Giornate Ematologiche Vicentine
X Edizione

La terapia dell'emofilia: Update 2023

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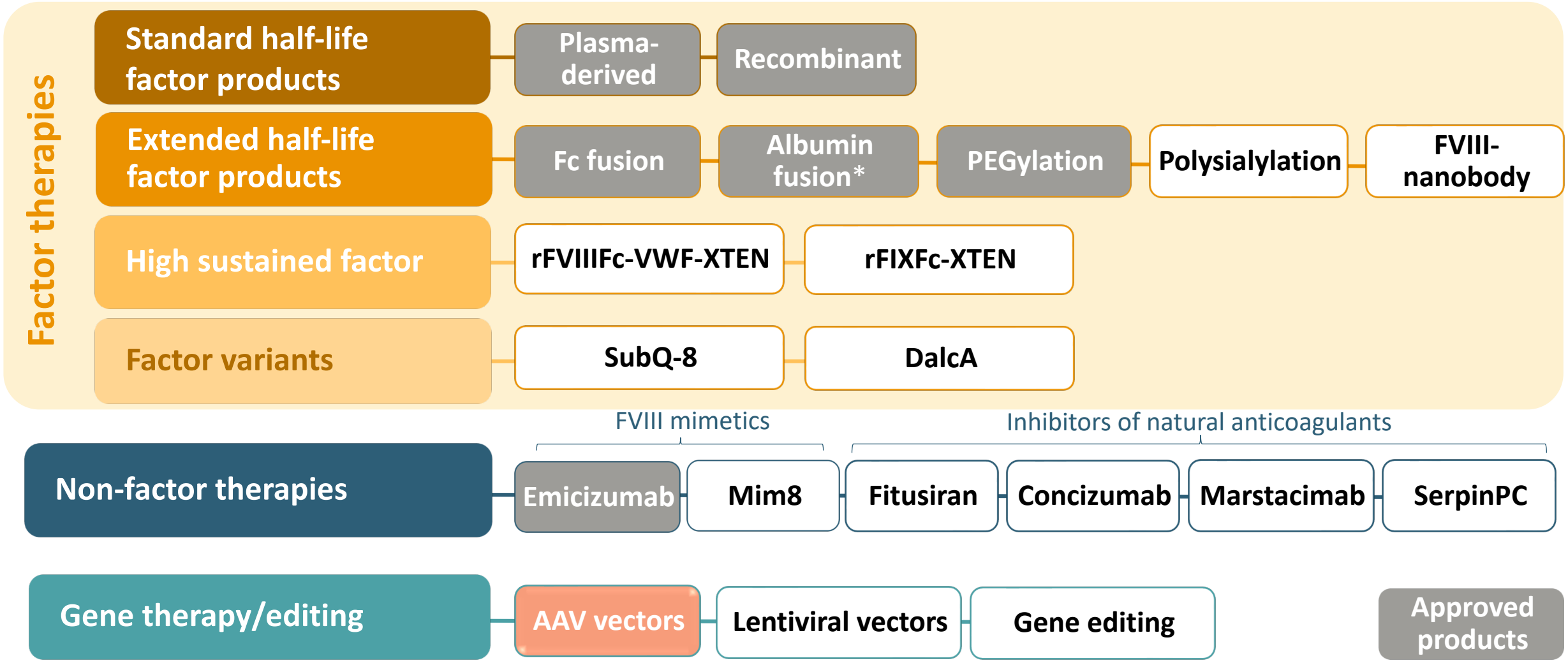
Disclosures

Giancarlo Castaman

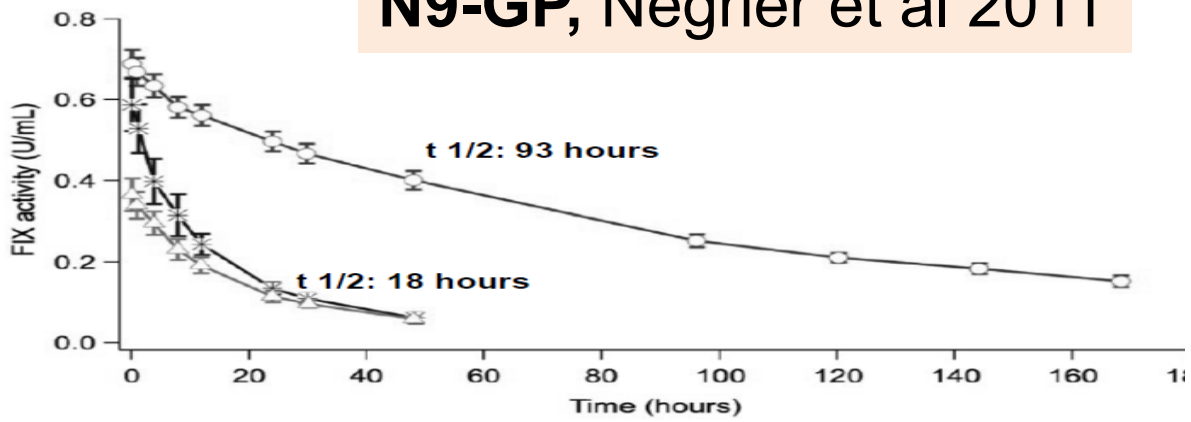
Employment	*NONE*
Research support	*NONE*
Scientific advisory board	*BIOMARIN-CSL-BEHRING, KEDRION-LFB, SHIRE-TAKEDA, NOVO NORDISK, PFIZER, ROCHE, UNIQURE, BAYER, SOBI*
Consultancy	*CSL BEHRING ROCHE - BIOMARIN*
Speakers bureau	*CSL-BEHRING, GRIFOLS, KEDRION-LFB, SHIRE-TAKEDA, WERFEN-IL, SOBI*
Major stockholder	*NONE*
Patents	*NONE*
Honoraria	*NONE*
Travel support	*NONE*
Other	*NONE*

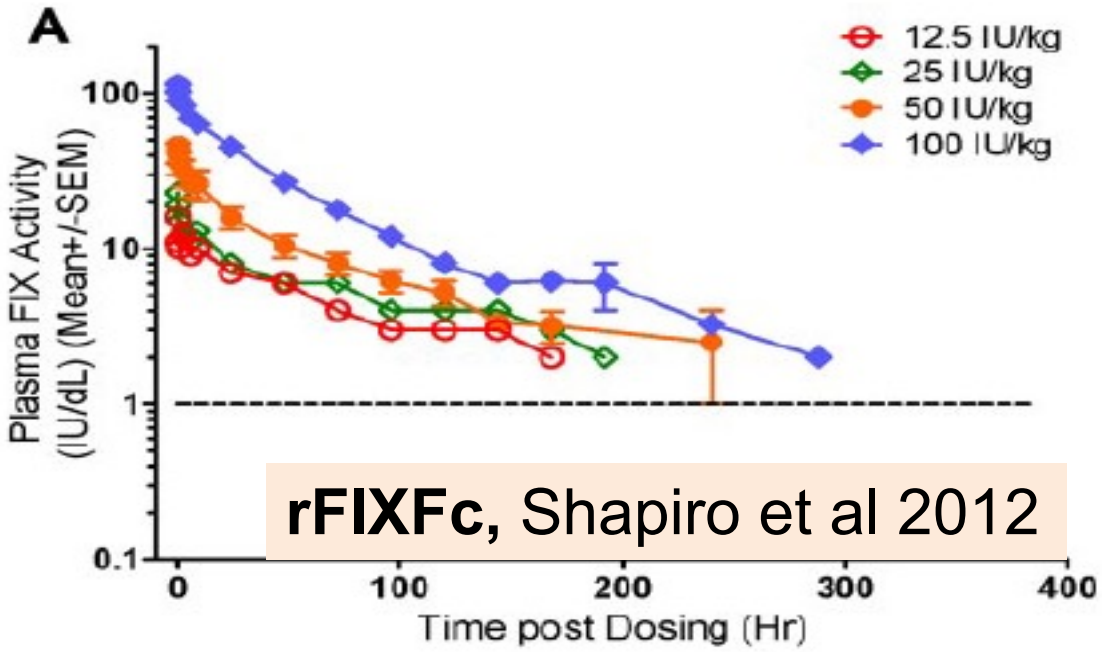
Available and upcoming hemophilia treatments



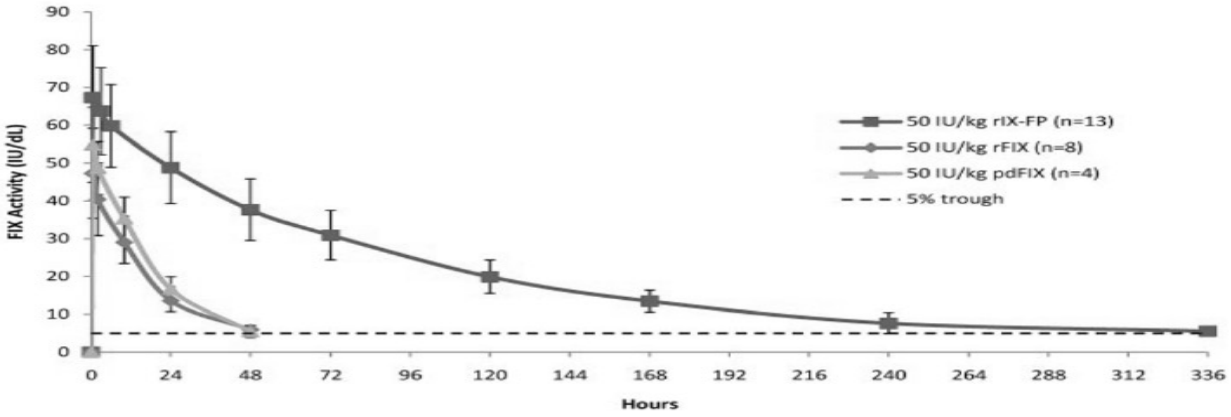
* Only for FIX

N9-GP, Negrier et al 2011





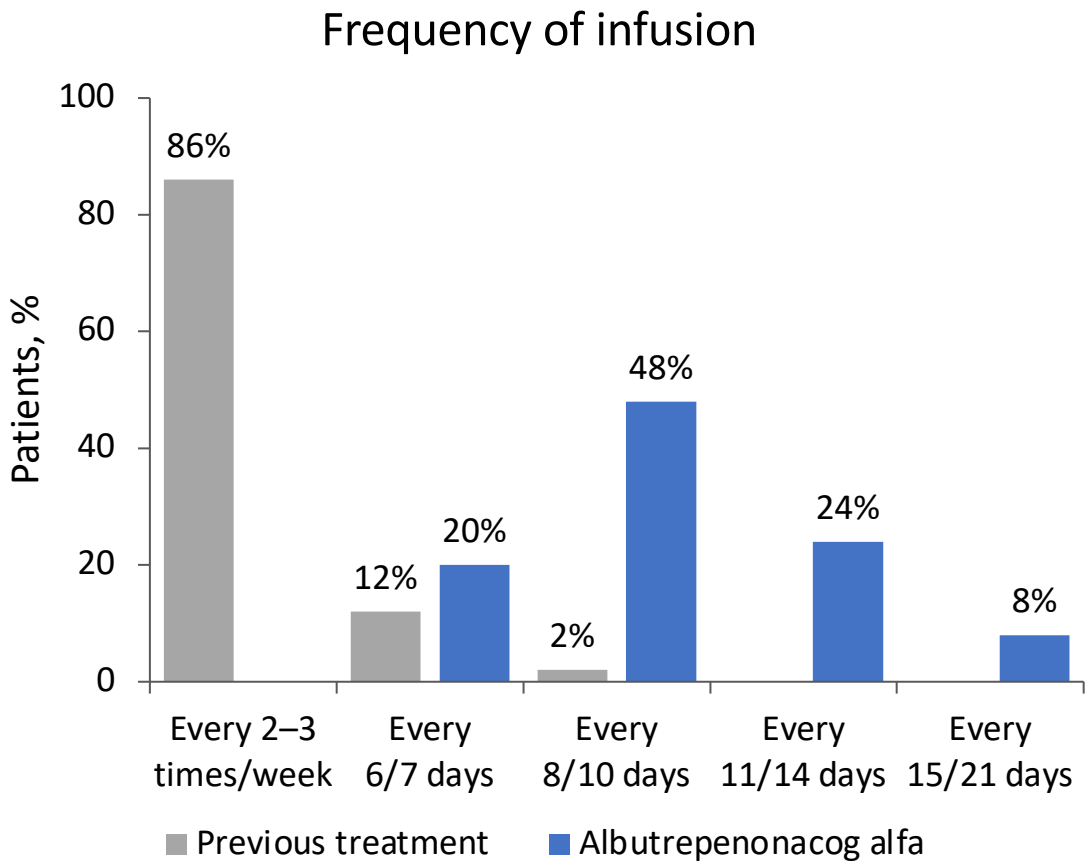
rFIXFc, Shapiro et al 2012



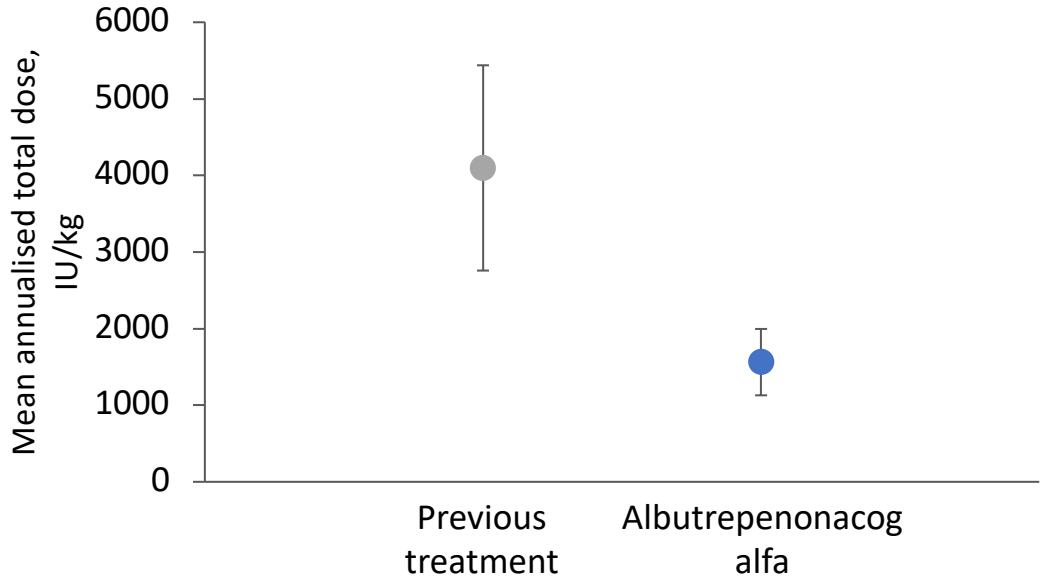
Albumin-fusion rFIX, Santagostino et al 2012

FIX half-life increased 3-5-fold
Prophylaxis feasible every
7-14 days

IDEAL STUDY: A REAL-WORLD ASSESSMENT OF TREATMENT REGIMENS, FACTOR IX TROUGH LEVELS AND CONCENTRATE CONSUMPTION IN HAEMOPHILIA B PATIENTS RECEIVING ALBUTREPENONACOG ALFA IN ITALY



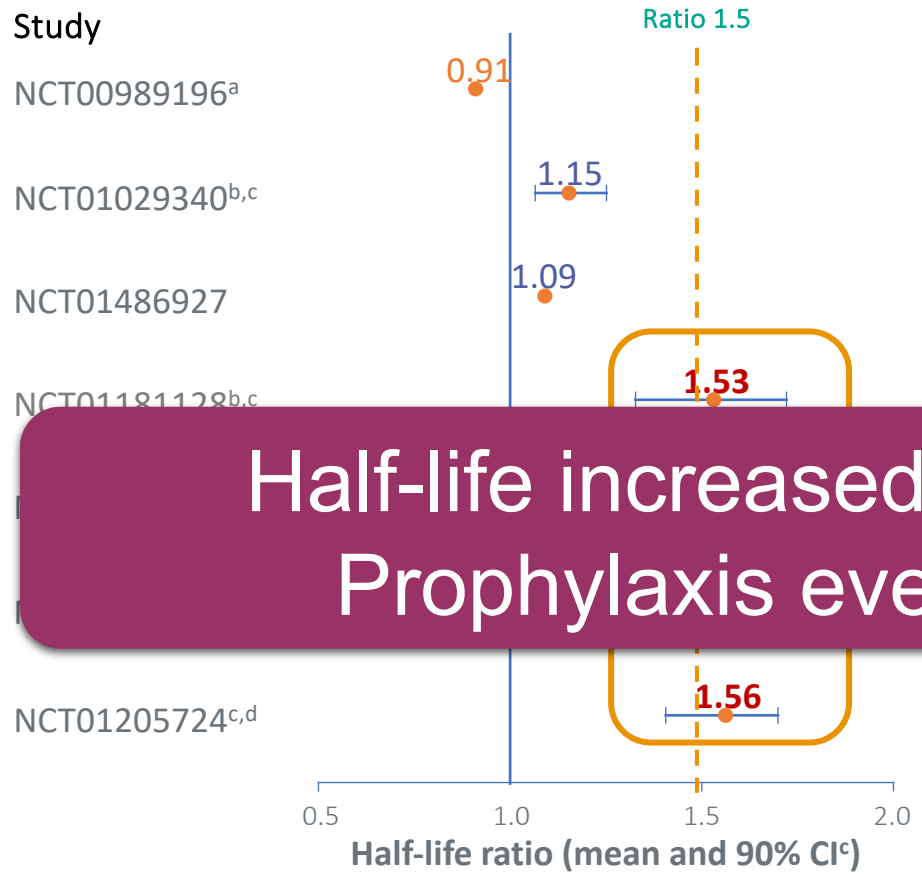
- Annualised total dose decreased from 4098.2±1340.10 IU/kg with previous treatment to 1562.6±433.23 IU/kg with albutrepenonacog alfa



- The prescribed dose remained reasonably stable during the entire 2-year observation period (range: 43.4 to 46.0 IU/kg)

