Il VWF ricombinante nella Malattia di von Willebrand

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Disclosures for Augusto B. Federici, MD

- **Consultancy:** Baxalta; CSL-Behring; Grifols; LFB-KEDRION; Octapharma; Werfen.
- **Honoraria:** Baxalta; CSL-Behring; Grifols; LFB-KEDRION; Octapharma; Werfen.
- **Speakers Bureau:** Baxalta; CSL-Behring; Grifols; LFB-KEDRION; Octapharma; Werfen.
- **Membership on an Entity's Board of Directors or Advisory Committee:** Baxalta; CSL-Behring; Grifols; LFB-KEDRION; Octapharma; Werfen.
- **Discussion of Off-Label:** Not Applicable.

Aims of Treatments of VWD patients
Correction of the VWF defects

To **correct the dual VWF defect** present in patients with the different VWD types:

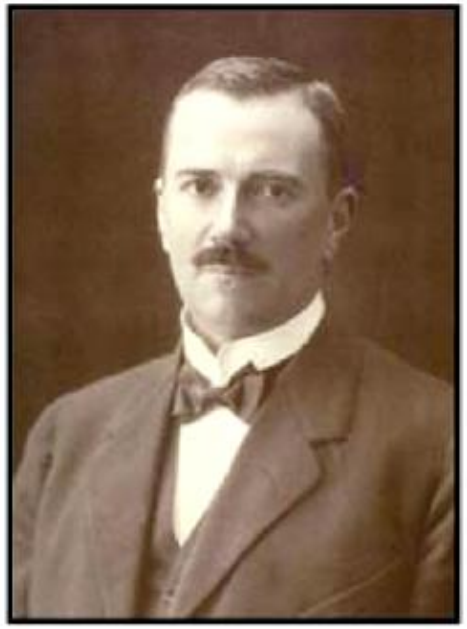
- **Impaired Platelet adhesion** and platelet-platelet interactions (PD-VWF assays)
- **Reduced levels of Factor VIII** that are associated with reduced or abnormal VWF

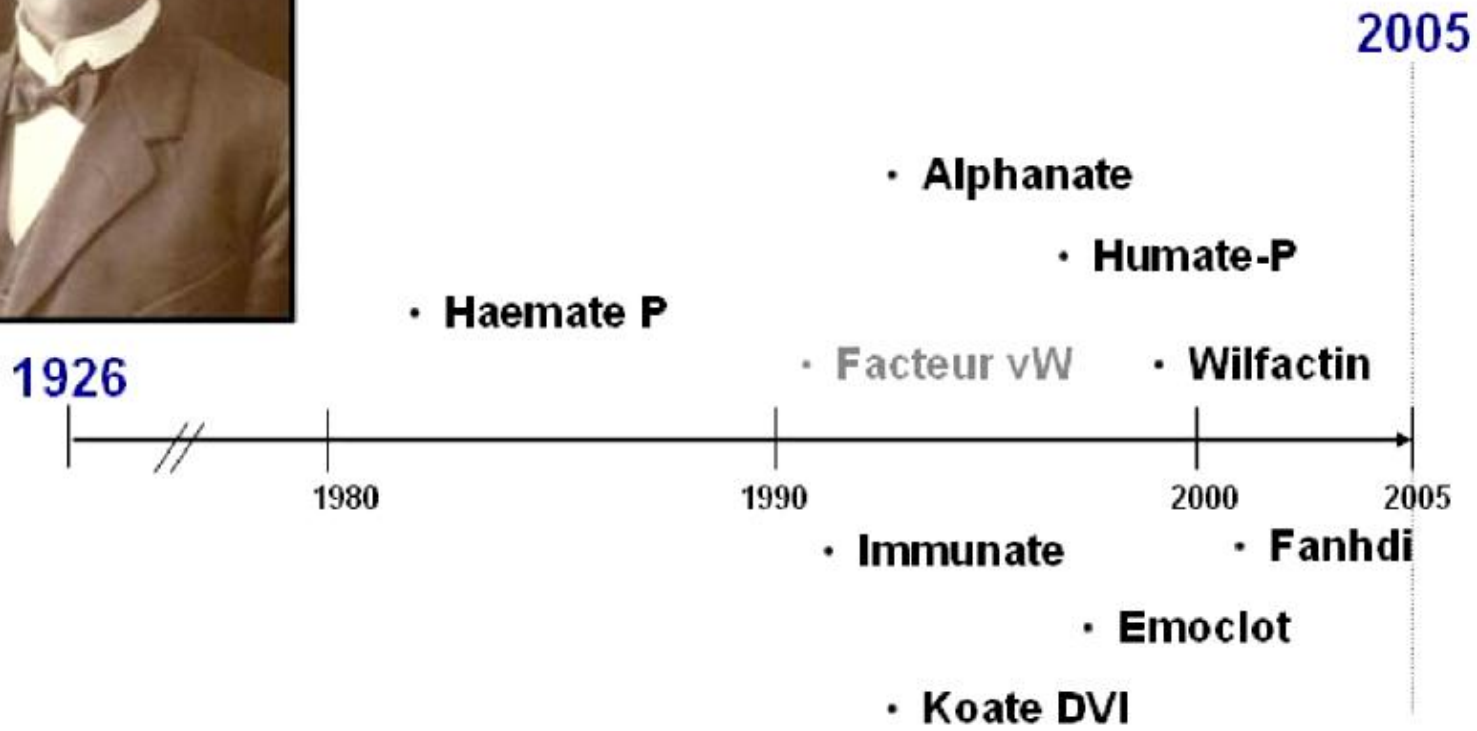
VWD therapeutic management

VWD treatment considerations	
Is desmopressin likely to be effective?	• Baseline VWF level >10%
	• Conduct desmopressin trial (IV, SC, IN)
	• Test VWF:Ag, VWF:RCo, and FVIII:C levels at 1, 2, and 4 hours
	• Positive response both FVIII and VWF > 50% after administration
Addition of adjunctive therapies	• Antifibrinolytics
	• For menorrhagia: oral contraceptive or levonorgestrel-releasing IUD
VWF concentrates	• If desmopressin response is inadequate
	• If desmopressin is required for several consecutive days
	• Dosing/product considerations
	VWF/FVIII ratio
	VWF multimer profile
	Dosing by VWF:RCo or FVIII:C
	Potential for prophylactic schedule
	Rare development of VWF alloantibodies

Lillicrap D, Blood 2013;122(23):3735-3740.

