Current Updates on Gene Therapy in Hemophilia

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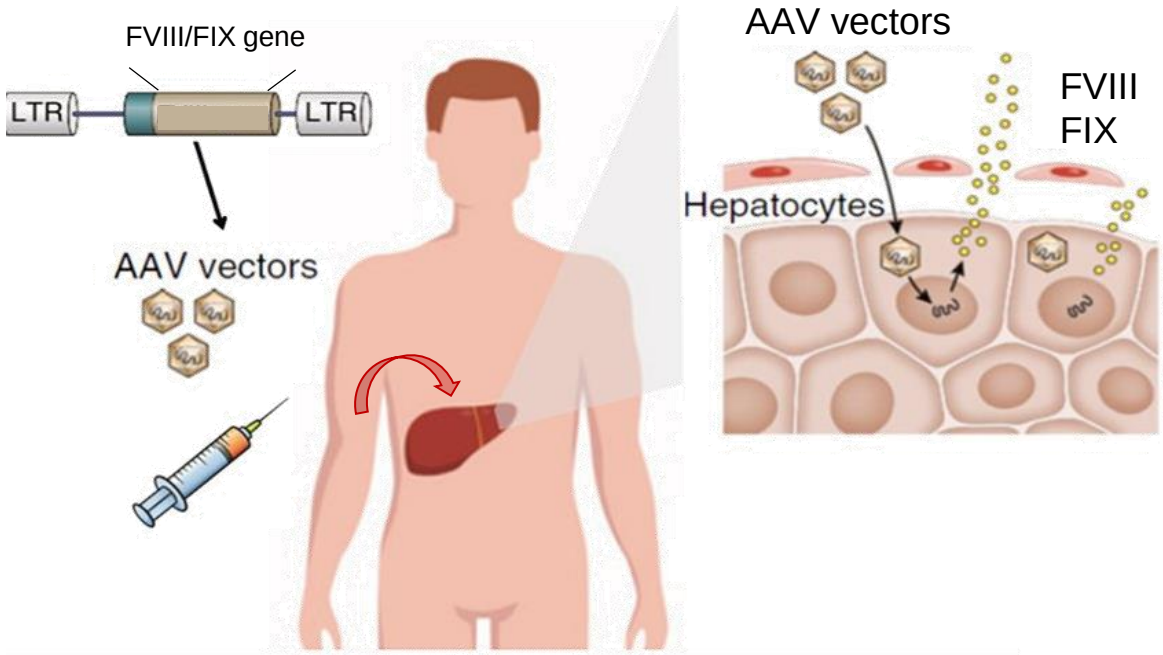
European Reference Network
Hematological Diseases (ERN EuroBloodNet)

Gene therapy definition

- Gene therapy is a novel method of treatment currently in use for a variety of genetic conditions
- The goal is to **introduce** an exogenous functional gene (**transgene**) into **target cells** using a vector (vehicle) to cure the disease with a single treatment



Nathwani AC. Hematology Am Soc Hematol Educ Program. 2022;2022:569-578

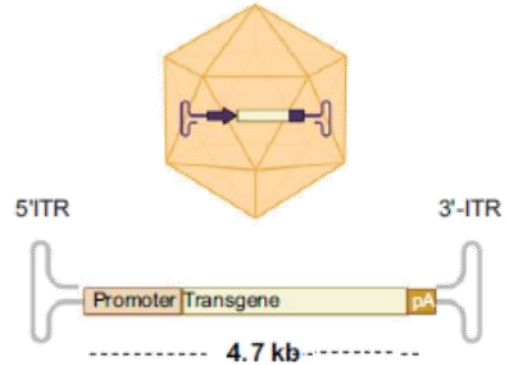
Gene therapy strategies

- The **success** of gene therapy depends on the development of **vectors** that can **efficiently introduce the therapeutic genes** into cell target
- **AAV vectors** are a leading platform for **safe and effective gene delivery**

Bulcha JT et al. Signal Transduct Target Ther. 2021;6:53; Maestro S et al. JHEP Rep. 2021;3:100300; Nathwani et al. Hum Gen Ther 2021;33:879-888

AAV, an appealing virus for gene therapy

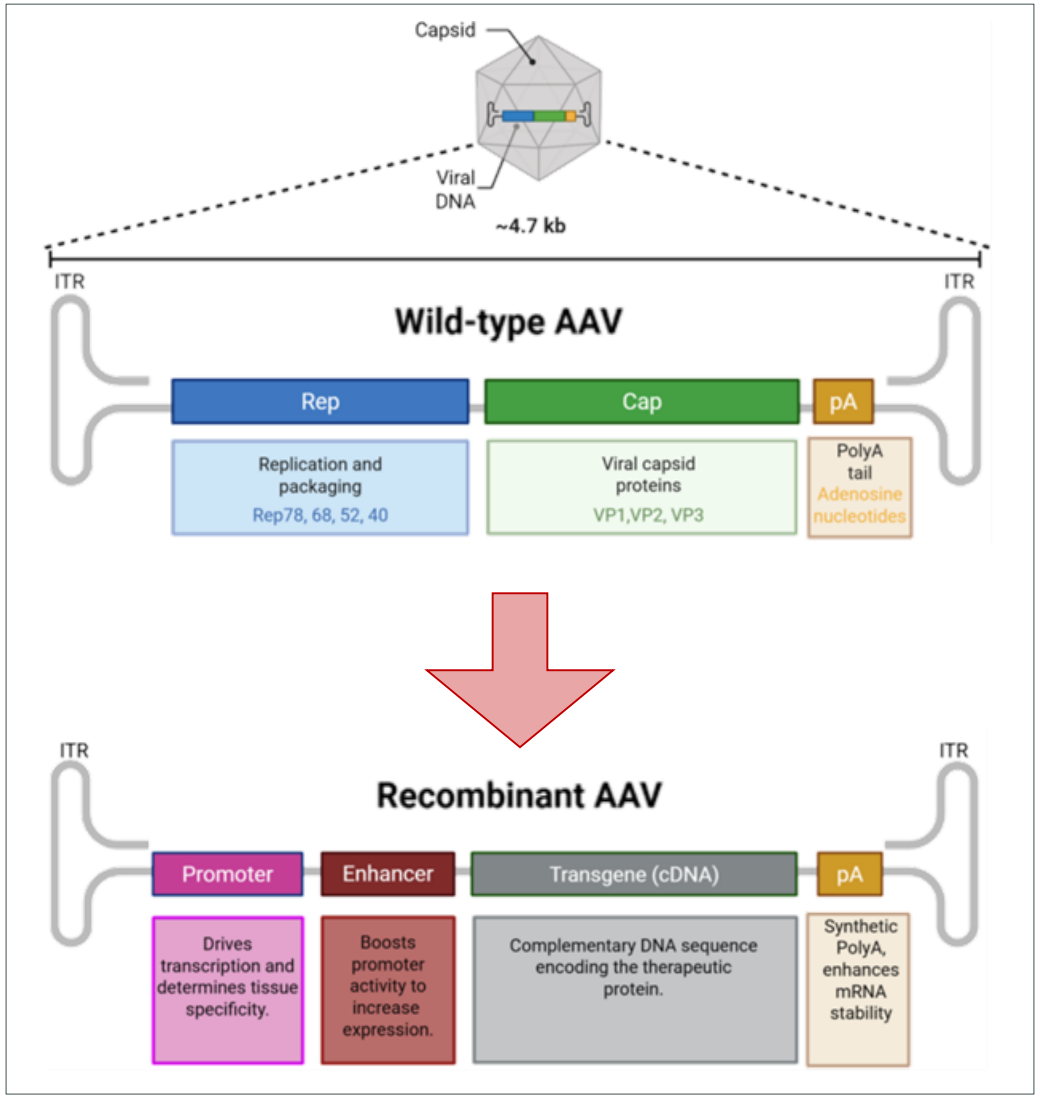
- **Single-stand DNA** virus of the **Parvovirus family**
- Non pathogenic
- Infect wide range of cell types and organs
- Transduce dividing and non-dividing cell
- Non- or minimally integrating vectors/low integration rate → this may limit their long-term expression, particularly into rapidly dividing cells



Bulcha JT et al. Signal Transduct Target Ther. 2021;6:53; Maestro S et al. JHEP Rep. 2021;3:100300; Nathwani et al. Hum Gen Ther 2021;33:879-888

