Supplemental data

This appendix has been provided by the authors to give readers additional information about the methodology and outcomes of the reported study.

Supplement to: **Recombinant von Willebrand factor prophylaxis in patients with severe von Willebrand disease: phase 3 study results**

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Supplemental methods

Patient exclusion criteria

Patients with type 2N VWD, pseudo VWD, or another hereditary or acquired coagulation disorder; VWF inhibitor and/or FVIII inhibitor historically or at screening; known hypersensitivity to any of the components of the study drugs; and/or a history of thromboembolic events were excluded.

Study treatment guidelines for breakthrough BEs

During the rVWF prophylaxis period, any breakthrough BEs could be treated with additional IV rVWF infusion(s) with or without rFVIII (antihemophilic factor [recombinant]; ADVATE®; Baxalta US Inc., a Takeda company, Lexington, MA, USA). The doses for rVWF and rFVIII were determined according to the bleeding type and severity and were adjusted based on the patient's clinical response and pharmacokinetics. Use of antifibrinolytics was permitted for treatment of minor or moderate breakthrough BEs when additional infusion of rVWF to treat the breakthrough BE was considered not necessary; antifibrinolytic therapy was to be recorded as concomitant medication.

Study treatment guidelines for surgery or dental procedures

Patients who required surgery or dental procedures during the study received rVWF with or without rFVIII to manage their surgical bleeding. IV rVWF infusions were administered preoperatively and, if required, intraoperatively and/or postoperatively.

Between 12 and 24 hours prior to surgery, a priming dose of rVWF was to be infused to allow endogenous FVIII levels to increase to ≥30 IU/dL (minor and/or oral surgery) or 60 IU/dL (major surgery), resulting in a priming dose of 40-60 IU/kg rVWF as a general guidance. An rVWF loading dose was to be administered within 3 hours before surgery. VWF and FVIII levels were to be assessed within 3 hours prior to surgery initiation, and if FVIII levels prior to the loading

dose administration were not ≥30 IU/dL (minor and/or oral surgery) or 60 IU/dL (major surgery), rFVIII was to be administered in addition to rVWF to raise FVIII:C to recommended levels.

The preoperative loading dose was calculated as the difference between the target peak and baseline plasma VWF:RCo levels divided by the incremental recovery (IR) (ΔVWF:RCo × body weight [kg] / IR) with IR at maximum concentration (C_{max}) defined as (C_{max}–$C_{predose}$) / dose (where $C_{predose}$ was defined as the plasma concentration before the rVWF infusion). If the IR was not available, an IR of 1.7 IU/dL per IU/kg was to be assumed, and the initial dose was calculated as follows: (100 – baseline plasma VWF:RCo) × body weight (kg) / 1.7. For minor and/or oral surgery, the IR for the preoperative priming dose was to be used to guide dosing, and the target peaks were 50-60 IU/dL VWF:RCo and 40-50 IU/dL FVIII. For major surgery, the target peaks were 100 IU/dL VWF:RCo and 80-100 IU/dL FVIII.

The surgery was to start only after normalization of the activated partial thromboplastin time. After the preoperative loading dose(s), patients who did not achieve the desired postinfusion recovery were to continue to receive rVWF, with or without rFVIII, as a bolus infusion, depending on VWF and FVIII levels. The perioperative and postoperative substitution regimen was to be individualized according to the PK results, the intensity and duration of the hemostatic challenge, and the institution's standard of care. Patients undergoing minor surgery were to be infused with rVWF every 12-24 hours or every other day, targeting >30 IU/dL (rVWF and FVIII) for at least the first 48 hours. Patients undergoing oral surgery were to be infused with rVWF at least once within the first 8-12 hours, targeting >30 IU/dL (rVWF and FVIII). Patients undergoing major surgery were to be infused with rVWF every 12-24 hours for at least the first 72 hours postsurgery, targeting VWF:RCo and FVIII trough activity >50 IU/dL, followed by further treatment beyond 72 hours for as long as deemed necessary by the investigator based on a trough target of >30 IU/dL for VWF:RCo and FVIII:C.