List of VWF concentrates used in Clinical practice (1982-2016)





2006-2016

BIOSTATE
WILATE

VONVENDI
(US)

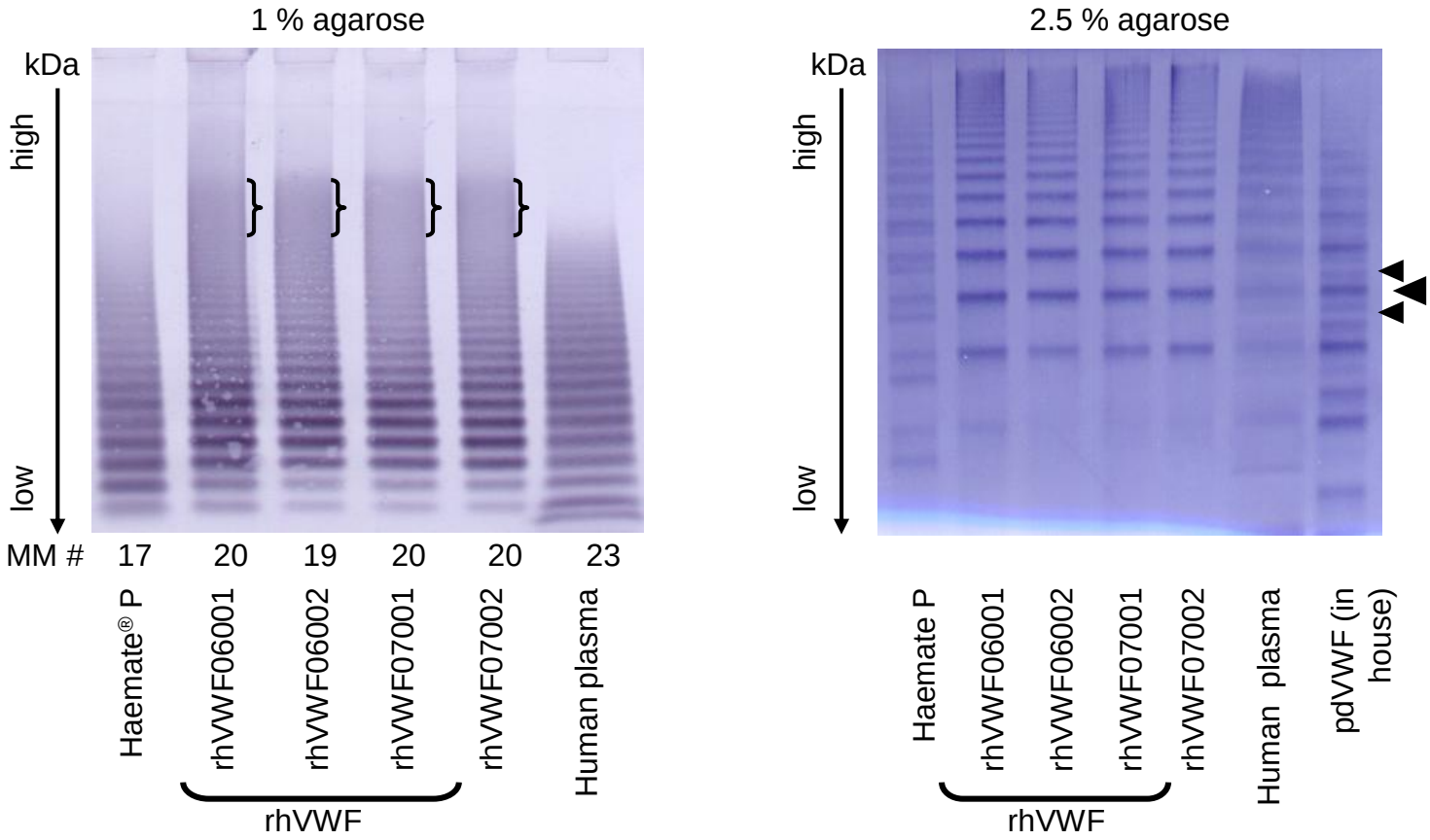
Recombinant VWF (rhVWF) Program Addressing the Medical Need for VWD

- **Medical need: a recombinant VWF option**
- **rhVWF is the largest and functionally most complex protein multimer ever produced by recombinant DNA technology**
- **rhVWF and ADVATE are coexpressed in Chinese hamster ovary cells**
  - **ADVATE is purified and rhVWF is removed**
- **rhVWF can be recovered, processed (propeptide cleavage), and purified**
- **rhVWF multimers are preserved**

rhVWF, recombinant human von Willebrand factor.

rhVWF Contains High and UHMW Multimers Usually Not Present in pdVWF

Multimer Analysis of rhVWF, PD-VWF Concentrates and VWF in Plasma



- Absence of ADAMTS13-mediated proteolytic fragments

UHMW, ultra high molecular weight.

Specific Activity of rhVWF Is Substantially Higher Than That of Plasma-Derived VWF and VWF/FVIII Products

	VWF:Ag (IU/mg protein)	VWF:RCo (IU/mg protein)	VWF:CBA/VWF:Ag (IU/IU)	VWF:RCo/VWF:Ag (IU/IU)
rhVWF	116 ± 7	134 ± 28	1.14 ± 0.16	1.16 ± 0.25
	n=7	n=7	n=3	n=7
Pasteurized pdVWF	17.9 ± 4.7	8.0 ± 1.7	0.84	0.51 ± 0.10
	n=12	n=7	n=1	n=12