AAV genome engineering

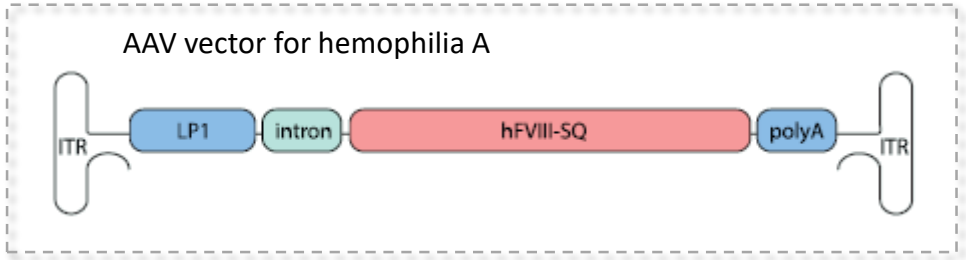
- AAV viruses are completely stripped of the **wild-type viral coding sequences** (*rep* and *cap* genes) that are **replaced with the gene of interest**, maintaining only the inverted terminal repeats (ITRs)
  - ITR sequence contains the origin of replication and packaging signal

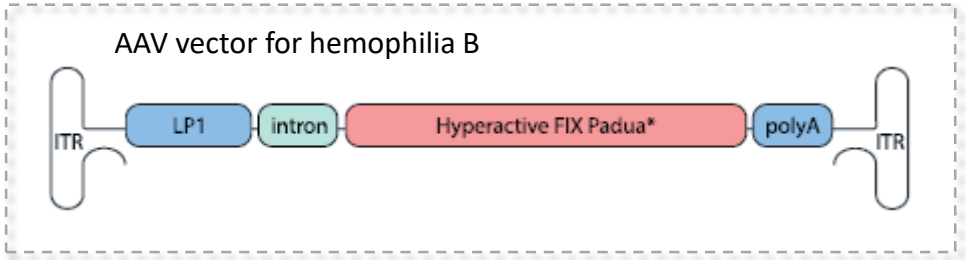


Nidetz NF et al Pharmacol Ther. 2020;207:107453; Li C and Samulski RJ. Nat Rev Genet. 2020;21:255-272; Figure from Simini G & Batty P. J Thromb Haemost. 2025;23:3781–3790.

Ideal vector for gene therapy

- **Highly precise and specific target cell delivery**
  - A liver-specific promoter
- **To improve expression levels**
  - Codon optimization of the transgene (*F8* or *F9*)
    - A key step in achieving high expression levels of the target gene
    - Synonymous mutations are introduced to promote efficient protein expression
  - **Pauda** "gain-of-function mutation" specific for *F9*



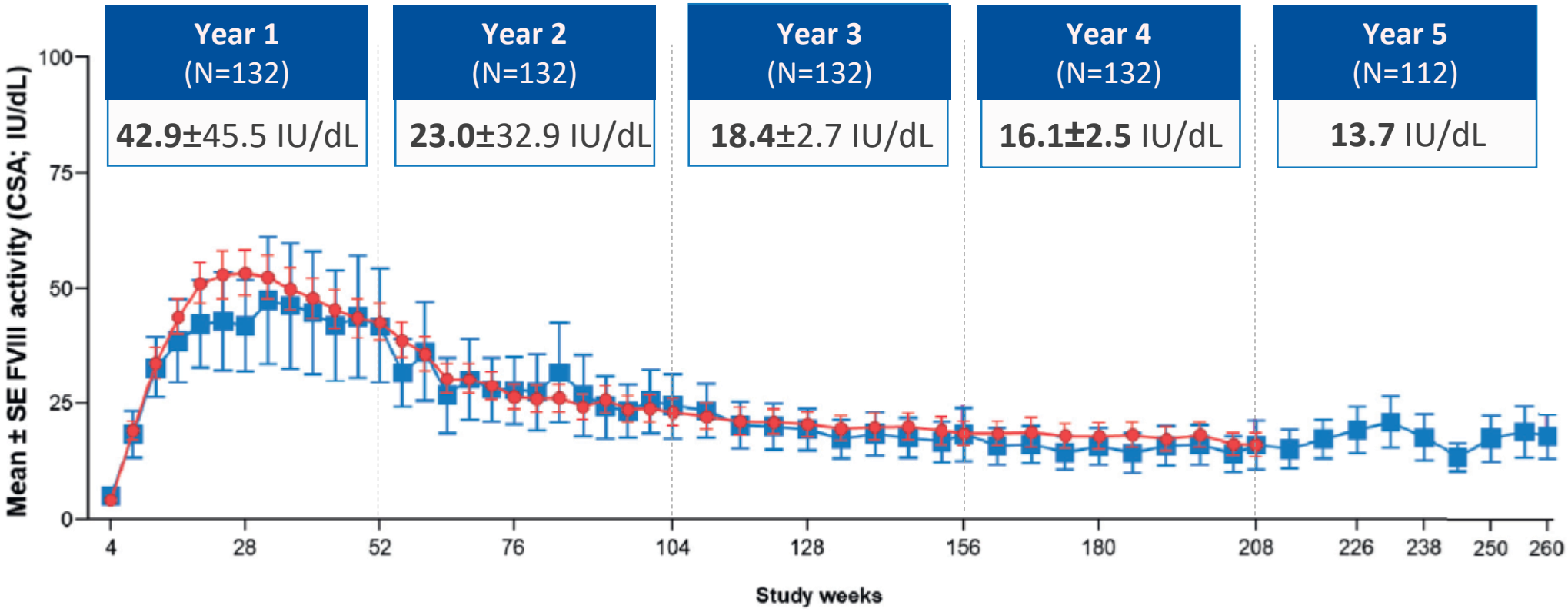


Nathwani et al. Hum Gen Ther 2021;33:879-888; Simioni P et al. N Engl J Med 2009;361:1671-1675

Efficacy Data of Valoctocogene Roxaparvovec

FVIII Expression **5 Years** after gene transfer - **Phase 3** Trial

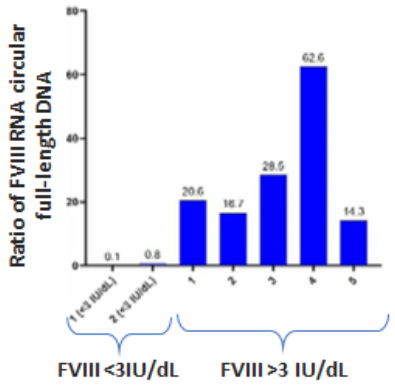
- **134 participants** received an infusion of **$6x10^{13}$** vg/kg of AAV5 vectors
- FVIII activity **declines most in year 1**, then **slows** in the following years **reaching a plateau**

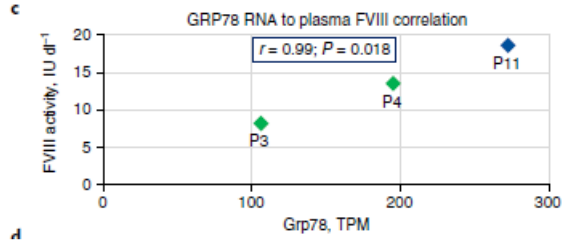


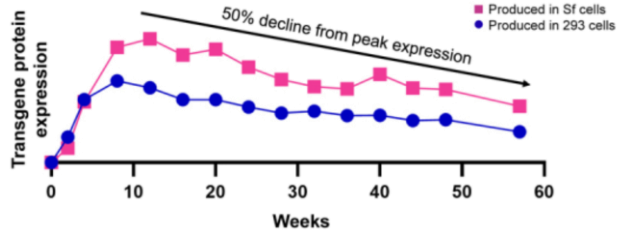
Leavitt AD et al Res Pract Thromb Haemost. 2025;102923 – ISTH Meeting 2025

Potential Causes for Declining FVIII Expression

- **Reduced transcription**: decline over time may result from decreased transcription of episomal vector DNA in hepatocytes
- Cellular folding capacity: higher basal BiP (GRP78) expression may enhance FVIII secretion efficiency
- **Transgene expression**: **epigenetic regulation** impacts transgene expression of vectors
- **Immune and inflammatory responses**: activation of $CD8^+$ T cells, NK cells, and Kupffer cells may downregulate translation