Currently half-life extension of FVIII limited to an average of 1.5 fold[1]

Half-life extension ratios of study FVIII vs. control FVIII for included studies[1]



Half-life increased 1.5 – 1.7-fold
Prophylaxis every 3-5 days

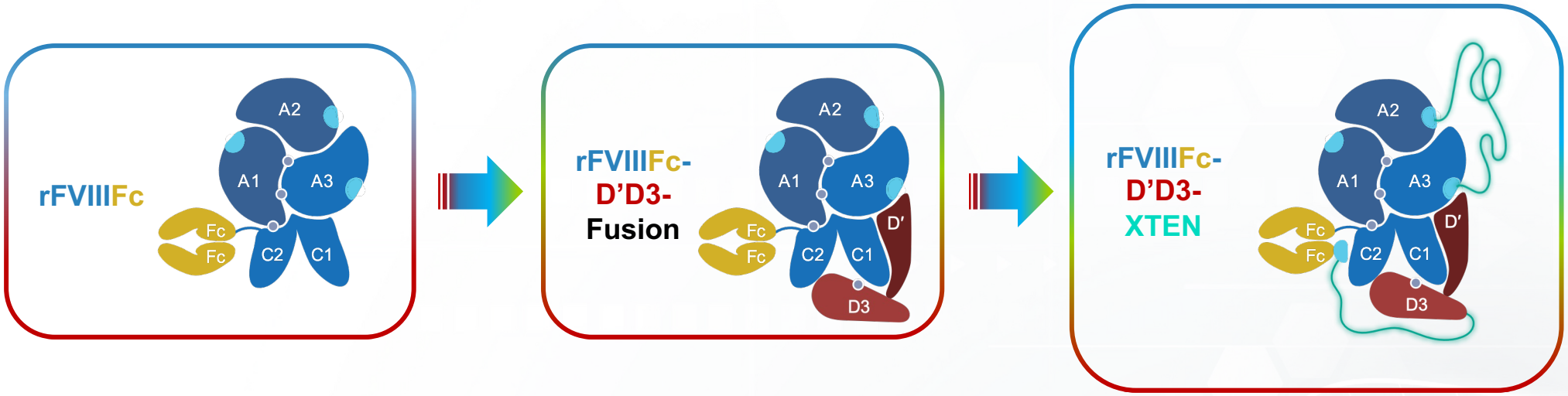
The maximal half-life extension for all modified FVIII molecules in recent clinical trials does not exceed 1.5-fold, which is attributed to the limiting effect of the VWF half-life

[a]No confidence intervals were available; [b]Geometric mean; [c]Indicates 95% CIs; [d]Multiple prior standard rFVIIIs were used as comparators in a single study

1. Mahlangu et al. *Haemophilia* 2018

Efanesoctocog alfa (rFVIIIFc-VWF-XTEN): molecular design[1,2]

- **Efanesoctocog alfa was developed by:**
  - Linking BDD rFVIIIFc via the IgG1 Fc domain to the FVIII-binding **D'D3 domain of VWF**
  - Inserting **2 XTEN polypeptides**: 1 inserted between the A2 and A3 domains, and 1 that links the D'D3 and Fc domains



- Similar to the thrombin-mediated release of FVIII from endogenous VWF, thrombin activation of efanesoctocog alfa leads to the release of **rFVIII-Fc*** from the D'D3 domain and XTEN linkers
  - The natural "on/off" regulation of FVIII is therefore maintained
  - Once activated, rFVIII-Fc* binds to phospholipids and FIXa, and participates in the clotting cascade

*Activation of efanesoctocog alfa by thrombin results in a rFVIII-Fc fusion protein

1. Seth Chhabra et al. *Blood* 2020 2. Konkle BA, et al. *N Engl J Med.* 2020

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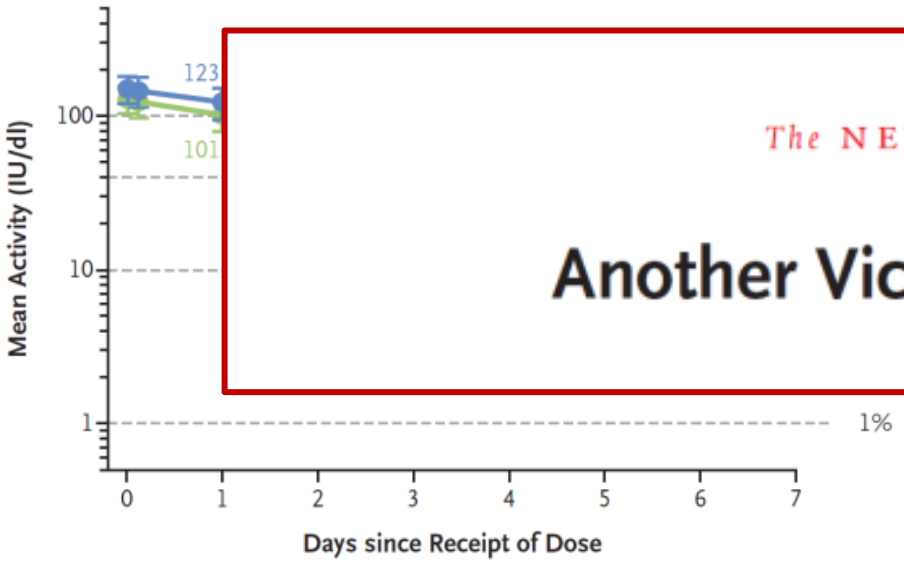
ORIGINAL ARTICLE

Efanesoctocog Alfa Prophylaxis for Patients with Severe Hemophilia A

PK 50 IU/Kg



Pharmacokinetic Variables	Sequential-Pharmacokinetic Subgroup (N=17)
	geometric mean (95% CI)
Half-life — hr	47.0 (42.3–52.2)
Maximum activity concentration — IU/dl	151 (137–167)
AUC_{0-tau} — hr×IU/dl	11,500 (10,200–13,000)
Clearance at steady state — ml/hr/kg	0.439 (0.390–0.493)
Incremental recovery — IU/dl per IU/kg	3.00 (2.71–3.31)

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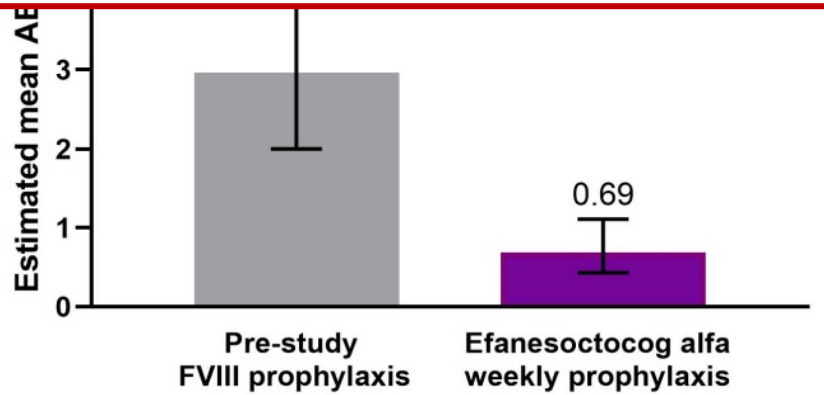
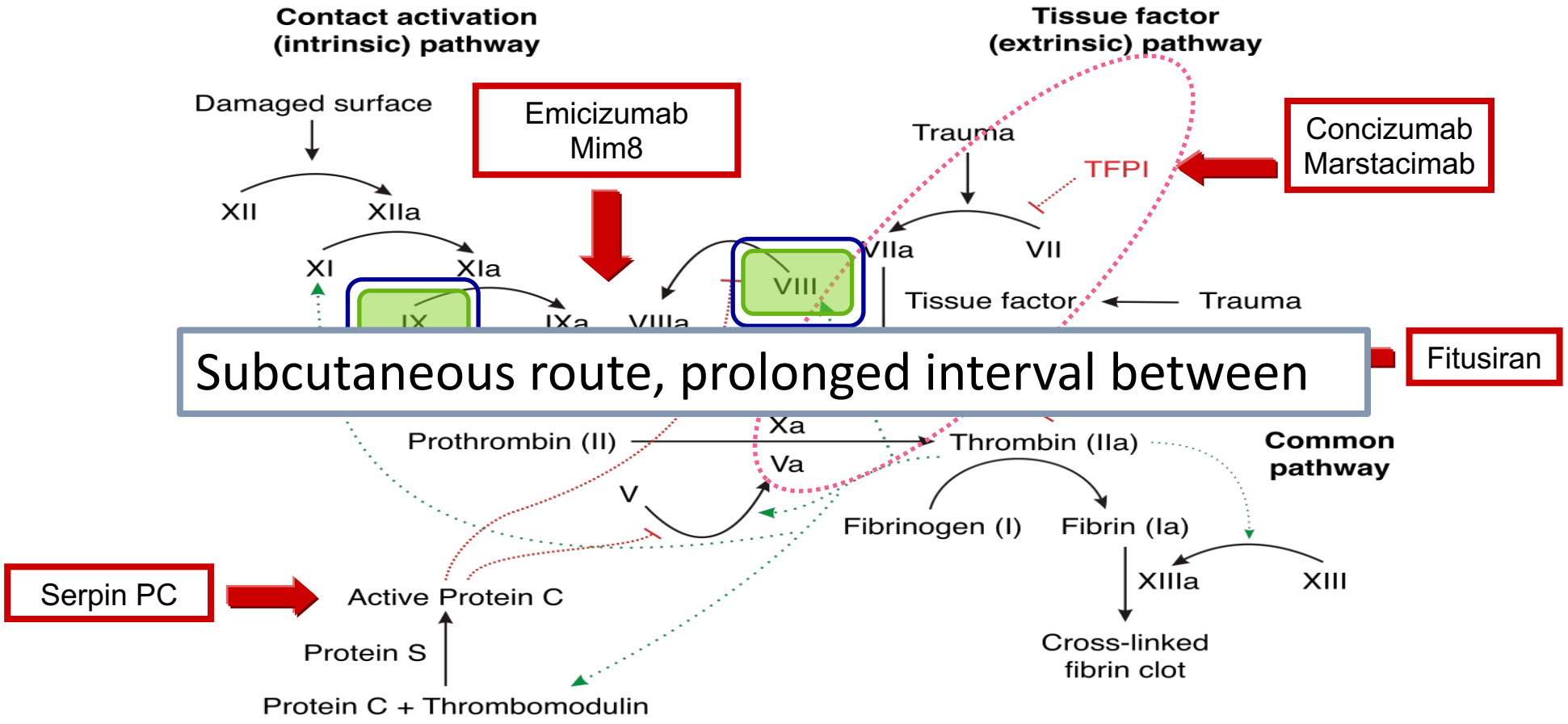
Another Victory for Patients with Hemophilia

Key Secondary Endpoint



NEJM January 26, 2023

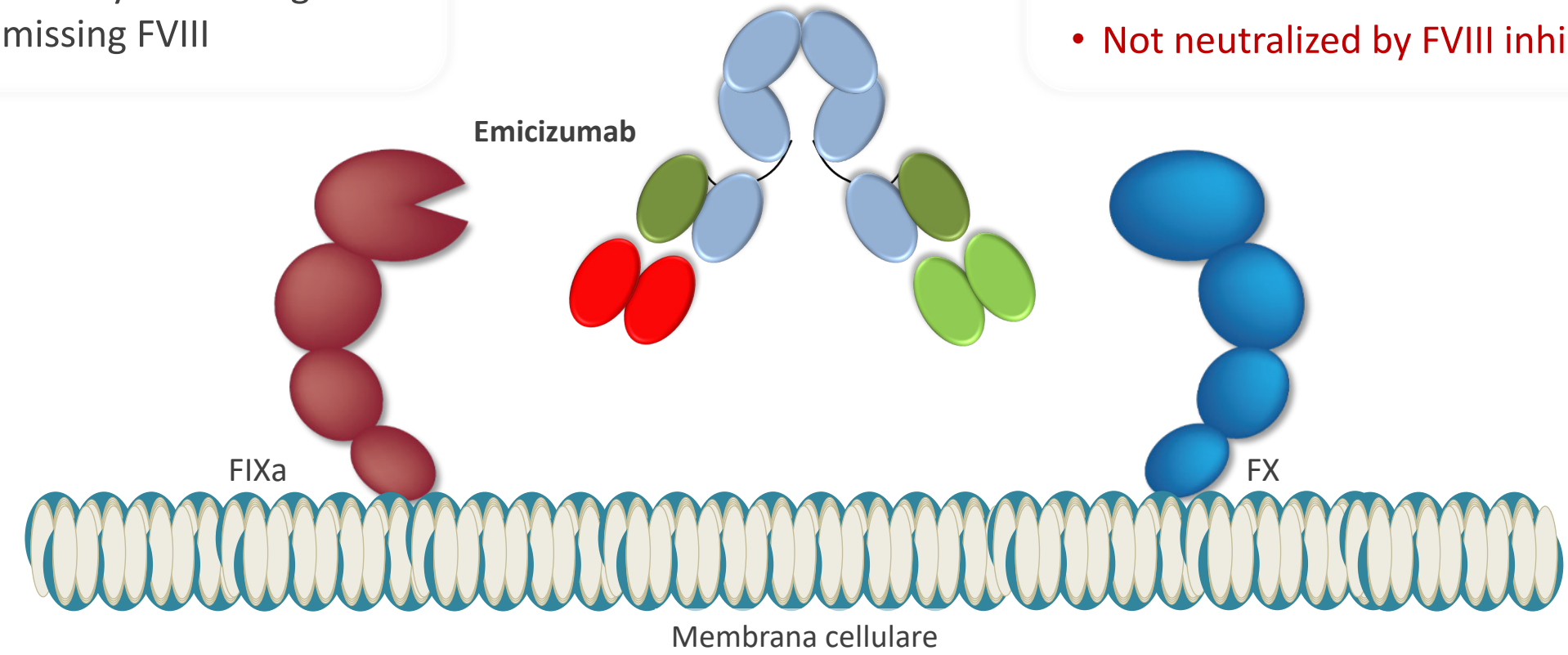
Non-factor therapies



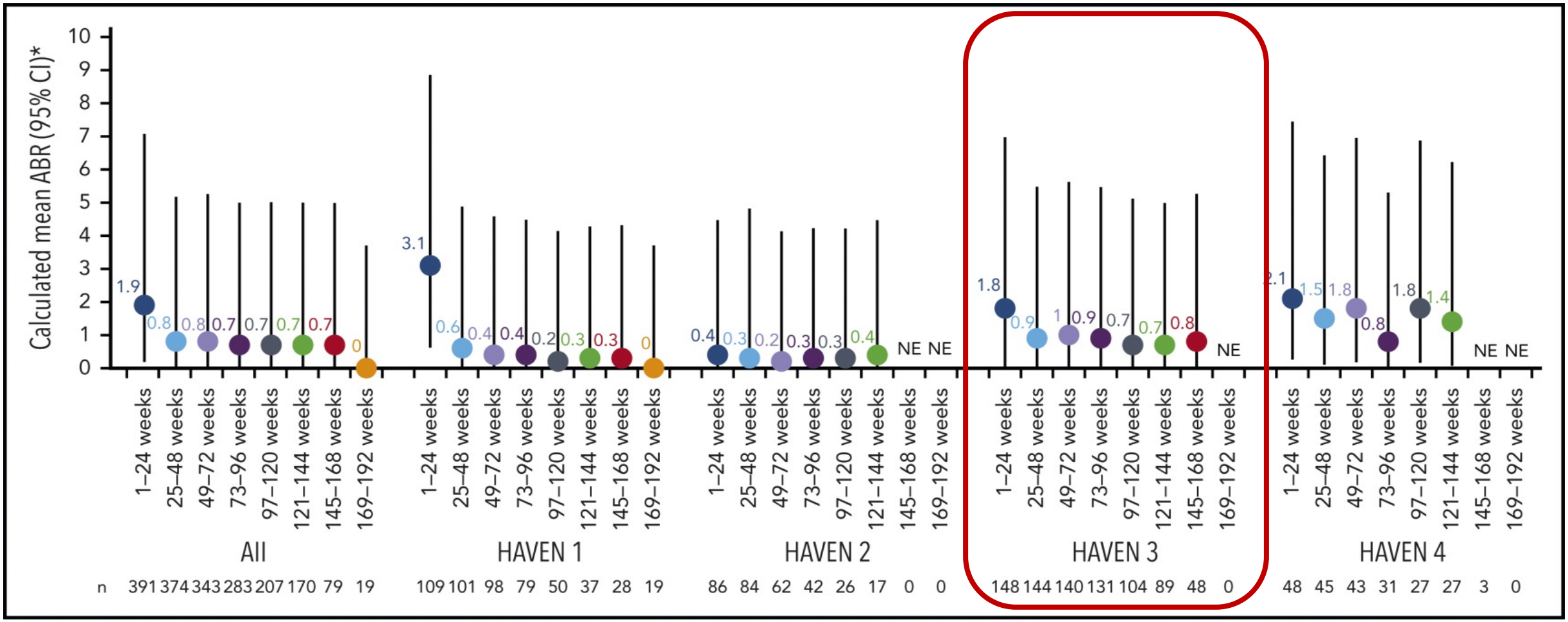
Emicizumab: a humanized, bi-specific mAb

- Emicizumab designed to bring together FIXa and FX, promoting coagulation by mimicking the role of missing FVIII

- Emicizumab has a half-life of 4-5 weeks
- Subcutaneous route every 1, 2 or 4 weeks
- Not neutralized by FVIII inhibitors



Long-term outcomes with emicizumab prophylaxis for hemophilia A with or without FVIII inhibitors from the HAVEN 1-4 studies



- There was a trend for decreasing ABR in each study over time
- With longer follow-up, the adult inhibitor/non-inhibitor ABRs decreased to be closer to the rates of paediatric patients, who tend to have lower ABR due to less damaged joints[1]

Callaghan et al, Blood 2021

Emicizumab in people with moderate or mild haemophilia A (HAVEN 6): a multicentre, open-label, single-arm, phase 3 study