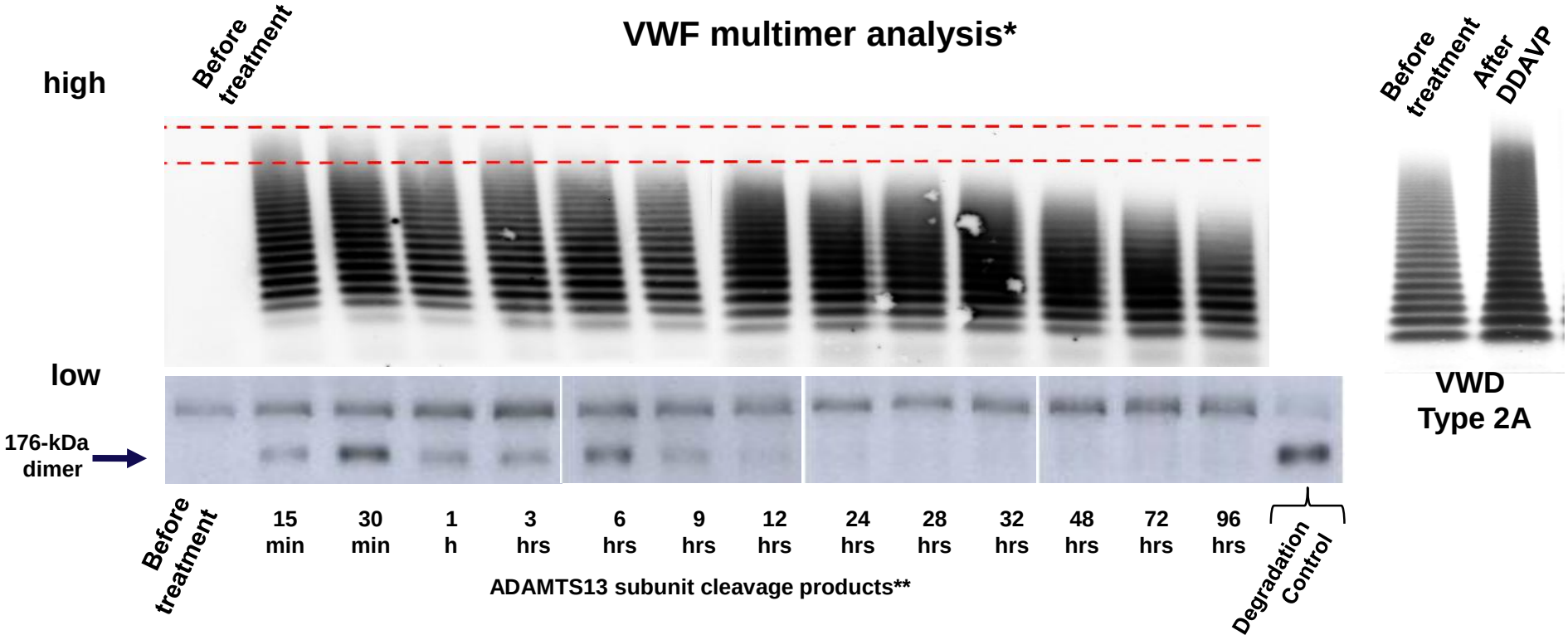
- The specific activity (VWF:RCo/protein) is substantially higher than that of pdVWF
  - pdVWF products contain other proteins, including human albumin, substantially lowering their specific activity
- rhVWF is a highly concentrated product with physiological VWF:RCo/VWF:Ag and VWF:CBA/VWF:Ag ratios
  - Contains more active VWF than pd concentrates

Ag, antigen; CBA, collagen binding activity; pd, plasma-derived;
pdVWF, plasma-derived von Willebrand factor; RCo, ristocetin cofactor.

Differences between rhVWF (VONVENDI) & plasma-derived VWF

rhVWF	pdVWF
Expressed in CHO cells	Synthesized in endothelial cells and megakaryocytes
Pro-peptide removal mediated in vitro through exposure of the pro-VWF to a second recombinant protein (the pro-peptide-processing enzyme furin)	Post-translational modification of pro-peptide removal occurs intra-cellularly during passage of the protein to the Golgi and post-Golgi compartments
No exposure to ADAMTS13 → intact VWF subunits → ultralarge VWF multimers present	Consists of VWF subunits that have been exposed to plasma ADAMTS13 → subunits cleaved at TYR^{1605}-MET^{1606} → Ultralarge VWF multimers absent
Glycosylation: ABO blood group glycans absent	Glycosylation: ABO blood group glycans present
	Plasma-derived VWF concentrate contain other proteins incl. ADAMTS13

Susceptibility of rhVWF to ADAMTS13 Cleavage in VWD Patients Allows Physiological Processing on Administration



* Low-resolution agarose (1% SeaKem®) / Samples adjusted to VWF:Ag content

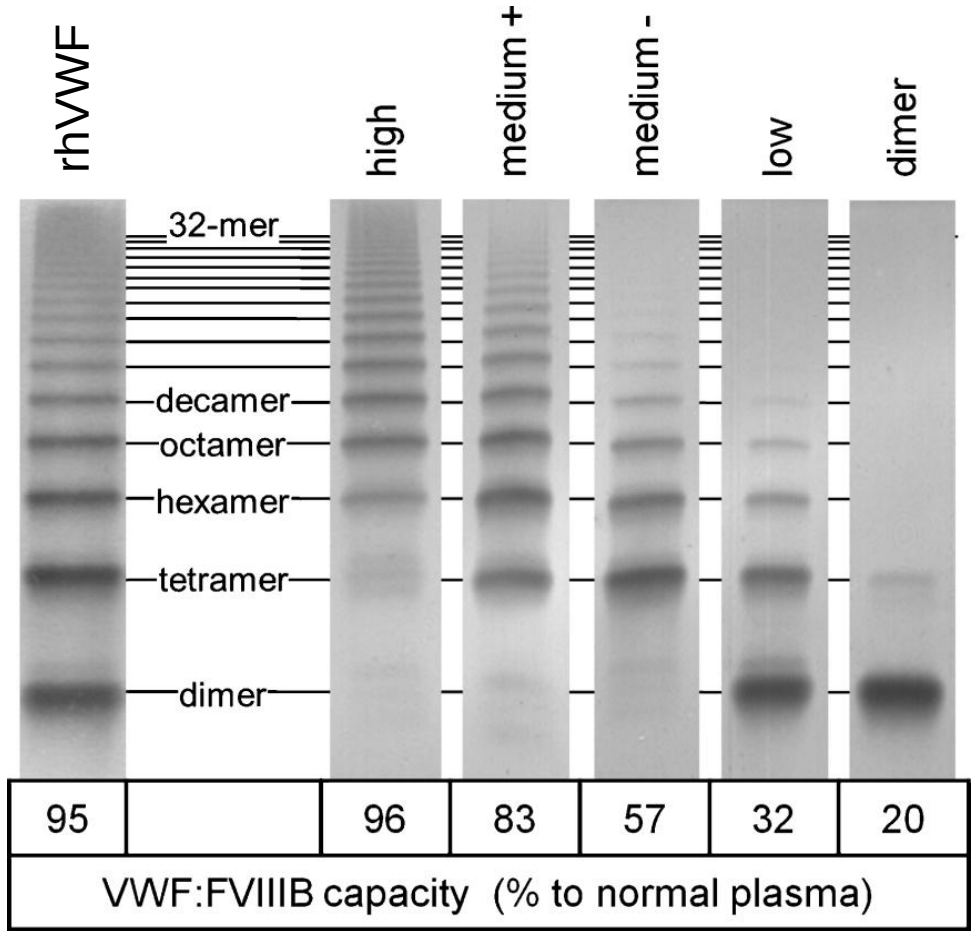
** SDS-PAGE / Immunoblot with polyclonal anti-VWF Ab / Samples undiluted