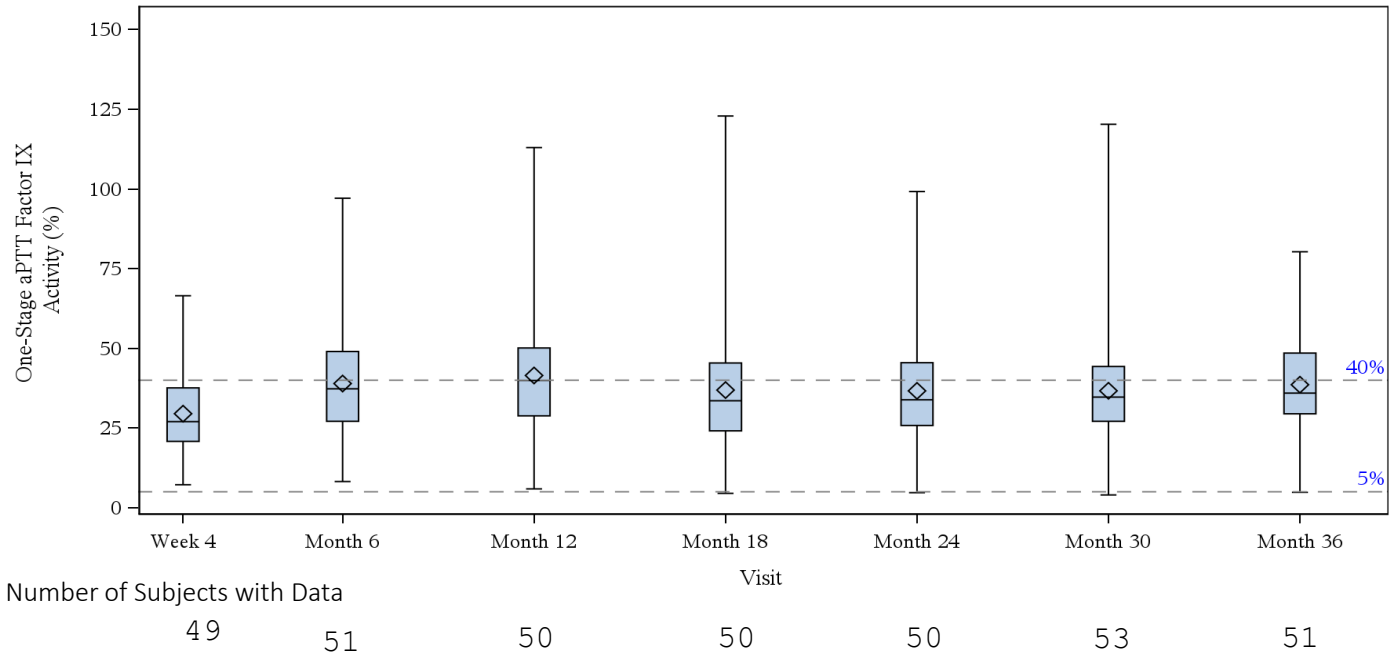
Ismail AM et al. 17th Annual Congress of EAHAD congress 2024; Fong S, et al. Nat Med. 2022;28:789-797; Handyside B, et al. Mol Ther. 2022;30:3570-3586; Butterfield JSS, et al. Mol Ther. 2022;30:3552-3569

Efficacy Data of Etranacogene dezaparvovec

FIX Expression **5 Years** after gene transfer - **Phase 3** Trial

- **54 participants** received an infusion of 2**x10^{13}** vg/kg of AAV5 vectors

Year 1	Year 2	Year 3	Year 4
41.5 IU/dL	**36.7** IU/dL	**38.6** IU/dL	**37.4** IU/dL



Overall stable FIX activity (36.1 IU/dL) levels over 5 years post-treatment

Leebeek FWG et al. 18th Annual Congress of EAHAD congress 2025; Pipe S ASH 2025 https://meetings-api.hematology.org/api/abstract/vmpreview/292697

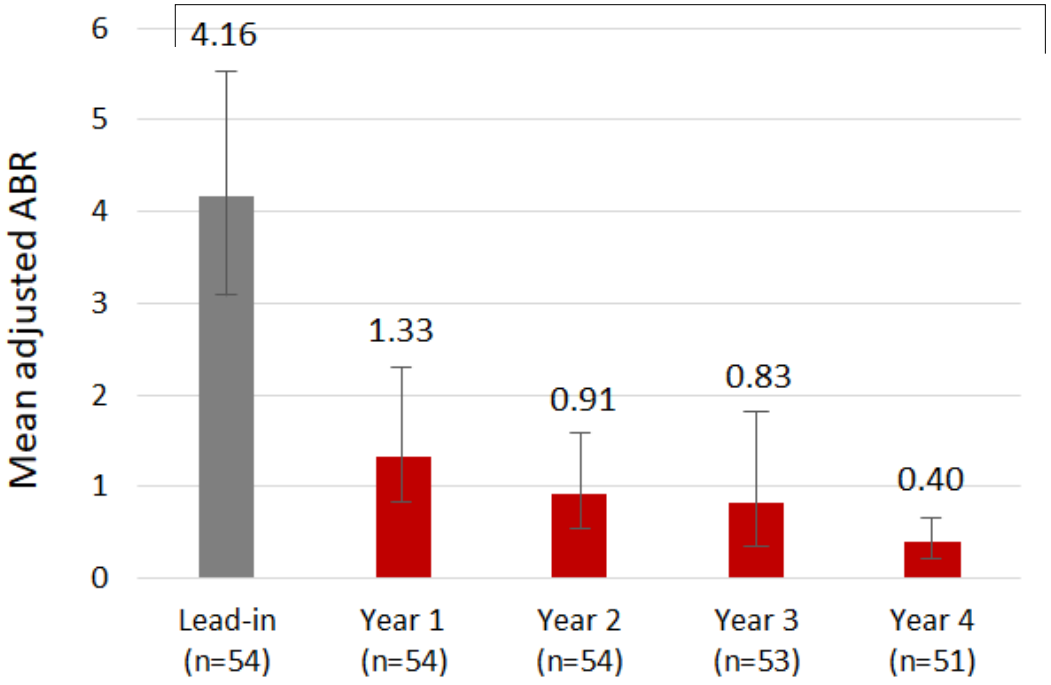
ABR and exogenous FIX use – Follow-up at year 4

- The mean **ABR** of **all bleeding** events show a reduction of **90%**
- The mean annualized **reduction in FIX consumption** rate was **96%**

46.3% (25/54) of participants received no FIX infusion over 4-year post gene therapy

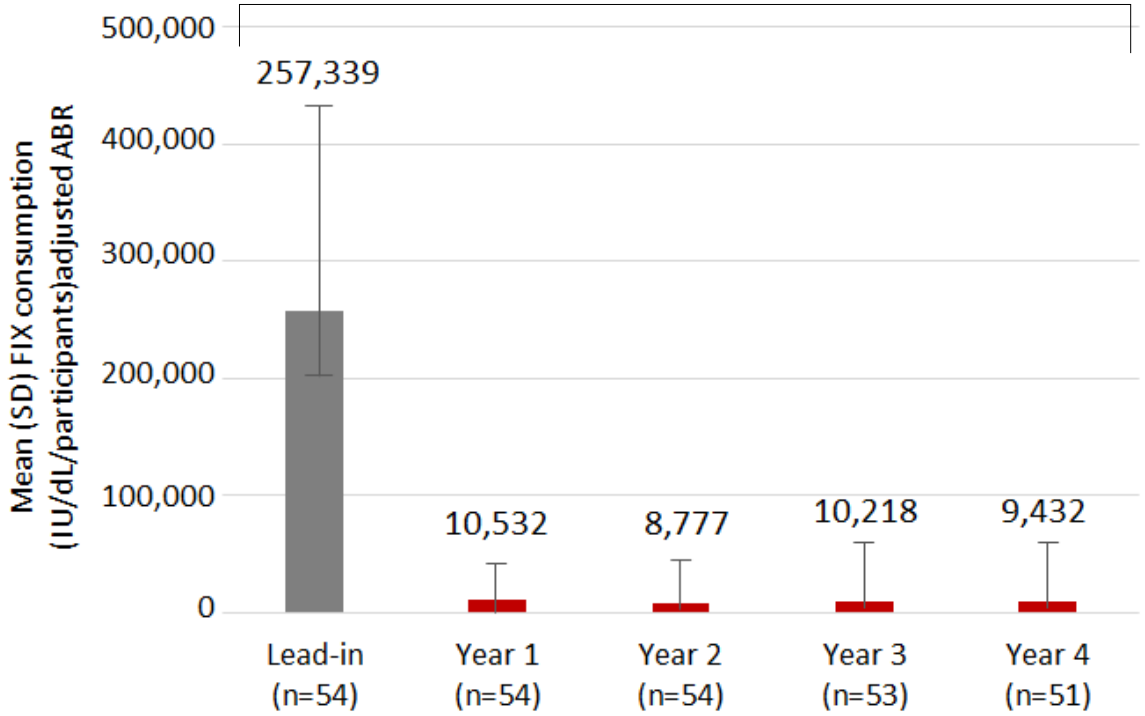
ABR (All bleeds)

