Immunogenicity Assessment of Emicizumab (Hemlibra® ▼)

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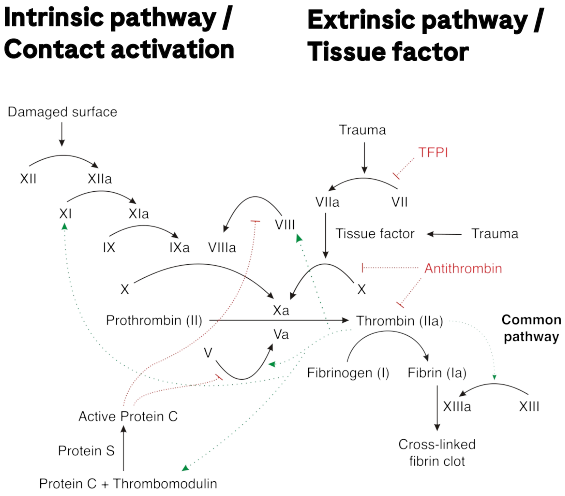
Haemophilia A
Background

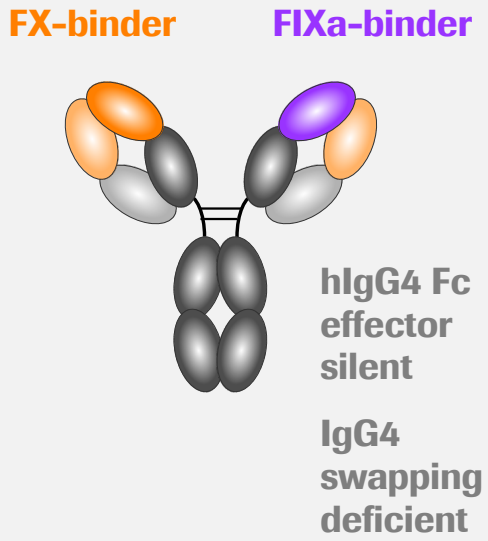
- Hemophilia A is a hereditary bleeding disorder caused by congenital absence or hypofunction of blood clotting factor VIII (FVIII)
- FVIII gene is located on the X chromosome
  - X-linked recessive inheritance of genetic defects, >99% of hemophilia patients are males
  - Prevalence of hemophilia A is approx. 1 in 5,000 males
- Main bleeding sites are intra articular, intramuscular, subcutaneous, intraoral, intracranial, gastrointestinal, and intranasal
- Repeated intra articular bleeding is a major factor that decreases health-related quality of life ⇨ progress to arthropathy and hemophilic arthropathy
- Severity of hemophilia A is classified in accordance with endogenous FVIII activity in the blood
  - Severe disease: FVIII activity <1% ⇨ about 70% of all patients with hemophilia A
  - Moderate disease between 1% and 5%
  - Mild disease: >5% and <40%



Emicizumab (Hemlibra® ▼)

Background

- Emicizumab is a humanized, bispecific IgG4 monoclonal antibody[1]
  - Bridges blood coagulation factors FIXa and FX to replace the function of FVIIIa in persons with haemophilia A (PwHA)
  - Optimized structure to minimize development of anti-emicizumab antibodies[2]
- Terminal half-life of ~30 days[3]
- Administered subcutaneously with high bioavailability[4]
- Demonstrated favorable safety and effective bleed prevention in pediatric and adult PwHA with or without FVIII inhibitors[5-11]
  - Approved in 108/96 countries for PwHA of all ages, with or without FVIII inhibitors, with once weekly (QW), every 2 weeks (Q2W), or every 4 weeks (Q4W) dosing

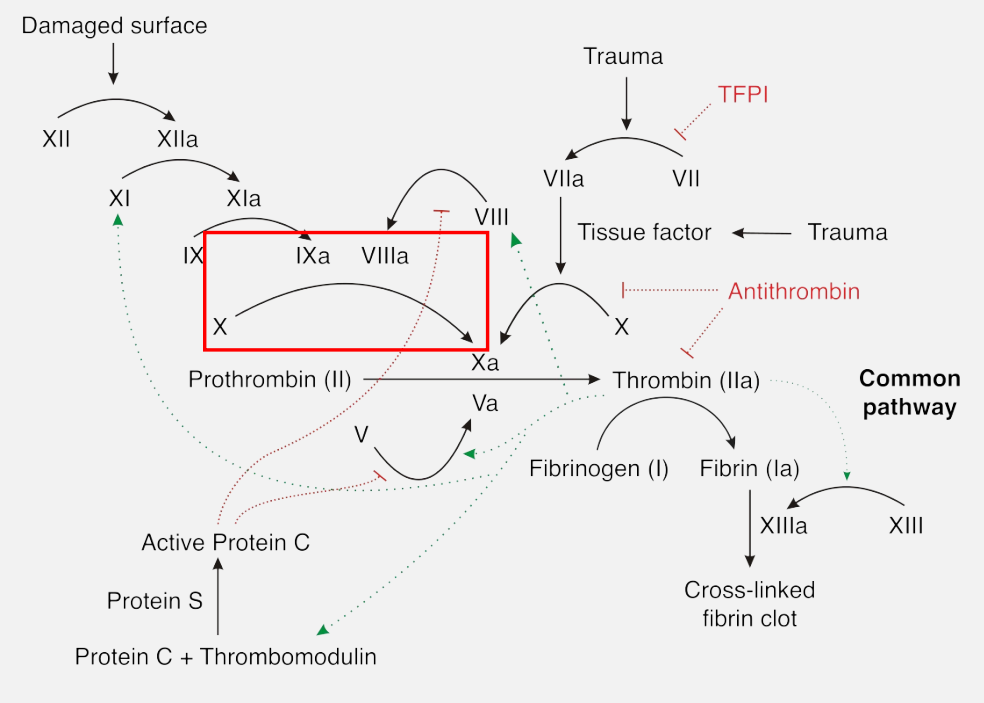


▼ This medicinal product is subject to additional monitoring. This will allow quick identification of new safety information. Healthcare professionals are asked to report any suspected adverse reactions. These should be reported to the Regulatory Authorities in your country according to your national requirements.

