μL for all mice.

Hemostatic response in the tail vein transection model with clot removal

In a second series of experiments using the TVT model, clots were dislodged at 15 and/or 30 min after injury. As for the procedure without clot removal, vehicle-treated FVIII-deficient mice displayed an initial bleeding arrest, but started to rebleed spontaneously several minutes later, resulting in 750±133 μL blood loss (N=11) (Figure 3). In contrast, wt mice showed minor blood loss (70±34 μL; N=12) and no spontaneous rebleeds were observed despite the wound being challenged twice (Figure 3). When FVIII was administered, we found that the lowest dose tested (2.5 IU FVIII/kg, resulting in 5 IU/dL in plasma) proved sufficient to arrest bleeding with no spontaneous rebleeds being observed in FVIII-treated mice upon dislodging the clot (2.5 or 5 IU/kg; N=14 combined) (Figure 3). At 2.5 IU/kg, blood loss was reduced to the level of wt mice (63±31 μL; N=7; *P*=1.0) (Figure 3).

When emicizumab was applied, mice rapidly produced a

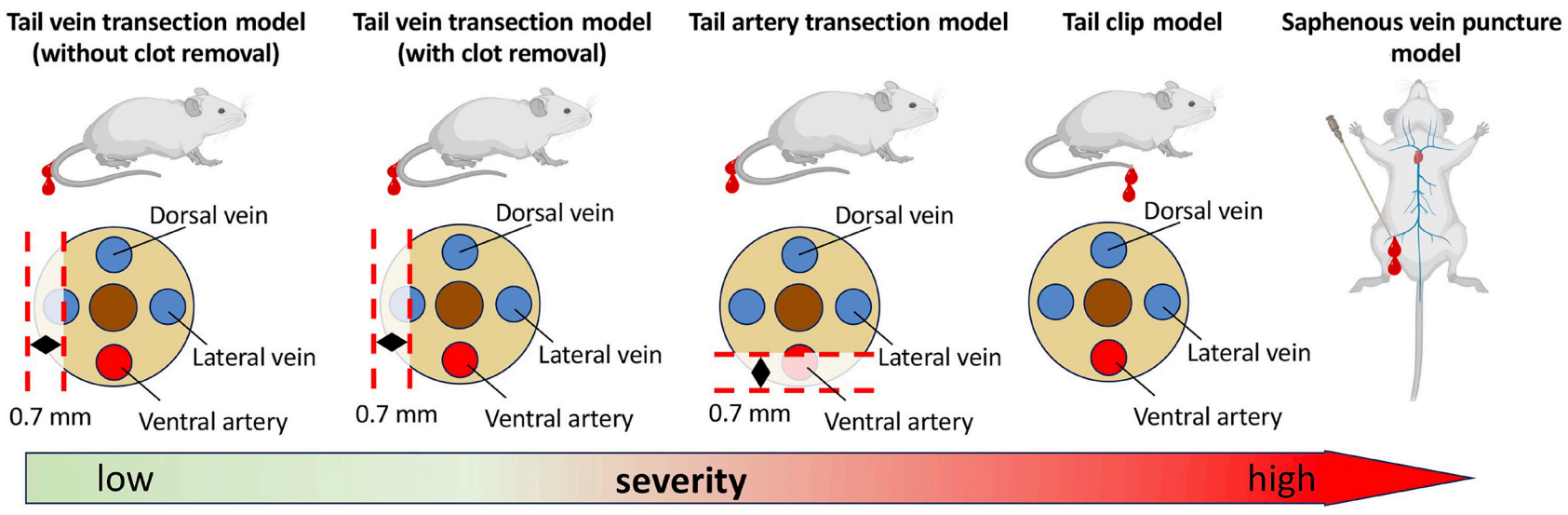


Figure 1. Different bleeding models used in this study. In the tail vein transection model, the lateral vein is transected in a template-dependent manner, allowing for a reproducible incision in terms of depth and location. In the tail artery transection model, the ventral artery is transected, also in a template-dependent manner to ensure reproducible incisions in terms of depth and location. In the tail clip model, the terminal 3 mm of the tail tip is severed. In the saphenous vein puncture model, the saphenous vein is punctured. Subsequently, every time a clot is formed, it is removed.

wound-occluding clot. However, all emicizumab-treated mice (N=6) presented spontaneous rebleeds following the first clot removal (Figure 3). Although blood loss was less than for vehicle-treated mice (565±165 μL; *P*=0.0037), it was significantly increased compared to FVIII-treated and wt mice (*P*<0.0001) (Figure 3).

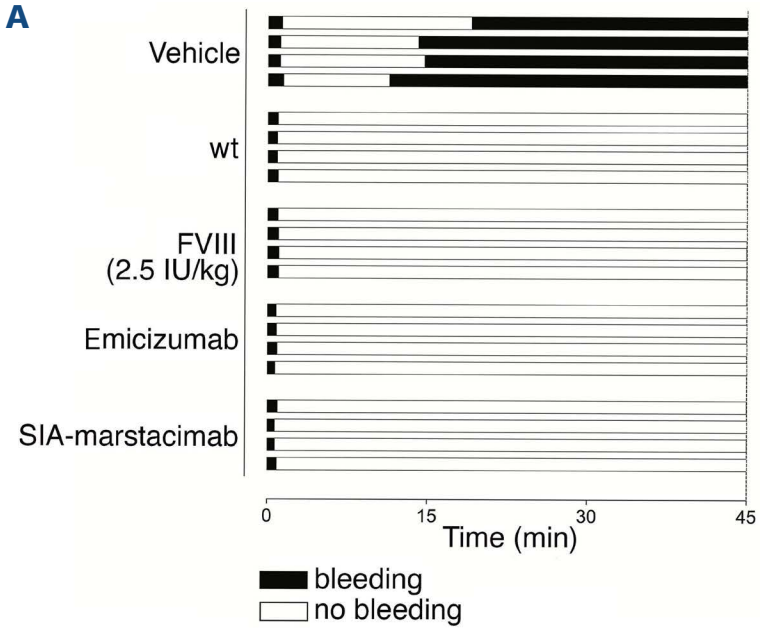
In SIA-marstacimab-treated mice, one of the 6 mice exhibited spontaneous rebleeds after the first clot removal (Figure 3). In terms of blood loss, SIA-marstacimab-treated mice were statistically similar to that of mice that received 2.5 IU/kg FVIII and wt mice: 100±81 μL (*P*=0.98) (Figure 3).

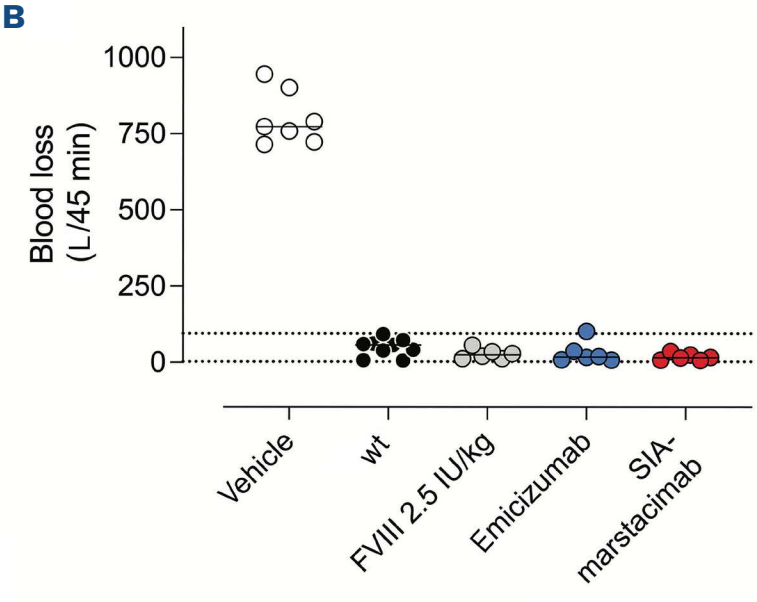
Hemostatic response in the tail artery transection model