90% reduction from baseline



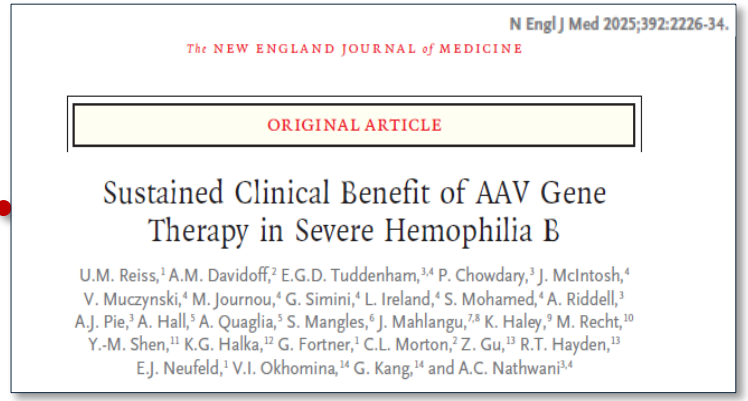
FIX consumption

96% reduction from baseline



Leebeek FWG et al. 18th Annual Congress of EAHAD congress 2025

Gene therapy in hemophilia B: 13-year follow-up

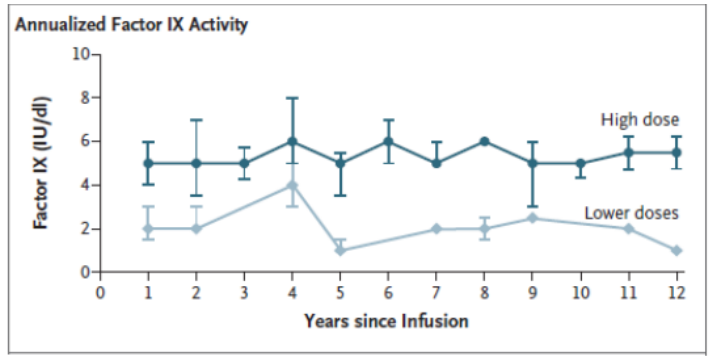

N Engl J Med 2025;392:2226-34.
The NEW ENGLAND JOURNAL of MEDICINE

ORIGINAL ARTICLE

Sustained Clinical Benefit of AAV Gene Therapy in Severe Hemophilia B

U.M. Reiss,[1] A.M. Davidoff,[2] E.G.D. Tuddenham,[3,4] P. Chowdary,[3] J. McIntosh,[4] V. Muczynski,[4] M. Journou,[4] G. Simini,[4] L. Ireland,[4] S. Mohamed,[4] A. Riddell,[3] A.J. Pie,[3] A. Hall,[5] A. Quaglia,[5] S. Mangles,[6] J. Mahlangu,[7,8] K. Haley,[9] M. Recht,[10] Y.-M. Shen,[11] K.G. Halka,[12] G. Fortner,[1] C.L. Morton,[2] Z. Gu,[13] R.T. Hayden,[13] E.J. Neufeld,[1] V.I. Okhomina,[14] G. Kang,[14] and A.C. Nathwani[3,4]

- **In 2014, patients with severe hemophilia B** received a single intravenous infusion of a serotype 8 pseudotyped **AAV vector** encoding a codon-optimized FIX transgene in **three different dose groups**
- **10 participants** were followed for a median of **13.0 years** (range, 11.1 to 13.8)
- **A single administration** of scAAV2/8-LP1-hFIXco gene therapy resulted in **durable FIX expression**



Dose cohort	Vector dose (vg/kg)	Median FIX activity		
		After 1 year	8 years	13 years
Low (n=2)	$2x10^{11}$	2%	1,9%	1,7%
Intermediate (n=2)	$6x10^{11}$	3-4%	2,3%	2,3%
High (n=6)	$2x10^{12}$	8-12%	5,1%	4,8%

Nathwani AC, et al. N Engl J Med 2011; 365:2357-65; Nathwani AC, et al. Blood 2018;132(suppl 1):491; Reiss UM et al. N Engl J Med 2025;392:2226-34

Safety Data

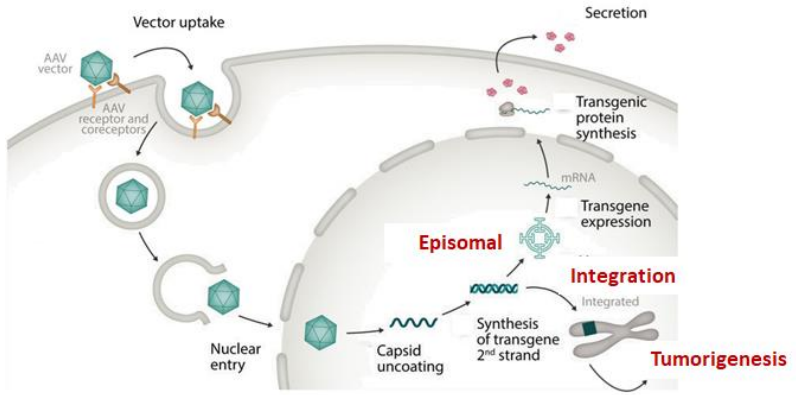
Liver-related complications

Short-term

- **Liver inflammation** is marker of immune response
- Elevation of liver enzymes – **ALT and AST**
- Prognostic of **loss of expression**

Long-term

- Risk of **hepatocellular cancer** due to **integration**



ALT: alanine aminotransferase; AST: aspartate aminotransferase;

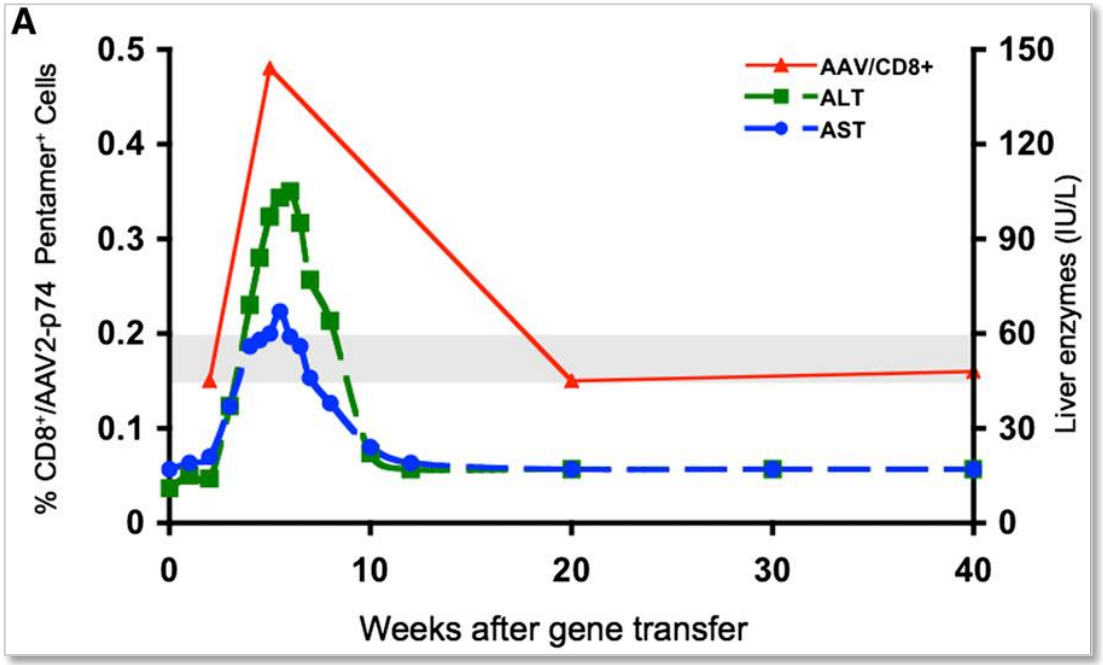
Miesbach W, et al. J Thromb Haemost. 2023;21(2):200-203; Madan B, et al. J Thromb Haemost. 2024;22(7):1880-1893; Pipe S, et al. N Engl J Med. 2023;388(8):706-718; Dhillon S. Drugs. 2024;84(4):479-486.

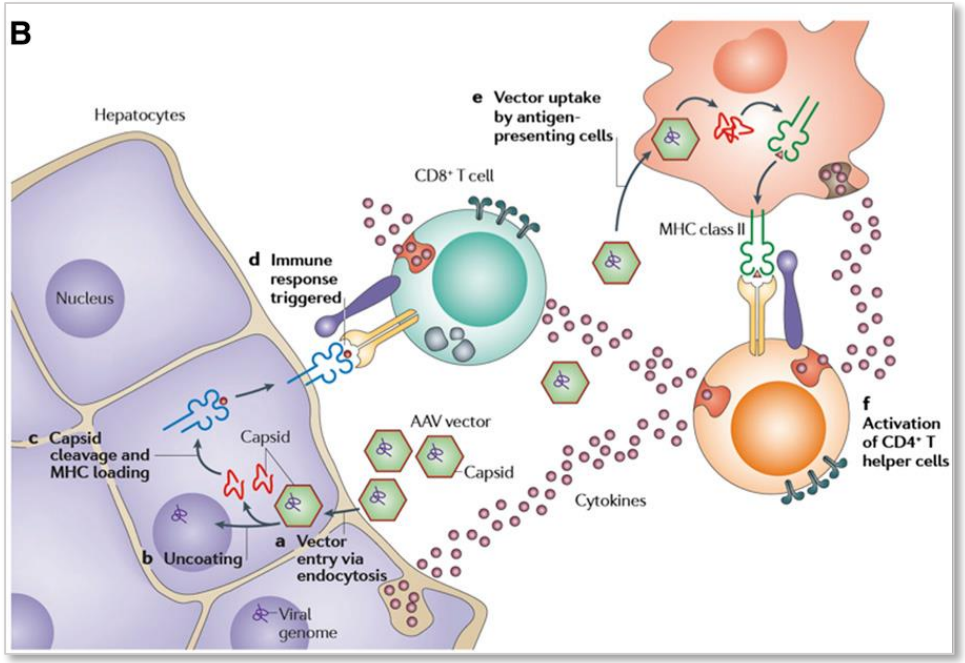
Short-term liver safety

- All clinical trials of gene therapy show an **early adverse events**
  - **Elevation of alanine aminotransferase** (ALT)
- Asymptomatic and transient
- May lead to reduced factor expression
- Treatable with **immunosuppressive agents**
  - The **duration** of corticosteroids and/or immunosuppressive **medication ranges from 6 weeks to 1 years**
  - Corticosteroid related adverse events were observed
    - The most frequent symptoms were acne, insomnia, Cushing syndrome and weight gain