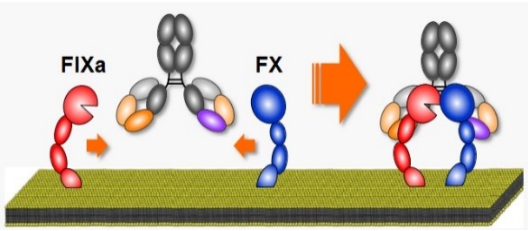
[1]Kitazawa T, et al. *Thromb Haemost* 2017;117:1348–57; [2]Sampei Z, et al. *PLoS One*. 2013;8:e57479; [3]Uchida N, et al. *Blood* 2016;127:1633–41; [4]Kotani N, et al. *Clin Pharmacol Drug Dev* 2018;doi:10.1002/cpdd.617; [5]Oldenburg J, et al. *New Engl J Med.* 2017;377(9):809–818; [6]Young et al. *Blood.* 2019;134(24):2127–2138; [7]Mahlangu J, et al. *N Engl J Med.* 2018;379(9):811–822; [8]Pipe et al. *Lancet Haematol.* 2019;6(6):E295–E305; [9]Yang R, et al. *Res Pract Thromb Haemost*. 2022 Mar 7;6(2):e12670. doi: 10.1002/rth2.12670. eCollection 2022 Feb; [10]Shima et al. *Haemophilia.* 2019;25(6):979–987; [11]Jiménez-Juste et al. *Res Prac Thromb Haemost.* 2020;4 (Suppl. 1)

Emicizumab

MoA



- **<u>Mode of action of emicizumab</u>:**
  - Emicizumab is a recombinant humanized anti-factor IXa (anti-FIXa) and anti-factor X (anti-FX) bispecific monoclonal IgG4 antibody for the treatment of hemophilia A
  - Emicizumab binds to activated blood coagulation factor IX (FIXa) and blood coagulation FX, and thereby mimics FVIII by promoting the activation of FX by FIXa and activating downstream hemostasis at the site of bleeding of PwHA who have hypofunctional levels or entirely lack FVIII, irrespective of the presence of FVIII inhibitors.

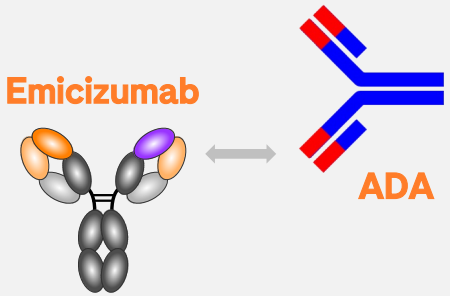


Emicizumab bioanalytical and immunogenicity testing strategy

Immunogenicity testing strategy

PK assessment

- Monitoring of dual-binding competent (active) emicizumab
  - by simultaneous binding of emicizumab <FIXa> and <FX> arms

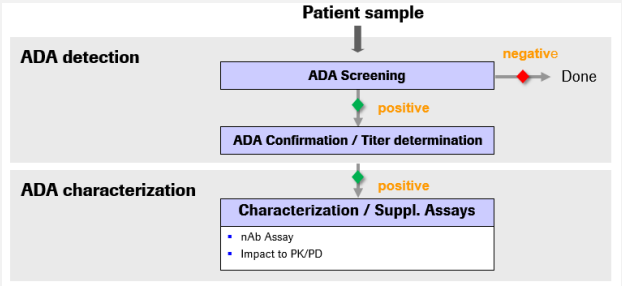


Clinical relevance

- Impact of ADAs on PK
- Impact on PD/efficacy
- Impact on safety

ADA assessment

- Tiered ADA testing approach using an initial screening assay followed by a confirmatory assay and a titering assay
- Evaluation of function-neutralizing potential of ADA
  - by consolidated data on ADA, PK, PD/efficacy
  - by emicizumab function-neutralizing ADA assay (nAb assay) (PMC requested by FDA)



Emicizumab bioanalytical and immunogenicity testing strategy

Assay strategy

PK Assay

- Monitoring of dual-binding competent (active) emicizumab
- Emicizumab–specific (FIXa and FX-specific) PK immunoassay (ELISA)
- Binding via anti-idiotypic antibodies as FIXa/FX surrogates

Clinical relevance

- Impact of ADAs on exposure
- PD markers:
  - FVIII-like activity via chromogenic assay
  - Activated partial thromboplastin time (aPTT)
- Efficacy / Annualized bleeding rate (ABR)
- Safety markers

ADA Assays

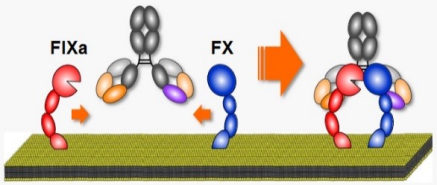
- Detection of ADA by bridging ADA immunoassay (ELISA) – ADA Screening and confirmation
- **Main performance characteristics of the Emicizumab ADA assay:**
  - **Cut-point** — **disease-population specific CP established using pre-study human plasma samples** (377 PwHA samples for screening assay; 100 PwHA samples for confirmation assay)
  - **Sensitivity** — **6.04 ng/mL ADA positive surrogate control** (polyclonal anti-idiotypic (FIXa and FX binder) antibody preparation)
  - **Drug tolerance** — **500 ng/mL of ADA positive surrogate control could be detected in presence of 40 µg/mL of emicizumab**

    100 ng/mL of ADA positive surrogate control in presence of 10 ug/mL emicizumab
- Detection of function-neutralizing ADA (nAb) by a chromogenic assay-based functional assay following immuno-enrichment of ADA

Emicizumab immunogenicity assay strategy

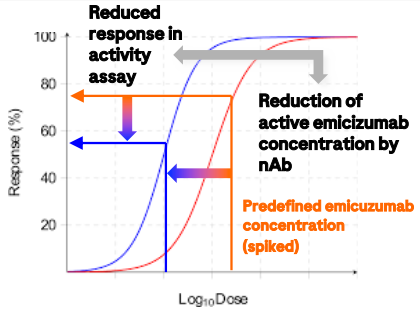
Function neutralizing ADA (nAb) assay

Activity Assay



nAb assay development (step A)

nAb Assay



- **<u>Strategy for emicizumab nAb assay</u>:**
  - nAb Assay strategy based on the therapeutic MoA
  - Activity measurement of emicizumab by a chromogenic assay (=potency assay) - based nAb assay