Dose modifications based on pre-infusion VWF/FVIII levels were to be performed as needed. For subsequent infusions following surgery, in case pre-infusion levels were not available prior to the consecutive infusion in a timely manner, pre-infusion levels from the previous dose could be used by the investigator for dosing guidance.

Binding and neutralizing antibody assessments

The presence of binding and neutralizing antibodies to VWF and FVIII was assessed at screening, initial PK/PD assessment, each scheduled follow-up visit, and the study completion visit. Testing was done prior to rVWF infusion and at least 72 hours after last rVWF infusion.

Binding antibodies to VWF were determined using an enzyme-linked immunosorbent assay (ELISA) with polyclonal antihuman IgG, IgM, and IgA. Three functional VWF assays (VWF collagen binding [VWF:CB], VWF:RCo, and VWF FVIII binding [VWF:FVIIIB]) were used to test for the presence of neutralizing anti-VWF antibodies, as measured by assays based on the Bethesda assay established for the quantitative analysis of FVIII inhibitors.[1-4] VWF:CB activity was measured using the Technozym VWF:CBA ELISA kit (#RB 13800; Technoclone, Vienna, Austria). The VWF:FVIIIB assay used the Asserachrom VWF: FVIIIB ELISA kit (#I 06438; Stago, Asnières sur Seine, France). The inhibitory VWF:RCo activity was measured using the VWF RCo assay (#506540; Siemens, Marburg, Germany) and the Behring Coagulation System analyzer (Siemens, Marburg, Germany). Endogenous FVIII inhibitor activity was determined using the Bethesda method with Nijmegen modification and the automated coagulation analyzer Sysmex CA 7000 (Sysmex, Kobe, Japan). Plasma was also analyzed for the presence of antibodies against CHO protein (total Ig), murine IgG, and human Furin (total Ig) using proprietary enzyme immunoassays.

One Bethesda unit (BU/mL) was defined as the amount of inhibitor that decreases the measured activity in the incubated test samples to 50% of the activity of the negative control.

The acceptance criterion for the VWF and FVIII assays was set to <25% for the relative SD. The results in residual activity of 25%-75% were used for calculation (ie, 75% activity was negative, 50% activity was 1 BU/mL, and 25% activity was 2 BU/mL). Because the test sample was not preheated to destroy endogenous activity, the inhibitor calculation was adjusted for the endogenous activity. If the endogenous activity was not available, below the limit of quantification was used.

PK and PD assessments

Initial and end-of-study PK and PD assessments were performed for each patient. Blood samples were analyzed for VWF activity using the VWF:RCo, VWF:CB, and VWF antigen assays, and for FVIII:C using the 1-stage clotting assay. In the Prior On-Demand group, PK/PD properties were evaluated with an initial PK/PD assessment after a washout period and with a steady-state PK/PD assessment at the end of the study. In the Switch group, the first PK/PD assessment was conducted after reaching steady state following rVWF prophylaxis, because no washout step was included to ensure uninterrupted VWF prophylaxis. A second steady-state PK/PD assessment was performed at study end.