Mahalangu J et al. N Engl J Med 2023;388:694-705; Pipe SW et al. N Engl J Med 2023;388:706-18; Cuker A, et al. N Engl J Med 2024;391:1108-18; Reiss UM et al. N Engl J Med 2025;392:2226-34

ALT elevation: why?





Activation of the innate and adaptative immune response (cytotoxic T cell response)

<u>Alternatives:</u> cellular stress (e.g., ER-stress) - direct injury from the vector

Courtesy of Prof. V. La Mura

ER: endoplasmatic reticulum

Manno CS et al. Nat Med. 2006;12:342-7; Mingozzi F et al. Blood 2013;122:23-36; Herzog RW et al. Mol Ther 2019; Fong S et al. Nat Med. 2022;28:789-814

Long-term liver safety

- **Malignancies** reported in **2 cases in hemophilia A** trials
- **Malignancies** reported in **4 cases in hemophilia B** trials

Hemophilia A	Hemophilia B
– Parotid carcinoma – B-cell acute lymphoblastic leukemia (B-ALL)	– Squamous cell carcinoma of the tonsil – Hepatocellular carcinoma – Lung adenocarcinoma – Prostate adenocarcinoma

In-depth molecular studies have shown that the development of various malignancies was not related to the gene therapy treatment

Approved Hemophilia gene therapy products

Hemophilia A

- **Valoctocogene roxaparvovec** (Roctavian)
  Available in US, Germany and Italy
- **Dalnacogene ponparvovec** (BBM-H901)
  Available in China

Hemophilia B

- **Etranacogene dezaparvovec** (Hemgenix)
  Available in US, EU, UK, Switzerland and Australia
- **Fidanacogene elaparvovec** (Beqvez)
  Withdrawn from the market

Gene Editing

- Gene editing can be used to correct small genetic defects or to insert therapeutic genes into predefined safe sites within the genome
  - In hemophilia, current gene-editing strategies under development rely on these safe genomic locations to integrate a functional copy of the F8 or F9 gene into cellular DNA
- Gene editing is still in clinical trials

Three Gene-Editing approaches in clinical development for Hemophilia

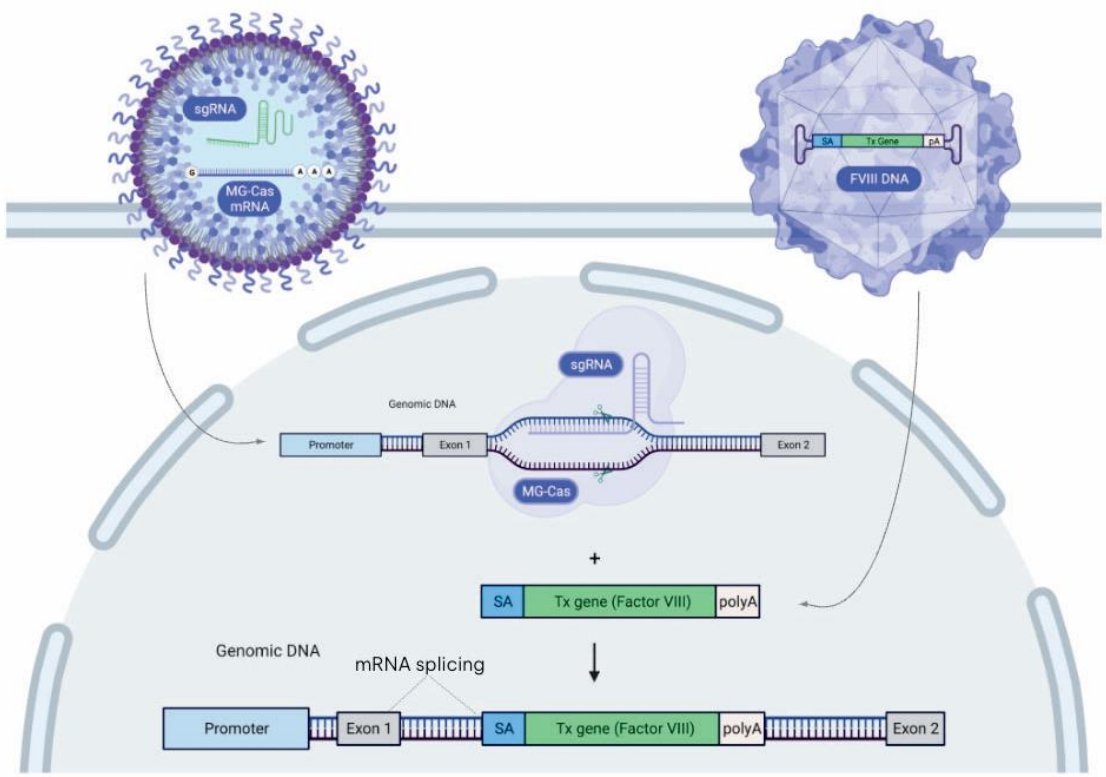
- **Hemophilia A**
  - **MGX-001** (Metagenomi)
    Preclinical studies
- **Hemophilia B**
  - **REGV131-LPN1265** (Regeneron)
    Phase 1/2 Clinical trial (NCT06379789)
  - **BE-101** (Be Biopharma)
    Clinical studies (NCT06611436)

Delivery via Dual infusions

- **Genome editing approach**
- Therapeutic gene delivered via AAV vectors
- Gene editing machinery deliverd via Lipid Nanoparticles (LPN)
  - **MGX-001** (Metagenomi)
  - **REGV131-LPN1265** (Regeneron)

LNP delivers nuclease mRNA and guide targeting albumin site

AAV delivers FVIII gene (donor DNA)



https://ir.metagenomi.co/static-files/81807947-be9c-4d3d-b5f7-0eeae86162b6

MGX-001 - Non-human primate (NHP) studies

- **MG nucleases** creates highly efficient cut at safe harbor locus **in albumin gene** (Metagenomi)
- **FVIII** donor DNA is **inserted at cut site**
- Strength of **albumin promoter provides high level of FVIII expression** even at low integration rates
- **Preclinical studies**
  - **Three NHPs** treated intravenously with **AAV8-cFVIII**-F2196K
  - After 5 weeks, **administration of liver-tropic LNPs** encapsulating **MG29-1 nuclease** mRNA and albumin-targeted gRNA
  - **Durable FVIII** activity demonstrated **out to 4.5 months**

Fig 1: All 3 animals maintain therapeutic levels of FVIII out to 4.5 months

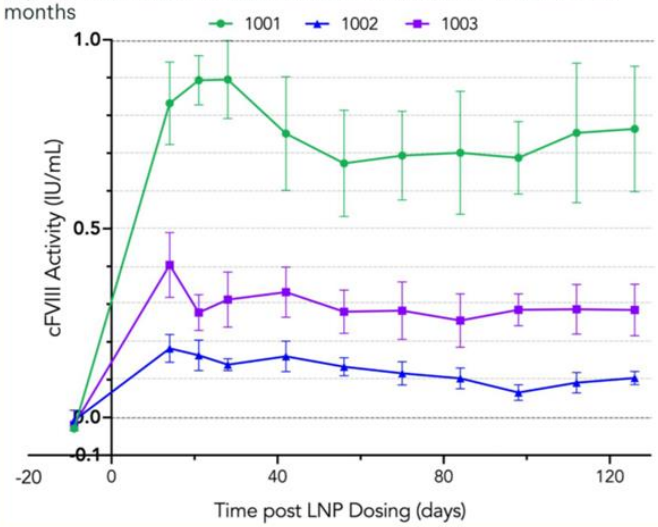


Table 1: Mean FVIII activity between 13-75% of normal is within the target therapeutic range of 10-150%