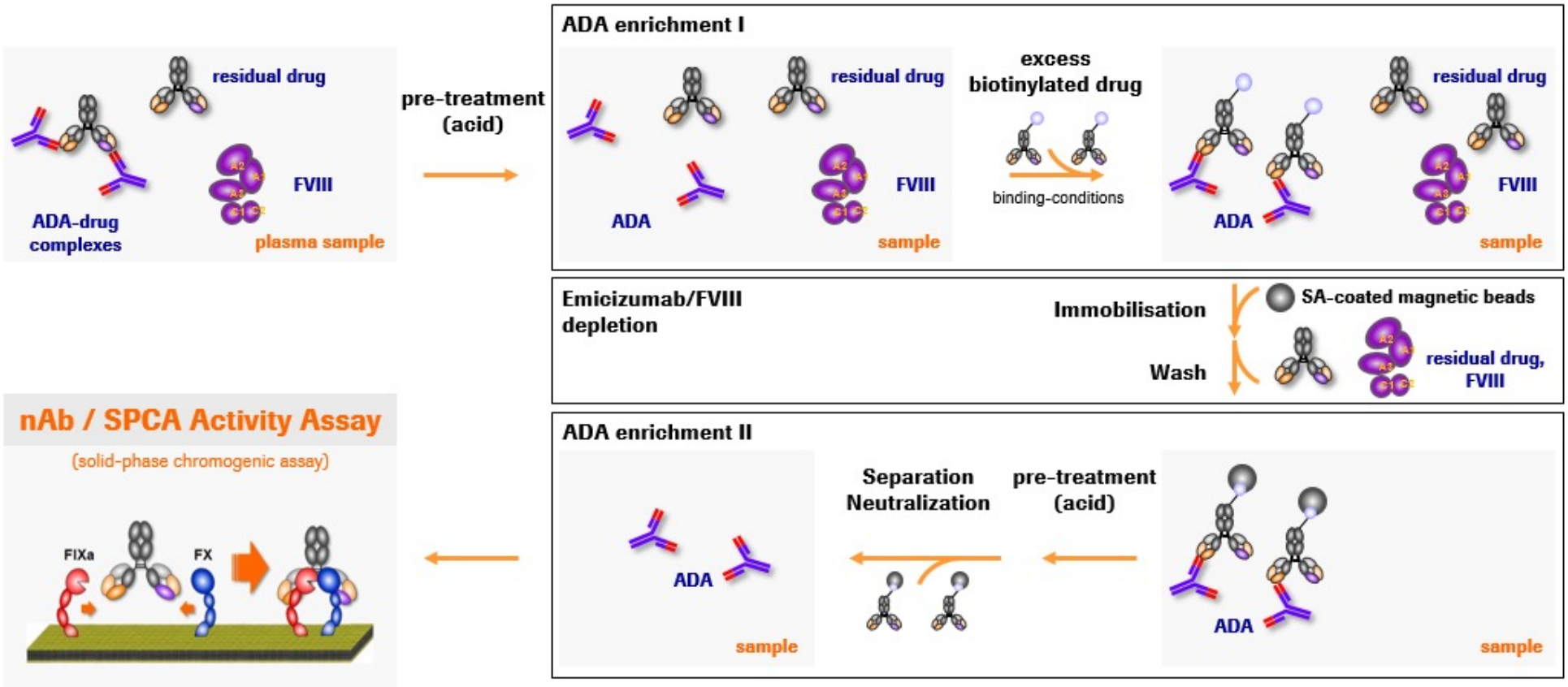
- **<u>Emicizumab nAb assay requirements</u>:**
  - High sensitivity of emicizumab activity detection to allow nAb-mediated activity reduction at low nADA levels
  - Optimization of chromogenic assay towards a suitable conc.-response curve

- **<u>Additional challenges for emicizumab nAb assay development</u>:**
  - Study samples from PwHA may also contain residual emicizumab and factor VIII which both generate a signal in the activity nAb assay
  - In the presence of residual emicizumab, nAbs are typically present as drug-ADA-complexes that are no longer active in the activity nAb assay

⇨ **Purification/enrichment of nAb is required in addition (step B)**

Emicizumab immunogenicity assay strategy

Function neutralizing ADA (nAb) assay



Emicizumab immunogenicity assay strategy

Function neutralizing ADA (nAb) assay

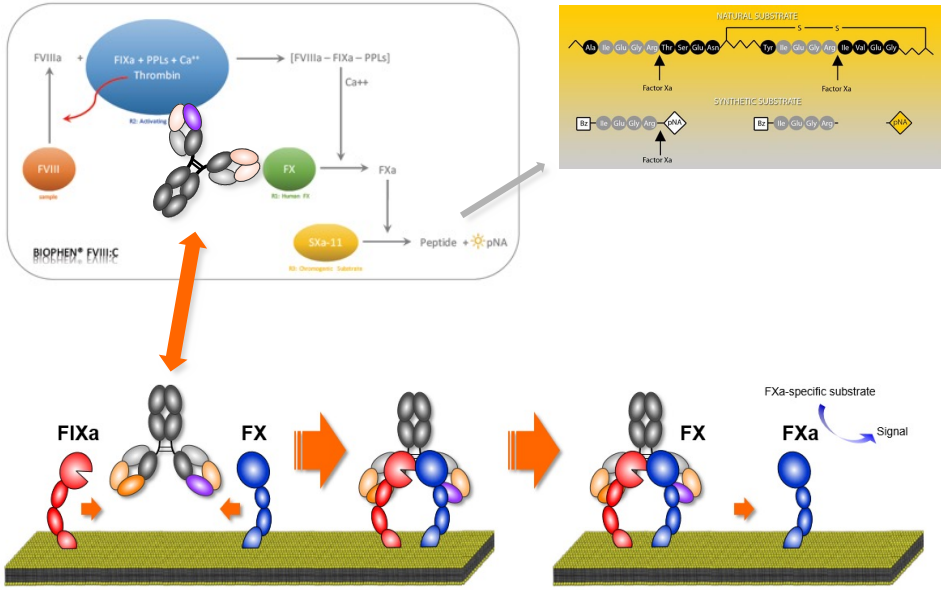
Chromogenic Assay (Biophen FVIII:C Assay)

