Immune Suppression following Gene Therapy in Hemophilia

Wolfgang Miesbach, MD, PhD

University Hospital of Frankfurt, Frankfurt, Germany

Presented at the World Federation of Hemophilia (WFH) Congress 2022, May 8–11, Montréal and Virtual

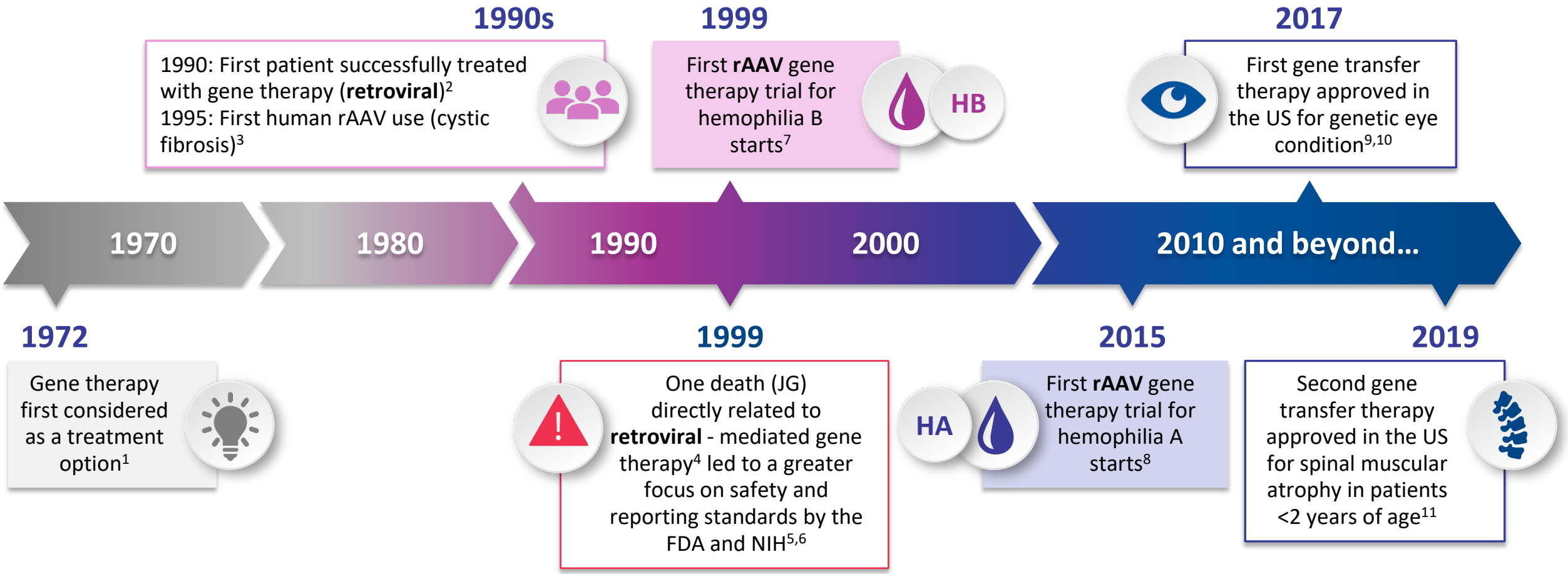
WFH 2022 WORLD CONGRESS

Disclosures for Wolfgang Miesbach

Conflict	Disclosure - if conflict of interest exists
Research Support	Bayer, BioMarin, Biotest, CSL Behring, Chugai, Freeline, LFB, Novo Nordisk, Octapharma, Pfizer, Roche, Sanofi, Takeda/Shire, and uniQure
Director, Officer, Employee	
Shareholder	
Honoraria	
Advisory Committee	Bayer, BioMarin, Biotest, CSL Behring, Chugai, Freeline, LFB, Novo Nordisk, Octapharma, Pfizer, Roche, Sanofi, Takeda/Shire, and uniQure
Consultant	

WFH
WORLD FEDERATION OF HEMOPHILIA
FÉDÉRATION MONDIALE DE L'HÉMOPHILIE
FEDERACIÓN MUNDIAL DE HEMOFILIA

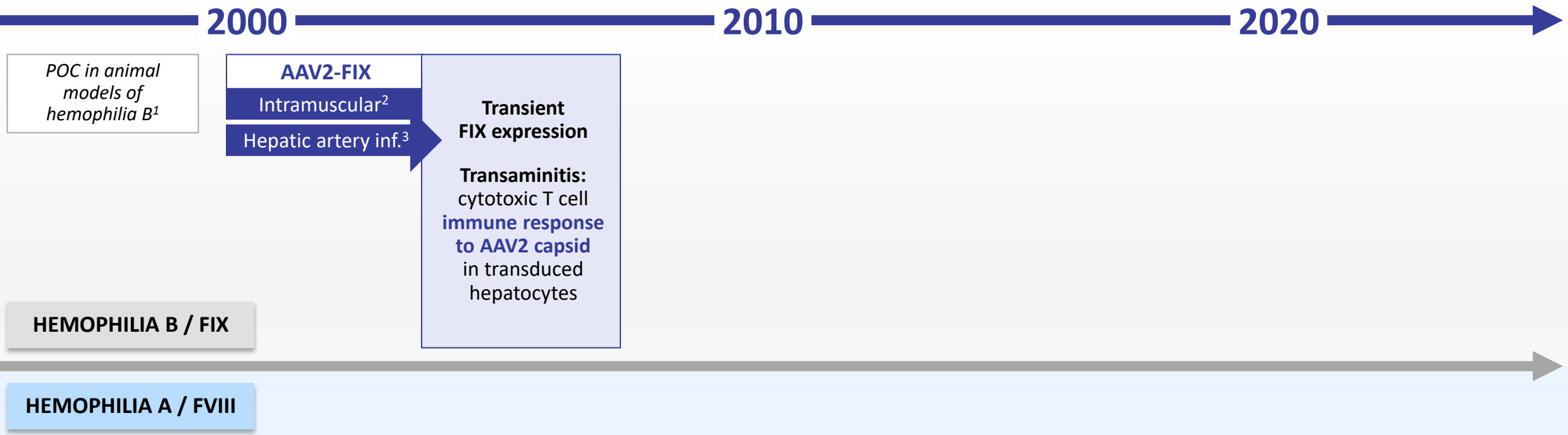
Brief history of gene therapy research



FDA, US Food and Drugs Administration;
HA, hemophilia A; HB, hemophilia B;
JG, Jesse Gelsinger;
NIH, National Institute of Health;
rAAV, recombinant adeno-associated virus