Supplementary tables and figures

Supplementary Table 1. Study sites that enrolled patients*

Country	Study site (primary investigator)
France	Hopital Cardiologique - CHU Lille, Lille (Sophie Susen)
	CHU Dijon - Hopital du Bocage, Dijon (Fabienne Volot)
Germany	Klinikum der Johann Wolfgang Goethe-Universitaet, Frankfurt (Wolfgang Miesbach)
	Medizinische Hochschule Hannover, Hannover (Andreas Tiede)
Italy	Azienda Ospedaliera Universitaria Policlinico Umberto I - Università di Roma La Sapienza, Rome (Antonio Chistolini)
	Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan (Flora Peyvandi)
Netherlands	Erasmus MC, Erasmus universitair Medisch Centrum, Rotterdam (Frank Leebeek)
Russian Federation	FSBI of Science "Kirov Scientific and Research Institute of Hematology and Blood Transfusion of FMBA", Kirov (Margarita Timofeeva)
Spain	Hospital Universitari i Politecnic La Fe, Valencia (Ana Rosa Cid Haro)
	Hospital General Universitario de Alicante, Alicante (Pascual Marco Vera)
Turkey	Ondokuz Mayis Univ. Med. Fac., Samsun (Canan Albayrak)
	Ege University Medical Faculty, Izmir (Can Balkan, Fahri Sahin)
	Istanbul University Cerrahpasa Medical Faculty, Istanbul (Osman Bulent Zulfikar)
United States	Indiana Hemophilia and Thrombosis Center, Indianapolis (Amy Shapiro)
	University of Colorado Hemophilia & Thrombosis Center, Aurora (Michael Wang)

*Centers that enrolled patients and provided permission to be named individually

Supplementary Table 2. rVWF prophylaxis (on-study) and treated spontaneous BEs and sABRs for patients with on-study treated spontaneous BEs

	Historical (within 12 months of study start)		rVWF prophylaxis (through month 12)		
Patient	**Number and location of spontaneous treated BEs**	**sABR**	**Number and location of spontaneous treated BEs**	**sABR**	**sABR % change from historical**
Prior On-Demand group					
Patient 1	1 oral mucosa, 1 sclera of the right eye, 1 menorrhagia	3	2 mucosal (nose), 1 other (bleeding menses), 2 menorrhagia, and 1 menstrual bleeding	5.78	93% ↑*†
Patient 2	4 right elbow, 1 right and left elbows, 1 inguinal area	6	2 mucosal (gum), 1 mucosal (mouth)	2.84	53% ↓
Switch group					
Patient 2	1 hemarthrosis	1	1 right ankle	0.98	2% ↓
Patient 4	46 nasal mucosa	46	10 mucosal (nose), 3 unknown	12.08	74% ↓
Patient 8	None	0	2 mucosal (gum), 2 mucosal (nose)	3.85	↑‡†

*Mostly driven by heavy menstrual bleeding episodes potentially due to hormonal changes and potential underreporting of menorrhagia episodes by the patient in the real-world setting, which were captured in the medical records and reported by the investigator as a part of the patient's VWD history and were used to derive the historical ABR in this study

†Changes in sABR appear to be related to variability in BEs over a period of 12 months

‡>70% compliant with prophylactic treatment and sufficient dosing confirmed by FVIII:C and VWF:RCo levels

Supplemental Table 3. Selected PK/PD parameters for single-dose and steady-state VWF:RCo and FVIII:C (PKFAS)