- Assay principle:
  - When activated by thrombin, FVIII:C forms an enzymatic complex with FIXa, phospholipids and Ca^{2+}, which activates FX to FXa
  - Activity of FXa is measured by a specific FXa chromogenic substrate (Sxa-11/S-2222™)

Emicizumab SPCA nAb assay:

- Emicizumab mimics action of FVIIIa therefore activating FX to FXa and is measured by specific cleavage of chromogenic substrate
- **Main performance characteristics of the emicizumab nAb assay:**
  - **Cut-point** — **established using 100 individual human plasma samples from healthy donors**
  - **Sensitivity** — **229 ng/mL nADA surrogate positive control**
  - **Drug tolerance** — **>70 µg/mL emicizumab at HPC (1200 ng/mL) and MPC (800 ng/mL) nADA concentration**

    tolerant to 50 µg/mL emicizumab at LPC (570 ng/mL) nADA concentration



Immunogenicity assessment of emicizumab

Phase 3/3b clinical studies included in the analysis

Study name	Population	No. of PwHA enrolled/evaluable	References
HAVEN 1	FVIII inhibitor adults/adolescents (≥12 years) – QW dosing	113/111	Oldenburg et al.[1]
HAVEN 2	FVIII inhibitor children (<12 years) – QW, Q2W, Q4W dosing	88/88	Young et al.[2]
HAVEN 3	Non-FVIII inhibitor adults/adolescents (≥12 year) – QW and Q2W dosing	152/151	Mahlangu et al.[3]
HAVEN 4	Non-FVIII inhibitor/inhibitor adults/adolescents (≥12 years) – Q4W dosing	48/48	Pipe et al.[4]
HAVEN 5	FVIII inhibitor adults/adolescents (≥12 years) – QW and 4W dosing	70/64	Yang et al.[5]
HOHOEMI	Non-FVIII inhibitor children (<12 years) – Q2W and Q4W dosing	13/13	Shima et al.[6]
STACEY	FVIII inhibitor adults/adolescents (≥12 years) – QW dosing	195/193	Jiménez-Juste et al.[7]

Σ 668 PwHA

- With the exception of the PK run-in cohort in HAVEN 4, participants received emicizumab prophylaxis starting with weekly loading doses of 3 mg/kg QW for 4 weeks, followed by maintenance doses of 1.5 mg/kg QW, 3 mg/kg Q2W, or 6 mg/kg Q4W. Participants in the HAVEN 4 run-in cohort received emicizumab 6 mg/kg Q4W, with no loading dose.
- [1]*New Engl J Med.* 2017;377(9):809–818; [2]*Blood.* 2019;134(24):2127–2138; [3]*N Engl J Med.* 2018;379(9):811–822; [4]*Lancet Haematol.* 2019;6(6):E295–E305; [5]*Res Pract Thromb Haemost*. 2022 Mar 7;6(2):e12670. doi: 10.1002/rth2.12670. eCollection 2022 Feb; [6]*Haemophilia.* 2019;25(6):979–987; [7]Res Prac Thromb Haemost. 2020;4 (Suppl. 1).

Immunogenicity assessment of emicizumab

Definition of ADA status

Subject ADA status	Sample ADA status	Definition
ADA-negative subject	ADA-negative for all samples	All pre-treatment (baseline) and post-dose samples were negative
	Treatment-unaffected ADA	Pre-treatment sample was positive and post-dose samples were either negative or positive with a <4-fold increase in ADA titre compared to baseline
ADA-positive subject	Treatment-induced ADA	Pre-treatment sample was negative and ≥1 post-dose samples was positive
	Treatment-boosted ADA	Pre-treatment sample was positive and ≥1 post-dose samples was positive with a ≥4-fold increase in ADA titre compared to baseline
	Transient ADA	Treatment-induced or –boosted ADAs detected only at 1 post-dose sample (excluding last sampling time point)
	Persistent ADA	Treatment-induced or –boosted ADAs detected at ≥2 post-dose samples (or detected only at last sampling time point)
	Neutralizing ADA	Treatment-induced or –boosted ADAs with in-vitro neutralizing capacity detected by Nab assay
	ADA with decreased exposure	ADA-positive subject with decreased emicizumab concentrations

Definitions adapted to harmonized nomenclature proposed by Shankar G. et al C. AAPS J. 2014 Jul;16(4):658-673.