1. Friedmann T, et al. Science 1972;175:949-55; 2. Blaese RM, et al. Science 1995;270:475–80; 3. Wang D, et al. Nat Rev Drug Discov 2019;18:358–78; 4. Sibbald B. CMAJ 2001;164:1612; 5. Cotrim AP, Baum BJ. Toxicol Pathol 2008;36:97-103; 6. Collins FS, Gottlieb S. N Engl J Med 2018;379:1393-95; 7. Hough C, Lillicrap D. J Thromb Haemost 2005;3:1195-1205; 8. Biomarin Pharmaceutical. Accessed July 22, 2019. https://www.biomarin.com/about/history/#1997; 9. Luxturna Prescribing Information. Spark Therapeutics, Inc. 2017; 10. FDA. Accessed January 8, 2020. https://www.fda.gov/news-events/press-announcements/fda-approves-novel-gene-therapy-treat-patients-rare-form-inherited-vision-loss; 11. Zolgensma Prescribing Information. AveXis, Inc. 2019

AAV gene therapy development for hemophilia



Schematic adapted from Dunbar CE, et al.[16]
AAV, adeno-associated viral vector; FVIII, factor VIII; FIX, factor IX; kb, kilobases; POC, proof of concept; pts, participants; SAE< serious adverse event

1. Nathwani AC, et al. Blood 2001;97:1258-65; 2. Manno CS, et al. Blood 2003;101: 2963-72; 3. Manno CS, et al. Nat Med 2006;12:342-47; 4. Nathwani AC, et al. N Engl J Med 2011; 365:2357-65; 5. Nathwani AC, et al. N Engl J Med 2014; 371:1994-2004; 6. Miesbach W, et al. Blood 2018;131:1022-31; 7. Von Drygalski A, et al. Blood Adv 2019;3:3241-47; 8. George LA, et al. N Engl J Med 2017;377:2215-27; 9. Nathwani AC, Tuddenham EGD. Br J Haematol 2020;191:573-78; 10. Rangarajan S, et al. N Engl J Med 2017;377:2519-30; 11. Pasi KJ, et al. Haemophilia 2021;27:947-56; 12. Ozelo MC, et al. N Engl J Med 2022;386:1013-25; 13. Visweshwar N, et al. Blood 2021;138:564–66; 14. https://wfh.org/fda-places-the-pfizer-sangamo-therapeutics-phase-3-affine-haemophilia-a-gene-therapy-study-on-clinical-hold/; 15. George LA, et al. N Engl J Med 2021;385:1961-73; 16. Dunbar CE, et al. Science 2018;359(6372):eaan4672

Managing transaminitis and cellular immune responses

- Immune-mediated injury is common in liver diseases
- A feature of many viral infections, autoimmunity and transplant rejection

The immune response to AAV vectors has characteristics of an **autoimmune hepatitis**

Classic profile:

- Raised AST/ALT
- Normal bilirubin, ALP
- Normal albumin
- Raised IgG
- F-actin positive

Considerations for management (**immunosuppression**):

- Strategy: **high-dose prednisolone** to control immune reaction but reduce quickly to minimize toxicities
  - Prednisolone 60 mg once daily initially, followed by tapering
  - PPI once daily (to protect GI tract)
  - Calcium and vitamin D (to avoid loss of bone density)
  - Consider second-line **steroid-sparing agents** if longer treatment is required

ALP, alkaline phosphatase; ALT, Alanine aminotransferase; AST, aspartate aminotransferase; FIX, factor IX; PPI, proton pump inhibitor

Czaja AJ, Freese DK. Hepatology 2002, 36:479–497. Luxon BA. Curr Gastroenterol Rep. 2006;8(1):83-88.

Managing transaminitis and cellular immune responses

- Immune-mediated injury is common in liver diseases
- A feature of many viral infections, autoimmunity and transplant rejection

The immune response to AAV vectors has characteristics of an **autoimmune hepatitis**

Classic profile:

- Raised AST/ALT
- Normal bilirubin, ALP
- Normal albumin
- Raised IgG
- F-actin positive

Considerations for management (**immunosuppression**):

- Strategy: **high-dose prednisolone** to control immune reaction but reduce quickly to minimize toxicities
  - Prednisolone
  - PPI once daily
  - Calcium and v
  - Consider seco is required

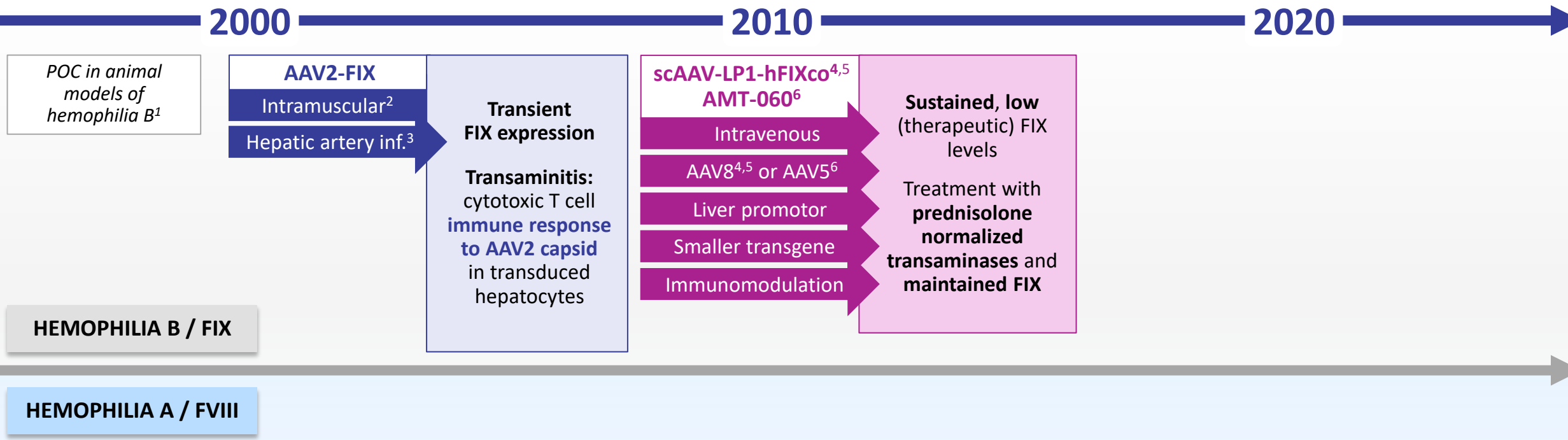
Alternative/second-line agents:

- Budesonide – better tolerated but requires metabolism in the liver to its active form
- Mycophenolate (caution: teratogenic)
- Calcineurin inhibitors (tacrolimus)

ALP, alkaline phosphatase; ALT, Alanine aminotransferase; AST, aspartate aminotransferase; FIX, factor IX; PPI, proton pump inhibitor

Czaja AJ, Freese DK. Hepatology 2002, 36:479–497. Luxon BA. Curr Gastroenterol Rep. 2006;8(1):83-88.