Lancet Haematology, January 27, 2023

72 patients with mild or moderate hemophilia A

	Treated bleeds	Treated joint bleeds	Treated spontaneous bleeds	Treated target joint bleeds	All bleeds
Model-based ABR (95% CI)				(0·03–0·40)	2·3 (1·67–3·12)
Calculated mean ABR (95% CI)†				(0·00–3·92)	2·3 (0·35–7·75)
Calculated median ABR (IQR)†				(0·00–0·00)	1·0 (0·00–3·11)
Calculated ABR range†				–3·21	0·00–21·04
Participants with zero bleeds, n (%)‡				4%)	24 (33%)§

Approved EMA February 2023

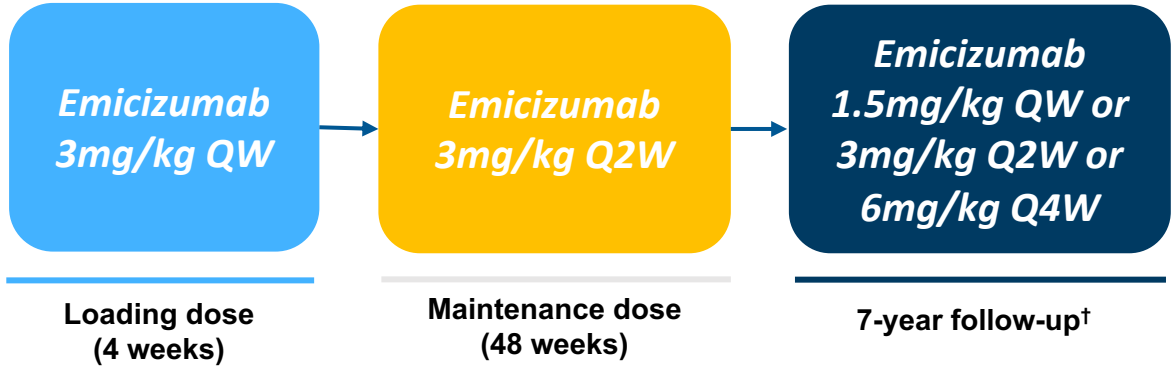
Median (range) follow-up time: 55·6 weeks (8·7–89·9). Compliance with bleed reporting on-study via the BMQ was >90%. ABR=annualised bleed rate. BMQ=bleed and medication questionnaire. *One participant (1%) had <24 weeks of follow-up, and 57 (79%) of 72 participants had ≥52 weeks of follow-up. †Calculated as: (number of bleeds/total number of days during the efficacy period) × 365·25. ‡At interim analysis (median follow-up time of 27·5 weeks), participants with zero bleeds were: 57 (80%) treated bleeds; 33 (47%) all bleeds; 64 (90%) treated joint bleeds; 68 (96%) treated spontaneous bleeds; 67 (94%) treated target joint bleeds.[28] §The pre-study bleed model-based ABR for all bleeds in the 33% of patients (n=24) who had no bleeds on study was 5·4 (95% CI 3·25–9·08).

Table 3: **Efficacy summary***

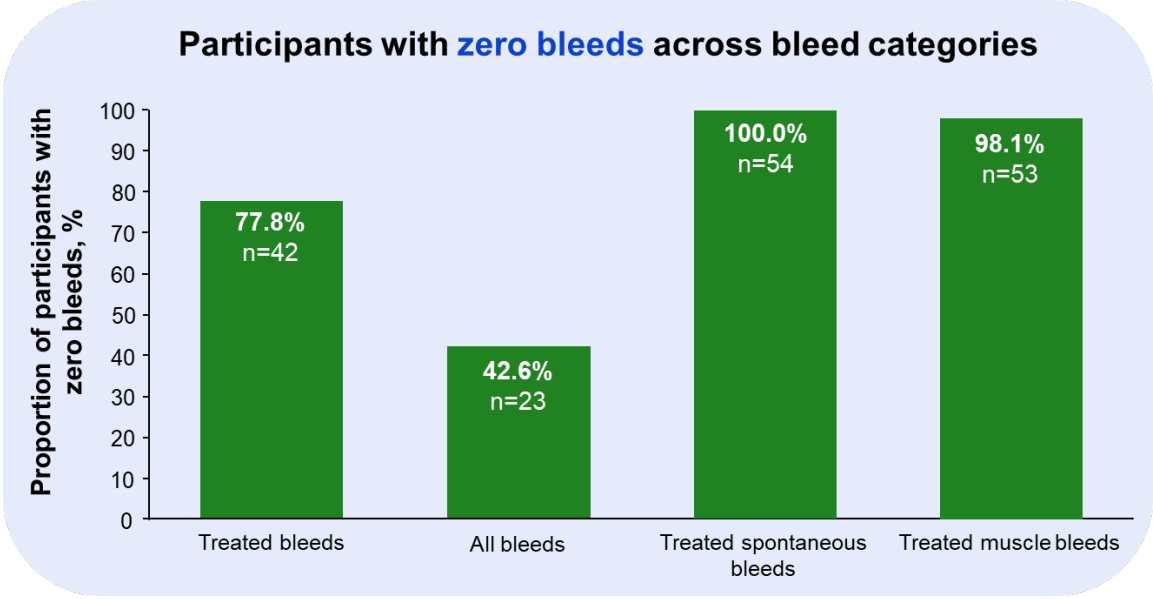
ABR for all bleeds 10.1 (95% CI 6.93 – 14.76) 24 weeks before
2.3 (95% CI 1.67 – 3.12) after a median follow-up of 55.6 weeks on emicizumab

HAVEN 7: Study design

- **A phase IIIb, multi-centre, open-label study of emicizumab in infants aged ≤12 months with severe HA without FVIII inhibitors (NCT04431726)[1]**



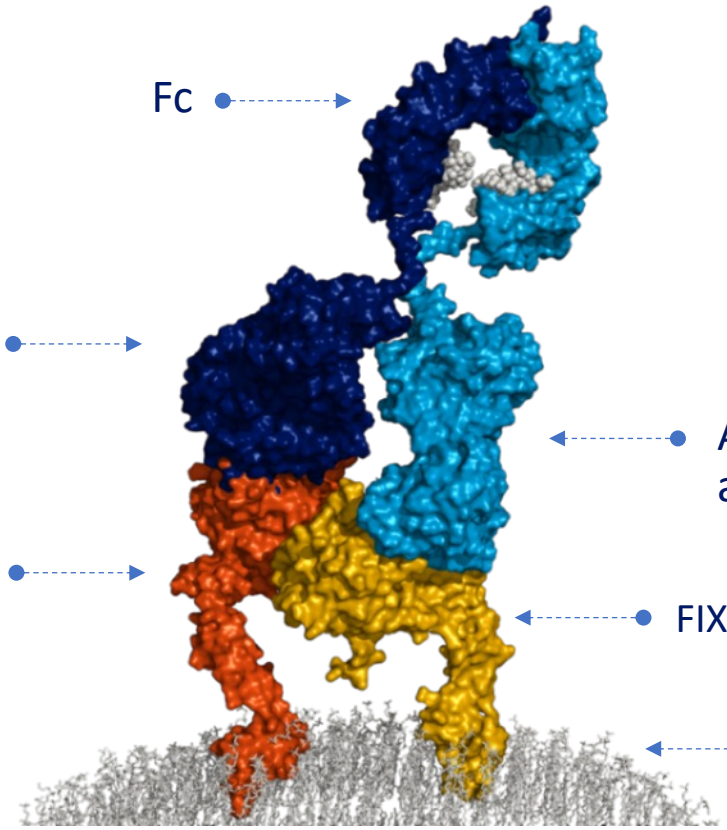
- **Endpoints included:**[1]
  - **Efficacy:** ABRs for treated bleeds, all bleeds, treated spontaneous bleeds, treated joint bleeds
  - **Safety:** AEs, AESIs including TEs and TMAs, immunogenicity
  - **PK/PD:** Plasma trough emicizumab concentrations
  - **Biomarkers:** FIX and FX antigen concentrations and effect of emicizumab on aPTT, among others



Participants had low model-based ABRs, and all participants had zero treated spontaneous bleeds; all treated bleeds were traumatic. PK analyses confirm that efficacious plasma concentrations were achieved and sustained in infants with HA[1]

Pipe S, et al. ASH 2022; Oral 187

New mimetics: the Mim8 molecule



Membrane localization; weak binding in the circulation to avoid systemic activation of coagulation

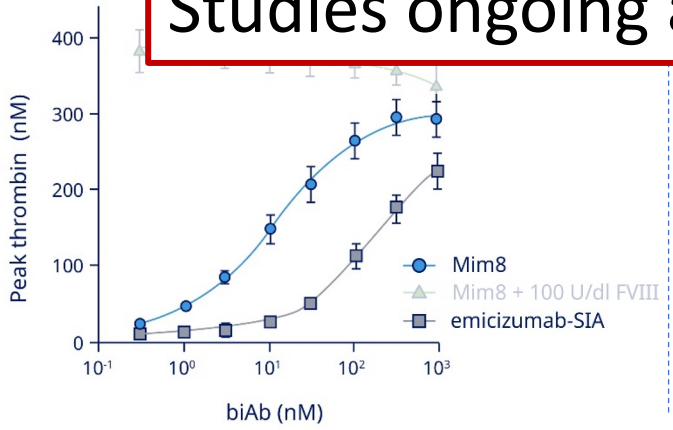
- Novel, next-generation FVIII mimetic
- For **subcutaneous prophylactic** treatment of haemophilia A with and without inhibitors
- Fully human IgG4 bispecific antibody
- **Unique binding epitopes to FIXa and FX**
  - **Strong stimulation of FIXa proteolytic activity**
  - **Facilitated delivery of FX to FIXa and exchange of FXa for FX**

Ostergaard et al, Blood 2021

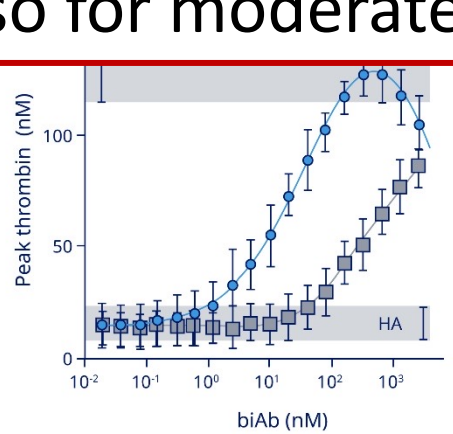
Fc, fragment crystallisable; FIX, factor IX; FIXa, activated factor IX; FVIII, factor VIII; FX, factor X; IgG, immunoglobulin G.

Mim8 displayed 13-18-fold enhanced potency over emicizumab SIA

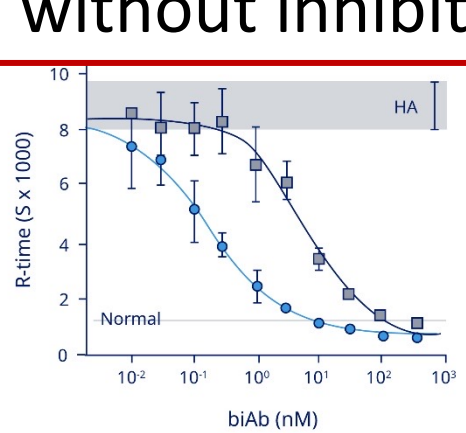
TGT: FXIa-triggered



TGT: TF-triggered



TEG: clot time



Studies ongoing also for moderate HA patients without inhibitors

mab SIA

Mim8 demonstrated to be **13-fold more potent** than emicizumab SIA in thrombin-generation test, triggered with FXIa

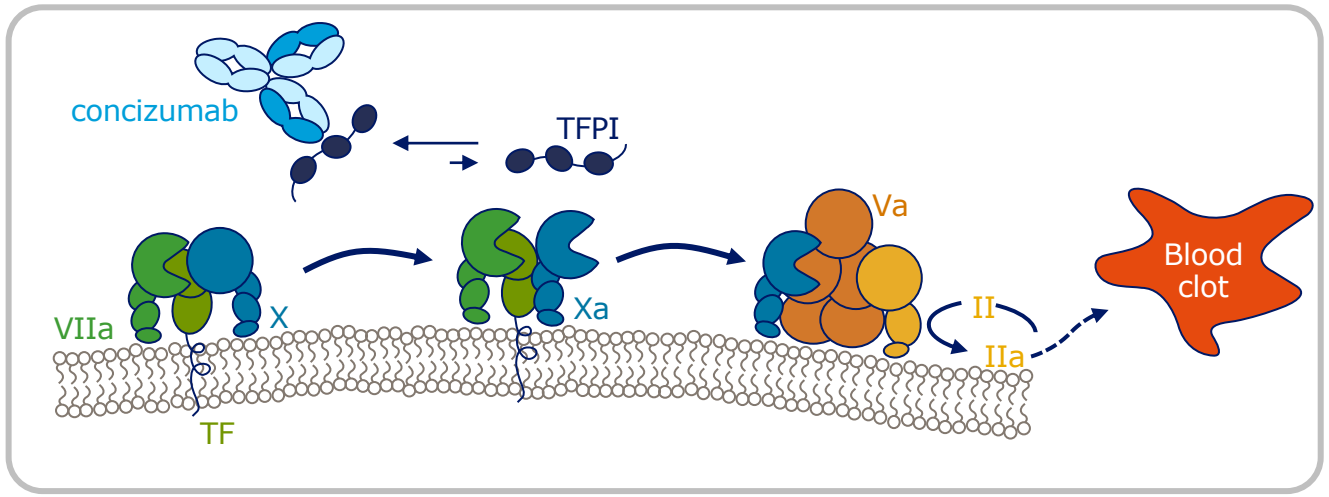
Mim8 normalized thrombin generation while emicizumab failed to do so in this setting, even at the highest concentration

Potency of Mim8 based on R-time was improved by **18-fold**, relative to emicizumab SIA

FXIa, activated factor XI; SIA, sequence-identical analog; TEG, thromboelastography; TF, tissue factor; TGT, thrombin generation test

Østergaard et al., Blood. 2021;blood.2020010331.doi: 10.1182/blood.2020010331.

Concizumab restores the hemostatic potential by binding TFPI while maintaining the rest of the coagulation system

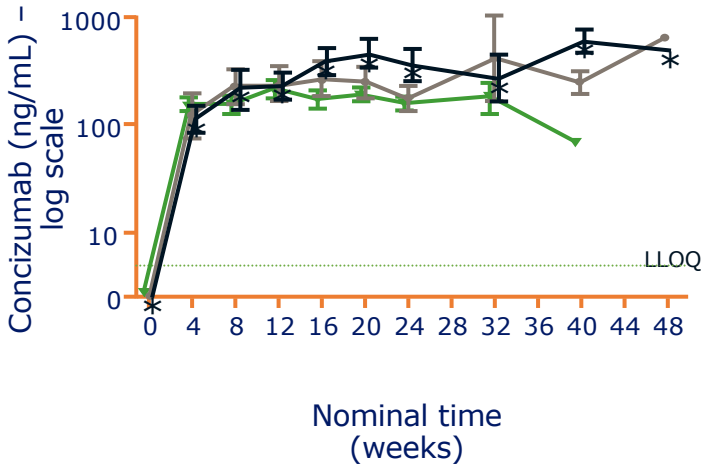


- High-affinity, humanised, monoclonal IgG4 antibody
- Inhibits TFPI, extending the haemostatic initiation phase
- Restores thrombin generation potential

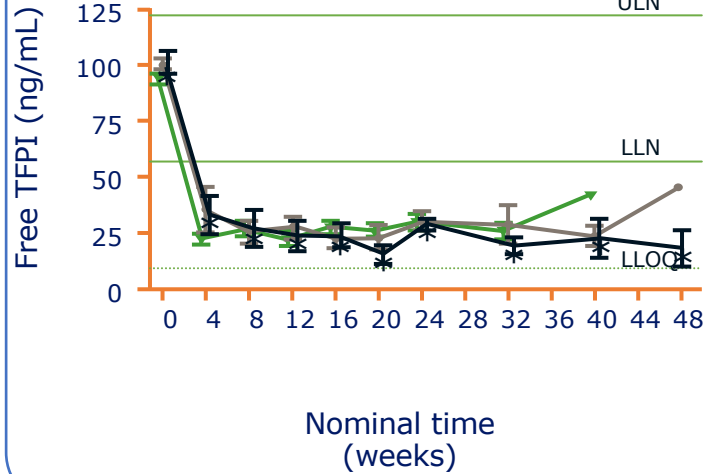
Daily SC route, meeting unmet need for prophylaxis in orphan-drug patients (e.g., Hemophilia B with inhibitors)

Free TFPI and concizumab concentration are inversely proportional and peak thrombin is within normal range

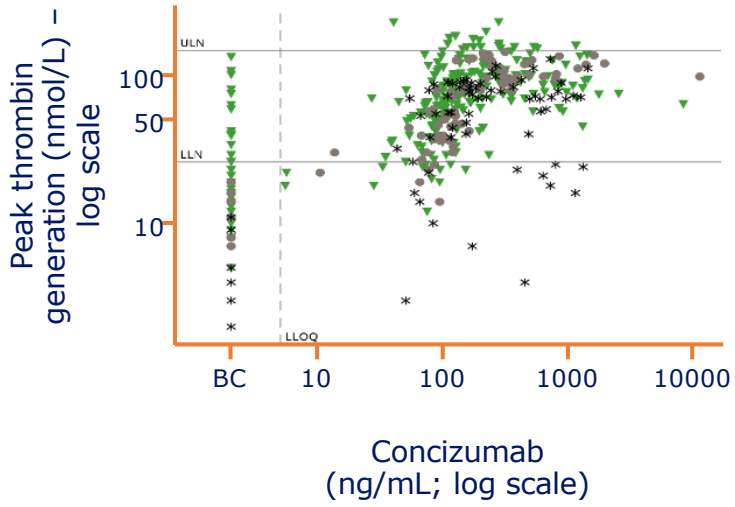
Concizumab concentration



Free TFPI



Peak thrombin generation



▼ HA ● HAwI ∗HBwI

Error bars are SE of the geometric mean. Number of subjects per combination of visit and haemophilia type are shown. Measurements from Visit 3 in explorer4 (inhibitor trial) are not included since measurements are not present in explorer5 (non-inhibitor trial).