Animal ID	INDELS in liver (d7)	FVIII gene integration frequency # (copies per 100 genomes)	Mean FVIII activity % of normal (d14 to d126)
1001	45%	2.9%	75% +/- 9
1002	50%	0.7%	13% +/- 4
1003	55%	1.4%	29% +/- 5

INDELS and integration frequency measured in liver biopsy at day 7
Data-cut off at 4.5 months, study remains ongoing

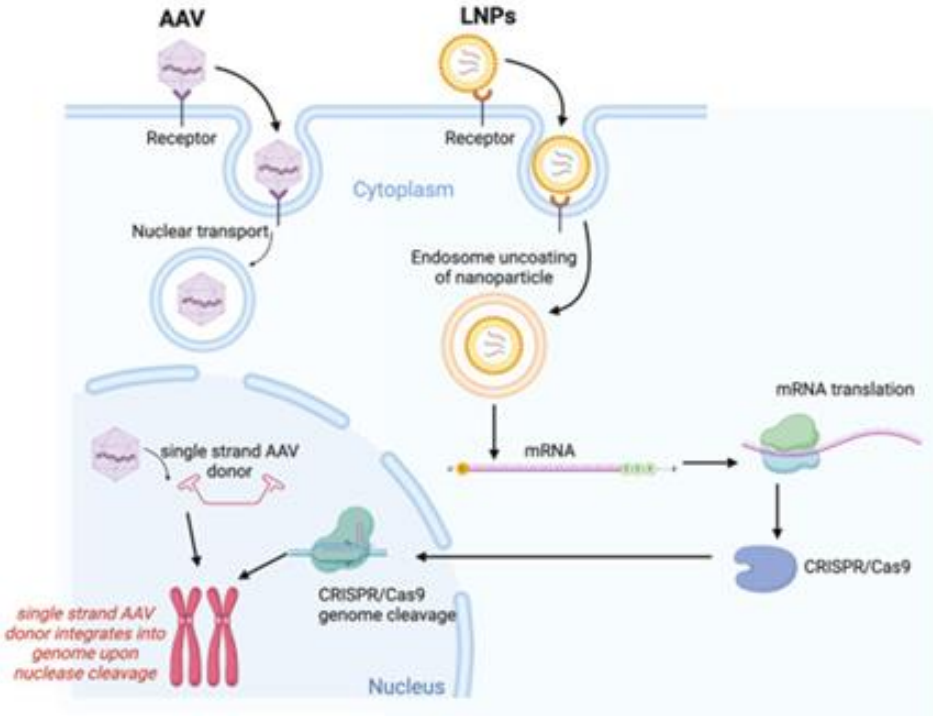
https://ir.metagenomi.co/node/6856/html

REGV131-LNP1265 – Phase 1/2 trial

- REGV131-LNP1265: A CRISPR/Cas9 based coagulation FIX Gene Therapy (Regeneron)
- FIX transgene targeted to the albumin locus in liver cells
- **Wild-type FIX** selected (instead of FIX-Padua) to **limit thrombotic risk** from high albumin-driven expression

- Stepwise in vivo delivery:
  - LNP carrying CRISPR gene-editing machinery
  - AAV8 vector delivering the F9 transgene

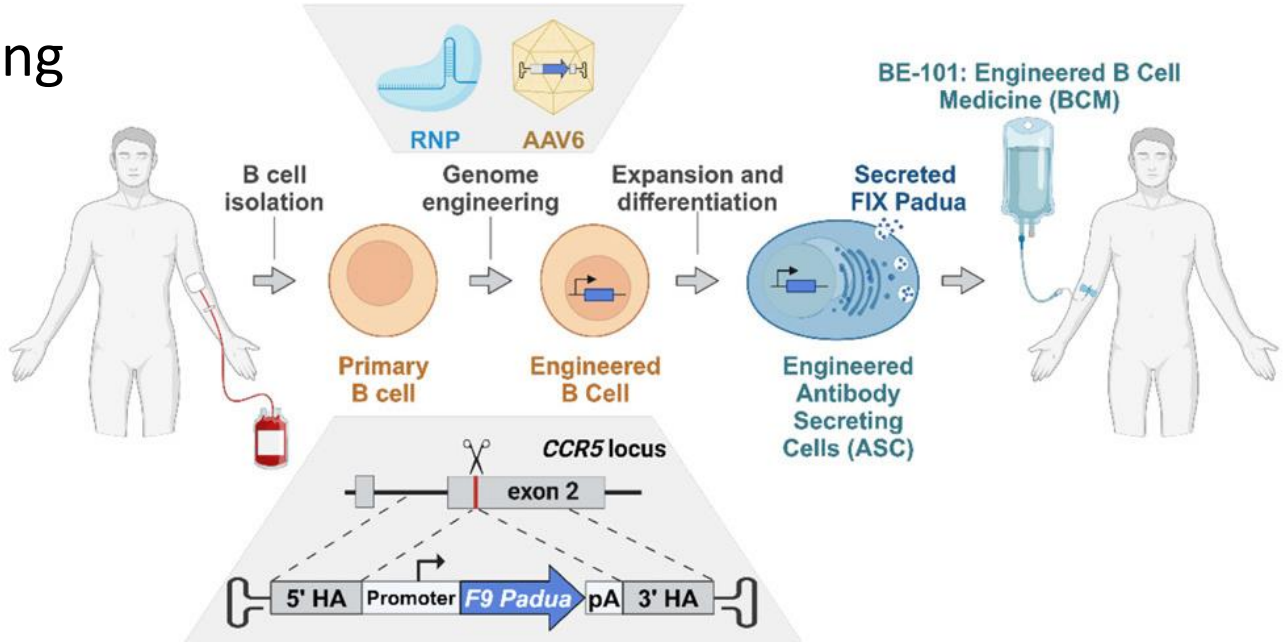
- A phase 1/2 dose escalation trial is ongoing



Herzog RW, et al. Mol Ther. 2025;33:2015-2034.

Ex-vivo gene editing with BE-101 (Be Biopharma) Phase 1/2 trial

- B-lineage cells genetically engineered *ex-vivo*
- CRISPR/Cas9 in combination with AAV-mediated homology-directed repair insert a human *F9* with Padua variant at CCR5 safe harbor locus
- Cells express and secrete FIX protein
- Phase 1/2 (BeCoMe-9 Study) is ongoing