Immunogenicty assessment of emicizumab

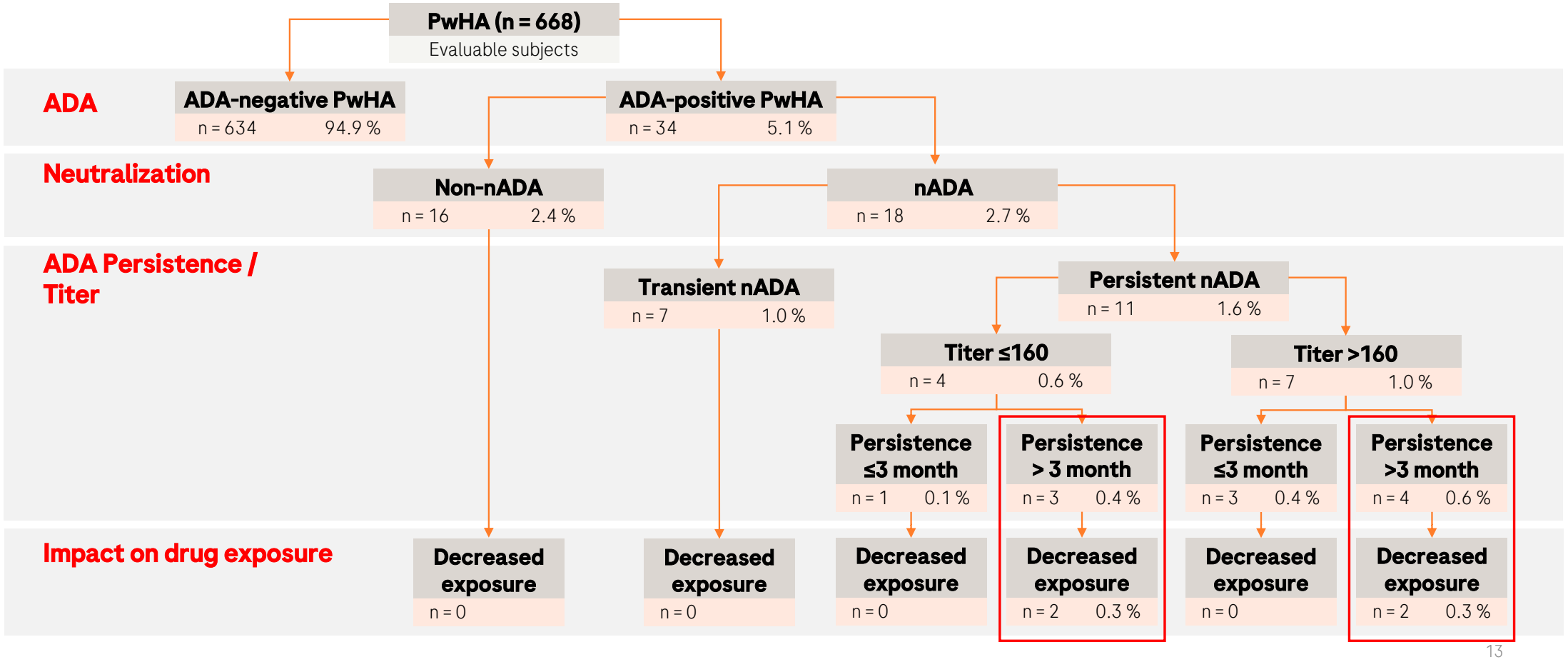
Summary – Immunogenicity status evaluated per study

	HAVEN 1	HAVEN 2	HAVEN 3	HAVEN 4	HAVEN 5	HOHOEMI	STASEY	Total
No. of PwHA evaluable	111	88	151	48	64	13	193	**668**
n (%) of PwHA, ADA+ at baseline	2 (1.8)	4 (4.5)	4 (2.6)	3 (6.3)	4 (6.3)	2 (15.4)	5 (2.6)	**24 (3.6)**
n (%) of ADA- PwHA	109 (98.2)	82 (93.2)	145 (96.0)	46 (95.8)	56 (87.5)	13 (100)	183 (94.8)	**634 (94.9)**
ADA- (all samples)	107 (96.4)	78 (88.6)	142 (94.0)	43 (89.6)	52 (81.3)	11 (84.6)	180 (93.3)	**613 (91.8)**
ADA- (treatment uneffected)	2 (1.8)	4 (4.5)	3 (2.0)	3 (6.3)	4 (6.3)	2 (15.4)	3 (1.6)	**21 (3.1)**
n (%) of ADA+ PwHA	2 (1.8)	6 (6.8)	6 (4.0)	2 (4.2)	8 (12.5)	0 (0)	10 (5.2)	**34 (5.1)**
ADA+ (treatment induced)	2 (1.8)	6 (6.8)	5 (3.3)	2 (4.2)	8 (12.5)	0 (0)	8 (4.2)	**31 (4.6)**
ADA+ (treatment boosted)	0 (0)	0 (0)	1 (0.7)	0 (0)	0 (0)	0 (0)	2 (1.0)	**3 (0.5)**
n (%) of PwHA with nADA	2 (1.8)	3 (3.4)	4 (2.6)	1 (2.1)	3 (4.7)	0 (0)	5 (2.6)	**18 (2.7)**
n (%) of PwHA with ADA with decreased exposure	1 (0.9)	2 (2.3)	0 (0)	0 (0)	1 (1.6)	0 (0)	0 (0)	**4 (0.6)**

Schmitt, C., Emrich, T., Chebon, S. Fernandez, E. Petry, C., Yoneyama, K., Kiiliainen, An, Howard, M, Niggli, M. Paz-Priel, I, Chang, T. *Haemophilia*. 2021 Nov;27(6):984-992.

Immunogenicty assessment of emicizumab

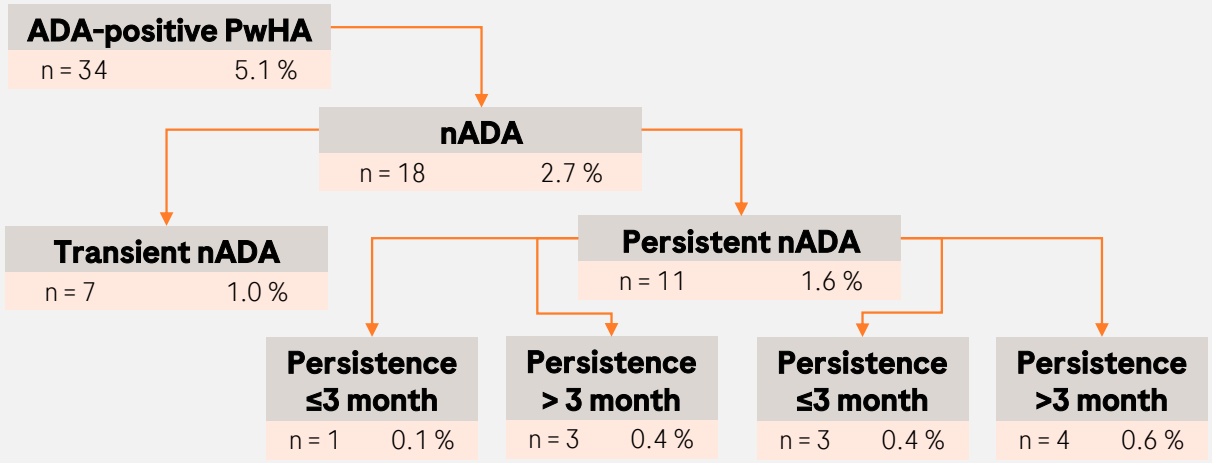
Summary – Overall immunogenicity status



Immunogenicty assessment of emicizumab

Titer, on-set and persistence of ADA detection

PwHA (N = 668)	Time to first treatment-induced or treatment-boosted ADA				
	≤1 month	>1 to 2 month	>2 to ≤3 month	>3 to ≤ 6 month	>6 month
ADA-positive PwHA (n = 34) [n (%)]	3 (8.8)	4 (11.8)	9 (26.5)	14 (41.2)	4 (11.8)
nADA-positive PwHA (n = 18) [n (%)]	2 (11.1)	2 (11.1)	7 (38.9)	6 (33.3)	1 (5.6)