HA, haemophilia A; HAwI, haemophilia A with inhibitors; HBwI, haemophilia B with inhibitors; LLN, lower limit of normal; LLOQ, lower limit of quantification; SE, standard error; TFPI, tissue factor pathway inhibitor; ULN, upper limit of normal.

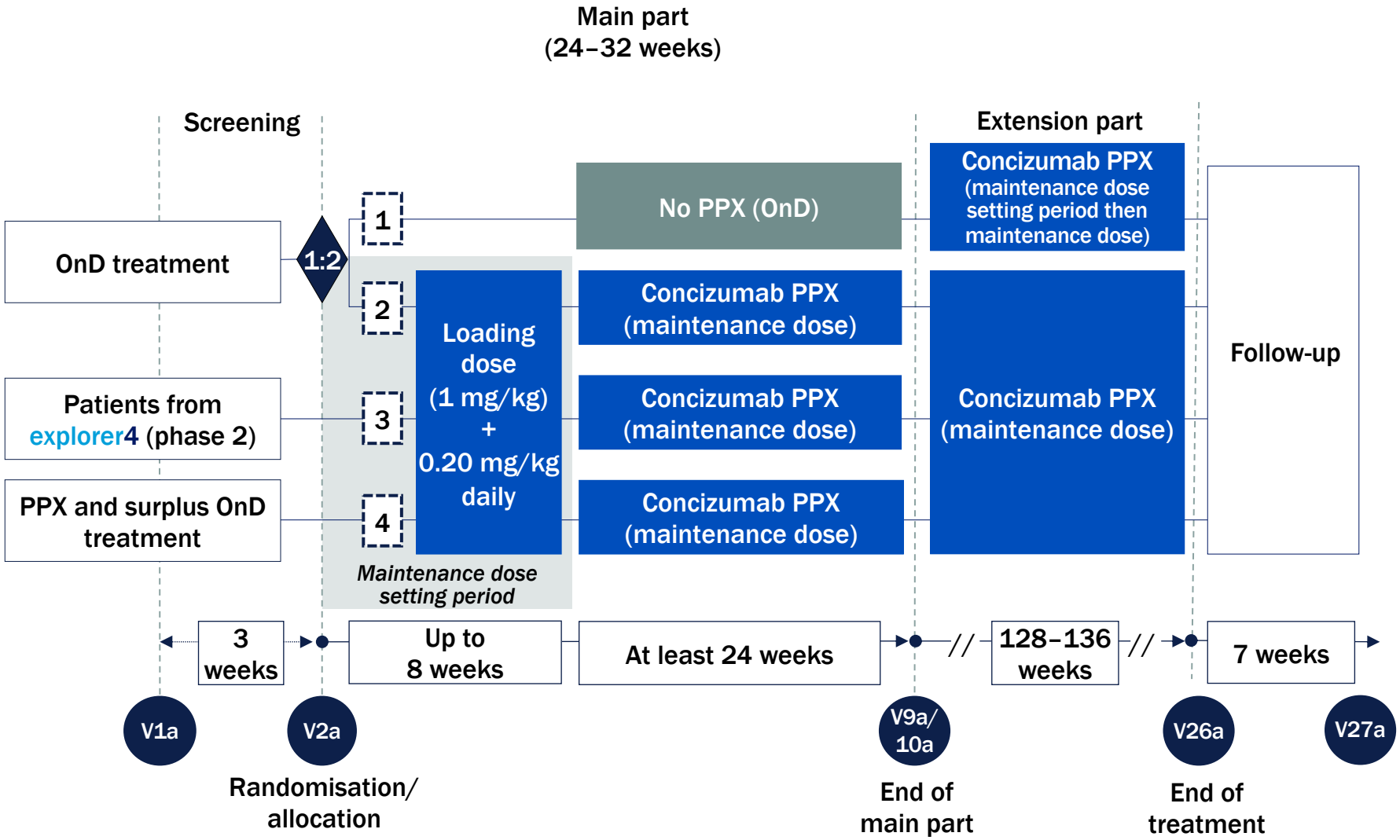
Shapiro AD et al. *Blood* 2019;134(22):1973–82.

The NEW ENGLAND JOURNAL of MEDICINE

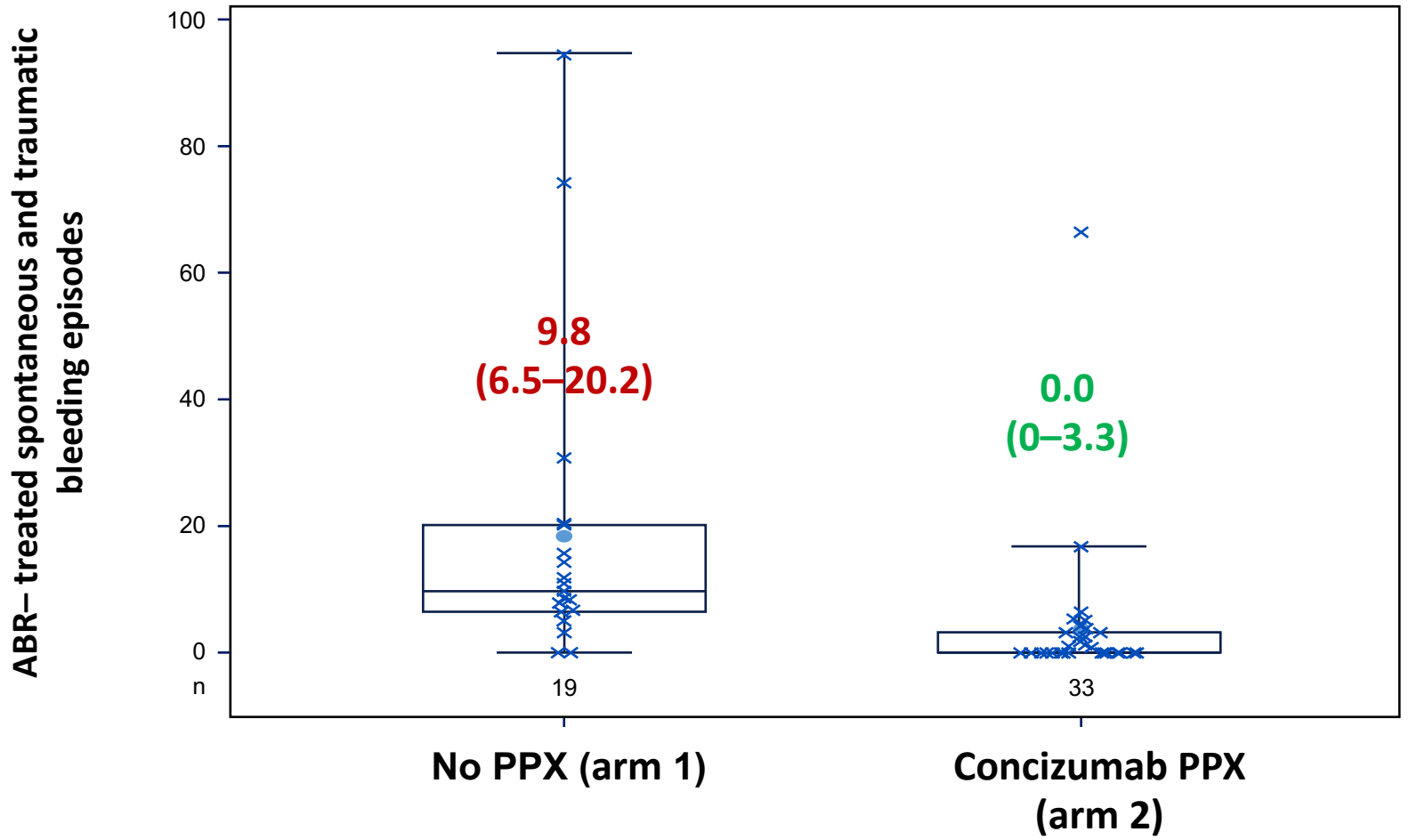
ESTABLISHED IN 1812 AUGUST 31, 2023 VOL. 389 NO. 9

Phase 3 Trial of Concizumab in Hemophilia with Inhibitors

T. Matsushita, A. Shapiro, A. Abraham, P. Angchaisuksiri, G. Castaman, K. Cepo, R. d'Oiron, M. Frei-Jones, A.-S. Goh, J. Haaning, S. Hald Jacobsen, J. Mahlangu, M. Mathias, K. Nogami, J. Skovgaard Rasmussen, O. Stasyshyn, H. Tran, K. Vilchevska, L. Villarreal Martinez, J. Windyga, C.W. You, N. Zozulya, B. Zulfikar, and V. Jiménez-Yuste, for the explorer7 Investigators*



Explorer7: ABRs
(n=140, HAwI and HBwI)

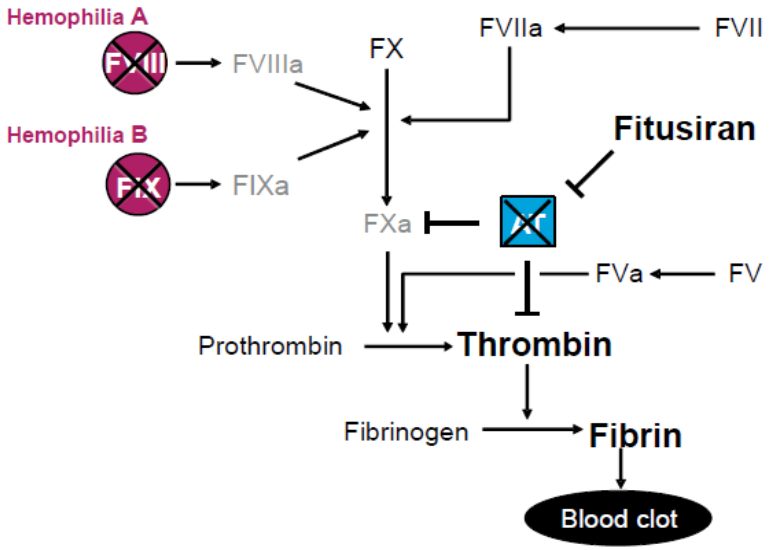


64% of patients on concizumab prophylaxis had **zero bleeding** (vs.11% in arm 1)

Trends to improved HRQoL (SF-36v2), significantly better scores in domains of general health, vitality, role-emotional and mental health

uste et al, ISTH 2022, LBA

FITUSIRAN (ALN-AT3)

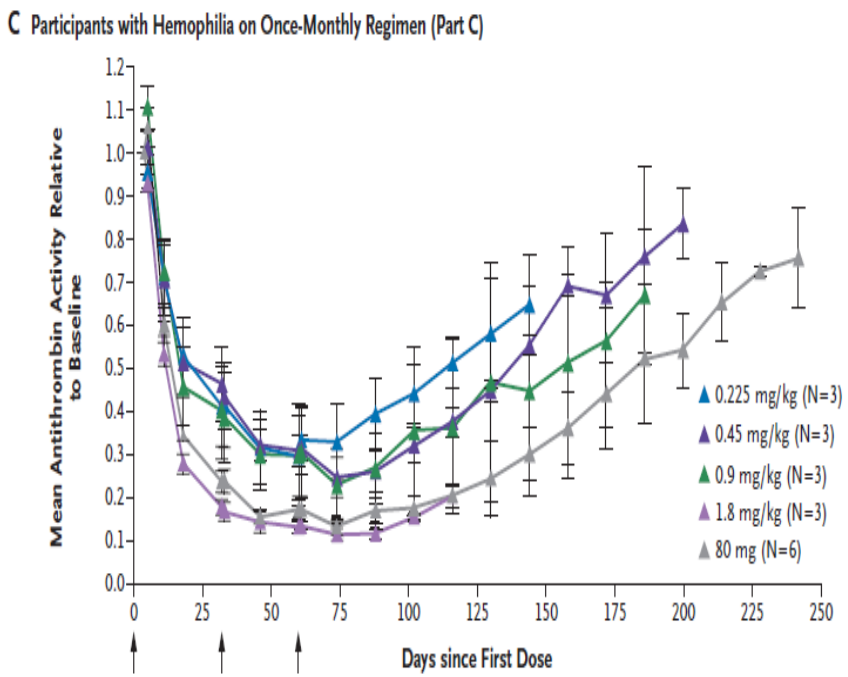


- ALN-AT3 is a siRNA designed to suppress liver synthesis of AT
- Interferes with mRNA of AT (coded for by *SERPINC1*)
- ALN-AT3 increases thrombin generation in hemophilic plasma when AT is reduced down to 25 %

ORIGINAL ARTICLE

Targeting of Antithrombin in Hemophilia A or B with RNAi Therapy

K.J. Pasi, S. Rangarajan, P. Georgiev, T. Mant, M.D. Creagh, T. Lissitchkov, D. Bevan, S. Austin, C.R. Hay, I. Hegemann, R. Kazmi, P. Chowdary, L. Gercheva-Kyuchukova, V. Mamonov, M. Timofeeva, C.-H. Soh, P. Garg, A. Vaishnaw, A. Akinc, B. Sørensen, and M.V. Ragni



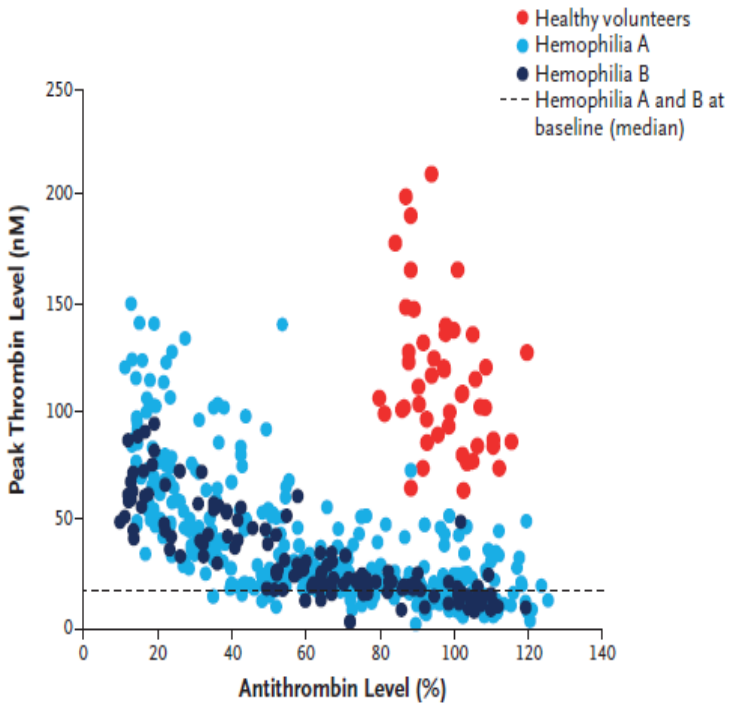


Figure 3. Relationship between Antithrombin Level and Thrombin Generation.

Sanofi Revises Fitusiran Dosing Regimen to Mitigate Risk of Vascular Thrombosis

Evaluation of five vascular events suggests the risk of vascular thrombotic events may be greater with antithrombin (AT) levels that have been reduced below 10% of normal levels

- Three study participants with AT levels below 10% suffered vascular thrombotic events. (Cerebrovascular accident, a cerebral infarct and a spinal vascular disorder)
- Two events in patients with AT levels of 10% to 20%.
- The incident rate of vascular thrombotic events per 100 patient years was the highest (5.91) in patients with AT levels < 10%
- Between 10% and 20%, the incident rate is 1.49 and above 20%, the incident rate is zero
- **Under the revised protocol, target AT levels of 15% to 35% have been implemented**
- Experience with the dosing of fitusiran so far suggests fitusiran hitting that range could reduce the risk of vascular thrombotic events.

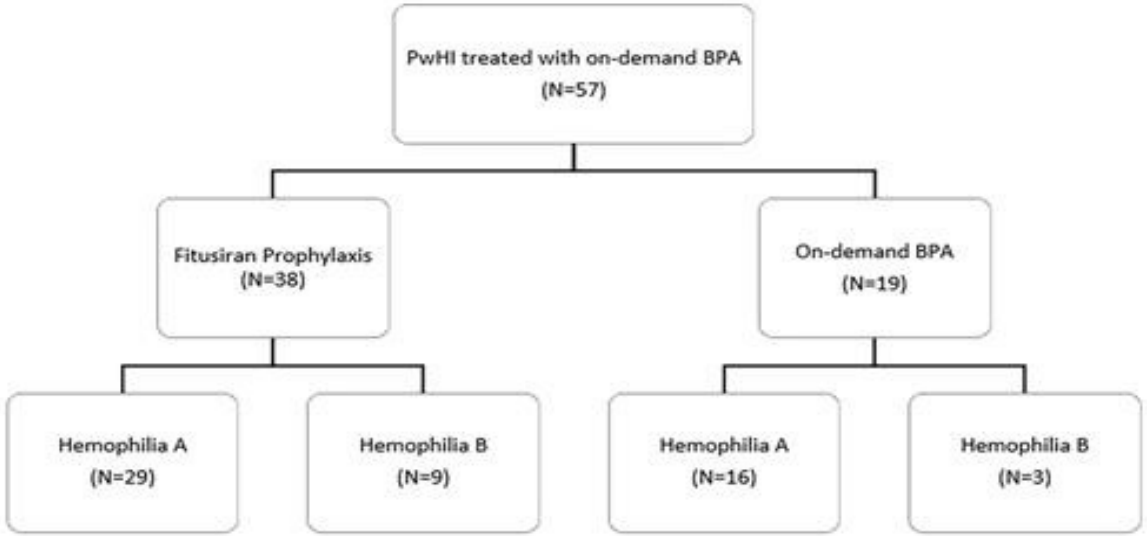
February 2021

Efficacy and Safety of Fitusiran Prophylaxis, an siRNA Therapeutic, in a Multicenter Phase 3 Study (ATLAS-INH) in People with Hemophilia A or B, with Inhibitors (PwHI)

ASH, December 12, 2021, 2:00 PM-4:00 PM

- Monthly sc administration di 80 mg in HA and HB with inhibitors
- **Median ABR for all bleeds 0.87**
- 1 episode of DVT
- Careful assessment of potential thrombotic risk

Figure 1: Study participant disposition



At the primary endpoint (9 months), eligible participants transitioned to the long-term study to receive ongoing fitusiran or to initiate fitusiran for those who were randomized to the on-demand arm.