In the TAT model, vehicle-treated FVIII-deficient mice shed more blood during the 30 min observation period compared to the 45 min observation period of the TVT model (904±135 μL; *P*=0.012 *vs*. TVT model) (Figure 4). In wt mice, blood loss in the TAT model was also significantly increased compared to the TVT model despite the shorter observation period (183±76 μL; *P*<0.0001 *vs*. TVT model). Nevertheless, no spontaneous rebleeds in the wt mice were observed once bleeding had stopped. When testing three different doses of FVIII (2.5-5-10 IU/kg), a dose-dependent hemostatic response was observed. Whereas spontaneous rebleeds were observed in mice treated with 2.5 IU/kg, none were observed in mice that received 5 or 10 IU/kg (N=30 in total) (Figure 4). Blood loss was hence reduced in a dose-dependent manner (*P* trend <0.0001). At 5 IU/kg, (i.e., 10 IU/mL) 8 out of 15 mice had blood loss in the same range of wt mice (≤285 μL), and the average blood loss at this dose was similar to that of wt mice (321±137 μL; N=15; *P*=0.156) (Figure 4).

Unexpectedly, all emicizumab-treated mice presented with spontaneous rebleeds after the initial bleeding arrest was obtained, similar to that of vehicle-treated mice (Figure 4). Indeed, no reduction in blood loss was observed (953±133 μL; N=9; *P*=0.988 *vs*. vehicle-treated mice).

Sequence-identical analog of marstacimab treatment was characterized by a variable response, with 6 of 11 mice showing no spontaneous rebleeds and 5 mice having spontaneous rebleeds following clot removal, compared to none of the 15 FVIII-treated mice at 5 IU/kg (*P*=0.0004) (Figure 4). In keeping with the variable response, average blood loss was quite variable between mice (257±239 μL; N=11), which was statistically similar to that of wt mice (*P*=0.874) and of FVIII-deficient mice that received 5 IU/kg (*P*=0.927). Overall, it appears that neither emicizumab nor SIA-marstacimab resemble FVIII in their capacity to arrest





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Comparison	*P*
Vehicle *vs.* FVIII 2.5 IU/kg	<0.0001
Vehicle *vs.* wild-type	<0.0001
Vehicle *vs.* emicizumab	<0.0001
Vehicle *vs.* SIA-marstacimab	<0.0001
FVIII 2.5 IU/kg *vs.* wild-type	0.908
FVIII 2.5 IU/kg *vs.* SIA-marstacimab	1.000
FVIII 2.5 IU/kg *vs.* emicizumab	0.996
Wild-type *vs.* emicizumab	0.959
Wild-type *vs.* SIA-marstacimab	0.717
Emicizumab *vs.* SIA-marstacimab	0.984

Figure 2. Tail vein transection model without clot removal. (A) Factor VIII (FVIII)-deficient mice were given intravenously vehicle, FVIII (2.5 IU/kg), emicizumab (5 mg/kg) or sequence-identical analog of marstacimab (SIA-marstacimab) (1 mg/kg). Estimated plasma concentrations at time of injury were 5 IU/dL FVIII, 55 μg/mL emicizumab or 16 μg/mL SIA-marstacimab. Wild-type (wt) mice were used as control. Mice were monitored for 45 minutes (min). During the 45-min observation time, periods of bleeding and bleeding arrest were noted. Bleeding patterns of 4 mice representative for each group are presented. (B) Blood loss for each individual mouse included in the study. (C) Statistical analysis of data presented in (B). Statistical analysis was performed via one-way ANOVA with Tukey's correction for multiple comparisons. Conditions that are above the threshold of statistical significance (*P*<0.05) are indicated in red.

bleeding in this model by forming a clot that resists spontaneous rebleeds.

Hemostatic response in the tail clip model

The tail clip model is the most frequently used bleeding test for hemophilic mice (Figure 5). In this model, vehicle-treated mice are consistently characterized by abundant blood loss (861±129 μL; N=18), and the wound starts rebleeding spontaneously several minutes after an initial arrest (Figure 5). In contrast, wt mice showed a rapid arrest of bleeding and no spontaneous rebleeds occurred during 30-min monitoring. Total blood loss for wt mice was 32±25 μL (N=19) (Figure 5). Four doses of FVIII were tested (2.5, 5, 7.5, and 10 IU/kg), revealing that both lower doses were associated with frequent rebleeds, whereas the dose of 7.5 IU/kg resulted in a complete arrest of bleeding, without any rebleeds being observed (Figure 5). A dose-dependent reduction of blood loss was obtained (*P* trend <0.0001) (Figure 5). From a statistical point of view, blood loss was similar to wt mice at a dose of 7.5 IU/kg (174±219 μL; N=8; *P*=0.135 *vs*. wt mice), whereas lower doses of 2.5 and 5 IU/kg were associated with increased blood loss compared to wt mice (792±96 μL; N=10; *P*<0.0001 and 477±180 μL; N=13; *P*<0.0001, respectively).

In agreement with our previous report, we observed a significant reduction in blood loss in emicizumab-treated mice compared to vehicle-treated mice (422±140 μL; N=14; *P*<0.0001 *vs*. vehicle) and similar to that of mice receiving 5 IU/kg (*P*=0.945) (Figure 5).[18] Similar to the FVIII dose at 5 IU/kg, emicizumab was associated with a temporary arrest of bleeding, and frequent rebleeds. Consequently, blood loss was significantly increased compared to wt mice (32±25 μL; N=19; *P*<0.0001 *vs*. emicizumab) or mice treated with 7.5 IU/kg FVIII (*P*=0.0005 *vs*. emicizumab) (Figure 5).

SIA-marstacimab-treated mice were also characterized by the presence of spontaneous rebleeds in all mice (8 of 8).

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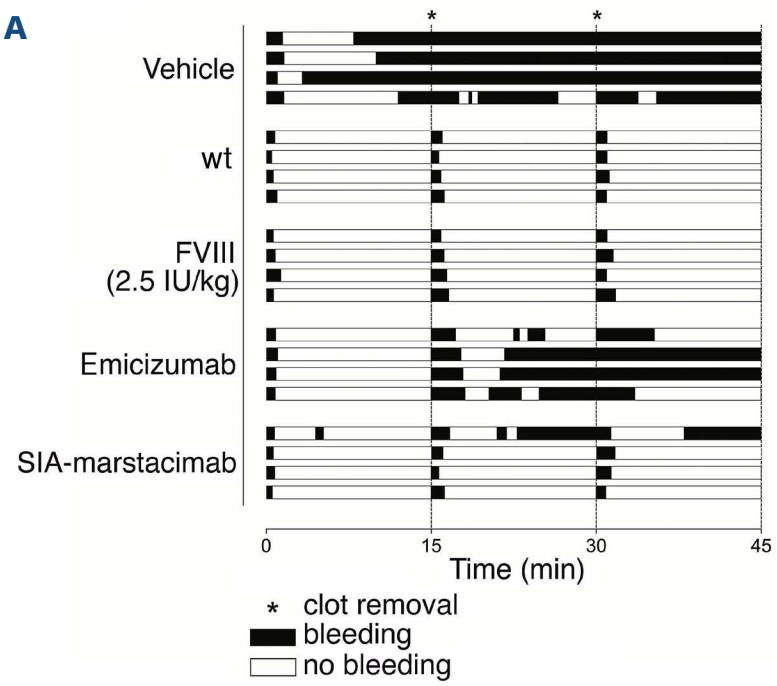