	Prior On-Demand group				Switch group			
	Initial assessment		Final assessment*		Initial assessment		Final assessment*	
	N = 12[†‡]		N = 9[†]		N = 10[†]		N = 7[†]	
PK parameter	Mean (SD)	Median (range)	Mean (SD)	Median (range)	Mean (SD)	Median (range)	Mean (SD)	Median (range)
VWF:RCo								
C_{max}, IU/dL	74.6 (16.1)	75.7 (37.3-105.8)	92.6 (37.1)	89.4 (41.6-148.7)	85.3 (40.9)	81.2 (35.6-167.4)	102.9 (44.7)	100.6 (46.7-176.6)
$AUC_{(0\text{-}inf)}$, IU*h/dL	1199 (467.8)	1178 (631-2150)	1561 (1298)[§]	1009 (460-4460)[§]	1242 (800.5)[§]	912.3 (692-2950)[§]	1662 (675.0)[§]	1314 (1230-2440)[§]
$AUC_{(0\text{-}inf)}$ / dose, [IU*h/dL]/[IU/kg]	23.6 (9.0)	23.1 (14.1-42.1)	30.9 (23.4)[§]	21.8 (9.8-83.4)[§]	23.7 (9.2)[§]	20.2 (13.3-39.6)[§]	27.5 (9.7)[§]	22.1 (21.7-38.7)[§]
$t_{½}$, h	17.2 (10.1)	16.0 (9.0-45.8)	-	-	-	-	-	-
IR at C_{max}, [IU/dL]/[IU/kg]	1.5 (0.3)	1.4 (0.8-2.1)	1.8 (0.5)	1.6 (0.9-2.7)	1.6 (0.4)	1.6 (1.2-2.1)	1.9 (0.3)	1.8 (1.6-2.3)
FVIII:C								
C_{max}, IU/dL	90.8 (32.1)	87 (46-158)	104.1 (35.1)	97.0 (63-169)	85.6 (21.5)	89.0 (53-118)	75.7 (37.3)	80.0 (22-112)
$AUC_{(0\text{-}tlast)}$, IU*h/dL	4949 (2436)	4524 (1670-11,300)	5984 (2490)[§]	5752 (2870-11400)[§]	4621 (1161)[§]	4327 (3530-6890)	5836 (1735)[§]	6175 (3960-7380)[§]
$AUC_{(0\text{-}tlast)}$ / dose, [IU*h/dL]/[IU/kg]	98.6 (47.5)	94.6 (33.8-221)	121.8 (45.4)[§]	116.1 (55.7-205)[§]	94.8 (26.7)[§]	88.5 (63.1-145)[§]	96.6 (23.6)[§]	101.8 (70.8-117)[§]
$C_{predose}$, IU/dL	8.8 (14.9)	2.0 (2-45)	22.1 (23.5)	11.0 (6-81)	30.0 (21.6)	24.0 (2-67)	28.0 (21.2)	21.0 (1-70)
T_{max}, h	ND	24.1 (12.0-46.3)	ND	24.5 (6.2-29.3)	ND	24.2 (9.8-30.0)	ND	24.1 (1.1-30.0)

For the PK/PD assessments, all patients in the Prior On-Demand group received rVWF doses between 44 and 55 IU/kg for the initial assessment and between 41 and 56 IU/kg for the final assessment, and the dosing frequency was twice weekly for 8 of 9 patients and 3 times weekly for the remaining patient.

For the initial and final PK/PD assessments, Switch patients received rVWF doses ranging from 24 to 74 IU/kg and from 24 to 77 IU/kg, respectively. At the initial assessment, Switch patients were taking rVWF doses twice weekly (8 of 10 patients), once weekly (1 patient), and 3 times weekly (1 patient). At the final assessment, Switch patients were taking rVWF doses twice weekly (4 of 7 patients), once weekly (1 patient who was also on the once-weekly regimen at the initial PK/PD assessment), and 3 times weekly (2 patients [the patient who was on the 3-times-weekly regimen at the initial PK/PD assessment and another patient who was on the twice-weekly regimen at the initial PK/PD assessment]).

AUC, area under the plasma concentration-time curve; $AUC_{(0\text{-}inf)}$, AUC from time 0 to infinity; $AUC_{(0\text{-}tlast)}$, AUC from 0 to the last measurable concentration; C_{max}, maximum plasma concentration; ND, not determined; T_{max}, time to reach the maximum concentration.

*At Month 12 visit, PK/PD blood samples taken at 10 time points post-infusion initiating on first day of Month 12 visit and finishing at 96 h post-infusion.