Table 1: Bleeding events in ATLAS-INH study (in efficacy period)

	Fitusiran 80 mg prophylaxis (N=38)	BPA on-demand (N=19)
All treated bleeds		
Estimated ABR (95% CI)	1.67 (1.01, 2.74)	18.07 (10.60, 30.81)
Rate ratio (95% CI)[a]	0.09 (0.04, 0.19)	
P-value[b]	<0.0001	
Observed ABR Median (IQR)	0.00 (0.00; 1.70)	16.80 (6.70; 23.50)
Treated spontaneous bleeds		
Estimated ABR (95% CI)	0.87 (0.49, 1.55)	15.68 (9.28, 26.47)
Rate ratio (95% CI)[a]	0.06 (0.03, 0.12)	
P-value[b]	<0.0001	
Observed ABR Median (IQR)	0.00 (0.00; 0.00)	13.40 (3.40; 21.80)
Treated joint bleeds		
Estimated ABR (95% CI)	1.35 (0.80, 2.28)	13.76 (7.95, 23.81)
Rate ratio (95% CI)[a]	0.10 (0.05, 0.21)	
P-value[b]	<0.0001	
Observed ABR Median (IQR)	0.0 (0.00; 1.70)	11.70 (3.40; 16.80)

[a]Fitusiran rate divided by BPA rate.
[b]P-value from a negative binomial regression model with treatment arm and randomization strata of number of bleeds in the 6 months prior to study (≤10, >10) as fixed effects, and the logarithm of the duration that each patient spends in the efficacy corresponding period matching the bleeding episode being analyzed as an offset variable (p-value versus null hypothesis of ratio=1).
The efficacy period is from Day 29 to Day 246, or the last day of bleeding follow up, whichever is the earliest.

Thrombosis with non-substitutive treatment

- Presence of cardiovascular risk factors (rebalancing agents)
- Concomitant treatment with replacement agents, at high-dose, for three or more days (Concizumab)
- Red

Mitigating the effect with specific guidance drastically reduced the risk

- High concizumab exposure levels in plasma
- aPCC for ≥ 24 hours and ≥ 100 U/kg/day for emicizumab

Haemophilia is an appropriate target for gene therapy

Haemophilia A and B are monogenic diseases[1]

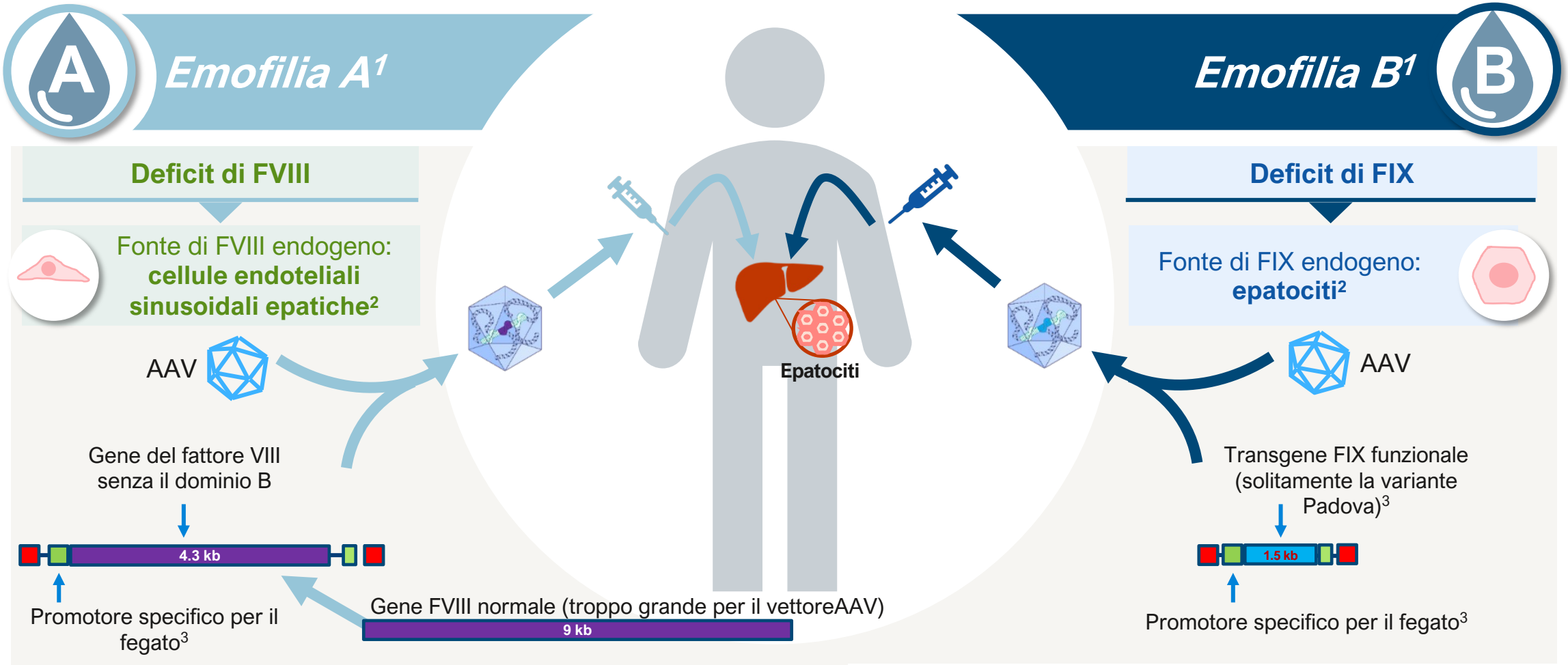
Well suited for correction by gene therapy[1]

- Large phenotypic improvement following modest factor increase
- Precise regulation not necessary

Efficacy readily assessable via factor level measurements and bleeding rates[2]

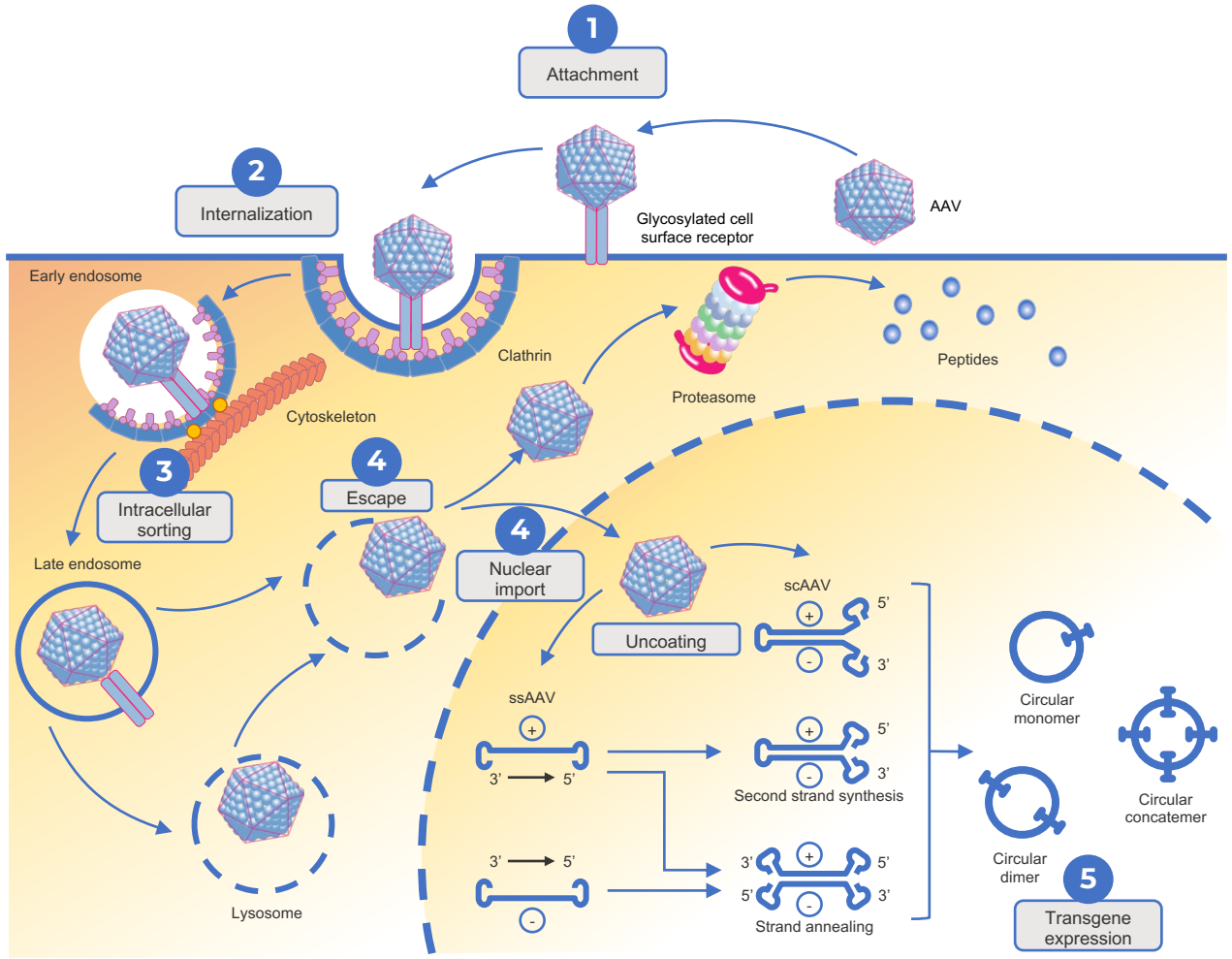
1. Perrin GQ, *et al*. *Blood* 2019; 133:407–414. 2. Arruda VR, Doshi BS. *Mediterr J Hematol Infect Dis* 2020; 12:e2020069.

Gli stessi approcci di terapia genica sono stati usati sia per l'emofilia A che per l'emofilia B



1. Arruda VR, Doshi BS. *Mediterr J Hematol Infect Dis* 2020; 12:e2020069. 2. Perrin GQ, *et al. Blood* 2019;133:407–414. 3. Batty P, Lillicrap D. *HemaSphere* 2021;5:3(e540)

Transduction of AAV-based gene therapy into cells is a multi-step process



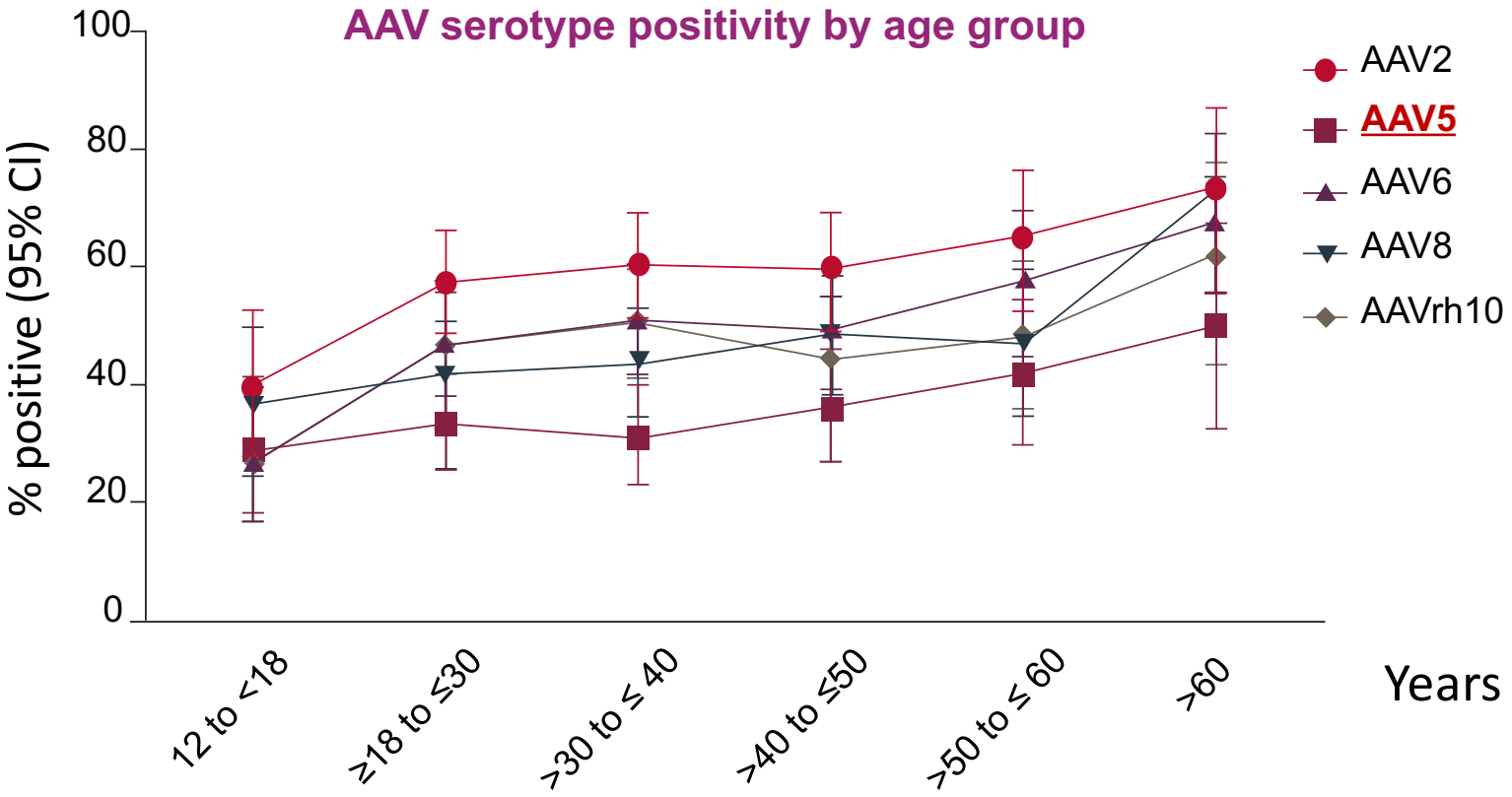
1 Attachment	Cell-surface attachment of AAV vectors is mediated by **primary glycan receptors** on the host cell and stabilized by secondary co-receptor(s)[2]
2 Internalization	Successful receptor recognition leads to internalization of the AAV vector by endocytosis [2]
3 Intracellular sorting	Intracellular sorting of AAV vectors takes place in endosomes and the Golgi [2]
4 Cytoplasmic escape and nuclear import	Prior to entering the nucleus, AAV particles escape the endosomal compartments (and/or trans-Golgi network) into the cytoplasm and accumulate around the perinuclear space [2]
5 Uncoating and transgene	The AAV genome is released from the capsid into the nucleus; ssAAV genomes must be converted to dsDNA before transcription, whereas scAAVs can immediately undergo transcription[2]

AAV, adeno-associated virus; dsDNA, double-stranded DNA; scAAV, self-complementary AAV; ssAAV, single-stranded AAV.

1. Wang D, et al. *Nat Rev Drug Discov* 2019; 18(5):358–378. 2. Dhungel BP, et al. *Trends Mol Med* 2020; 27(2):172–184.

Global seroprevalence of pre-existing immunity against AAV serotypes increases with age

- Prospective study in 546 participants with hemophilia A enrolled at 19 sites in 9 countries



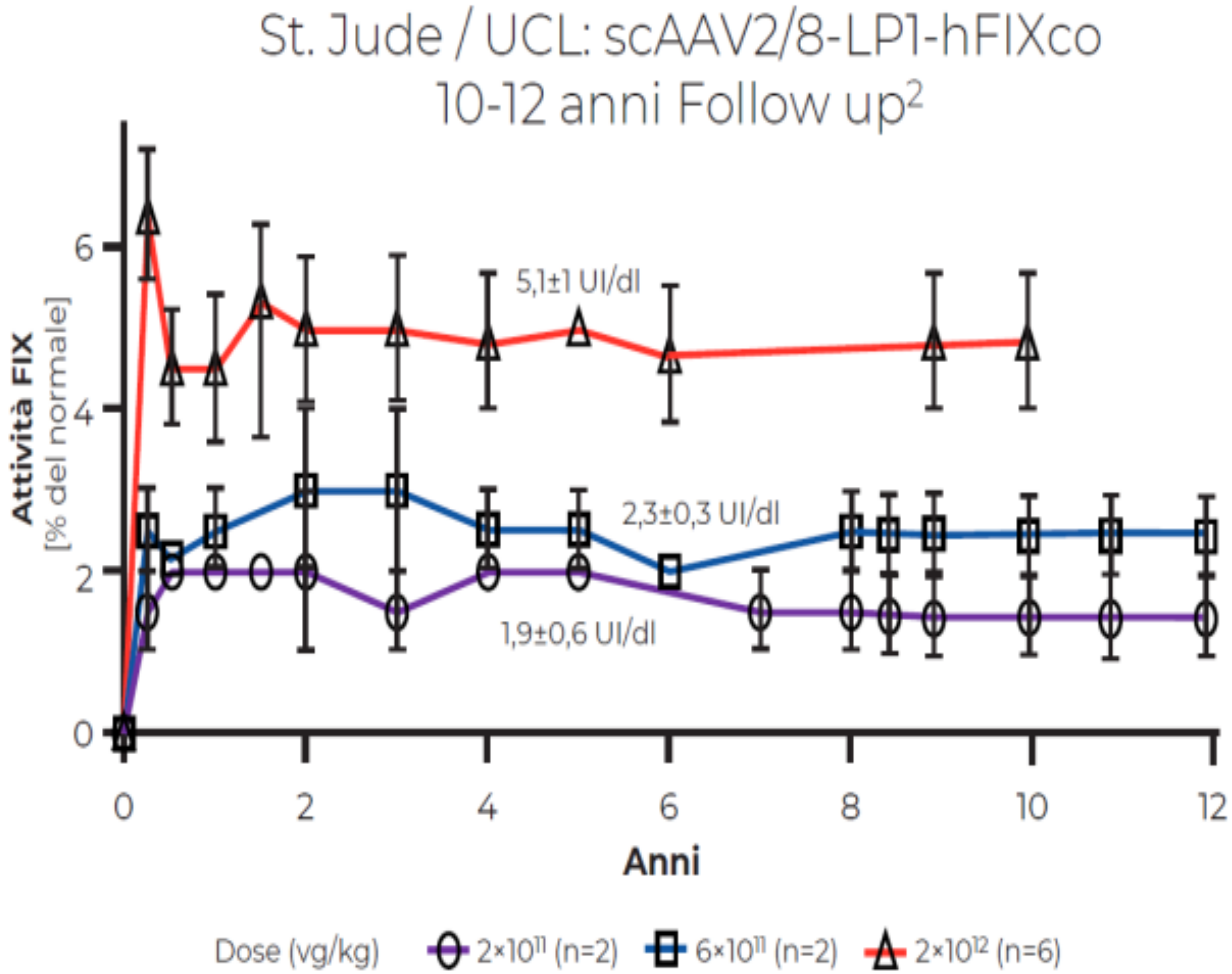
Klamroth R et al. Hum Gene Ther 2022;33:432-41

Hemophilia B gene therapy: general aspects

- The 1.5 kb FIX cDNA is easily packaged into a range of viral vectors, with expression mediated by liver-specific regulatory elements targeting the native site of FIX production.