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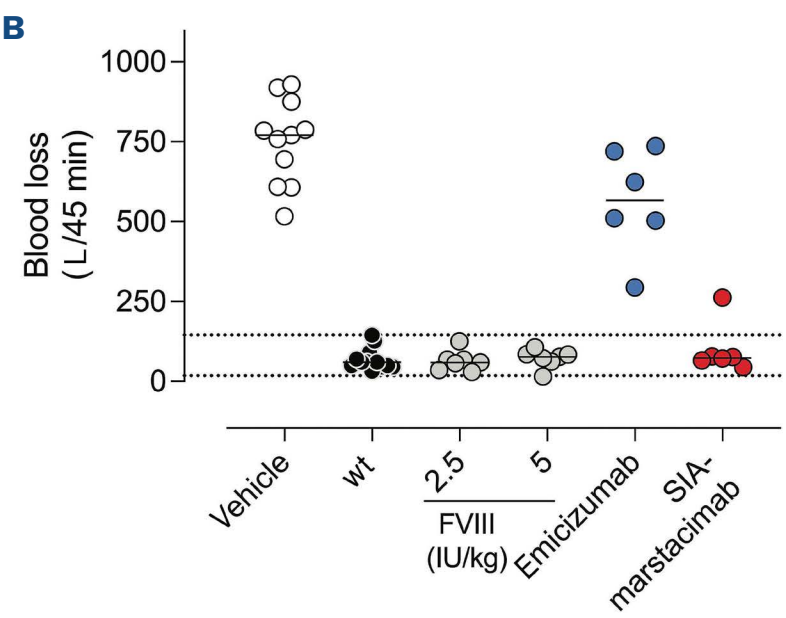

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Comparison	*P*
Vehicle *vs.* FVIII 2.5 IU/kg	<0.0001
Vehicle *vs.* FVIII 5 IU/kg	<0.0001
Vehicle *vs.* wild-type	<0.0001
Vehicle *vs.* emicizumab	0.0037
Vehicle *vs.* SIA-marstacimab	<0.0001
FVIII 2.5 IU/kg *vs.* FVIII 5 IU/kg	1.000
FVIII 2.5 IU/kg *vs.* wild-type	1.000
FVIII 2.5 IU/kg *vs.* emicizumab	<0.0001
FVIII 2.5 IU/kg *vs.* SIA-marstacimab	0.980
FVIII 5 IU/kg *vs.* wild-type	1.000
FVIII 5 IU/kg *vs.* emicizumab	<0.0001
FVIII 5 IU/kg *vs.* SIA-marstacimab	0.994
Wild-type *vs.* emicizumab	<0.0001
Wild-type *vs.* SIA-marstacimab	0.987
Emicizumab *vs.* SIA-marstacimab	<0.0001

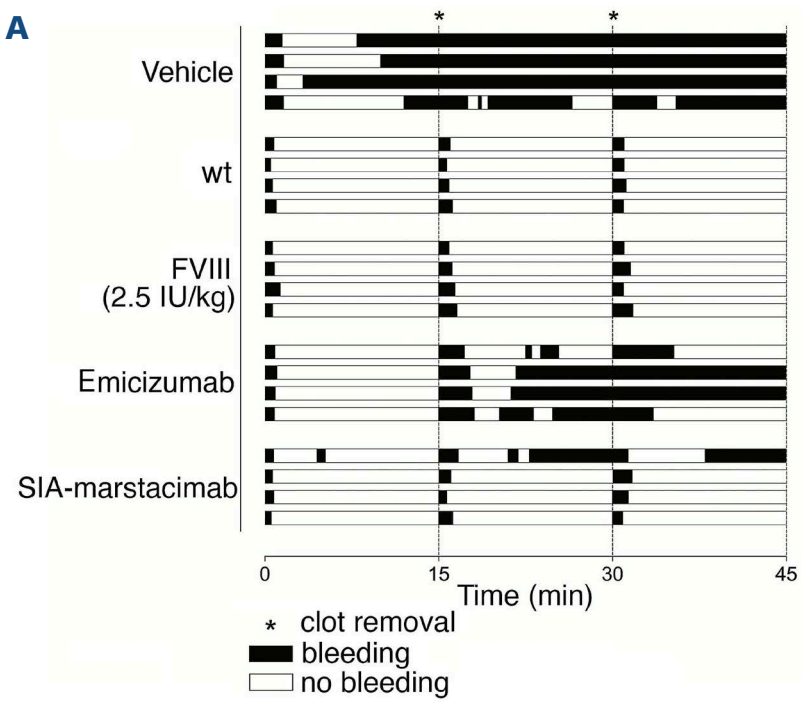
Figure 3. Tail vein transection model with clot removal. (A) Factor VIII (FVIII)-deficient mice were given intravenously vehicle, various doses of FVIII (2.5 and 5 IU/kg), emicizumab (5 mg/kg) or sequence-identical analog of marstacimab (SIA-marstacimab) (1 mg/kg). Estimated plasma concentrations at time of injury were 5 IU/dL FVIII, 10 IU/dL FVIII, 55 μg/mL emicizumab or 16 μg/mL SIA-marstacimab. Wild-type (wt) mice were used as control. If mice were not bleeding at 15 minutes (min) and/or 30 min, clots were dislodged. Mice were monitored for 45 min. During the 45-min observation time, periods of bleeding and bleeding arrest were noted. Bleeding patterns of 4 mice representative for each group are presented. For FVIII-treated mice, data for 4 mice receiving the lowest dose are depicted. (B) Blood loss for each individual mouse included in the study. (C) Statistical analysis of data presented in (B). Statistical analysis was performed via one-way ANOVA with Tukey's correction for multiple comparisons. Conditions that are above the threshold of statistical significance (*P*<0.05) are indicated in red.

Nevertheless, blood loss was less pronounced compared to emicizumab (149±126 μL), and was similar to that of mice treated with 7.5 IU/kg (*P*=1.00) or wt mice (*P*=0.340) (Figure 5).

Hemostatic response in the saphenous vein puncture model

The SVP model differs from the previous three models in that the read out is the number of clots formed over a 30-min period rather than the amount of blood shed. Vehicle-treated FVIII-deficient mice produce only few clots over this 30-min period, much less than wt mice (2±1 clots/30 min; N=20 and 26±2 clots/30 min; N=6; *P*<0.0001) (Figure 6). FVIII treatment was associated with a dose-dependent increase in the number of clots (*P* trend <0.0001). Although lower doses of FVIII allowed for an increased number of clots compared to vehicle-treated mice (5±2 clots/30 min [N=7] and 13±3 clots/30 min [N=9] for 2 and 10 IU/kg, respectively), a similar number of clots compared to wt mice was only obtained at a dose of 25 IU/kg (23±5 clots/30 min; N=18; *P*=0.565 *vs*. wt mice) (Figure 6).