AAV gene therapy development for hemophilia



Schematic adapted from Dunbar CE, et al.[16]
AAV, adeno-associated viral vector; FVIII, factor VIII; FIX, factor IX; kb, kilobases; POC, proof of concept; pts, participants; SAE< serious adverse event

1. Nathwani AC, et al. Blood 2001;97:1258-65; 2. Manno CS, et al. Blood 2003;101: 2963-72; 3. Manno CS, et al. Nat Med 2006;12:342-47; 4. Nathwani AC, et al. N Engl J Med 2011; 365:2357-65; 5. Nathwani AC, et al. N Engl J Med 2014; 371:1994-2004; 6. Miesbach W, et al. Blood 2018;131:1022-31; 7. Von Drygalski A, et al. Blood Adv 2019;3:3241-47; 8. George LA, et al. N Engl J Med 2017;377:2215-27; 9. Nathwani AC, Tuddenham EGD. Br J Haematol 2020;191:573-78; 10. Rangarajan S, et al. N Engl J Med 2017;377:2519-30; 11. Pasi KJ, et al. Haemophilia 2021;27:947-56; 12. Ozelo MC, et al. N Engl J Med 2022;386:1013-25; 13. Visweshwar N, et al. Blood 2021;138:564–66; 14. https://wfh.org/fda-places-the-pfizer-sangamo-therapeutics-phase-3-affine-haemophilia-a-gene-therapy-study-on-clinical-hold/; 15. George LA, et al. N Engl J Med 2021;385:1961-73; 16. Dunbar CE, et al. Science 2018;359(6372):eaan4672

scAAV2/8-LP1-hFIXco: codon-optimized FIX in AAV8-pseudotyped capsid

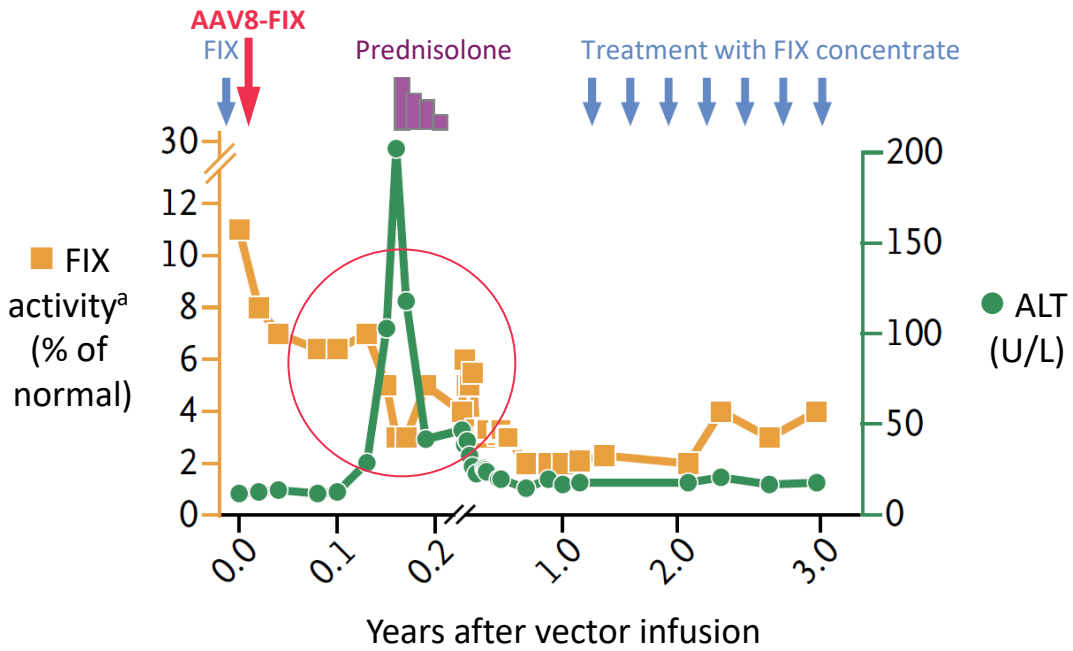
Liver-directed AAV8 vector (**scAAV2/8-LP1-hFIXco**) developed to improve transduction efficiency

10 patients with severe hemophilia B and absence of pre-existing antibodies against AAV8 capsid

3 doses: 2E11 vg/kg, 6E11 vg/kg, or 2E12 vg/kg

- Transient increases in ALT occurred in 4 of 10 participants (4 of 6 high-dose participants)
- Prednisolone initiated at 60 mg/day followed by gradual dose tapering
- FIX levels declined by 50 to 70% compared with values before the onset of the elevated ALT
  - …especially if prednisolone started >2 days after ALT increase
- No patient had a recurrent episode of elevated ALT

Participant 5 (high dose, 2E12 vg/kg)



"Prednisolone was effective in limiting the hepatocellular toxicity as well as preserving the expression of transgenic factor IX, especially when such treatment was initiated early"

[a]FIX coagulation activity determined with the use of a one-stage clotting assay

ALT, Alanine aminotransferase; FIX, factor IX; P, prednisolone; vg, vector genomes