Conclusions

- Majority of ADAs (88.2%; n = 30/34) were first detected within 6 month of treatment initiation with emicizumab
- 41.2% (n = 14/34) of PwHA who developed ADA had transient ADAs (data not shown)
- Most ADA-positive PwHA (70.6%; n = 24/34) had maximum ADA titres ≤ 160 (data not shown)
- Highest ADA titre (10,400) was observed in a subject with decreased emicizumab exposure

Immunogenicty assessment of emicizumab

Impact of ADAs on efficacy

	ADA-negative PwHA (N = 634)	ADA-positive PwHA (N = 30)	ADA-positive PwHA with decreased exposure (N = 4)
Duration of efficacy period Median (IQR), weeks	**103** [84-150]	**103** [78-160]	**57** [16; 65;80;50]
Annualized rate of treated bleeding events Model-based [95% CI]	**0.9** [0.79-1.11]	**0.7** [0.37-1.33]	**14.1** [23.0; 28.0; 0; 5.3]
Proportion of participants with zero treated bleeds n (%) [95% CI]	**374 (59.0)** [55.1-62.9]	**16 (53.3)** [34.3-71.7]	**1 (25.0)** [0.6-80.6]
Annualized rate of all bleeding events Model-based [95% CI]	**2.3** [1.99-2.60]	**1.2** [0.75-1.91]	**15.2** [23.0; 28.0; 0; 7.4]
Proportion of participants with zero all bleeds n (%) [95% CI]	**224 (35.3)** [31.6-39.2]	**7 (23.3)** [9.9-42.3]	**1 (25.0)** [0.6-80.6]

Conclusions

- Comparable median efficacy period for ADA-negative and ADA-positive PwHA
- Emicizumab was highly effective in controlling bleeding events in PwHA with and without ADA (excluding ADA-positive PwHA with decreased exposure; all 4 ADA-positive PwHA with decreased exposure were also nADA-positive)

Immunogenicty assessment of emicizumab

Impact of ADAs on safety

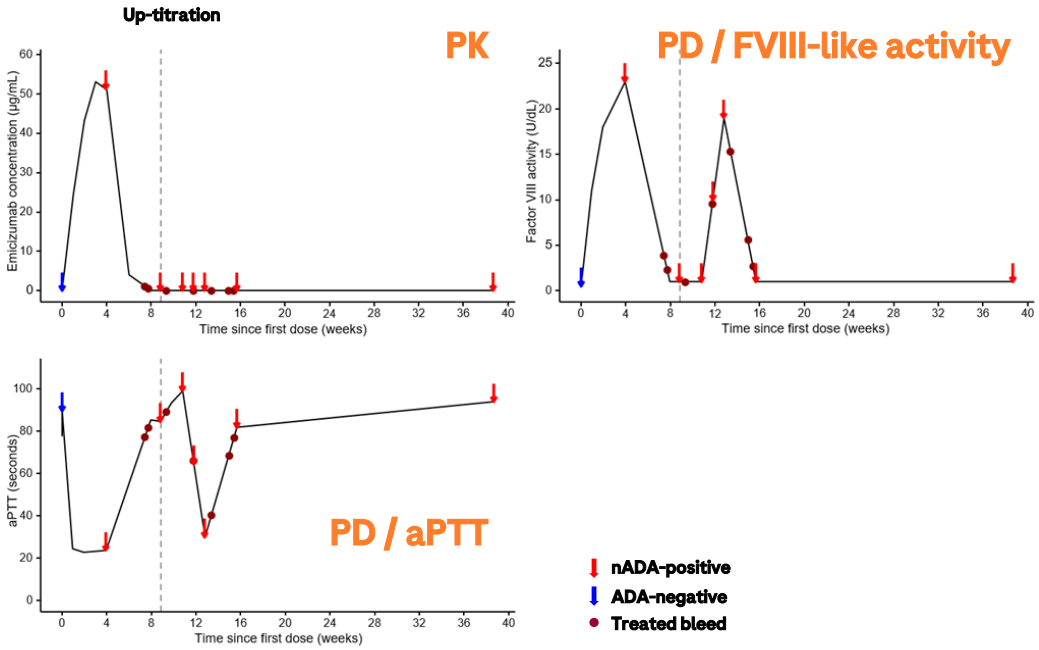
	ADA-negative PwHA (N = 634)	ADA-positive PwHA (N = 34)
Duration of exposure period Median (IQR), weeks	**103** [83-148]	**100** [55-159]
PwHA with at least one AE, n (%)	**575 (90.7)**	**31 (91.2)**
PwHA with at least one drug-related AE, n (%)	**187 (29.5)**	**11 (32.4)**
PwHA with at least one SAE, n (%)	**122 (19.2)**	**7 (20.9)**
PwHA with at least one drug-related SAE, n (%)	**6 (0.9)**	**1 (2.9)**
PwHA with at least one ISR, n (%)	**132 (20.8)**	**10 (29.4)**
PwHA with at least one hypersensitivity, anaphylactic or anaphylactoid reaction, n (%)	**2 (0.3)**	**0 (0)**

Conclusions

- Safety profile of PwHA with and without detected ADAs does not differ
- The presence of nADA in ADA-positive PwHA did not affect the safety profile of emicizumab

Immunogenicty assessment of emicizumab

Case details of PwHA with neutralizing ADAs with decreased exposure – Participant A (HAVEN 2)