Both emicizumab and SIA-marstacimab treatment resulted in a marked increase in the number of clots compared to vehicle-treated FVIII-deficient mice, with the number of clots/30 min being 12±4 (N=10) and 14±6 (N=6), respectively (Figure 6). As such, this number of clots was similar to that obtained using FVIII at a dose of 10 IU/kg (*P*=0.995



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Comparison	*P*
Vehicle *vs.* FVIII 2.5 IU/kg	0.0003
Vehicle *vs.* FVIII 5 IU/kg	<0.0001
Vehicle *vs.* FVIII 10 IU/kg	<0.0001
Vehicle *vs.* wild-type	<0.0001
Vehicle *vs.* emicizumab	0.988
Vehicle *vs.* SIA-marstacimab	<0.0001
FVIII 2.5 IU/kg *vs.* FVIII 5 IU/kg	<0.0001
FVIII 2.5 IU/kg *vs.* FVIII 10 IU/kg	<0.0001
FVIII 2.5 IU/kg *vs.* wild-type	<0.0001
FVIII 2.5 IU/kg *vs.* emicizumab	<0.0001
FVIII 2.5 IU/kg *vs.* SIA-marstacimab	<0.0001
FVIII 5 IU/kg *vs.* FVIII 10 IU/kg	0.171
FVIII 5 IU/kg *vs.* wild-type	0.156
FVIII 5 IU/kg *vs.* emicizumab	<0.0001
FVIII 5 IU/kg *vs.* SIA-marstacimab	0.927
FVIII 10 IU/kg *vs.* wild-type	1.00
FVIII 10 IU/kg *vs.* emicizumab	<0.0001
FVIII 10 IU/kg *vs.* SIA-marstacimab	0.890
Wild-type *vs.* emicizumab	<0.0001
Wild-type *vs.* SIA-marstacimab	0.874
Emicizumab *vs.* SIA-marstacimab	<0.0001

Figure 4. Tail artery transection model. (A) Factor VIII (FVIII)-deficient mice were given intravenously vehicle, various doses of FVIII (2.5, 5, and 10 IU/kg), emicizumab (5 mg/kg) or sequence-identical analog of marstacimab (SIA-marstacimab) (1 mg/kg). Estimated plasma concentrations at time of injury were 5 IU/dL FVIII, 10 IU/dL FVIII, 20 IU/dL FVIII, 55 μg/mL emicizumab or 16 μg/mL SIA-marstacimab. Wild-type (wt) mice were used as control. If mice were not bleeding at 15 minutes (min), clots were dislodged. Mice were monitored for 30 min. During the 30-min observation time, periods of bleeding and bleeding arrest were noted. Bleeding patterns of 4 mice representative for each group are presented. For FVIII-treated mice, data for mice receiving the two lowest doses are depicted. (B) Blood loss for each individual mouse included in the study. (C) Statistical analysis of data presented in (B). Statistical analysis was performed via one-way ANOVA with Tukey's correction for multiple comparisons. Conditions that are above the threshold of statistical significance (*P*<0.05) are indicated in red.

and *P*=0.990, respectively).
Together, these data indicate that the SVP model requires the highest concentration of FVIII (25 IU/kg) to achieve wt-like hemostasis relative to the bleeding models tested. In addition, emicizumab and SIA-marstacimab display a similar pro-hemostatic effect as a FVIII dosed at 10 IU/kg in this SVP model.

Discussion

The aim of the present study was to compare the activity of the bispecific antibody emicizumab and a sequence identical analog of the anti-TFPI antibody marstacimab (SIA-marstacimab) to FVIII in a series of bleeding models. Our data demonstrate that there is no consistent FVIII equivalence for emicizumab nor SIA-marstacimab between the different models.
The clinical management of hemophilia A has long relied on the use of FVIII concentrates, and broad consensus has been achieved on which level of FVIII activity would be required for specific situations, like for the prophylactic prevention of spontaneous joint bleeds, for surgery or for particular physical activities (Figure 7).[19-22] Specific FVIII

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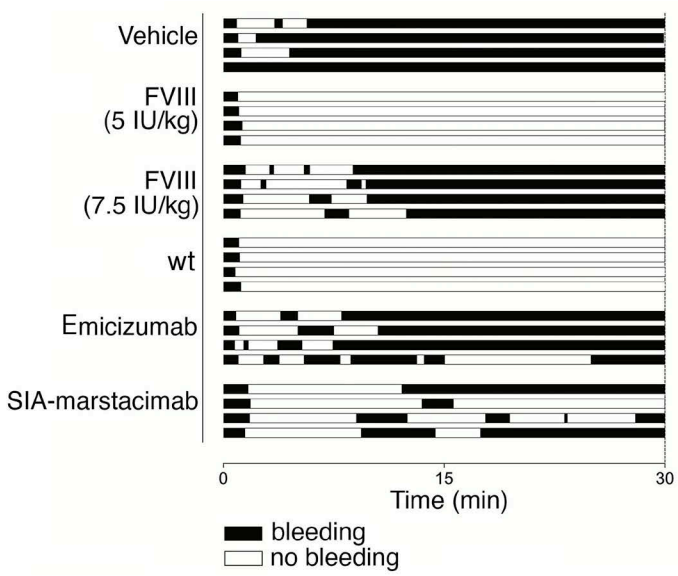

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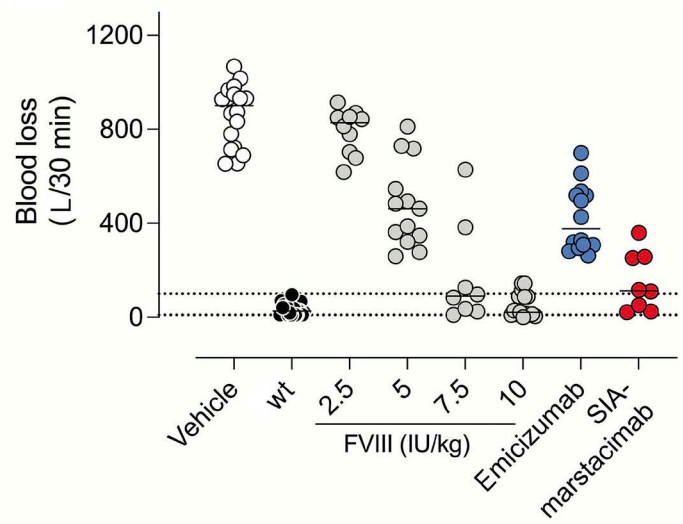

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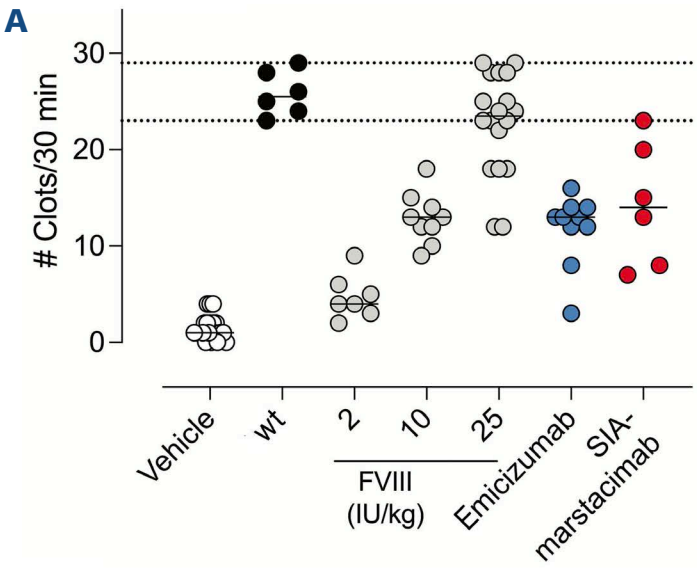
Comparison	*p*-value
Vehicle *vs.* FVIII 2.5 IU/kg	0.856
Vehicle *vs.* FVIII 5 IU/kg	<0.0001
Vehicle *vs.* FVIII 7.5 IU/kg	<0.0001
Vehicle *vs.* FVIII 10 IU/kg	<0.0001
Vehicle *vs.* wild-type	<0.0001
Vehicle *vs.* emicizumab	<0.0001
Vehicle *vs.* SIA-marstacimab	<0.0001
FVIII 2.5 IU/kg *vs.* FVIII 5 IU/kg	<0.0001
FVIII 2.5 IU/kg *vs.* FVIII 7.5 IU/kg	<0.0001
FVIII 2.5 IU/kg *vs.* FVIII 10 IU/kg	<0.0001
FVIII 2.5 IU/kg *vs.* wild-type	<0.0001
FVIII 2.5 IU/kg *vs.* emicizumab	<0.0001
FVIII 2.5 IU/kg *vs.* SIA-marstacimab	<0.0001
FVIII 5 IU/kg *vs.* FVIII 7.5 IU/kg	<0.0001
FVIII 5 IU/kg *vs.* FVIII 10 IU/kg	<0.0001
FVIII 5 IU/kg *vs.* wild-type	<0.0001
FVIII 5 IU/kg *vs.* emicizumab	0.945
FVIII 5 IU/kg *vs.* SIA-marstacimab	<0.0001
FVIII 7.5 IU/kg *vs.* FVIII 10 IU/kg	0.346
FVIII 7.5 IU/kg *vs.* wild-type	0.135
FVIII 5 IU/kg *vs.* emicizumab	0.0005
FVIII 7.5 IU/kg *vs.* SIA-marstacimab	1.000
FVIII 10 IU/kg *vs.* wild-type	1.000
FVIII 10 IU/kg *vs.* emicizumab	<0.0001
FVIII 10 IU/kg *vs.* SIA-marstacimab	0.636
Wild-type *vs.* emicizumab	<0.0001
Wild-type *vs.* SIA-marstacimab	0.340
Emicizumab *vs.* SIA-marstacimab	<0.0001