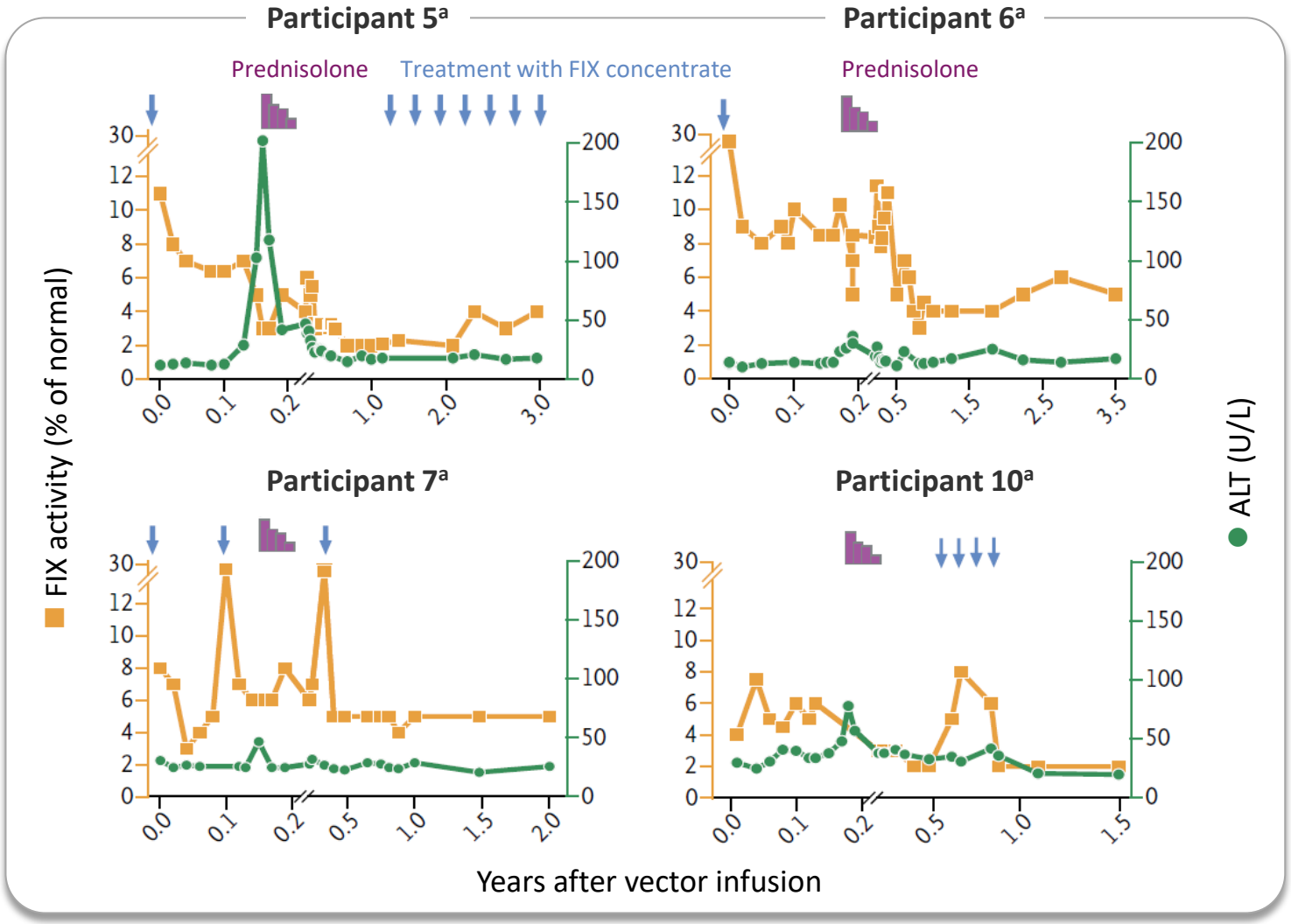
Nathwani AC, et al. N Engl J Med 2014;371:1994-2004

scAAV2/8-LP1-hFIXco: codon-optimized FIX in AAV8-pseudotyped capsid

- However, **associations between ALT elevation, FIX decline, and prednisolone treatment varied** among participants

- Despite early episodes of ALT elevations in some participants, there was long-term endogenous FIX expression at therapeutic steady-state levels of 1–6%[1]

- Latest follow-up[2]:
  - all 10 participants have stable transgenic FIX activity through up to 10 years
  - significant reductions in annual FIX concentrate usage and frequency of spontaneous bleeding