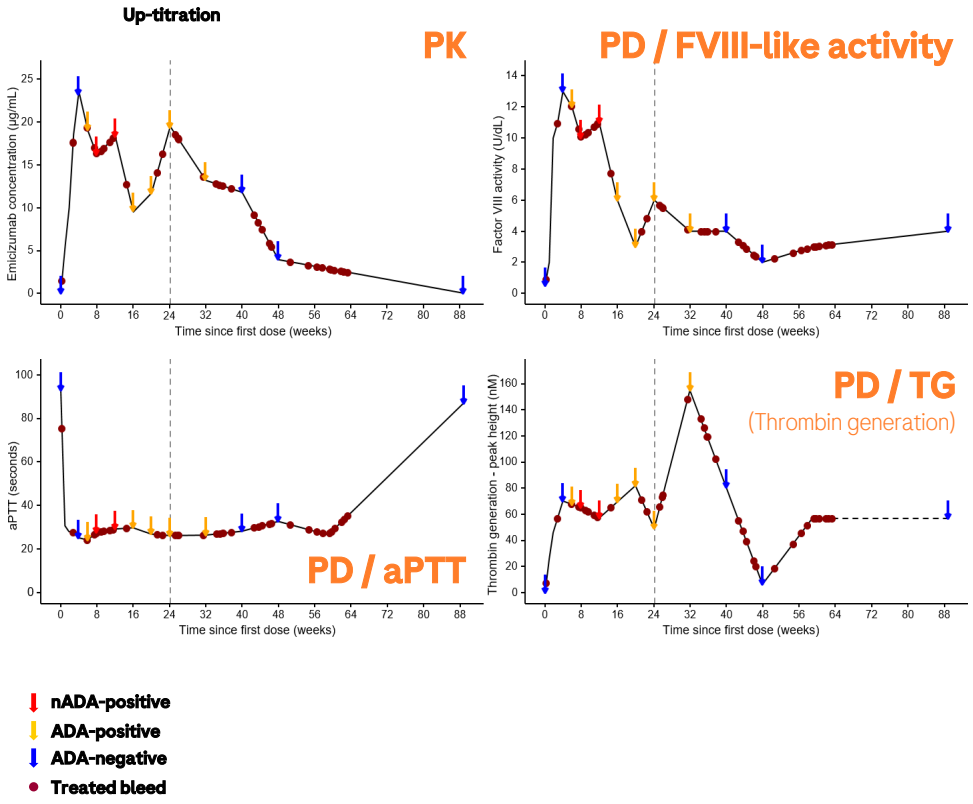


Observation

- ADA detected (titre, 160) at w4 of emicizumab prophylaxis at the end of the loading-dose period.
- ADA titre increased to 10,200 at w9. All ADA samples all ADA samples were neutralizing in vitro.
- Emicizumab concentration declined at w4 below detectable levels.
- At w4, FVIII-like activity declined to ~1 U/dL and activated partial thromboplastin time (aPTT) reverted back to the baseline (elevated) value (85.2 s).
- At w9, the participant was up-titrated from 6 mg/kg every 4 weeks (Q4W) to 3 mg/kg once per week (QW).
- Participant received multiple doses of recombinant factor FVIIa around the time of up-titration to treat bleeds.
- The participant discontinued emicizumab treatment at w15 due to loss of efficacy.

Immunogenicty assessment of emicizumab

Case details of PwHA with neutralizing ADAs with decreased exposure – Participant B (HAVEN 1)

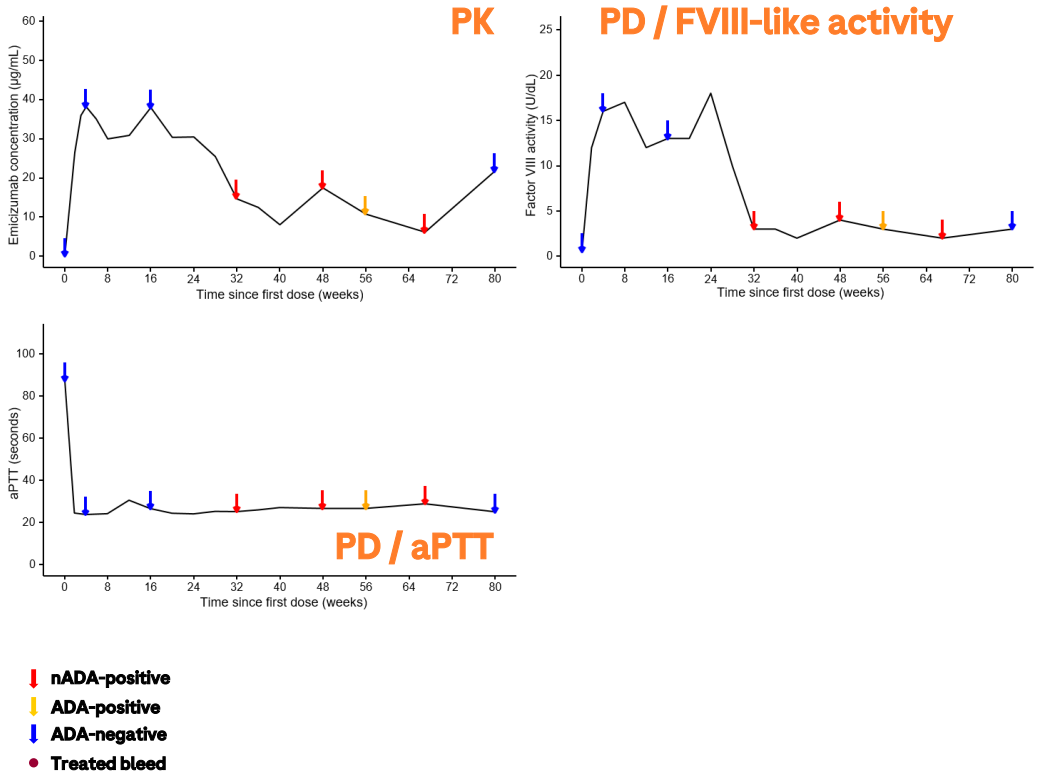


Observation

- ADA detected (titre, 10) at w6 of emicizumab treatment.
- ADA titre increased to 80 at w12 to w20 before declining to 20 at w32. From w40 the participant became ADA-negative.
- Emicizumab concentration reached a maximum (23.6 µg/mL) at w4, and declined thereafter. Despite up-titration, emicizumab trough concentrations continued to decline, reaching approximately 4 µg/mL at w48.
- Similar kinetic and profile was observed for FVIII-like activity.
- At w24, the participant was up-titrated from 3 mg/kg.
- Despite up-titration, emicizumab trough concentrations continued to decline, reaching approximately 4 µg/mL at w48.
- The participant discontinued emicizumab treatment due to personal preferences at w64.