- ULMW multimers also found in the circulation after DDAVP treatment
- ULMW multimers disappeared over time, similar to the disappearance in patients treated with DDAVP
- ADAMTS13-specific cleavage product appeared 15 minutes after administration

Ab, antibody; DDAVP, desmopressin acetate; SDS-PAGE, sodium dodecyl sulfate–polyacrylamide gel electrophoresis; ULMW, ultra-low molecular weight.

FVIII Binding Capacity in Dependence of VWF Multimerization Degree

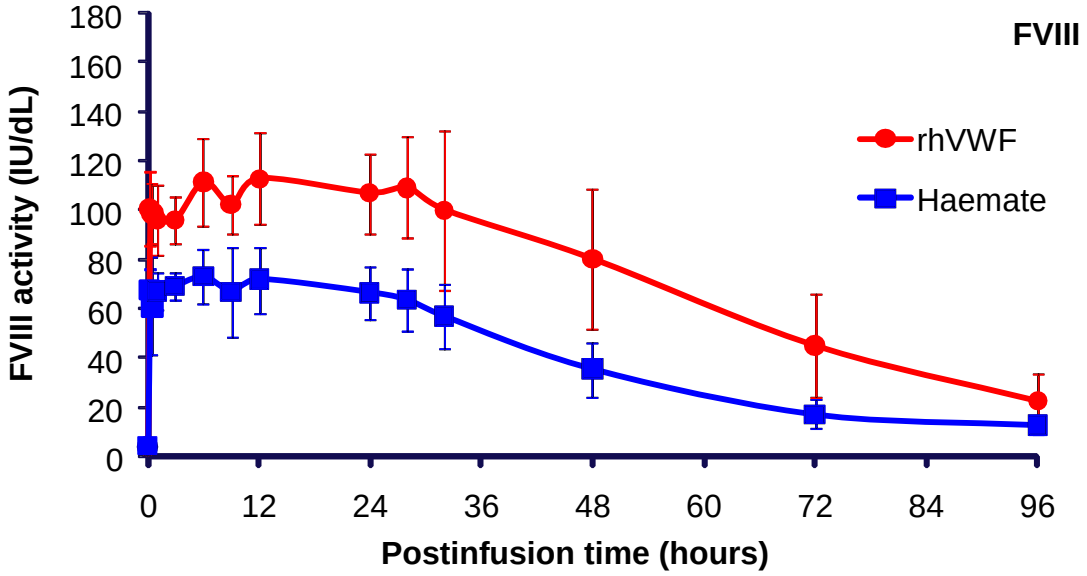
- **Each VWF monomer contains one binding site for FVIII**
  - **Each monomer should be able to bind one FVIII molecule**
- **Implies that larger multimers can bind more FVIII than smaller ones**
- **Dependency of FVIII binding capacity on the multimerization degree of VWF was analyzed**
  - **Gradual decrease in FVIII binding capacity for fractions with lower molecular weight multimers**
  - **VWF dimer as the smallest possible unit retained an FVIII binding capacity of 20% relative to normal human plasma**



Turecek PL et al. *Semin Thromb Hemost*. 2010;36:510-521.

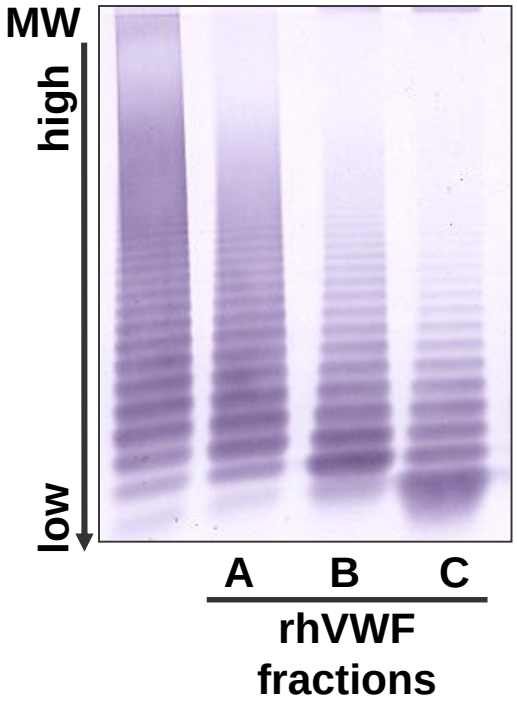
Improved Stabilization of FVIII in Circulation (cont)

- **Enhanced FVIII stabilization leads to longer time at effective FVIII levels**
- **Enables use of rhVWF alone to treat bleeding event – reduces risk of thrombosis due to high FVIII levels compared with current VWF:FVIII products**



Generation of rhVWF Fractions of Varying Multimer Size

VWF multimer number
(1% agarose)