- FIX is naturally synthesized in the hepatocytes

Terapia genica di St. Jude / UCL



The NEW ENGLAND JOURNAL of MEDICINE

ESTABLISHED IN 1812 DECEMBER 22, 2011 VOL. 365 NO. 25

Adenovirus-Associated Virus Vector–Mediated Gene Transfer in Hemophilia B

Attività FIX stabile e dose-dipendente >10 anni

studio condotto dal St. Jude Children's Research Hospital di Londra che, utilizzando un vettore AAV2/8-LP1-hFIXco alla dose di 2^{11} vg/kg, hanno trattato **10 pazienti** tra il 2010 e il 2012. **Tutti hanno ottenuto un'espressione stabile di FIX, i cui livelli si sono mantenuti in un periodo di follow-up di 10 anni, associandosi a riduzioni significative dell'utilizzo di FIX esogeno e della frequenza di sanguinamenti spontanei**

Improving Hemophilia B gene therapy

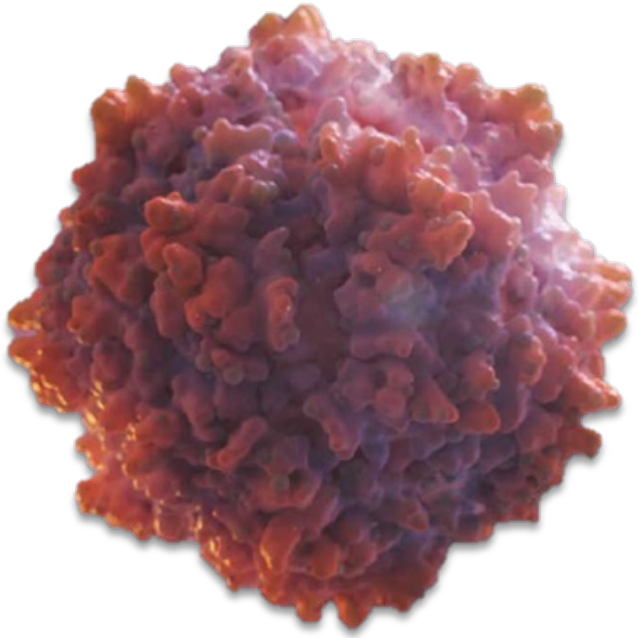
- FIX level with wild-type gene therapy usually between 2-3 to 10 U/dL

- *The discovery of a gain-of-function FIX variant (FIX Padua, p.R338L)* has further enhanced the potential for attaining therapeutic FIX activity levels with moderate vector doses

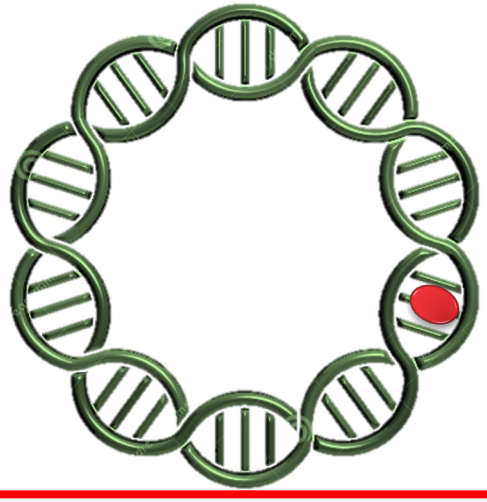
- This R338L missense mutant increases the specific activity of the molecule approximately 7-fold, *without evidence of increased immunogenicity*

AMT-060/AMT-061

AAV5 capsid



Liver-specific promoter & human FIX gene[3]



AMT-061
AGG to CTG in gene resulting in **R338L** in protein

AMT-060 – wildtype
AMT-061 – Padua variant
(expected 6-7x increase in activity)

- Low prevalence of pre-existing neutralizing antibodies able to impact clinical outcomes[1,4]
- Previously tested in humans without sign of cellular immune activation[2]

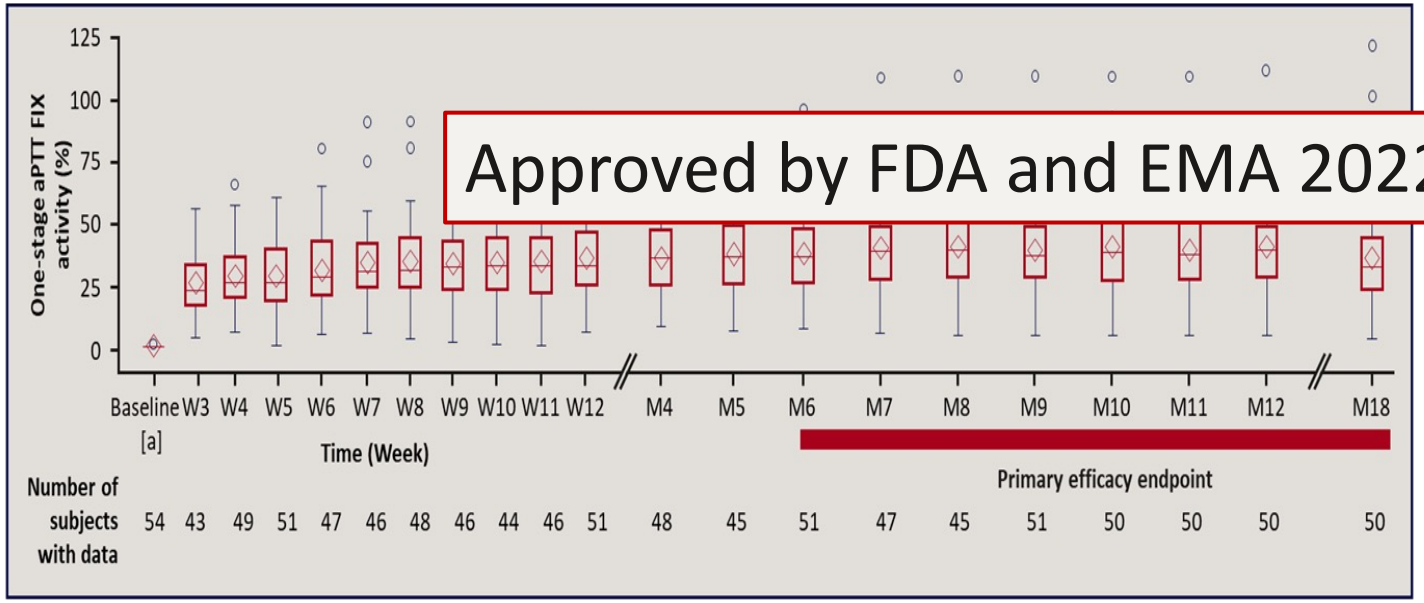
[1]Boutin et al, *Human Gen Ther* 2010; 21(6):704-12. [2]D'Avola et al, *Journal of Hepatology* 2016; doi: http://dx.doi.org/10.1016/j.jhep.2016.05.012.
[3]Nathwani et al. *NEJM* 2014; 371:1994-2004. [4]Majowicz et al, ASGCT 2018

The NEW ENGLAND JOURNAL of MEDICINE

ORIGINAL ARTICLE

Gene Therapy with Etranacogene Dezaparvovec for Hemophilia B

S.W. Pipe, F.W.G. Leebeek, M. Recht, N.S. Key, G. Castaman, W. Miesbach, S. Lattimore, K. Peerlinck, P. Van der Valk, M. Coppens, P. Kampmann, K. Meijer, N. O'Connell, K.J. Pasi, D.P. Hart, R. Kazmi, J. Astermark, C.R.J.R. Hermans, R. Klamroth, R. Lemons, N. Visweshwar, A. von Drygalski, G. Young, S.E. Crary, M. Escobar, E. Gomez, R. Kruse-Jarres, D.V. Quon, E. Symington, M. Wang, A.P. Wheeler, R. Gut, Y.P. Liu, R.E. Dolmetsch, D.L. Cooper, Y. Li, B. Goldstein, and P.E. Monahan



Uncontaminated central laboratory data (the visit did not occur within 10 days of exogeneous FIX use). FIX levels beginning with the Week 3 assessment were used in the analysis. Subjects with 0 uncontaminated central-laboratory post-AMT-061 values had change from baseline assigned to zero for this analysis and had their post-baseline values set equal to their baseline value. Baseline FIX was imputed based on subject's historical haemophilia B severity documented on the case record form. If the patient had documented severe FIX deficiency (FIX plasma level <1%), their baseline FIX activity level is imputed as 1%. If the subject had documented moderately severe FIX deficiency (FIX plasma level ≥1% and ≤ 2%), their baseline FIX activity level was imputed as 2%.

Mean (SD; min, max) FIX activity was 39.0 IU/dL (±18.7; 8.2, 97.1) at 6 months and 36.9 IU/dL (±21.4; 4.5, 122.9) at 18 months

At 6 months, mean (SD) change from baseline was 37.77 (18.78) with a p-value <0.0001; at 18 months the change from baseline was 35.72 (21.46) with a p-value <0.0001.

aPTT, activated partial thromboplastin time; FIX, factor IX; M, month; SD, standard deviation; W, Week.

1. Miesbach W, et al. Oral presentation. EHAD 2022.

February 23, 2023

Approved by FDA and EMA 2022 and 2023

54 patients with FIX ≤ 2 U/dL

Also patients with pre-existing AAV5 Nabs responded

HOPE-B results showed less variability, with more stable factor expression

bleed reduced by **77%**

- ✓ Prophylaxis discontinued in **96.3%**
- ✓ Significant reduction in annualized FIX consumption by **97%(p<0.001)**

AEs:

- **Transaminase elevations requiring steroid treatment in 17%**
- Other common TRAEs: headache (**15%**), and influenza-like illness (**13%**)

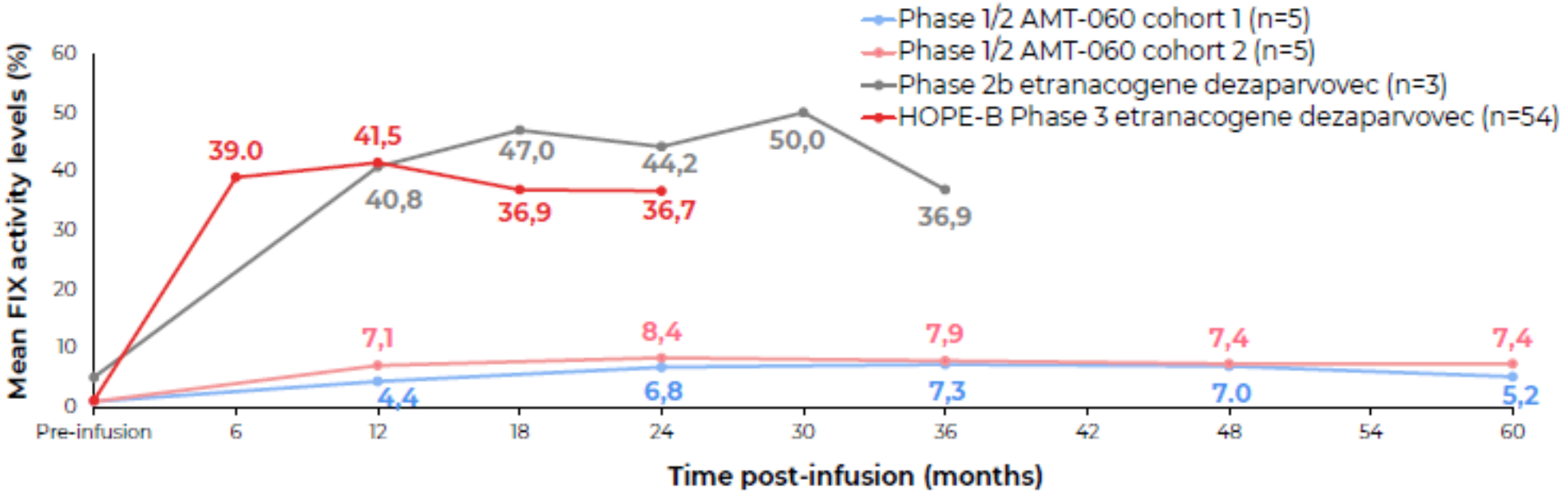
One SAE of **HCC**; occurred after the 6-month data cut and was determined **unlikely to be treatment related**

Durability of response after long-term follow-up in the Phase 1/2 study of AMT-060, and Phase 2b and 3 studies of etranacogene dezaparvovec in haemophilia B

Wolfgang Miesbach[1], Michael Recht[2], Nigel S. Key[3], Krupa Sivamurthy[4], Paul E. Monahan[4], Steven W. Pipe[5], on behalf of study investigators

[1]University Hospital Frankfurt, Frankfurt, Germany; [2]Yale University School of Medicine, New Haven, CT, USA; [3]University of North Carolina, Chapel Hill, NC, USA; [4]CSL Behring, King of Prussia, PA, USA; [5]University of Michigan, Ann Arbor, MI, USA

Figure 2. Mean FIX activity levels over time in the clinical trials of AMT-060 and etranacogene dezaparvovec[a]



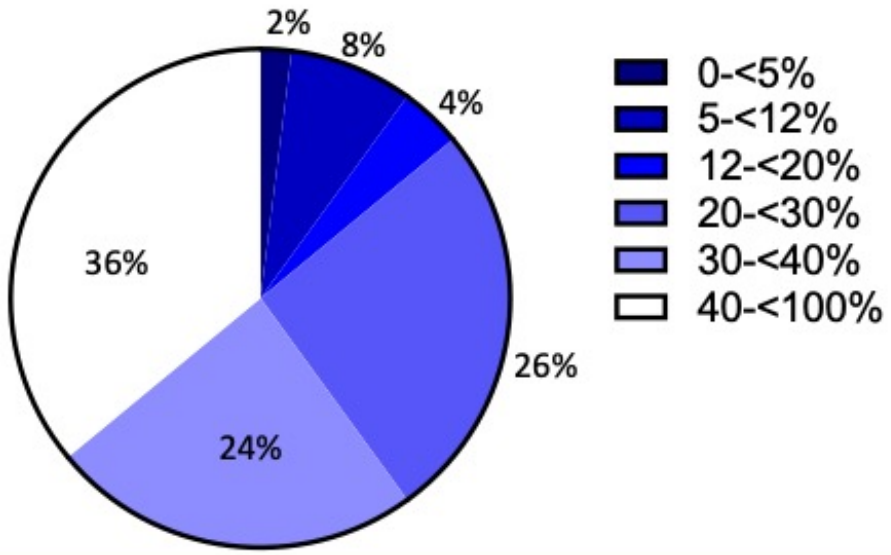
[a] Phase 1/2 AMT-060 and Phase 2b had no lead-in period; Phase 3 HOPE-B had a ≥6-month lead-in period where participants received FIX prophylaxis. For Phase 2b, baseline FIX expression was 0–2%, but no washout of infused FIX was required prior to Day 1; n=3 for all timepoints except pre-infusion (n=1), 18 months (n=2), and 36 months (n=2). For HOPE-B Phase 3, n=54 at pre-infusion, n=51 at 6 months, and n=50 at 12, 18 and 24 months.
FIX, factor IX.

Poster presented at the 16th Annual Congress of the European Association for Haemophilia and Allied Disorders(EAHAD), 7–10 February 2023.

Rate of expression of FIX transgene

MOST PARTICIPANTS EXPRESSED FIX ACTIVITY LEVELS IN THE MILD-NORMAL RANGE AT MONTH 24

Proportion of participants within FIX activity ranges[a] (n=50)[b]



2 participants did not express endogenous FIX following administration of etranacogene dezaparvovec

- **one with the highest AAV5 NAb titer of 3212,**
- **one who received only ~10% of the planned dose [AAV5 NAb+]**

98% of participants who responded to treatment expressed FIX activity within the mild-normal range

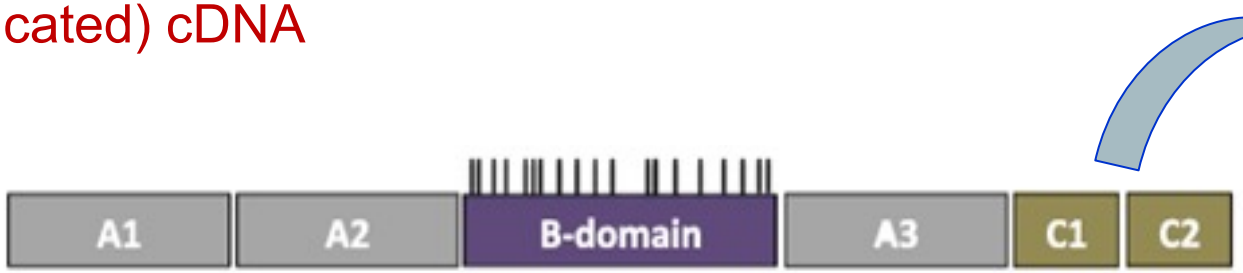
[a] Based on One-stage FIX activity levels, and only uncontaminated samples were included in analysis, i.e., blood sampling did not occur within 5 half-lives of exogenous FIX use

[b]. From 54 patients dosed, 1 patient died at month 15 (unrelated to treatment), 3 patients had FIX activity levels contaminated by exogenous FIX use including the 2 who did not stop prophylaxis due to lack of efficacy and 1 who used on-demand FIX <5 half-lives before month 24 visit.