Figure 5. Tail clip model. (A) Factor VIII (FVIII)-deficient mice were given intravenously vehicle, various doses of FVIII (2.5, 5, 10, and 20 IU/kg), emicizumab (5 mg/kg) or sequence-identical analog of marstacimab (SIA-marstacimab) (1 mg/kg). Estimated plasma concentrations at time of injury were 5 IU/dL FVIII, 10 IU/dL FVIII, 20 IU/dL FVIII, 40 IU/dL, 55 μg/mL emicizumab or 16 μg/mL SIA-marstacimab. Wild-type (wt) mice were used as control. Mice were monitored for 30 minutes (min). During the 30-min observation time, periods of bleeding and bleeding arrest were noted. Bleeding patterns of 4 mice representative for each group are presented. For FVIII-treated mice, data for mice receiving 5 or 7.5 IU/kg are depicted. (B) Blood loss for each individual mouse included in the study. (C) Statistical analysis of data presented in (B). Statistical analysis was performed via one-way ANOVA with Tukey's correction for multiple comparisons. Conditions that are above the threshold of statistical significance (*P*<0.05) are indicated in red.

plasma and concentrate standards are available to validate FVIII concentrations in either one-stage or chromogenic activity assays.[23,24] Alternatively, thrombin generation assays or other global hemostatic assays have been used to assess FVIII activity.[25-28] These same assays are now being applied to quantify the effect of non-factor agents, like emicizumab or anti-TFPI antibodies.[29-31] Do these assays generate a reliable FVIII equivalence? Most likely, they do not.[10] If we consider emicizumab, the limiting factor in its functioning is FIXa, contrary to the tenase-complex in which activated FVIII is the limiting component. However, the amounts of FIXa that will be generated in thrombin generation assays are fully dependent on the concentrations of TF or FXIa that are used to initiate the reactions. Indeed, emicizumab has quite different FVIII-like activity when comparing the activated partial thromboplastin time (aPTT), chromogenic FVIII assay or thrombin generation assays. Between these assays, the FVIII equivalence may vary between more than 100% in the aPTT to approximately 10% in TF-initiated and 5% in FXIa-initiated thrombin generation (*Online Supplementary Figure S1*).[12,32,33] It is difficult to give precise numbers since the dose-response of FVIII and emicizumab are non-parallel. Similarly, anti-TFPI antibodies have variable activity in TF-initiated thrombin generation assays (i.e., depending on the amount of TF used to initiate the assay), while they have virtually no activity in FXIa-initiated thrombin generation assays (*Online Supplementary Figure S1*). Thus, *in vitro* assays do not constitute the appropriate conditions to assign a FVIII equivalence to emicizumab or anti-TFPI antibodies.

Rather than using *in vitro* assays, we considered the possibility that *in vivo* models could provide an alternative approach to determine FVIII equivalence for non-factor agents. *In vivo* assessment differs from the *in vitro* approaches in that all hemostatic participants are present, including all pro- and anti-coagulant factors, cellular and vascular components and flow. It is important, however, to be aware of limitations of such mouse models. Emicizumab does not bind murine factors IX and X, and human factors need to be infused to obtain emicizumab activity. It should be noted that when FIX and/or FX are given in the absence of emicizumab, no procoagulant effect is observed in the tail clip bleeding model.[18] Also, combining emicizumab with either FIX or FX alone does not result in any reduction in blood loss.[18,34] Furthermore, spiking murine FIX-deficient plasma with different ratios of human and murine recombinant FIX results in similar clotting times.[18] Moreover, the presence of human FIX and FX does not alter the efficacy by which FVIII corrects bleeding in mice.[18] Based on these data, it seems unlikely that the presence of human factors