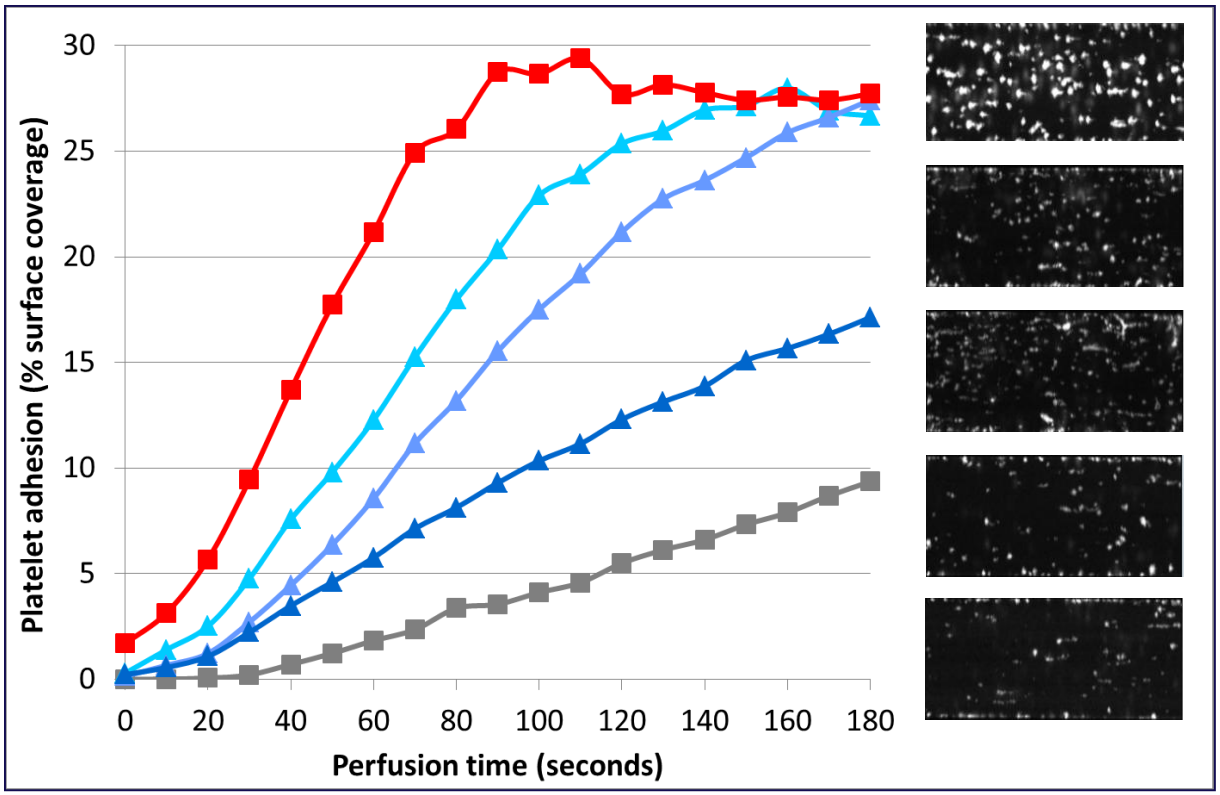


VWF activity parameters

		VWF:Ag/ protein (IU/mg)	VWF:RCo/ VWF:Ag (IU/IU)
BAX111		116 ± 7 n=7	1.16 ± 0.25 n=7
rhVWF fraction	A	133	0.420
	B	131	0.088
	C	149	0.026

- rhVWF fractions of varying multimer size were generated by size exclusion chromatography
- Multimer size contributes to VWF functional activity

VWF Size-Dependent Platelet Adhesion



Shear rate of 1500 s^{-1} (60 dyn/cm^2)

- Multimer size of rhVWF contributes to platelet adhesion properties

First Recombinant VWF Concentrate: Summary

- Dependable manufacturing
  - **Pure rhVWF concentrate**
    - Formulated in absence of animal or human components
  - **Intact VWF subunits**
- Longer half-life than for pdVWF
- Consistent multimer composition = more predictable therapeutic effects
  - **Platelet binding properties depend on the multimeric size of rhVWF**
  - **rhVWF contains the hemostatic most effective UHMW multimers**
    - Improved FVIII stabilizing effect than for pdVWF
- rhVWF effectively promoted platelet adhesion to collagen – even under shear stress

UHMW, ultra-high molecular weight.

First Recombinant VWF Concentrate: Summary (cont)

- **Physiologically degradable**
  - **rhVWF is a substrate for ADAMTS13 that is as good as plasmatic VWF; it can also be cleaved under shear stress**
  - **Low amounts of ADAMTS13 (<1% of normal human plasma) are sufficient to rapidly cleave rhVWF and remove UHMW portions of multimers**

- **Therapeutic potential**
  - **Dosing rhVWF with ADVATE will allow better control of the initial levels of both FVIII and VWF**
  - **Dosing rhVWF alone will allow better control of FVIII levels, reducing the risk of thrombotic events due to high FVIII levels**

Objectives: Phase 3
rhVWF in VWD

Primary Objective

- Hemostatic efficacy of rhVWF:rFVIII and/or rhVWF alone for treatment of bleeding episodes

Secondary Objectives

- Pharmacokinetics (rhVWF alone and with rFVIII)
- Tolerability and safety of rhVWF
- Exploratory
  - Changes in health-related quality of life
  - Subjective hemostatic efficacy rating

Primary End Point: High Treatment Success Rate

	Subjects With Treatment Success	
Full analysis set	N of N (%)	90% CI for proportion
Prospective efficacy rating excluding GI bleeds	18/18 (100)	84.7 – 100.0
Prospective efficacy rating including GI bleeds	20/20 (100)	86.1 – 100.0
All bleeds	22/22 (100)	87.3 – 100.0

- **Definition of primary end point: Number/proportion of subjects with treatment success for treated bleeding episodes based on estimated versus actual number of infusions**
- **Benchmark: Humate-P claims 97% treatment success**
  http://labeling.cslbehring.com/PI/US/Humate-P/EN/Humate-P-Prescribing-Information.pdf

CI, confidence interval; GI, gastrointestinal.

Secondary Efficacy End Points: Bleeding Episodes Rated Good or Excellent

	Bleeding Episodes With an Efficacy Rating of Excellent or Good	
Full analysis set	N of N (%)	90% CI for proportion
Prospective efficacy rating excluding GI bleeds	126 (100)	97.7 – 100.0
Prospective efficacy rating including GI bleeds	130 (100)	97.7 – 100.0
All bleeds	192 (100)	98.5 – 100.0

- **186 (97%) of all bleeds treated with rhVWF:rFVIII and rhVWF were rated excellent**
- **The majority of bleeds (81.8%) was resolved with 1 infusion**
- **The maximum number of infusions was 4**

Secondary Efficacy End Points (cont): Infusions per Bleeding Episode and Dose per Bleeding Episode

Number of Infusions Required for the Treatment of a Bleeding Episode (Secondary Efficacy Outcome Measure) (Study 071001: Full Analysis Set)

Infusions per Bleeding Episode	N[a]	Mean	SD	Median	90% CI for Median	Minimum	Maximum
Prospectively estimated	130	1.2	0.61	1.0	1.0 – 1.0	1	6
Actual[b]	130	1.2	0.47	1.0	1.0 – 1.0	1	3
Actual – total	192	1.2	0.56	1.0	1.0 – 1.0	1	4

Actual Dose [IU/kg] per Bleeding Episode (Secondary Efficacy Outcome Measure (Study 071001: Full Analysis Set)

Treatment	Analyte	N[a]	Mean	SD	Median	90% CI for Median	Minimum	Maximum
rhVWF:rFVIII or rhVWF	rhVWF	174	57.4	30.27	48.2	43.9 – 50.2	23.8	184.9

[a] N = Number of bleeding episodes
[a] Number of actual infusions for prospectively estimated ones.