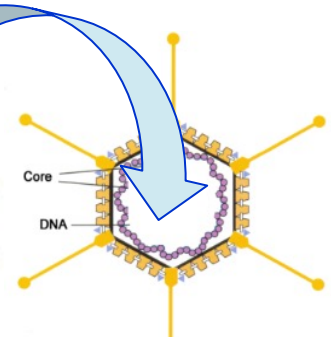
AAV5, adeno-associated virus serotype 5; FIX, factor IX; LS, least squares; NAb, neutralising antibody;

Hemophilia A gene therapy: general aspects

- The size of the native FVIII cDNA of ~9 kb precludes packaging into clinically applicable vectors, and thus all current FVIII transgene constructs utilize a B domain-deleted (or truncated) cDNA

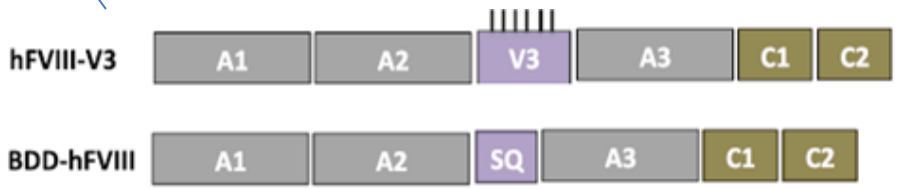


FVIII

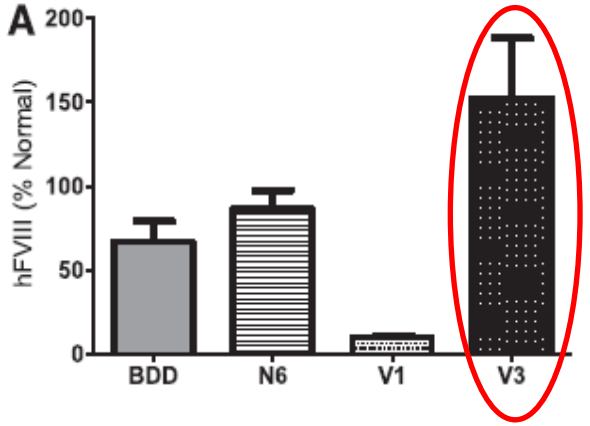


Viral vector

Codon optimized



The animal models showed an increase in FVIII expression levels



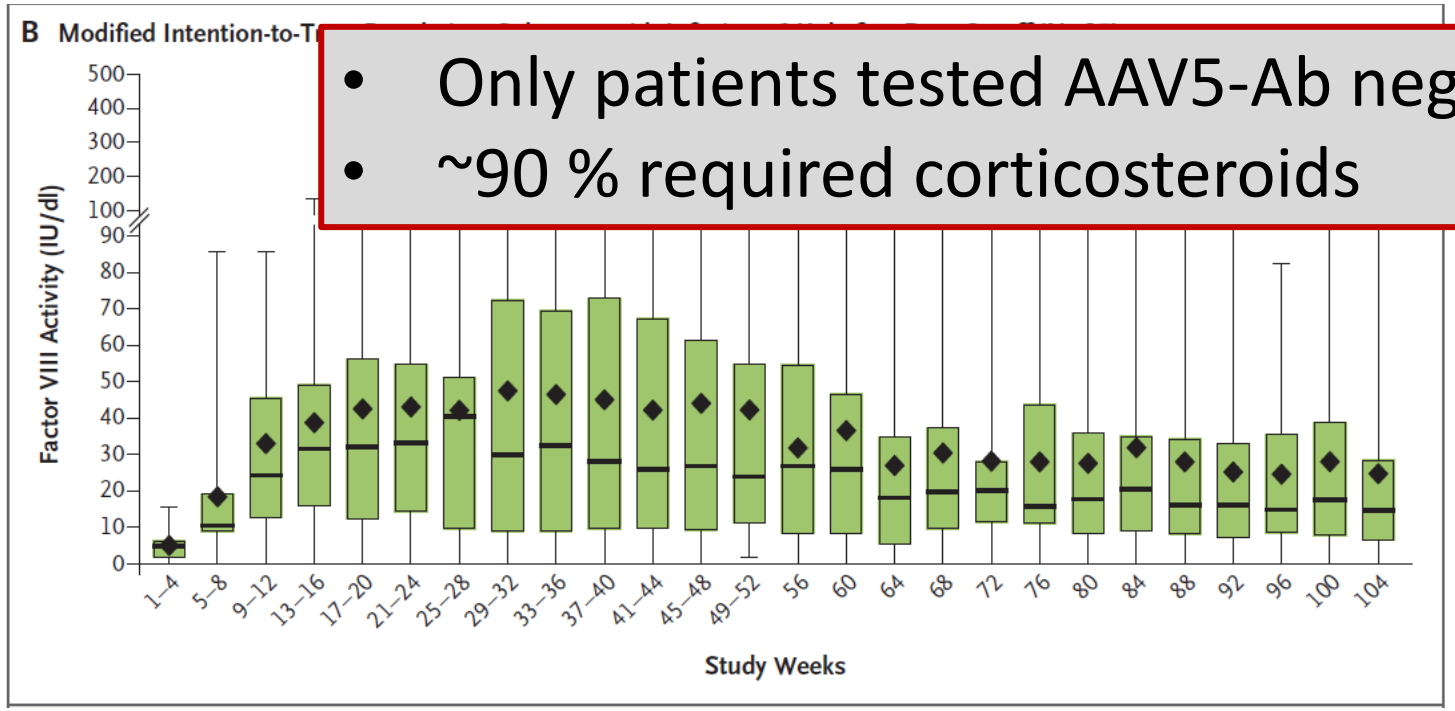
- Replacement of the FVIII B domain with a 17 amino acid peptide containing 6 glycosylation sequences has also been demonstrated to enhance FVIII trafficking and secretion

The NEW ENGLAND JOURNAL of MEDICINE

ESTABLISHED IN 1812 MARCH 17, 2022 VOL. 386 NO. 11

Valoctocogene Roxaparvovec Gene Therapy for Hemophilia A

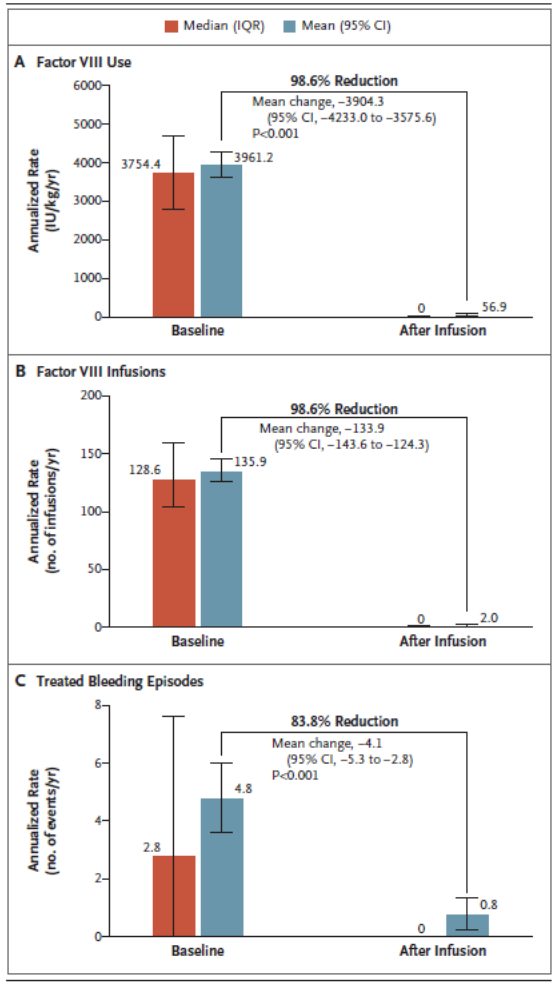
M.C. Ozelo, J. Mahlangu, K.J. Pasi, A. Giermasz, A.D. Leavitt, M. Laffan, E. Symington, D.V. Quon, J.-D. Wang, K. Peerlinck, S.W. Pipe, B. Madan, N.S. Key, G.F. Pierce, B. O'Mahony, R. Kaczmarek, J. Henshaw, A. Lawal, K. Jayaram, M. Huang, X. Yang, W.Y. Wong, and B. Kim, for the GENEr8-1 Trial Group*



- Only patients tested AAV5-Ab negative
- ~90 % required corticosteroids

Mean

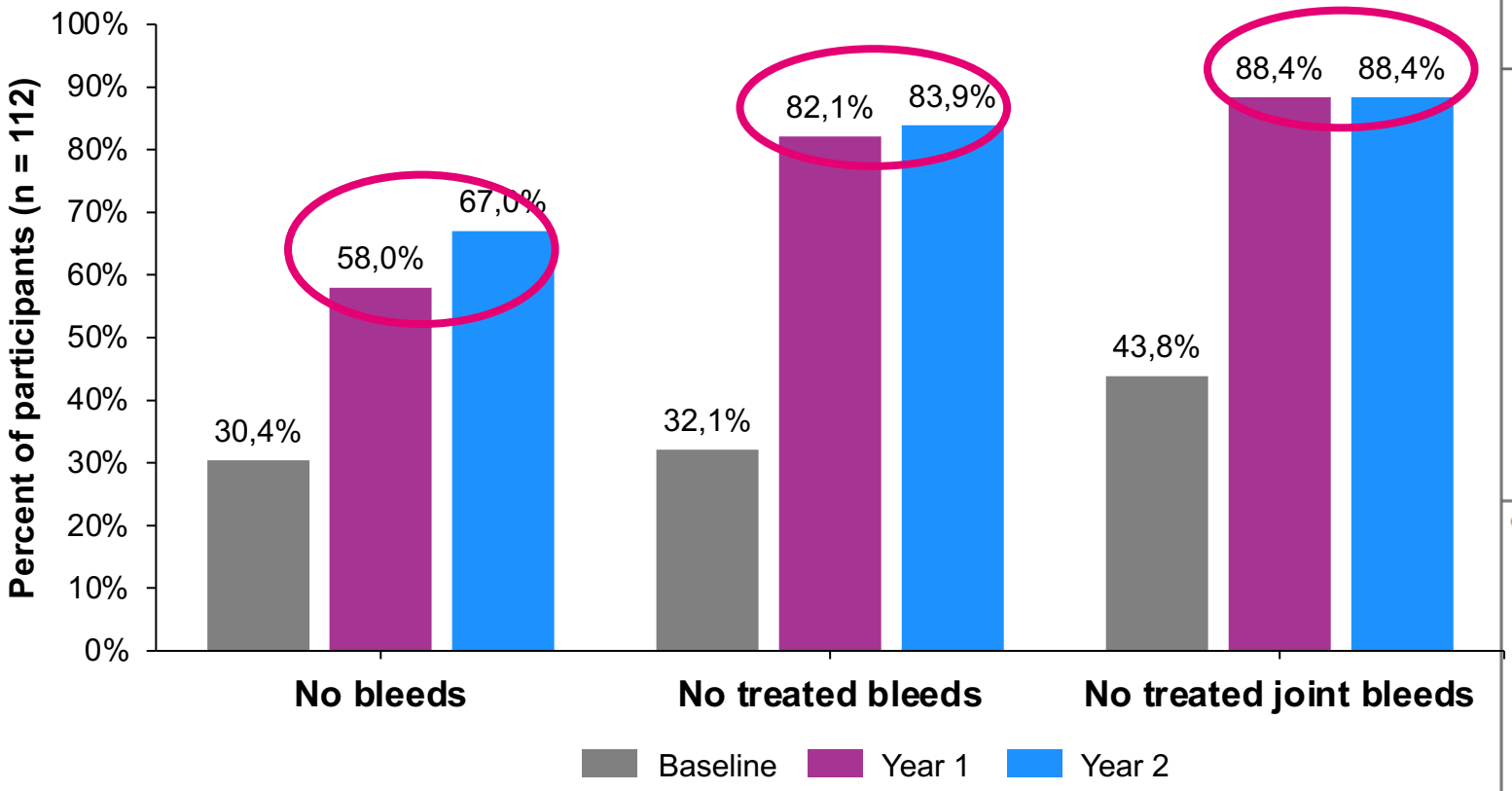
Median

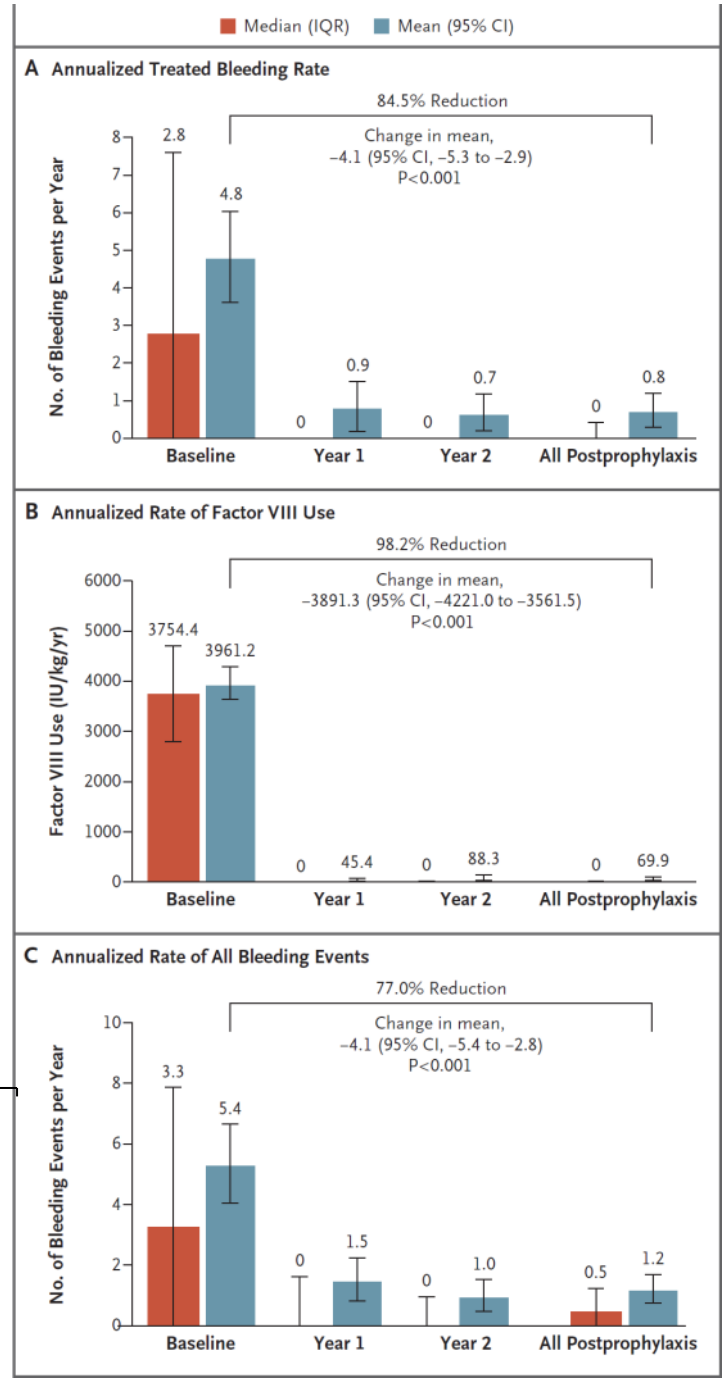


The NEW ENGLAND JOURNAL of MEDICINE

ORIGINAL ARTICLE

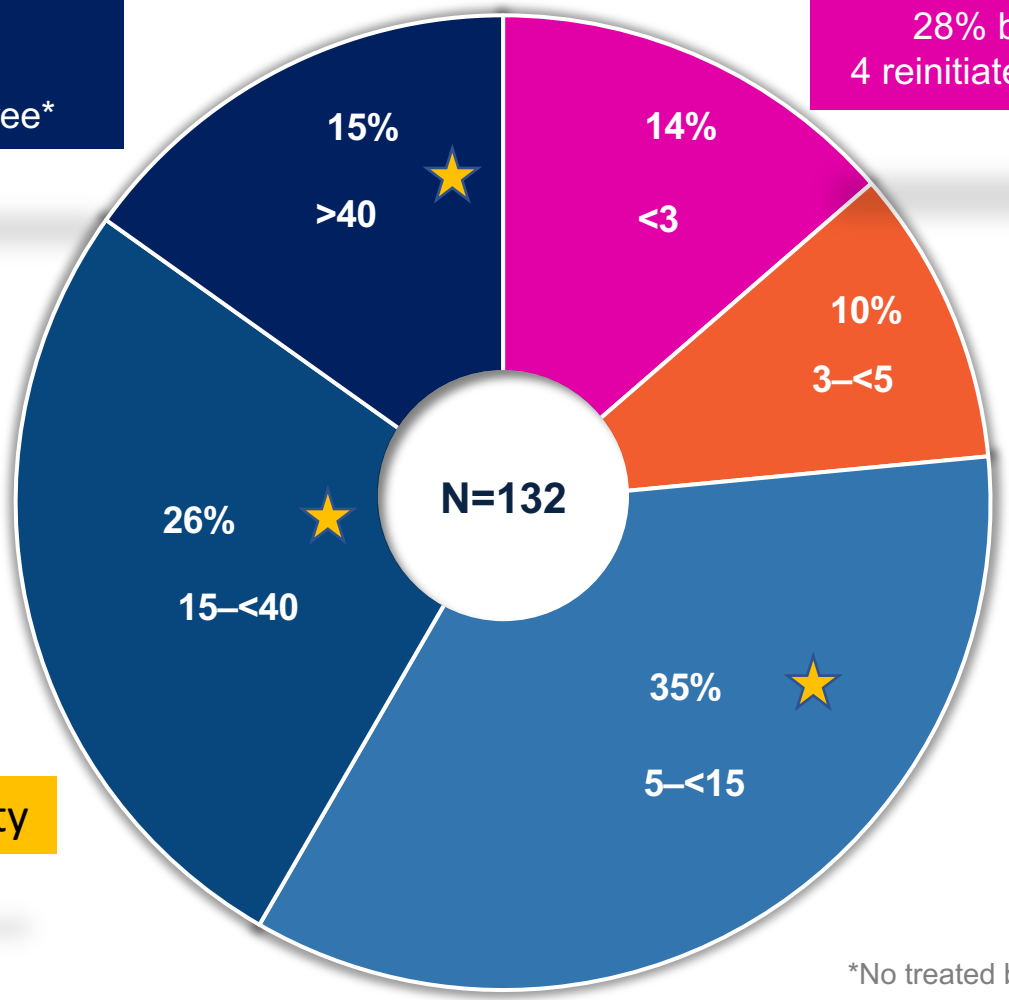
Two-Year Outcomes of Valoctocogene Roxaparvovec Therapy for Hemophilia A