A

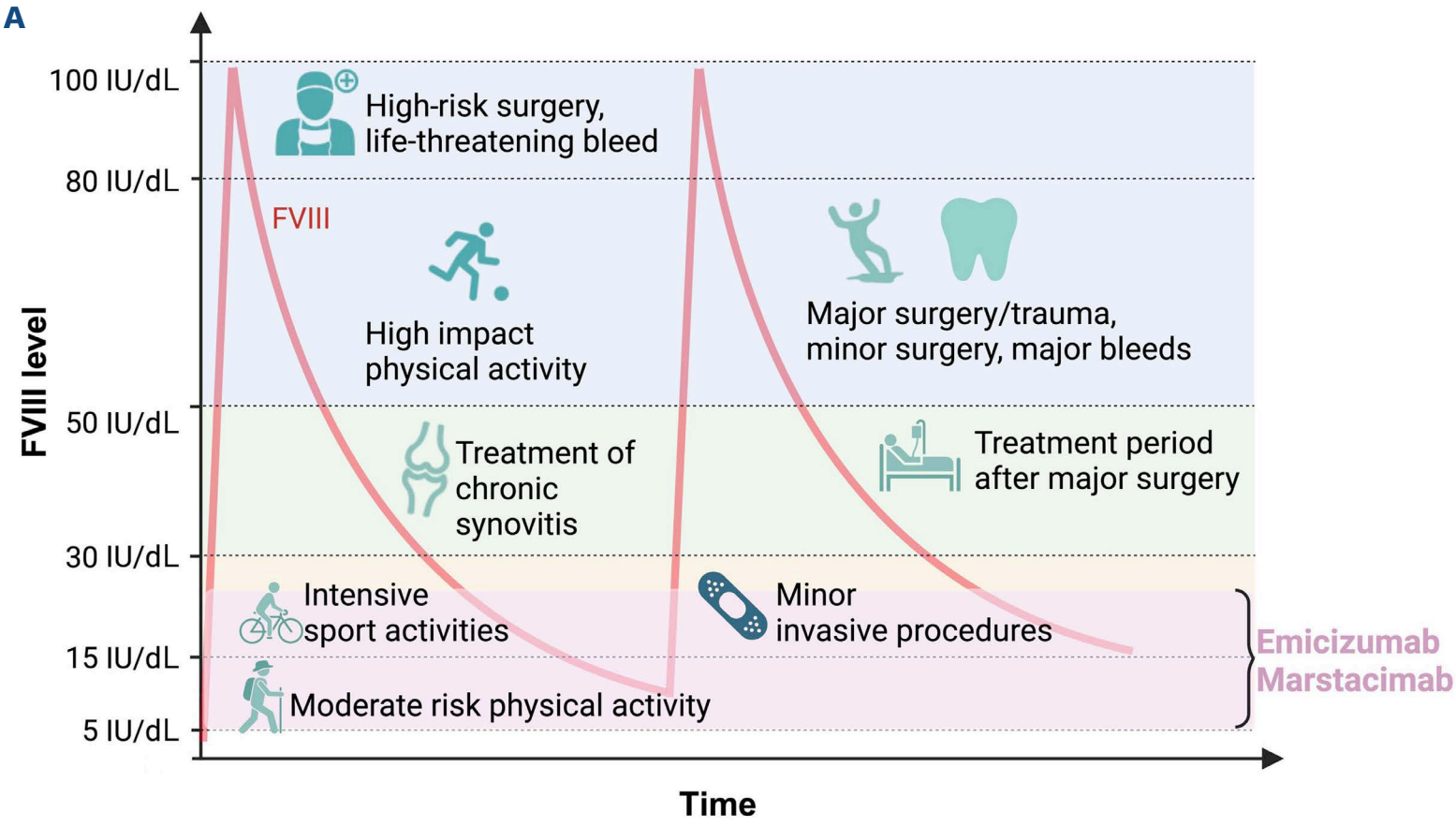


B

Comparison	*P*
Vehicle *vs.* FVIII 2 IU/kg	0.439
Vehicle *vs.* FVIII 10 IU/kg	<0 0001
Vehicle *vs.* FVIII 25 IU/kg	<0.0001
Vehicle *vs.* wild-type	<0.0001
Vehicle *vs.* emicizumab	<0.0001
Vehicle *vs.* SIA-marstacimab	<0.0001
FVIII 2 IU/kg *vs.* FVIII 10 IU/kg	0.0008
FVIII 2 IU/kg *vs.* FVIII 25 IU/kg	<0.0001
FVIII 2 IU/kg *vs.* wild-type	<0.0001
FVIII 2 IU/kg *vs.* emicizumab	0.0042
FVIII 2 IU/kg *vs.* SIA-marstacimab	0.0003
FVIII 10 IU/kg *vs.* FVIII 10 IU/kg	<0.0001
FVIII 10 IU/kg *vs.* wild-type	<0.0001
FVIII 10 IU/kg *vs.* emicizumab	0.995
FVIII 10 IU/kg *vs.* SIA-marstacimab	0.990
FVIII 25 IU/kg *vs.* wild-type	0.565
FVIII 25 IU/kg *vs.* emicizumab	<0.0001
FVIII 25 IU/kg *vs.* SIA-marstacimab	0.0002
Wild-type *vs.* emicizumab	<0.0001
Wild-type *vs.* SIA-marstacimab	<0.0001
Emicizumab *vs.* SIA-marstacimab	0.838

Figure 6. Saphenous vein puncture model. (A) Factor VIII (FVIII)-deficient mice were given intravenously vehicle, various doses of FVIII (2, 10, and 25 IU/kg), emicizumab (5 mg/kg) or sequence-identical analog of marstacimab (SIA-marstacimab) (1 mg/kg). Plasma concentrations at time of injury were 4 IU/dL FVIII, 20 IU/dL FVIII, 50 IU/dL FVIII, 55 μg/mL emicizumab or 16 μg/mL SIA-marstacimab. Wild-type (wt) mice were used as control. Mice were monitored for 30 minutes (min). Presented is the number of clots over 30 min generated for each individual mouse included in the study. (B) Statistical analysis of data presented in (A). Statistical analysis was performed via one-way ANOVA with Tukey's correction for multiple comparisons. Conditions that are above the threshold of statistical significance (P<0.05) are indicated in red.

A



B

Model	FVIII (IU/kg) needed to correct bleeding to wt level	Emicizumab response
Tail vein transection (no clot removal)	2.5	Blood loss similar to that of wt mice and FVIII-deficient mice treated with FVIII at 2.5 IU/kg.
Tail vein transection (with clot removal)	2.5	Blood loss reduced compared to vehicle-treated mice. Upon clot removal, rebleeds are observed in all mice. Such rebleeds are absent in mice treated with FVIII at 2.5 IU/kg.
Tail artery transection	5	Similar bleeding pattern and blood loss as vehicle-treated mice.
Tail clip	7.5	Blood loss is similar to that of mice treated with FVIII (5 IU/kg). Following initial bleeding arrest, spontaneous rebleeds in all mice. Such rebleeds are absent in mice treated with FVIII at 7.5 IU/kg.
Saphenous vein puncture	25	Significantly increased clot formation relative to vehicle-treated mice, similar to mice treated with FVIII at 10 IU/kg.

C

Model	FVIII (IU/kg) needed to correct bleeding to wt level	SIA-marstacimab response
Tail vein transection (no clot removal)	2.5	Blood loss similar to that of wt mice and FVIII-deficient mice treated with FVIII at 2.5 IU/kg.
Tail vein transection (with clot removal)	-	Blood loss similar to that of wt mice. Spontaneous rebleeds observed in 1 of 6 mice. Such rebleeds are absent in mice treated with FVIII at 2.5 IU/kg.
Tail artery transection	5	Blood loss similar to mice treated with FVIII at 5 IU/kg and wt mice. Upon clot removal, spontaneous rebleeds observed in 6 of 11 SIA-marstacimab-treated mice (compared to 0 of 15 mice treated at 5 IU/kg).
Tail clip	7.5	Blood loss is similar to that of mice treated with FVIII at 7.5 I U/kg and wt mice. After initial bleeding arrest, spontaneous rebleeds in all mice, which is not observed for FVIII-treated mice.
Saphenous vein puncture	25	Significantly increased clot formation relative to vehicle-treated mice, similar to mice treated with FVIII at 10 IU/kg.

Continued on following page.

Figure 7. Overview of the protective levels of factor VIII. (A) Schematic depiction on the potential relation between factor VIII (FVIII) levels and the extent of protection they provide under different conditions, based on consensus proposed in several guidance articles.[19-22] Note that these values may vary between individuals. Red line simulates plasma levels of FVIII following intravenous infusion; pink horizontal bar mimics steady-state levels of emicizumab and marstacimab during subcutaneous prophylactic treatment. Based on the data generated in this study, it appears that emicizumab and sequence-identical analog of marstacimab (SIA-marstacimab) display a model-dependent, variable FVIII equivalence that does not seem to exceed 20 IU/dL (as is observed in the saphenous vein puncture model). (B) Summary of the hemostatic responses upon emicizumab treatment in the various bleeding models. (C) Summary of the hemostatic responses upon SIA-marstacimab treatment in the various bleeding models.

IX and X in combination with the murine counterparts alters clot formation in mice. Despite its potential limitations, this mouse model has been accepted as a valid model to explore the *in vivo* activity of emicizumab.[34-37] Another potential limitation relates to SIA-marstacimab, which targets the Kunitz-2 domain of both human and murine TFPI with similar affinity. However, whereas humans express two isoforms of TFPI (TFPIα and TFPIβ), mice also express a third isoform, i.e., TFPIγ, which is expressed more abundantly than both other isoforms.[38] Consequently, the efficacy by which SIA-marstacimab interferes with TFPI-mediated inhibition of FVIIa and FXa may differ from the situation in humans. It should be noted that residual TFPI activity or antigen levels were not measured in the current study. Since marstacimab recognizes murine TFPI with an apparent affinity of <1 nM[14] and the antibody plasma concentration is >100-fold above the Kd-value (16 μg/mL corresponds to 106 nM), more than 99% of the TFPI and antibody are expected to be in complex.