Secondary Efficacy End Points (cont): Product Usage per Bleeding Episode

- 1st infusion per protocol: rhVWF + rFVIII

Investigational Product Usage per Bleeding Episode (Study 071001: Full Analysis Set)

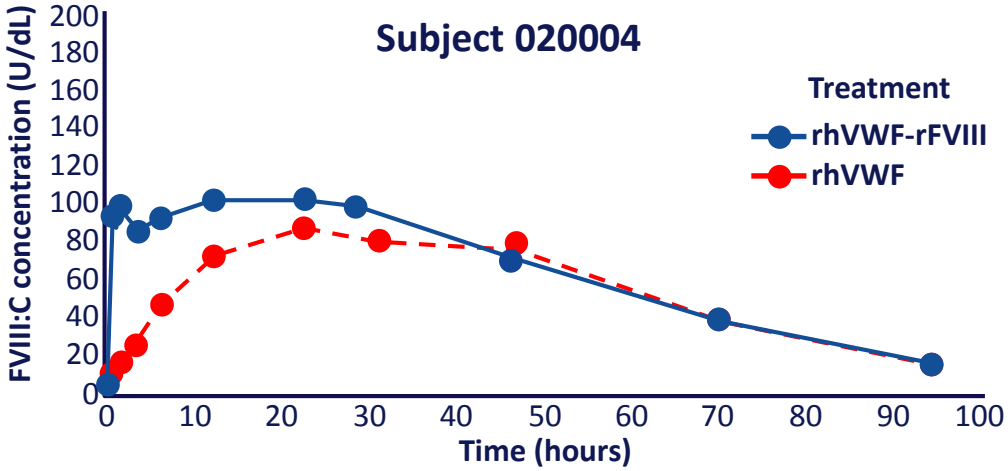
Infusion	rhVWF:rFVIII n of N (%)	rhVWF only n of N (%)
1st	182 of 192 (94.8)	10 of 192 (5.2)
2nd	14 of 35 (40.0)	21 of 35 (60.0)
3rd	2 of 10 (20.0)	8 of 10 (80.0)
4th	0 of 1 (0.0)	1 of 1 (100.0)

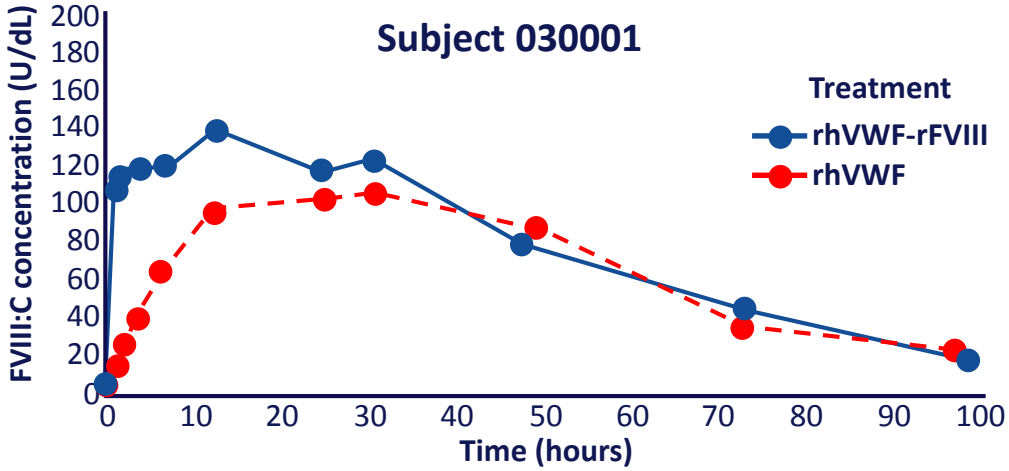
Treatment summary of all bleeding episodes.

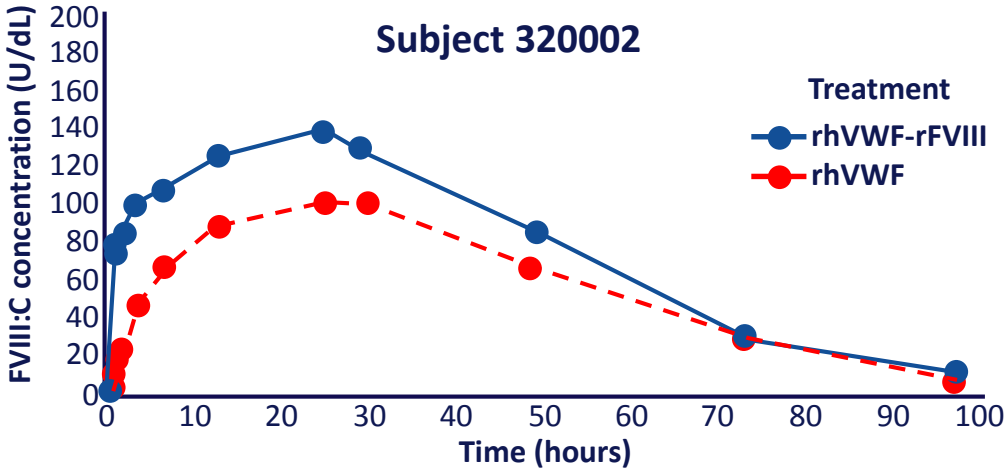
	No. bleeding episodes	Total no. infusions	Median (range) no. infusions/bleed	Median (range) VWF:RCo dose (IU/kg)	Median (range) rFVIII dose (IU/kg)	% bleeds (N = 192) rated* excellent or good (n excellent/good)
Subject VWF type						
Type 3	175	219	1 (1-4)	48.2 (23.8-184.9)	33.6 (16.6-129.3)	100% (171/4)
Type 2A	16	18	1 (1-2)	50.2 (32.9-90.2)	32.5 (23.7-38.6)	100% (14/2)
Type 2N	1	1	1 (1-1)	54.3 (54.3-54.3)	NA†	100% (1/0)
Bleed severity						
Minor	122	132	1 (1-3)	43.3 (25.2-158.2)	33.5 (17.6-86.2)	100% (119/3)
Moderate	61	89	1 (1-4)	52.7 (23.8-184.9)	36.9 (16.6-129.3)	100% (59/2)
Major/severe	7	15	2 (1-3)	100.0 (57.5-135.0)	39.0 (25.0-42.3)	100% (6/1)
Unknown	2	2	1 (1-1)	33.4 (33.1-33.8)	23.3 (23.1-23.6)	100% (2/0)
Bleed site‡						
Joint	59	66	1 (1-3)	48.2 (23.8-139.6)	34.9 (16.6-129.3)	100% (57/2)
Gastrointestinal	6	10	1 (1-2)	60.0 (53.6-121.1)	33.2 (19.3-49.4)	100% (5/1)
Mucosal	106	121	1 (1-4)	43.3 (23.8-184.9)	30.6 (16.6-61.3)	100% (103/3)
Other§	37	57	1 (1-4)	52.2 (25.2-184.9)	36.8 (17.6-86.2)	100% (36/1)
Bleed cause						
Spontaneous	165	255	1 (1-4)	46.5 (23.8-184.9)	33.6 (16.6-86.2)	100% (160/5)
Traumatic	26	30	1 (1-3)	51.9 (25.2-139.6)	35.8 (17.6-129.3)	100% (26/0)
Unknown	1	3	3 (3-3)	125.5 (125.5-125.5)	50.3 (50.3-50.3)	100% (0/1)