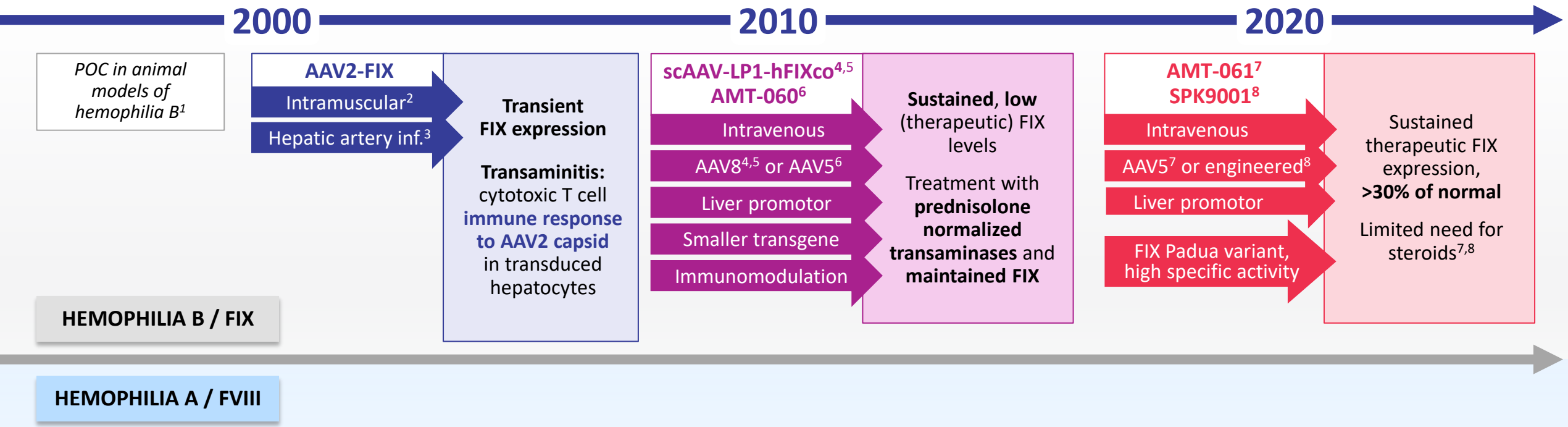


[a]These participants received the high dose, 2E12 vg/kg

ALT, Alanine aminotransferase; FIX, factor IX

1. Nathwani AC, et al. N Engl J Med 2014;371:1994-2004;
2. Nathwani AC, Tuddenham EGD. Br J Haematol 2020;191:573-78

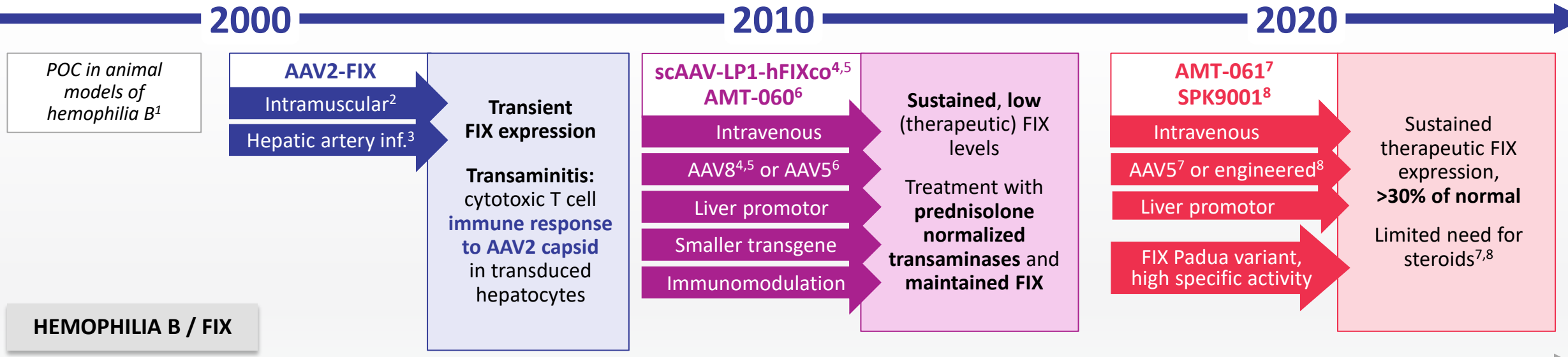
AAV gene therapy development for hemophilia



Schematic adapted from Dunbar CE, et al.[16]
AAV, adeno-associated viral vector; FVIII, factor VIII; FIX, factor IX; kb, kilobases; POC, proof of concept; pts, participants; SAE< serious adverse event

1. Nathwani AC, et al. Blood 2001;97:1258-65; 2. Manno CS, et al. Blood 2003;101: 2963-72; 3. Manno CS, et al. Nat Med 2006;12:342-47; 4. Nathwani AC, et al. N Engl J Med 2011; 365:2357-65; 5. Nathwani AC, et al. N Engl J Med 2014; 371:1994-2004; 6. Miesbach W, et al. Blood 2018;131:1022-31; 7. Von Drygalski A, et al. Blood Adv 2019;3:3241-47; 8. George LA, et al. N Engl J Med 2017;377:2215-27; 9. Nathwani AC, Tuddenham EGD. Br J Haematol 2020;191:573-78; 10. Rangarajan S, et al. N Engl J Med 2017;377:2519-30; 11. Pasi KJ, et al. Haemophilia 2021;27:947-56; 12. Ozelo MC, et al. N Engl J Med 2022;386:1013-25; 13. Visweshwar N, et al. Blood 2021;138:564–66; 14. https://wfh.org/fda-places-the-pfizer-sangamo-therapeutics-phase-3-affine-haemophilia-a-gene-therapy-study-on-clinical-hold/; 15. George LA, et al. N Engl J Med 2021;385:1961-73; 16. Dunbar CE, et al. Science 2018;359(6372):eaan4672

AAV gene therapy development for hemophilia



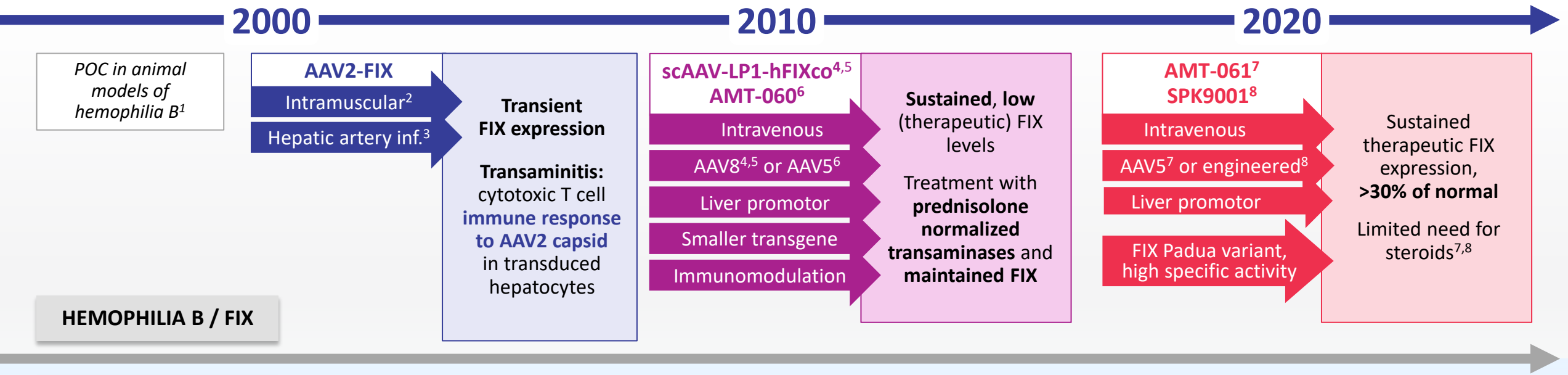
Hemophilia A has been more challenging

- *FVIII protein is inefficiently synthesised in hepatocytes*
- *FVIII cDNA is too large (7 kb) to be packaged into AAV (maximum packaging capacity 5 kb)*[9]

Schematic adapted from Dunbar CE, et al.[16]
AAV, adeno-associated viral vector; FVIII, factor VIII; FIX, factor IX; kb, kilobases; POC, proof of concept; pts, participants; SAE< serious adverse event

1. Nathwani AC, et al. Blood 2001;97:1258-65; 2. Manno CS, et al. Blood 2003;101: 2963-72; 3. Manno CS, et al. Nat Med 2006;12:342-47; 4. Nathwani AC, et al. N Engl J Med 2011; 365:2357-65; 5. Nathwani AC, et al. N Engl J Med 2014; 371:1994-2004; 6. Miesbach W, et al. Blood 2018;131:1022-31; 7. Von Drygalski A, et al. Blood Adv 2019;3:3241-47; 8. George LA, et al. N Engl J Med 2017;377:2215-27; 9. Nathwani AC, Tuddenham EGD. Br J Haematol 2020;191:573-78; 10. Rangarajan S, et al. N Engl J Med 2017;377:2519-30; 11. Pasi KJ, et al. Haemophilia 2021;27:947-56; 12. Ozelo MC, et al. N Engl J Med 2022;386:1013-25; 13. Visweshwar N, et al. Blood 2021;138:564–66; 14. https://wfh.org/fda-places-the-pfizer-sangamo-therapeutics-phase-3-affine-haemophilia-a-gene-therapy-study-on-clinical-hold/; 15. George LA, et al. N Engl J Med 2021;385:1961-73; 16. Dunbar CE, et al. Science 2018;359(6372):eaan4672

AAV gene therapy development for hemophilia



Hemophilia A has been more challenging

- *FVIII protein is inefficiently synthesised in hepatocytes*
- *FVIII cDNA is too large (7 kb) to be packaged into AAV (maximum packaging capacity 5 kb)*[9]