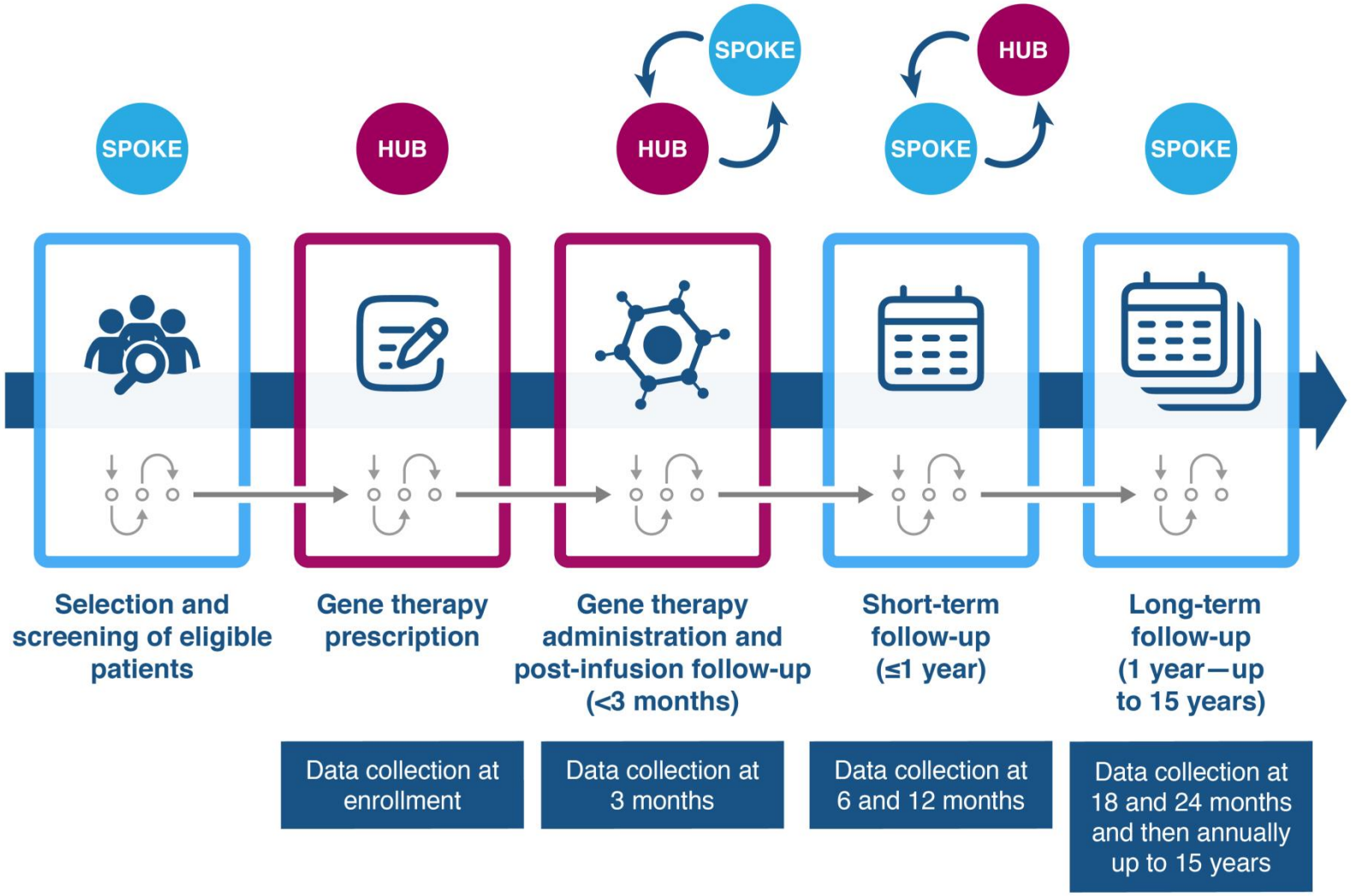


Liu H, et al. Mol Ther 2025; 33:S1525-0016(25)00729-4; Pipe SW, et al. Blood 2024;144: 25931–25932.

Experience at Angelo Bianchi Bonomi Hemophilia center in Milan

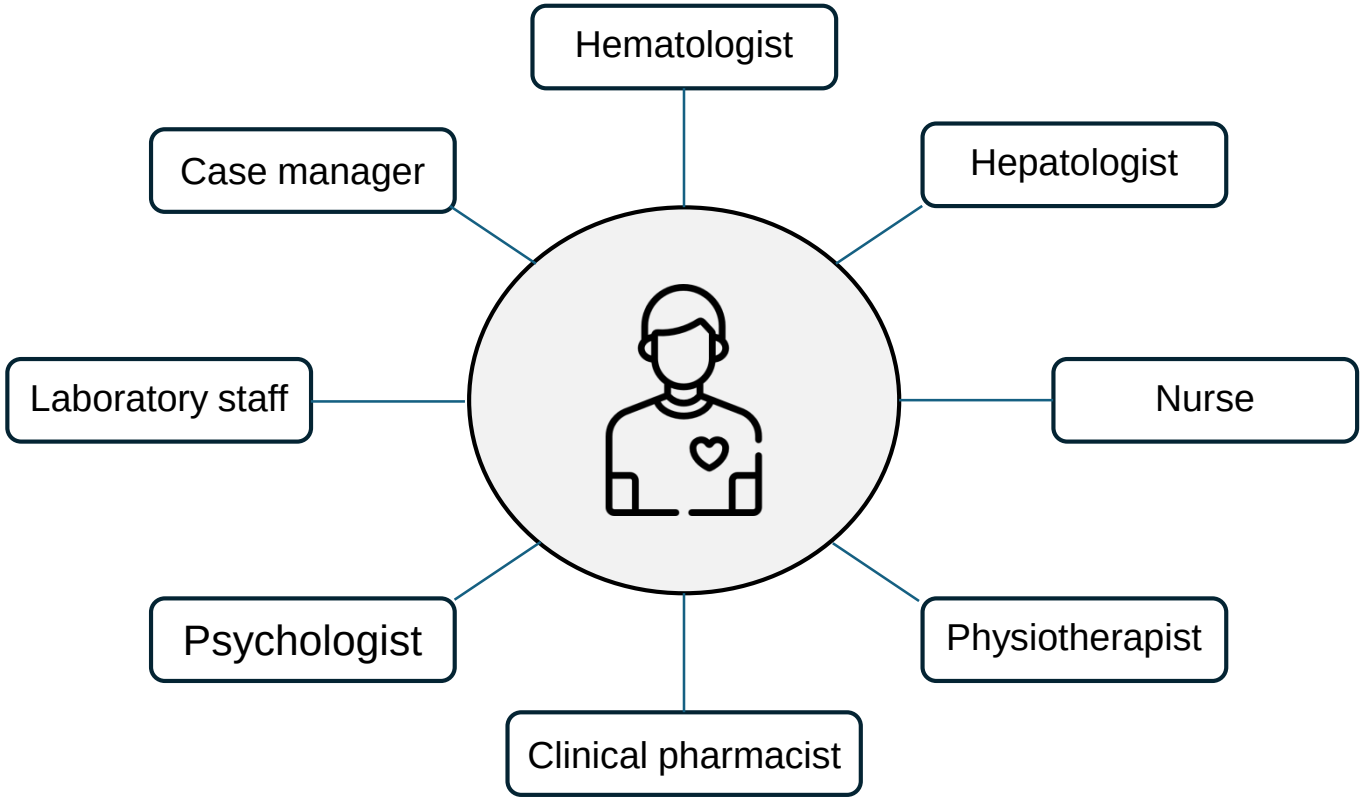
European Reference Network | Hematological Diseases (ERN EuroBloodNet)

The gene therapy journey



The importance of engaging a multidisciplinary team

- Multidisciplinary care model is a key condition for **ensuring maximum benefit** from gene therapy and **providing optimal levels of treatment and care**
- Success depends on **active communication and interdisciplinary data sharing**

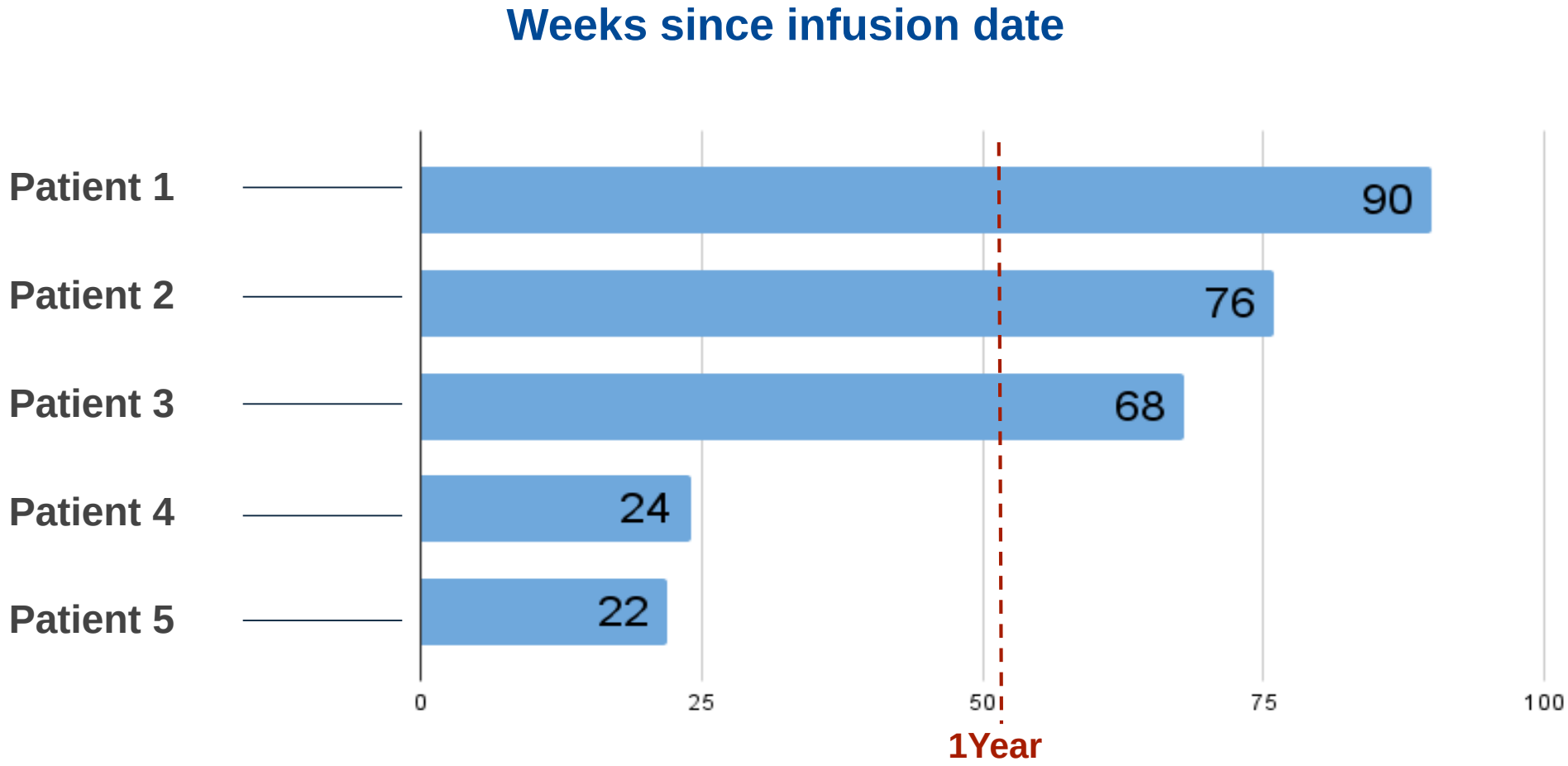


Miesbach W, et al. Haemophilia. 2022;28:e12-e14

Baseline characteristics of patients with **hemophilia A**

	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5
Age at infusion (yr)	24	38	48	38	46
Prophylaxis	Yes	Yes	Yes	Yes	Yes
Treatment type	EHL FVIII	EHL FVIII	Emicizumab	EHLFVIII	EHL FVIII