By comparing different bleeding models, it became evident that each model requires a different dose of FVIII in order to correct bleeding to the level of wt mice, indicating that the severity of the injury was model-dependent (Figure 7). The model that required the lowest amount of FVIII for correction was the TVT model, in which a single tail vein is transected. At the lowest dose tested (2.5 IU/kg, which corresponds to 5 IU/dL) full correction of bleeding was obtained. When transecting the tail artery, a higher dose of FVIII was needed, i.e., 5 IU/kg. It should be noted that the vein and artery were transected in an identical manner, indicating that higher concentrations of FVIII are needed to correct an arterial bleed compared to a venous bleed. An even higher dose of FVIII (7.5 IU/kg) was required to correct bleeding to the level of wt mice in the tail clip model. In this model, all tail veins and the artery are being transected, albeit at a smaller diameter compared to the TVT and the TAT models. Importantly, bleeding was not only corrected in terms of the amount of blood shed in each of these models, but the clots that were generated were stable and no spontaneous rebleeds were observed. The highest dose of FVIII to achieve wt-level hemostasis was needed in the SVP model, with 25 IU/kg (i.e., 50 IU/dL). Although it relates to a venous injury, it concerns a large vein (larger than the lateral veins in the tail) that is challenged with continuous removal of occlusive clots that are being formed. Together, these bleeding models cover a wide range of severity. The amounts of FVIII needed for full correction in these models to a large extent overlap with those needed for bleeding protection in patients under a spectrum of conditions (Figure 7A). The notion that different doses of FVIII are required in each model further provides an opportunity to assess the potential FVIII equivalence of non-factor agents by using these different models.

Previously, both emicizumab and marstacimab had been tested for their capacity to correct for FVIII deficiency in animal models (cynomolgus monkey and mice for emicizumab and mice for marstacimab).[17,18,39,40] However, only for emicizumab has there been an attempt to define a FVIII equivalence in a single bleeding model: Ferrière *et al.* determined a potential FVIII equivalence of emicizumab corresponding to 5 IU FVIII/kg in terms of blood loss in the tail clip model.[18] No dose-response of emicizumab was observed in that particular study, with a similar reduction in blood loss at emicizumab levels between 25 and 185 μg/mL.[18]

In the present study, emicizumab and SIA-marstacimab were both significantly reducing blood loss compared to vehicle-treated mice in the TVT and tail clip models. Particularly, in the TVT model without clot removal, their efficacy was similar to that of wt mice, with a complete arrest of bleeding within 2 min after injury. In addition, SIA-marstacimab also reduced blood loss in the TAT model, contrary to emicizumab. Nevertheless, we noticed an important difference compared to FVIII, in that treatment with either emicizumab or SIA-marstacimab was characterized by the occurrence of more or less frequent rebleeds in the TVT and TAT models upon clot removal and in the tail clip model (Figure 7B, C). The reason for the presence of these rebleeds is still unclear, but is perhaps related to a different clot morphology. Indeed, it seems that, in mice, emicizumab-derived clots are more fragile and lack elasticity due to an altered fibrin-network structure.[37] Current studies are ongoing to identify whether such differences in clot structure are also present upon treatment with anti-TFPI antibodies.

While SIA-marstacimab appeared to reduce blood loss more effectively than emicizumab in our mouse models, it is important to emphasize that this study was not designed to evaluate or compare the clinical efficacy of these two antibodies. Our goal has not been to determine the maximum efficacious value (E_{max}) for each drug or the concentration that produces 50% of their maximum effect (EC_{50}). Therefore, these findings should not be interpreted

as evidence of the superiority of one antibody over the other in terms of efficacy during prophylactic treatment in patients. We believe that our mouse models are not sufficiently humanized to allow such a comparison, and clinical trials and real-life data are more equipped to compare efficacy of these different treatment options. Instead, we actually used these models to compare both agents to FVIII, hoping to identify a FVIII equivalence for each of the molecules. However, depending on the model that was used, a different FVIII equivalence was determined, irrespective of the model severity or whether emicizumab or SIA-marstacimab was considered. The reason for this is probably quite similar to that which is seen in the *in vitro* assays, despite the presence of the complete hemostatic system in the *in vivo* models. In FVIII-mediated coagulation, FVIII is the limiting factor (based on its low concentration of <1 nM and its rather unstable nature once activated), since concentrations of phosphatidyl-serine containing membranes, FIXa and FX, are well in excess over FVIII during coagulation. In contrast, emicizumab and anti-TFPI antibodies are strictly dependent on the local availability of FIXa and TFPI, respectively. FIXa generation will be different between locations and will depend on the severity of the injury. TFPI availability is variable according to location, with more TFPI activity on venous endothelial cells compared to arterial endothelial cells.[41] The capacity of emicizumab and anti-TFPI antibodies to correct hemostasis will, therefore, be controlled by the location and severity of an injury. Therefore, it is unlikely that a consistent FVIII equivalence for these agents can be attributed.

Disclosures

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Contributions

TS, GM, MC, HM, IP, FS and MK performed experiments and analyzed data. TD and CB contributed to the experimental design and data analysis. CVD, ODC, CC, VM and PJL are responsible for data analysis and conception, and the design and supervision of the study. PJL wrote the manuscript. All authors contributed to the final editing of the manuscript.

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Data-sharing statement

Data are available upon reasonable request to the corresponding author.

References

1. Weyand AC, Pipe SW. New therapies for hemophilia. Blood. 2019;133(5):389-398.
2. Kitazawa T, Igawa T, Sampei Z, et al. A bispecific antibody to factors IXa and X restores factor VIII hemostatic activity in a hemophilia A model. Nat Med. 2012;18(10):1570-1574.
3. Oldenburg J, Mahlangu JN, Kim B, et al. Emicizumab prophylaxis in hemophilia A with inhibitors. N Engl J Med. 2017;377(9):809-818.
4. Mahlangu J, Oldenburg J, Paz-Priel I, et al. Emicizumab prophylaxis in patients who have hemophilia A without Inhibitors. N Engl J Med. 2018;379(9):811-822.
5. Callaghan MU, Negrier C, Paz-Priel I, et al. Long-term outcomes with emicizumab prophylaxis for hemophilia A with or without FVIII inhibitors from the HAVEN 1-4 studies. Blood. 2021;137(16):2231-2242.
6. Mahlangu J, Iorio A, Kenet G. Emicizumab state-of-the-art update. Haemophilia. 2022;28(Suppl 4):103-110.
7. Matsushita T, Shapiro A, Abraham A, et al. Phase 3 trial of concizumab in hemophilia with inhibitors. N Engl J Med. 2023;389(9):783-794.
8. Mahlangu J, Luis Lamas J, Cristobal Morales J, et al. Long-term safety and efficacy of the anti-tissue factor pathway inhibitor marstacimab in participants with severe haemophilia: phase II study results. Br J Haematol. 2023;200(2):240-248.
9. Mahlangu JN, Lamas JL, Morales JC, et al. A phase 1b/2 clinical study of marstacimab, targeting human tissue factor pathway inhibitor, in haemophilia. Br J Haematol. 2023;200(2):229-239.
10. Lenting PJ. Laboratory monitoring of hemophilia A treatments: new challenges. Blood Adv. 2020;4(9):2111-2118.
11. Lenting PJ, Denis CV, Christophe OD. Emicizumab, a bispecific antibody recognizing coagulation factors IX and X: how does it actually compare to factor VIII? Blood. 2017;130(23):2463-2468.
12. Leksa NC, Aleman MM, Goodman AG, Rabinovich D, Peters R, Salas J. Intrinsic differences between FVIIIa mimetic bispecific antibodies and FVIII prevent assignment of FVIII-equivalence. J Thromb Haemost. 2019;17(7):1044-1052.
13. Bi L, Lawler AM, Antonarakis SE, High KA, Gearhart JD, Kazazian HH Jr. Targeted disruption of the mouse factor VIII gene produces a model of haemophilia A. Nat Genet. 1995;10(1):119-121.
14. Apgar JR, Benard S, Carven GJ, et al. Tissue factor pathway inhibitor antibodies and uses thereof. USPTO; 2017, WO201729583A2. https://patents.google.com/patent/US20170073428 Accessed March 18, 2024.
15. Johansen PB, Tranholm M, Haaning J, Knudsen T. Development of a tail vein transection bleeding model in fully anaesthetized haemophilia A mice - characterization of two novel FVIII molecules. Haemophilia. 2016, 22(4):625-631.
16. Pastoft AE, Lykkesfeldt J, Ezban M, Tranholm M, Whinna HC, Lauritzen B. A sensitive venous bleeding model in haemophilia A mice: effects of two recombinant FVIII products (N8 and