Schematic adapted from Dunbar CE, et al.[16]
AAV, adeno-associated viral vector; FVIII, factor VIII; FIX, factor IX; kb, kilobases; POC, proof of concept; pts, participants; SAE< serious adverse event

1. Nathwani AC, et al. Blood 2001;97:1258-65; 2. Manno CS, et al. Blood 2003;101: 2963-72; 3. Manno CS, et al. Nat Med 2006;12:342-47; 4. Nathwani AC, et al. N Engl J Med 2011; 365:2357-65; 5. Nathwani AC, et al. N Engl J Med 2014; 371:1994-2004; 6. Miesbach W, et al. Blood 2018;131:1022-31; 7. Von Drygalski A, et al. Blood Adv 2019;3:3241-47; 8. George LA, et al. N Engl J Med 2017;377:2215-27; 9. Nathwani AC, Tuddenham EGD. Br J Haematol 2020;191:573-78; 10. Rangarajan S, et al. N Engl J Med 2017;377:2519-30; 11. Pasi KJ, et al. Haemophilia 2021;27:947-56; 12. Ozelo MC, et al. N Engl J Med 2022;386:1013-25; 13. Visweshwar N, et al. Blood 2021;138:564–66; 14. https://wfh.org/fda-places-the-pfizer-sangamo-therapeutics-phase-3-affine-haemophilia-a-gene-therapy-study-on-clinical-hold/; 15. George LA, et al. N Engl J Med 2021;385:1961-73; 16. Dunbar CE, et al. Science 2018;359(6372):eaan4672

AAV gene therapy development for hemophilia

2000 → 2010 → 2020

POC in animal models of hemophilia B[1]

HEMOPHILIA B / FIX

AAV2-FIX
- Intramuscular[2]
- Hepatic artery inf.[3]

→ **Transient FIX expression**

Transaminitis: cytotoxic T cell **immune response to AAV2 capsid** in transduced hepatocytes

scAAV-LP1-hFIXco[4,5] **AMT-060**[6]
- Intravenous
- AAV8[4,5] or AAV5[6]
- Liver promotor
- Smaller transgene
- Immunomodulation

→ **Sustained, low** (therapeutic) FIX levels

Treatment with **prednisolone normalized transaminases** and **maintained FIX**

AMT-061[7] **SPK9001**[8]
- Intravenous
- AAV5[7] or engineered[8]
- Liver promotor
- FIX Padua variant, high specific activity

→ Sustained therapeutic FIX expression, **>30% of normal**

Limited need for steroids[7,8]

HEMOPHILIA A / FVIII

Hemophilia A has been more challenging
- *FVIII protein is inefficiently synthesised in hepatocytes*
- *FVIII cDNA is too large (7 kb) to be packaged into AAV (maximum packaging capacity 5 kb)*[9]

- B-domain deleted FVIII
- Liver-specific promoter
- Different serotypes & doses

valoctocogene roxaparvovec[10]	Sustained therapeutic FVIII, >15%; steroids used **5 years' follow-up to date in Phase 1/2**[11] **(N=13)** **2-3 years in Phase 3 (N=134)**[12]
giroctocogene fitelparvovec[13]	Steroids used; **high FVIII levels** in Phase 3 **led to clinical hold** due to concern about thrombosis risk[14]
SPK-8011[15]	Phase 2 (N=18), steroids used; 2 lost FVIII due to immune response **despite steroids**[15]

Schematic adapted from Dunbar CE, et al.[16]
AAV, adeno-associated viral vector; FVIII, factor VIII; FIX, factor IX; kb, kilobases; POC, proof of concept; pts, participants; SAE< serious adverse event

1. Nathwani AC, et al. Blood 2001;97:1258-65; 2. Manno CS, et al. Blood 2003;101: 2963-72; 3. Manno CS, et al. Nat Med 2006;12:342-47; 4. Nathwani AC, et al. N Engl J Med 2011; 365:2357-65; 5. Nathwani AC, et al. N Engl J Med 2014; 371:1994-2004; 6. Miesbach W, et al. Blood 2018;131:1022-31; 7. Von Drygalski A, et al. Blood Adv 2019;3:3241-47; 8. George LA, et al. N Engl J Med 2017;377:2215-27; 9. Nathwani AC, Tuddenham EGD. Br J Haematol 2020;191:573-78; 10. Rangarajan S, et al. N Engl J Med 2017;377:2519-30; 11. Pasi KJ, et al. Haemophilia 2021;27:947-56; 12. Ozelo MC, et al. N Engl J Med 2022;386:1013-25; 13. Visweshwar N, et al. Blood 2021;138:564–66; 14. https://wfh.org/fda-places-the-pfizer-sangamo-therapeutics-phase-3-affine-haemophilia-a-gene-therapy-study-on-clinical-hold/; 15. George LA, et al. N Engl J Med 2021;385:1961-73; 16. Dunbar CE, et al. Science 2018;359(6372):eaan4672