February 23, 2023

Study 270-301 | Participant distribution by week 104 CSA FVIII level



FVIII <3
N=18
28% bleed-free*
4 reinitiated prophylaxis

FVIII >40
N=20
100% bleed-free*

FVIII 3 - <5
N=13
77% bleed-free*
1 reinitiated prophylaxis

FVIII 15 - <40
N=35
97% bleed-free*

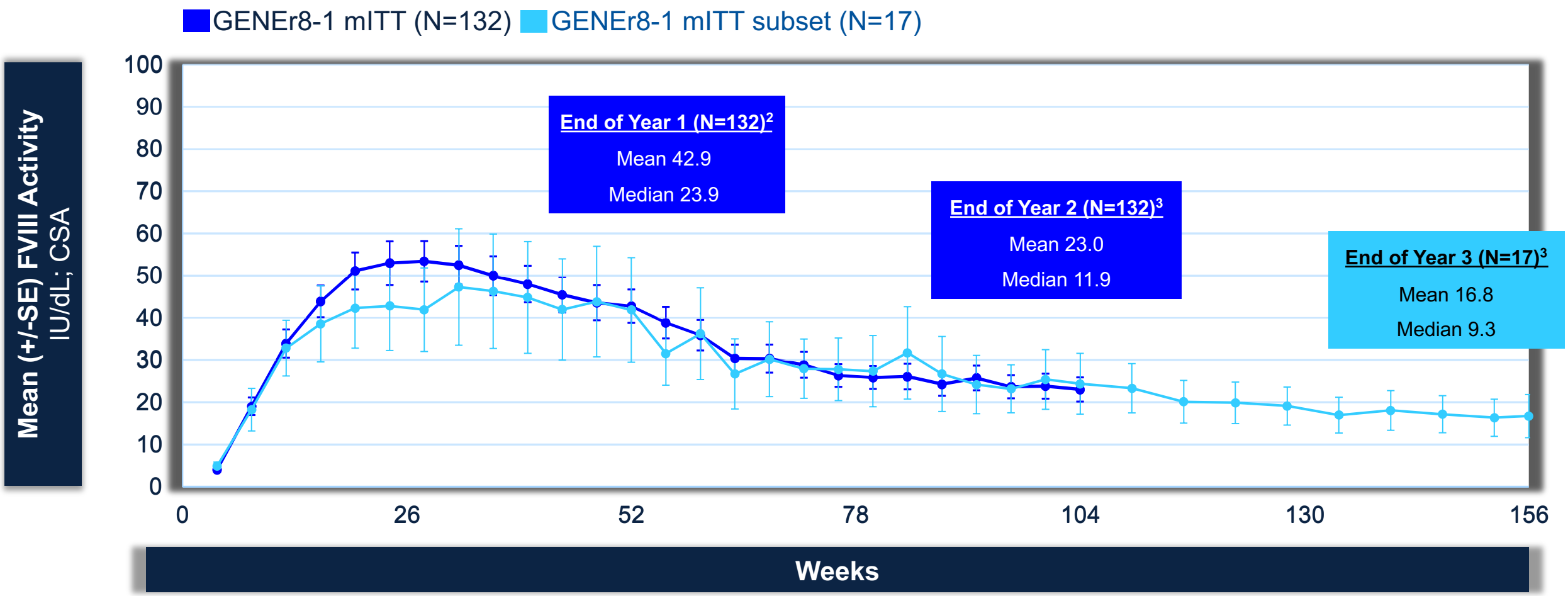
FVIII 5 - <15
N=46
85% bleed-free*
1 reinitiated prophylaxis

★ 76% > 5 U/dL FVIII activity

FVIII, factor VIII; CSA, chromogenic substrate assay.
*No treated bleeds within the past year of follow-up and not on prophylaxis.

Mahlangu J *et al.* Presented at EAHAD Virtual Congress. Feb 2022

Study 270-301 | FVIII activity over time[1]



FVIII, factor VIII; mITT, modified intent-to-treat population; SE, standard error; CSA, chromogenic substrate assay
Missing FVIII values are imputed as follows: smaller of adjacent non-missing values; 0 if participant has discontinued study; linear extrapolation if there are no subsequent valid values

1. Ozelo M *et al.* Presented at ISTH Virtual Congress. July 17–21, 2021; 2. Mahlangu J *et al.* Presented at EAHAD Virtual Congress. Feb 2022; 3. BioMarin data on file

Safety alerts in gene therapy for hemophilia

- **Hepatotoxicity**
  Increased liver enzymes

- ***Thrombotic events***
  Two thrombotic events in HA and HB trials

- ***Genotoxicity***

FIX	
AAV5-hFIXco-Padua	Hepatocellular carcinoma
AAV8.sc-TTR-FIXR338L	Acinar cell carcinoma of parathyroid gland
FVIII	
AAV5-hFVIII-SQ	Squamous cell carcinoma of tonsil
AAV5-hFVIII-SQ	B-cell ALL Ph positive

Valoctocogene roxaparvovec regulatory status[1–5]

EUROPEAN MEDICINES AGENCY
SCIENCE MEDICINES HEALTH

- Orphan drug designation from the European Commission[4]
- Priority Medicines (PRIME) scheme from the European Medicines Agency (EMA)[5]
- AAV5 total antibody assay received a CE-mark under the current EU IVD Directive (IVDD) in Jan 2022
- **CHMP positive opinion** received June 23, 2022[6]
- European Commission (EC) **Conditional Marketing Authorization**, August 24, 2022[7]

AIFA
AGENZIA ITALIANA DEL FARMACO

- Value dossier submitted, November 2022

FDA U.S. FOOD & DRUG ADMINISTRATION

- Submitted, under evaluation

1. BioMarin Receives Orphan Drug Designation From FDA for First AAV-Factor VIII Gene Therapy, BMN 270, for Patients With Hemophilia A [press release]. March 1, 2016; 2. FDA Grants Breakthrough Therapy Designation for BioMarin's Valoctocogene Roxaparvovec (formerly BMN 270), an Investigational Gene Therapy for Hemophilia A [press release]. October 26, 2017; 3. https://www.aruplab.com/news/2-20-2020/aav5-cdx-hemophelia-gene-therapy-treatment; 4. BioMarin Receives European Orphan Drug Designation for BMN 270, First Investigational AAV-Factor VIII Gene Therapy for Patients with Hemophilia A [press release]. March 24, 2016; 5. BioMarin Receives Access to Priority Medicines (PRIME) Regulatory Support from EMA for BMN 270 Gene Therapy in Hemophilia A [press release]. February 1, 2017; 6. https://www.ema.europa.eu/en/medicines/human/summaries-opinion/roctavian; 7. https://investors.biomarin.com/2022-08-24-First-Gene-Therapy-for-Adults-with-Severe-Hemophilia-A,-BioMarins-ROCTAVIAN-TM-valoctocogene-roxaparvovec-,-Approved-by-European-Commission-EC. Accessed August 2022

Gene therapy for all?

- **Limitations:**
  - Children
  - Chronic liver disease
  - Inhibitors
  - At present, it is expected no more than 20-25% of HA and 40 % HB patients are eligible
- **Open issues:**
  - Management/Prevention transaminitis
  - Expression duration
  - Long-term safety (rate of integration)
  - Expression outcome unpredictable
  - ……

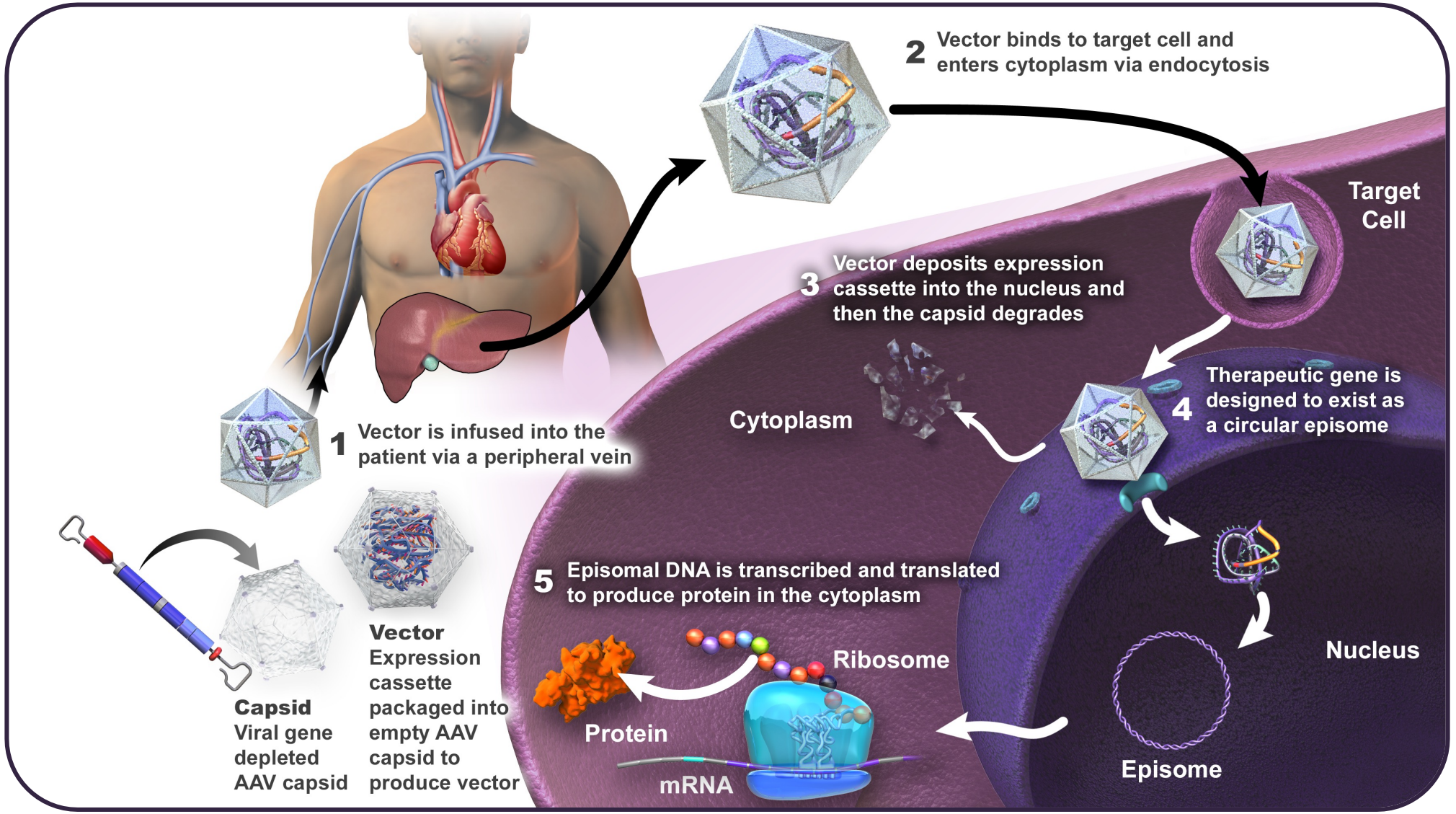
Conclusions

- Non-substitutive agents have made **prophylaxis** feasible in inhibitor patients
- They could enhance and favors prophylaxis in patients without inhibitors, rescuing also adult patients with poor adherence (venous access, elbow arthropathy…)
- They do not abolish the need for replacement therapy (breakthrough bleeding, surgery…)
- The use of by-passing agents and FVIII/FIX is critical in careful management of these situations
- Careful evaluation of **thrombotic risk** must be made against the availability of efficacious options
- Guidance provided efficacious in attenuating this risk
- The role of HTC/Hemophilia consultant is central and all efforts should be made to provide education, training, a costant supervision to the patients and caregivers

Conclusions for gene therapy

- Big steps forward, potential for cure, “prophylaxis enhancer”
  - Available clinical trials provided information for transgene expression likely at therapeutic levels long-term for hemophilia B, medium-term for hemophilia A
  - Abolition of bleeding events in most, together with concentrate consumption
  - Population eligible limited, transaminitis in hemophilia A
  - High costs, need for long-term reimbursement plans (by results? Pay back?)
  - Other technologies in development (e.g., Lentivirus, CRISPR/Cas9…)
  - Careful evaluation within the available therapeutic option scenario

AAV-Mediated Gene Transfer Mechanism of Action

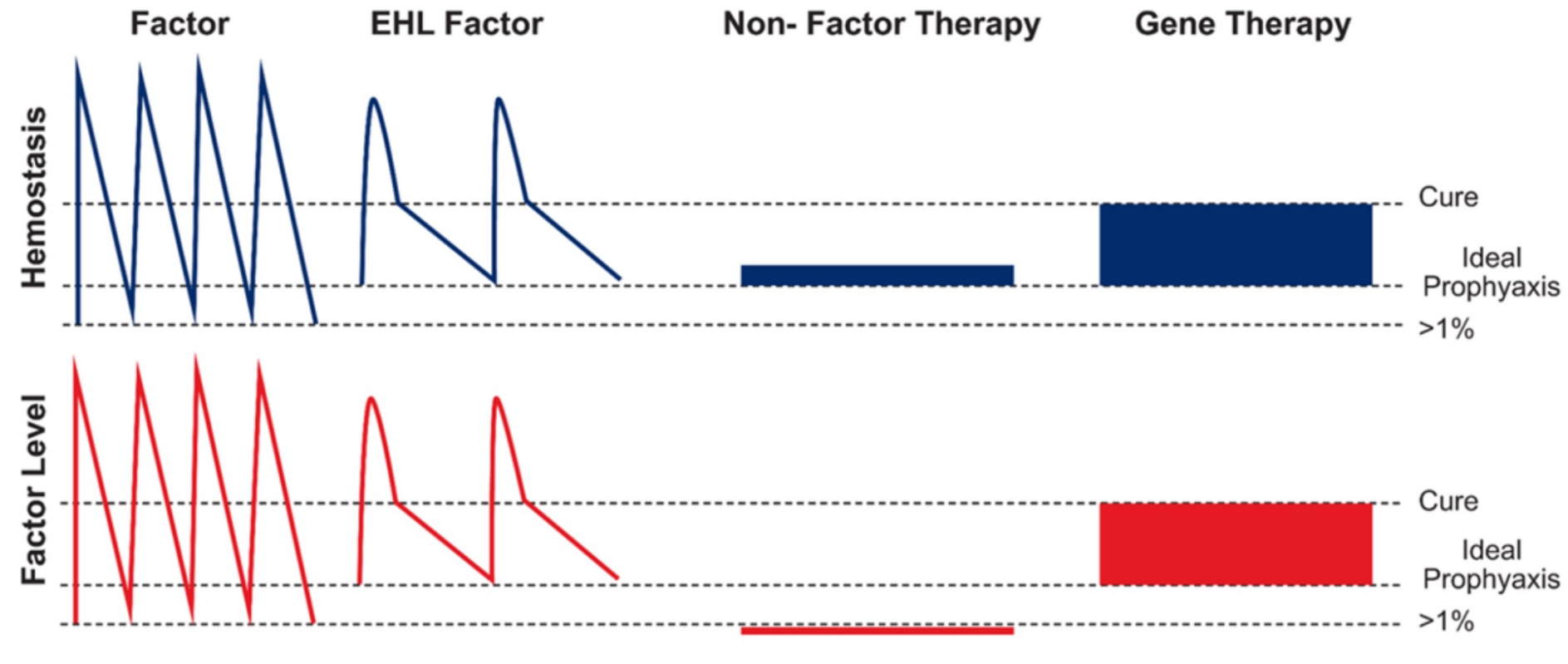


1. Coura RdS *et al. Genet Mol Biol* 2008;31:1–11; 2. Sen D *et al. Sci Rep* 2013;3:1832

From Clinical trials to Real World…

- The first licensed hemophilia *gene therapy products will be in clinics soon*, and the uptake of this new treatment will then depend upon a complex combination of
- *patient satisfaction with current therapies*
- *uncertainties surrounding long-term gene therapy outcomes*
- *payment options*

The Benefits of Gene Therapy



From: Arruda et al., Blood, 2017

Predictability of transgene expression level and durability

- Probably the most critical questions that patients will ask about potential outcomes of hemophilia gene therapy are:

- **what level of factor will I achieve with gene therapy?**

- **how long will the effect last?**

a) In human trials to date, there is significant variability in the factor levels that in some instances is as high as >10-fold (0.20 to >2.00 IU/mL)

b) In terms of durability of transgene expression,Human FIX gene therapy studies in adult patients are now ~ 10 years post-single administration, with minimal evidence of a decline in plasma FIX levels

Somewhat in contrast, the results of the longest duration human FVIII gene transfer trial using an AAV5 vector has shown a significant decline in FVIII levels during the first 2 years, but with more stable pattern up to 4 years post-vector delivery.