Gill JC et al, Blood 2015;126(17):2038-2046.

PK50 FVIII:C Concentration Over Time

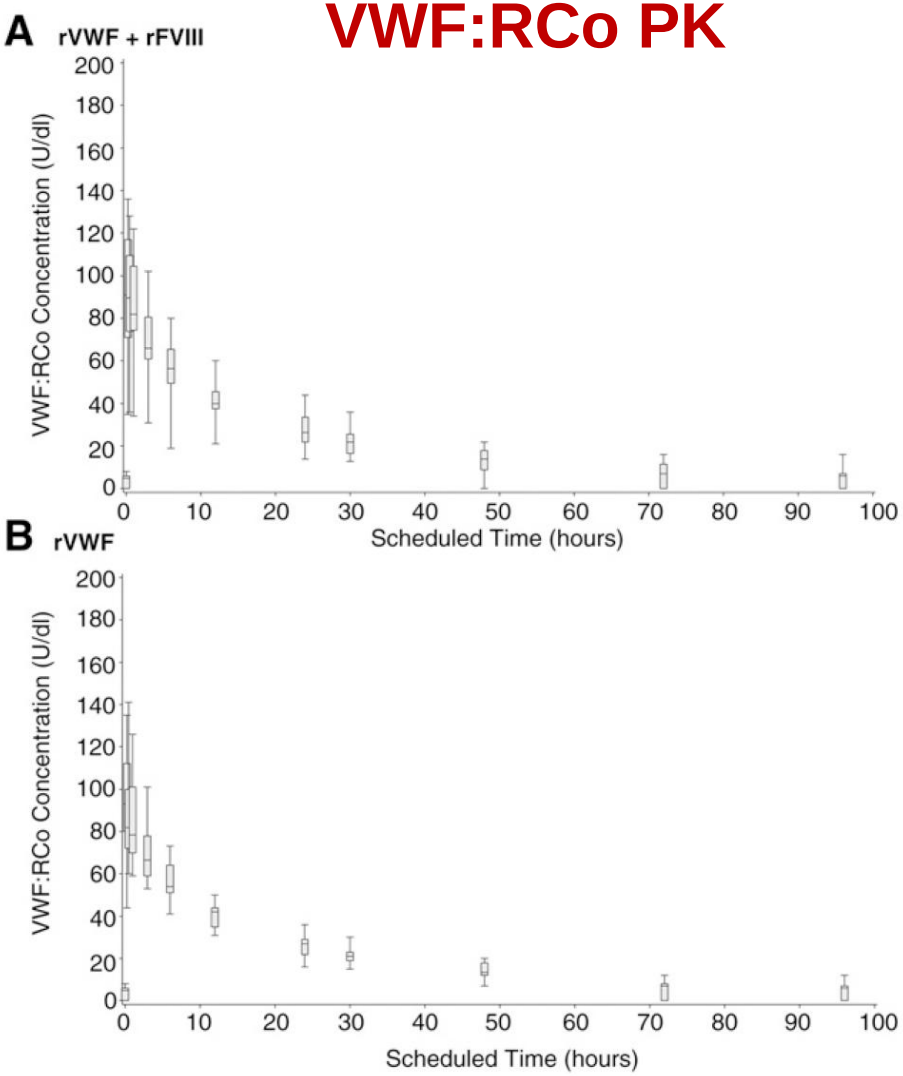




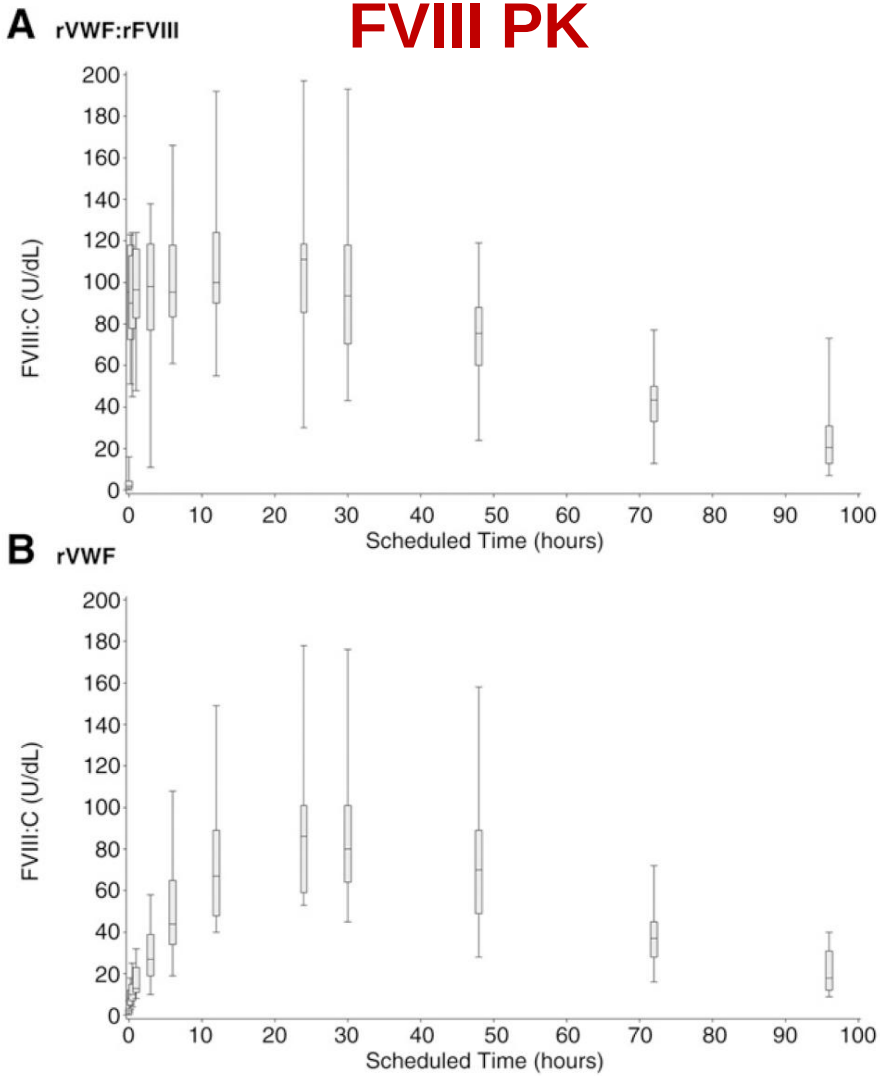


PK of rVWF ± rFVIII in VWD patients.

VWF:RCo PK



FVIII PK



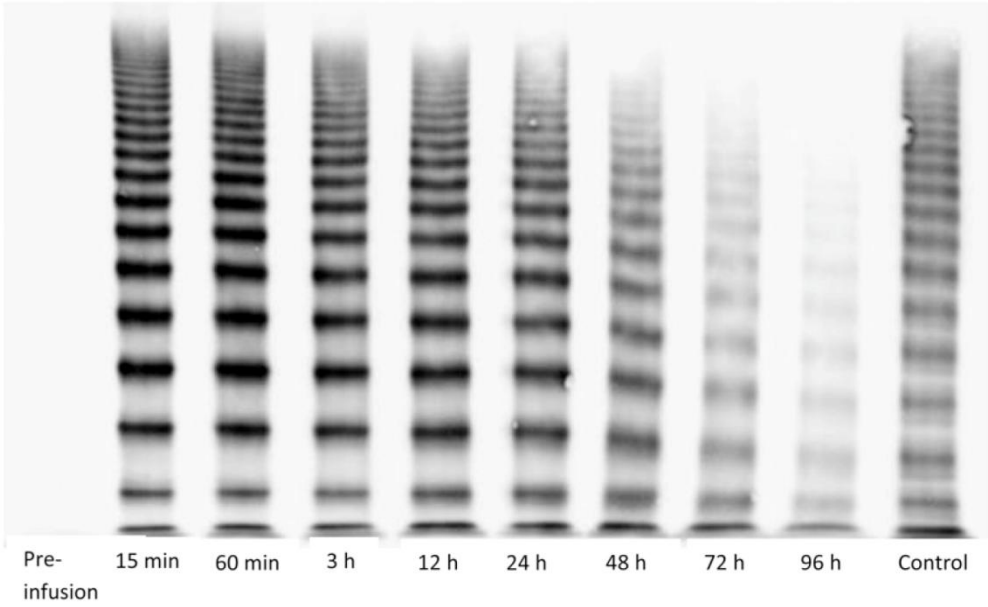
Gill JC et al, Blood 2015;126(17):2038-2046.

VWF multimer activity.

A Proportion of large VWF multimers

Time point	Median % (range)
Within 1 h pre-infusion	0.0% (0-24)
15 +/- 5 min post-infusion	30.0% (20-40)
30 +/- 5 min post-infusion	29.5% (25-35)
60 +/- 5 min post-infusion	29.5% (22-38)
3 +/- 0.5 h post-infusion	26.0% (19-32)
6 +/- 0.5 h post-infusion	25.0% (17-37)
12 +/- 0.5 h post-infusion	21.5% (12-26)
24 +/- 0.5 h post-infusion	14.0% (8-27)
30 +/- 0.5 h post-infusion	8.5% (4-23)
48 +/- 0.5 h post-infusion	7.5% (3-14)
72 +/- 0.5 h post-infusion	3.0% (1-5)
96 +/- 0.5 h post-infusion	2.0% (1-7)

B VWF multimers and degradation products pre- and post-infusion of rVWF in a subject with type 3 VWD.



Gill JC et al, Blood 2015;126(17):2038-2046.

PK Summary

- $T_{1/2}$ of rhVWF appears to be slightly longer than $T_{1/2}$ of pdVWF products published[1]
- $T_{1/2}$ of rhVWF in clinical phase 3 is consistent with $T_{1/2}$ in clinical phase 1
- IR of rhVWF is comparable to that of pdVWF products
- PK is not dependent regardless of whether rhVWF is given alone or in combination with rFVIII
- Pretreatment and end-of-study repeated PK results are in close agreement
- PK50 and PK80 results are comparable

1. Kessler CM et al. *Thromb Haemost*. 2011;106:279-288.

Safety Summary

- **Overal, rhVWF was safe and well tolerated**
- **No development of inhibitory and total anti-VWF binding antibodies**
- **No development of inhibitory antibodies to FVIII**
- **No development of antibodies to Chinese hamster ovary proteins, mouse immunoglobulin G, and recombinant Furin**
- **No thrombotic events**

rhVWF Summary

- **rhVWF is the first pure rVWF concentrate**
- **rhVWF was safe and well tolerated in phase 1 and 3 studies**
- **rhVWF has the potential to provide a better treatment option for patients with VWD**
- **Based on these positive results, it would be also interesting to assess this novel rhVWF in other clinical conditions characterized by abnormal VWF (AVWS)**

Questions?

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