Patients treated with **Valoctocogene roxaparvovec** Experience to date



Post-Gene Therapy Infusion Outcomes

EFFICACY

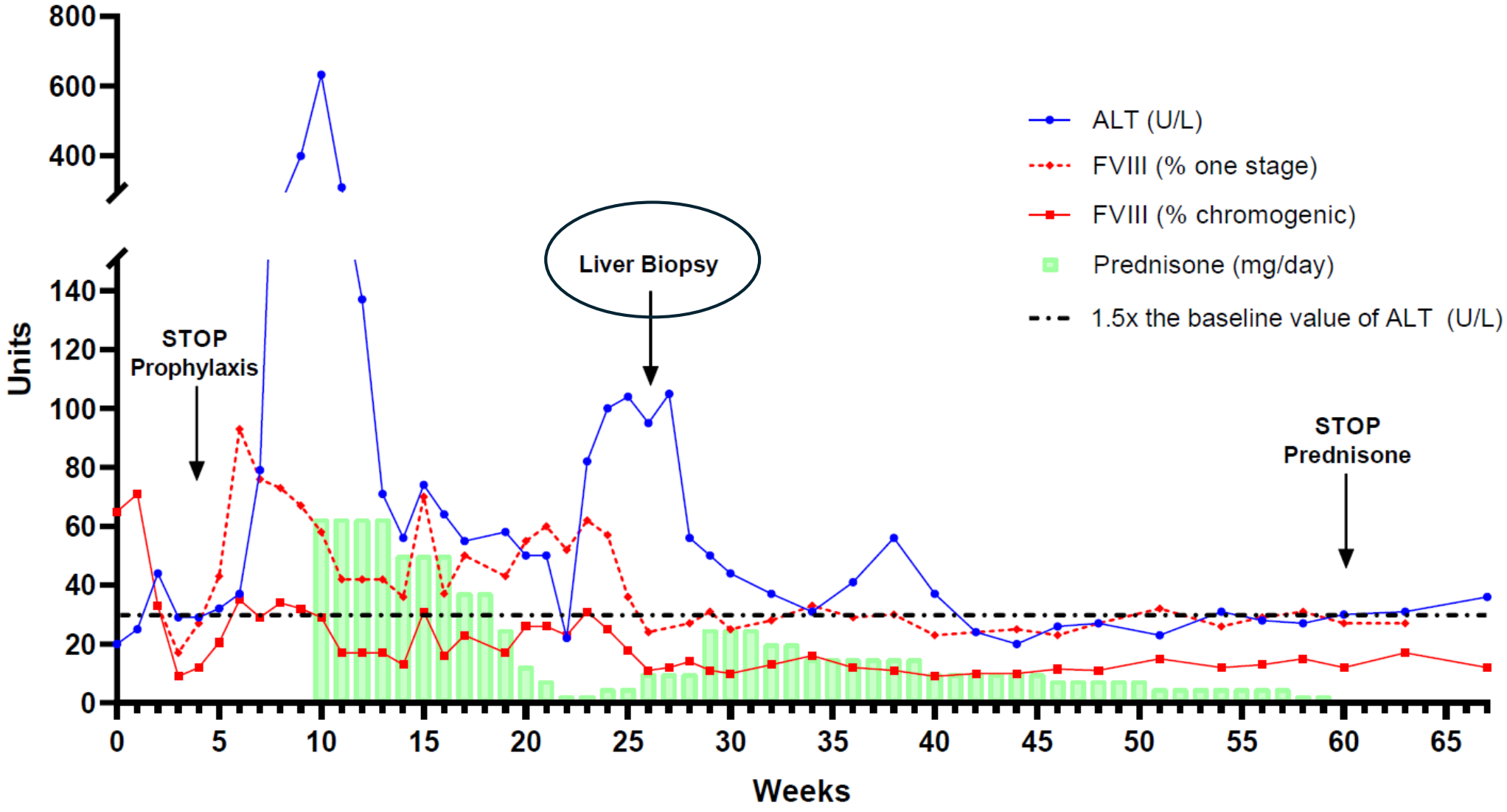
- **FVIII transgene expression:**
  - Detected 3 weeks after infusion
- **High variability in FVIII activity levels:**
  - At Week 26, ranged from 11% to 234% (chromogenic assay)
- **Prophylaxis Discontinuation:**
  - Stopped within 3–4 weeks after infusion

Post-Gene Therapy Infusion Outcomes

SAFETY

- No allergic reactions
- **Elevated liver enzymes reported in two patients**
  - Treated with corticosteroids (one patient for one year, second patient treatment ongoing)
- FVIII levels ≥150 IU/dL observed in two patients
  - **No thrombotic events**
  - No anticoagulant therapy
- No malignancies

Management of elevated transaminases with steroid therapy

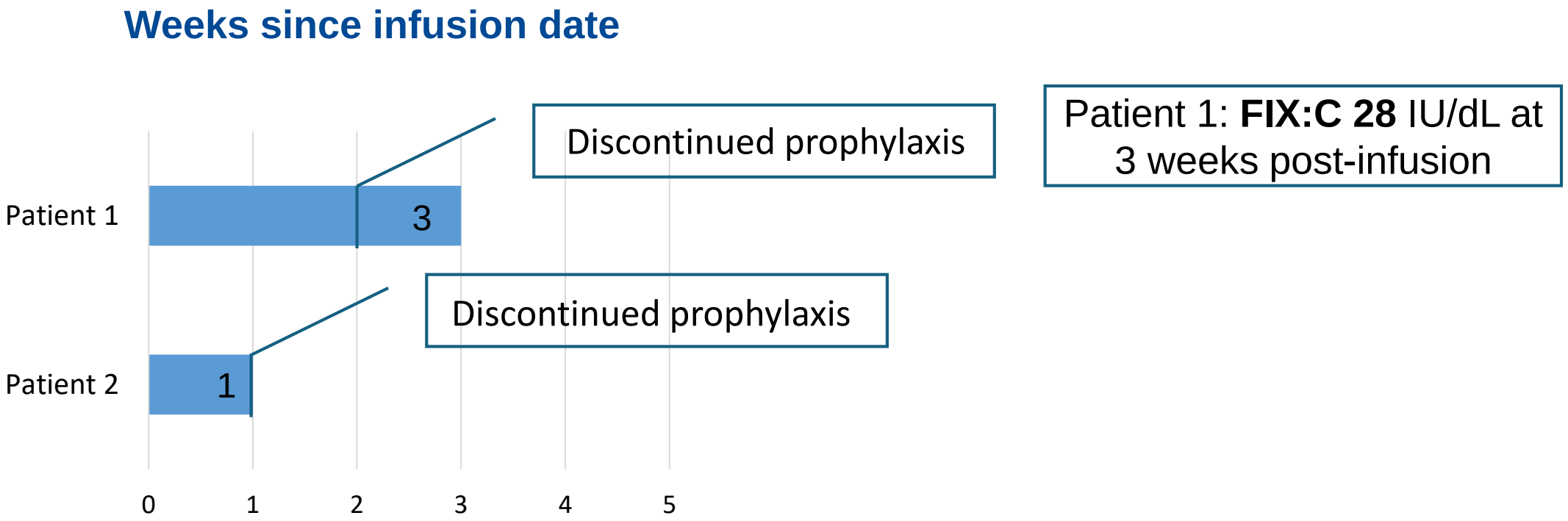


La Mura, Peyvandi under review

Baseline characteristics of patients with **hemophilia B**

	Patient 1	Patient 2
Age at infusion (yr)	64	47
Prophylaxis	Yes	Yes
Treatment type	EHL FIX	EHL FIX

Patients treated with **Etranacogene dezaparvovec** Experience to date



Conclusion

- Gene therapy in Hemophilia enables long term clotting factor producion
- Reduces bleeding and need for replacement therapy
- Ongonging work could improve safety and durability