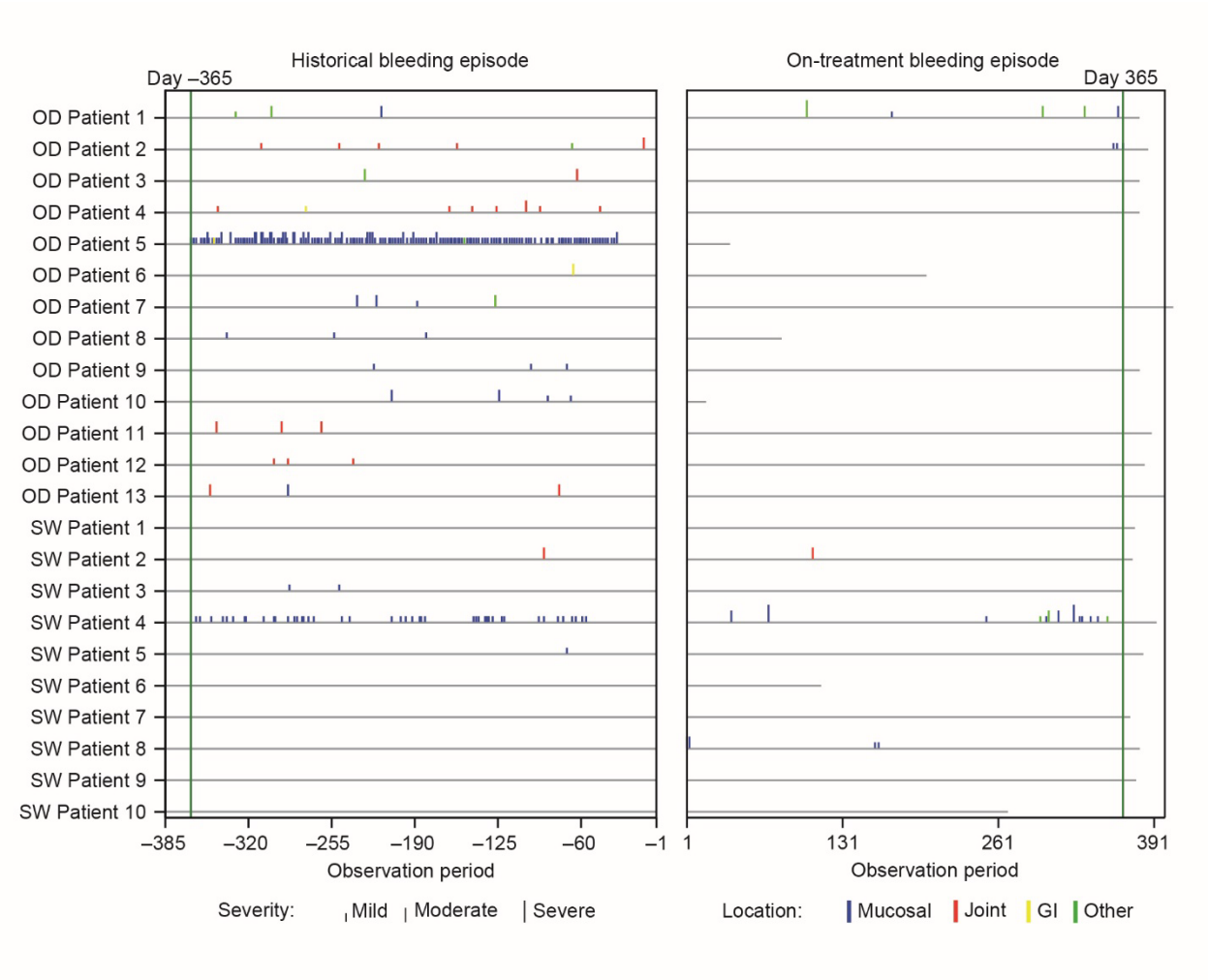
†N is the number of patients who had PK/PD assessments, but not all parameters could be calculated for all patients.

‡One patient provided PK/PD samples pre-dose and 30 minutes post dose, but not for the noncompartmental evaluation.

§Values are $AUC_{(0\text{-}96\ hours)}$.

Supplemental Figure 1. Historical and on-study treated spontaneous BEs by patient.

In the Prior On-Demand (OD) Patient 3 had 2 historical BEs and Patients 11 had 3 historical BEs with missing severity grading. The missing severity grading for these 5 episodes were imputed as “moderate”. “Other” includes BEs with the following locations: historical bleeds – menorrhagia, eye, skin, and soft tissue; on-study bleeds – menorrhagia and other.



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