Advate®). Haemophilia. 2012;18(5):782-788.
17. Pittman DD, Rakhe S, Bowley SR, Jasuja R, Barakat A, Murphy JE. Hemostatic efficacy of marstacimab alone or in combination with bypassing agents in hemophilia plasmas and a mouse bleeding model. Res Pract Thromb Haemost. 2022;6(2):e12679.
18. Ferriere S, Peyron I, Christophe OD, et al. A hemophilia A mouse model for the in vivo assessment of emicizumab function. Blood. 2020;136(6):740-748.
19. Berntorp E, Astermark J, Bjorkman S, et al. Consensus perspectives on prophylactic therapy for haemophilia: summary statement. Haemophilia. 2003;9 Suppl 1:1-4.
20. Srivastava A, Santagostino E, Dougall A, et al. WFH Guidelines for the Management of Hemophilia, 3rd edition. Haemophilia. 2020;26 Suppl 6:1-158.
21. Mancuso ME, Bidlingmaier C, Mahlangu JN, Carcao M, Tosetto A. The predictive value of factor VIII/factor IX levels to define the severity of hemophilia: communication from the SSC of ISTH. J Thromb Haemost. 2018;16(10):2106-2110.
22. Iorio A, Iserman E, Blanchette V, et al. Target plasma factor levels for personalized treatment in haemophilia: a Delphi consensus statement. Haemophilia. 2017;23(3):e170-e179.
23. Raut S, Daniels S, Heath AB, SSC Sub-Committee on Factor. Value assignment of the WHO 8th International Standard for factor VIII, concentrate (07/350). J Thromb Haemost. 2012;10(6):1175-1176.
24. Hubbard AR, Hamill M, Beeharry M, Bevan SA, Heath AB, SSC Sub-Committees on Factor VIII/Factor IX and von Willebrand factor of ISTH. Value assignment of the WHO 6th International Standard for blood coagulation factor VIII and von Willebrand factor in plasma (07/316). J Thromb Haemost. 2011;9(10):2100-2102.
25. Bukkems LH, Valke L, Barteling W, et al. Combining factor VIII levels and thrombin/plasmin generation: a population pharmacokinetic-pharmacodynamic model for patients with haemophilia A. Br J Clin Pharmacol. 2022;88(6):2757-2768.
26. Maseide RJ, Berntorp E, Nummi V, Lassila R, Tjonnfjord GE, Holme PA. Bleeding phenotype of patients with moderate haemophilia A and B assessed by thromboelastometry and thrombin generation. Haemophilia. 2021;27(5):793-801.
27. Milos M, Coen Herak D, Mahmoud Hourani Soutari N, et al. Overall hemostasis potential and aPTT-clot waveform analysis as powerful laboratory diagnostic tools for identification of hemophilia A patients with unexpected bleeding phenotype. Int J Lab Hematol. 2021;43(2):273-280.
28. Dargaud Y, Wolberg AS, Gray E, et al. Proposal for standardized preanalytical and analytical conditions for measuring thrombin generation in hemophilia: communication from the SSC of the ISTH. J Thromb Haemost. 2017;15(8):1704-1707.
29. Waters EK, Sigh J, Friedrich U, Hilden I, Sorensen BB. Concizumab, an anti-tissue factor pathway inhibitor antibody, induces increased thrombin generation in plasma from haemophilia patients and healthy subjects measured by the thrombin generation assay. Haemophilia. 2017;23(5):769-776.
30. Patel-Hett S, Martin EJ, Mohammed BM, et al. Marstacimab, a tissue factor pathway inhibitor neutralizing antibody, improves coagulation parameters of ex vivo dosed haemophilic blood and plasmas. Haemophilia. 2019;25(5):797-806.
31. Verhagen MJA, Valke L, Schols SEM. Thrombin generation for monitoring hemostatic therapy in hemophilia A: a narrative review. J Thromb Haemost. 2022;20(4):794-805.
32. Adamkewicz JI, Chen DC, Paz-Priel I. Effects and Interferences of emicizumab, a humanised bispecific antibody mimicking activated factor VIII cofactor function, on coagulation assays. Thromb Haemost. 2019;119(7):1084-1093.
33. Jenkins PV, Bowyer A, Burgess C, et al. Laboratory coagulation tests and emicizumab treatment A United Kingdom Haemophilia Centre Doctors' Organisation guideline. Haemophilia. 2020;26(1):151-155.
34. Shimizu K, Nakajima Y, Takami E, Nakano H, Nogami K. Additional factor X enhances emicizumab-driven coagulation function in patients with hemophilia A and hemophilia A mice. Thromb Haemost. 2025, 125(1):21-32.
35. Ostergaard H, Lund J, Greisen PJ, et al. A factor VIIIa-mimetic bispecific antibody, Mim8, ameliorates bleeding upon severe vascular challenge in hemophilia A mice. Blood. 2021;138(14):1258-1268.
36. Janbain M, Enjolras N, Bolbos R, Brevet M, Bordet JC, Dargaud Y. Haemostatic effect of adding tranexamic acid to emicizumab prophylaxis in severe haemophilia A: a preclinical study. Haemophilia. 2021;27(6):1002-1006.
37. Sefiane T, Maynadie H, Ettingshausen CE, et al. Differences in venous clot structures between hemophilic mice treated with emicizumab versus factor VIII or factor VIIIFc. Haematologica. 2024;109(6):1836-1848.
38. Maroney SA, Ferrel JP, Collins ML, Mast AE. Tissue factor pathway inhibitor-gamma is an active alternatively spliced form of tissue factor pathway inhibitor present in mice but not in humans. J Thromb Haemost. 2008;6(8):1344-1351.
39. Muto A, Yoshihashi K, Takeda M, et al. Anti-factor IXa/X bispecific antibody (ACE910): hemostatic potency against ongoing bleeds in a hemophilia A model and the possibility of routine supplementation. J Thromb Haemost. 2014;12(2):206-213.
40. Muto A, Yoshihashi K, Takeda M, et al. Anti-factor IXa/X bispecific antibody ACE910 prevents joint bleeds in a long-term primate model of acquired hemophilia A. Blood. 2014;124(20):3165-3171.
41. Crawley J, Lupu F, Westmuckett AD, Severs NJ, Kakkar VV, Lupu C. Expression, localization, and activity of tissue factor pathway inhibitor in normal and atherosclerotic human vessels. Arterioscler Thromb Vasc Biol. 2000;20(5):1362-1373.