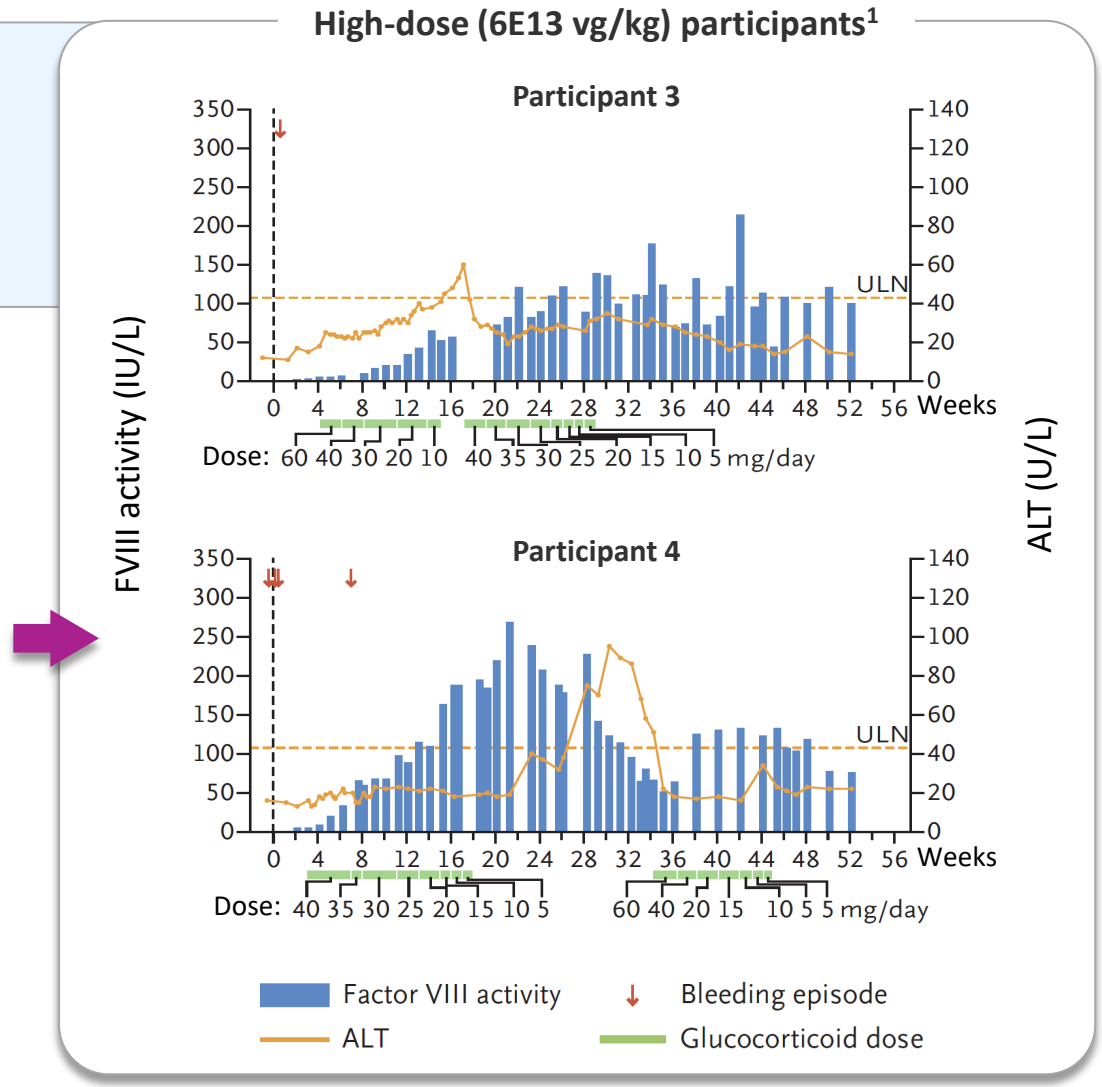
Valoctocogene roxaparvovec: B-domain deleted FVIII in AAV5 capsid

To address the size constraints and inefficient FVIII expression from native *FVIII* gene, **AAV5-hFVIII-SQ** is designed with a reduced size (**B-domain-deleted**) FVIII expression cassette, liver-specific promoter and AAV5 vector

15 men with severe hemophilia A and no anti-AAV5 antibodies;

4 doses from 6E12 vg/kg to 6E13 vg/kg[1]

- Participants in the high-dose cohort received prophylactic prednisolone 40 mg/day, tapering from week 3 to week 17 or longer
- 73.3% (11/15) had ALT elevations above ULN
- No clear/consistent association between FVIII, resolution of ↑ALT, and prednisolone use (e.g. Participant 3)
- One participant (Participant 4) showed ↓FVIII activity with ↑ALT
  - ALT started to decline before initiation of therapeutic prednisolone
  - FVIII activity level subsequently increased
  - No bleeding was reported
- Reductions in bleeding and FVIII use maintained long-term
  - At latest follow-up, all participants dosed at 4E13 or 6E13 vg/kg remain off prophylaxis[a] 4 or 5 years after gene transfer, respectively[2]



[a]FVIII replacement or emicizumab

ALT, alanine aminotransferase; FVIII, factor VIII; ULN, upper limit of normal range (ALT); vg, vector genomes

1. Rangarajan S, et al. N Engl J Med 2017;377:2519-30; 2. Pasi KJ, et al. Haemophilia 2021;27:947–56

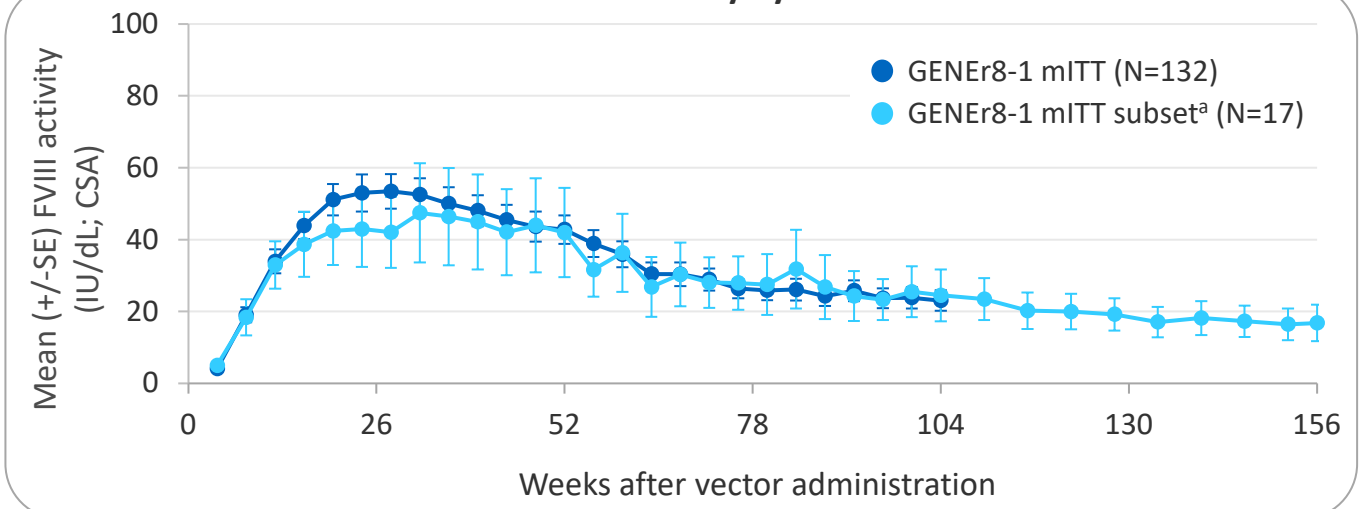
Phase 3 valoctocogene roxaparvovec trial (GENEr8-1)

Open-label, single-group, multicenter, phase 3 study[1]

134 men with severe hemophilia A and no anti-AAV5 antibodies; 6E13 vg/kg dose

- 89% had ↑ALT ≥1.5 baseline or ULN; 83% received **reactive** prednisolone 60 mg/day (or other immunosuppressants if contraindicated), tapering over ≥8 weeks[2]
- Some ↑ALT (particularly those occurring early) with positive interferon gamma ELISpot
- Reductions in bleeding and FVIII use maintained to date
- Sustained FVIII ~20% by CSA and >30% by OSA[2]