Immunogenicty assessment of emicizumab

Case details of PwHA with neutralizing ADAs with decreased exposure – Participant C (HAVEN 2)

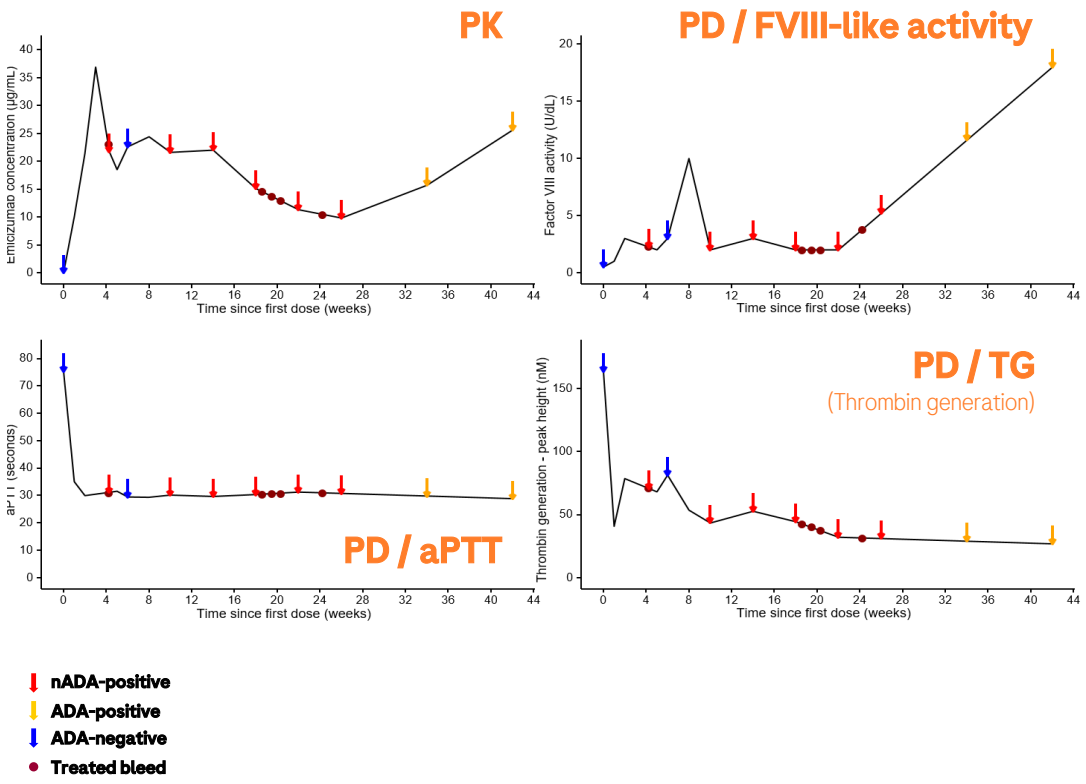


Observation

- ADA detected (titre, 80) at w32 and during ~ 1y (tire, 40-80) of emicizumab treatment. ADA samples were neutralizing in vitro with the exception of Week 56.
- Participant became ADA-negative at w80.
- Emicizumab concentration declined to ~ 15 µg/mL and FVIII-like activity below 5 U/dL were observed from w32 onwards.
- aPTT was unaffected (emicizumab concentrations >15 µg/mL).
- At w80 participant had become ADA-negative, with restoration of anticipated emicizumab plasma concentrations.
- The participant had not experienced any bleeds (treated or all) throughout the study duration and remained on study. The participant completed the study and continued to commercial emicizumab.

Immunogenicty assessment of emicizumab

Case details of PwHA with neutralizing ADAs with decreased exposure – Participant D (HAVEN 5)



Observation

- ADA detected (titre, 20) at w4 of emicizumab treatment. Participant tested ADA positive again at w10 (titre, 80) onwards and with a peak titre of 640 reached at w16, decreasing by w26. ADA samples up to w26 were nADA-positive in vitro.
- After initial peak prior w4, emicizumab concentrations stabilized for a few weeks, but with detection of ADAs declined to a minimum of 10 µg/mL.
- After w26, emicizumab concentrations and in parallel FVIII-like activity began to rise even in the presence of ADAs. aPTT remained unaffected.
- The participant had an ABR (treated bleed) of 5.3, experiencing four treated bleeds between w18 and w24.

Immunogenicity assessment of emicizumab

Summary and conclusions

- A extensive bioanalytical and risk-based immunogenicity testing strategy has been developed for immunogenicity assessment of emicizumab in PwHA.
- The immunogenicity testing strategy is based on consolidation of ADA/nADA/PK/PD analysis for identification of clinical relevant immunogenicity.
- The immunogenicity assessment in 7 clinical phase 3/3b studies shows that emicizumab is associated with a low incidence of ADAs in PwHA. A large proportion of ADAs is transient in nature and/or of low titre.
- Detection of ADAs and especially those with function-neutralizing potential is an essential supportive component in understanding impact of ADAs to PK and PD/efficacy. However, purely analytical detection of ADAs only, especially nADAs, has only limited impact on clinical management of PwHA. Simultaneous analysis of drug exposure and PD markers is key for identification of clinically important ADAs.

Acknowledgement

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Elena Fernadez
Stefan Zoellner
Roland Staack
Eginhard Schick

Team members of the Emicizumab Project Team

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Doing now what patients need next