FVIII activity by CSA[2]



ALT elevations[2]

AEs of post-baseline ALT elevation	n	%
ALT increased (# participants)	**119**	**88.8**
Potential Hy's law case	0	0
Total no. of ALT elevations (# events)	385	
Grade 1	327/385	84.9
Grade 2	45/385	11.7
Grade 3	13/385	3.4
Serious AEs of ALT elevations	2/385	0.5

All Grade 3 events were managed with corticosteroids and resolved

No Grade 4 or higher ALT elevations have occurred

No clinically important signs of hepatocellular injury or liver dysfunction

[a]Participants with ≥156 weeks post-gene transfer at data cut

AE, adverse event; ALT, alanine aminotransferase; CSA, chromogenic substrate assay; FVIII, factor VIII; LLOQ, lower limit of quantification; mITT, modified intent-to-treat population; OSA, one-stage assay; vg, vector genomes

1. Ozelo MC, et al. N Engl J Med 2022;386:1013-25;
2. Mahlangu J, et al. EAHAD 2022, oral presentation

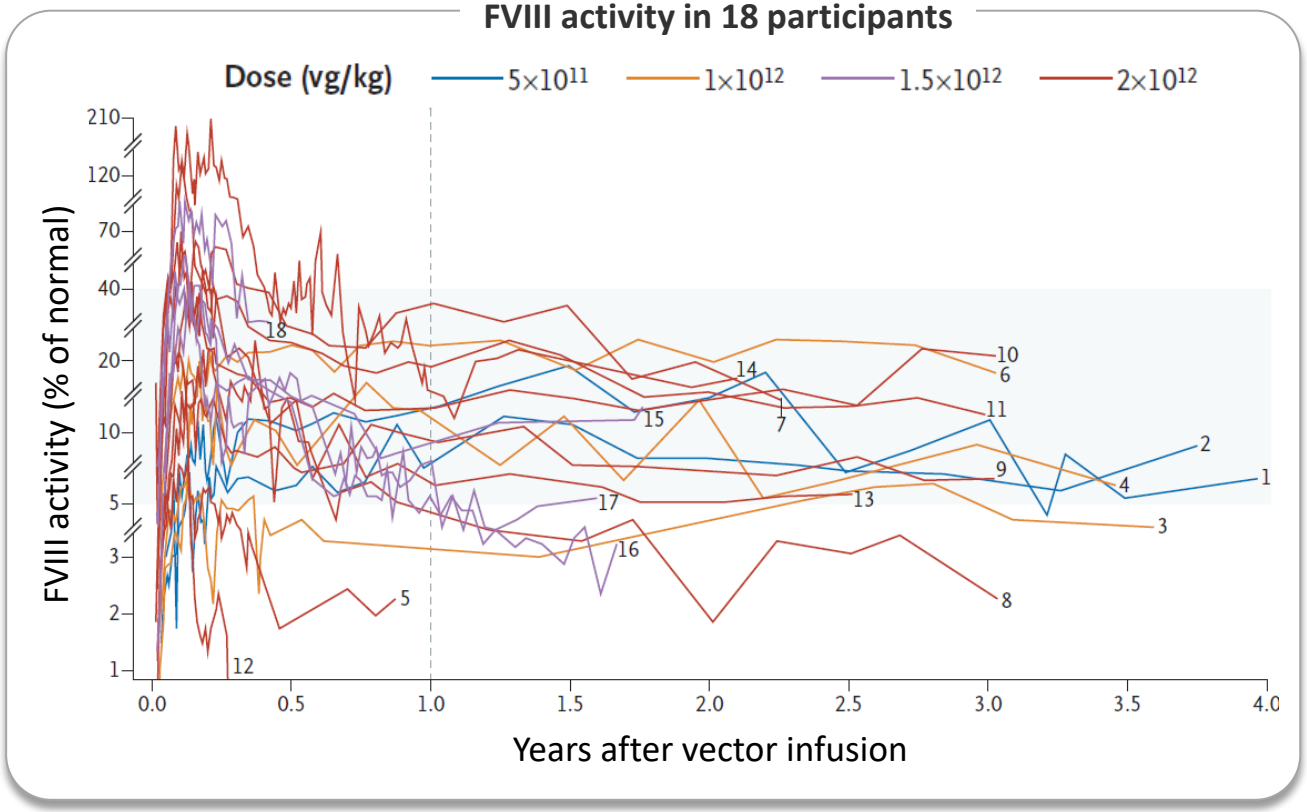
SPK-8011: B-domain deleted FVIII in AAV3-based capsid

SPK-8011 contains a **codon-optimized human FVIII gene** under the control of a liver-specific promoter with an **AAV3-based bioengineered capsid**

18 men with hemophilia A (FVIII ≤2% of normal), with SPK200 neutralizing antibody titers of 1:5 or less

Dosed at between 5E11 and 2E12 vg/kg

- Most received prednisolone **reactively**
  - 5 participants received **prophylactic** prednisolone 2 to 4 weeks after vector administration; this did not prevent ALT elevations
- 61% (11/18) participants had ALT elevations above ULN
- 33% (6/18) had ALT elevations and cellular immune responses
- 2 participants **lost expression after a cellular immune response against the SPK200 capsid**



ALT, alanine aminotransferase; FVIII, factor VIII; ; OSA, one-stage assay; ULN, upper limit of normal; vg, vector genomes

George LA, et al. N Engl J Med 2021;385:1961-73

What do we still not know about ALT elevations?

- Why are there differences in what we've seen among studies so far?
- Does this have implications when considering who is eligible for gene therapy?
- Can steroid regimens be improved?
- How should risks of steroid use be weighted vs. longer term prospects of success?
- What do patients need to know ahead of gene therapy from an immunosuppression point of view?

ALT, alanine aminotransferase

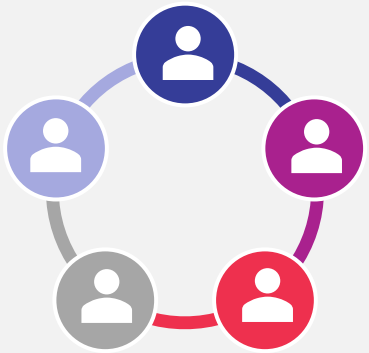
Efforts to advance our understanding

Basic research studies seeking to better understand mechanisms

- Efforts ongoing
- Challenges with inter-species translatability and availability of liver biopsy samples from clinical trials

Clinical studies evaluating immunosuppression regimens

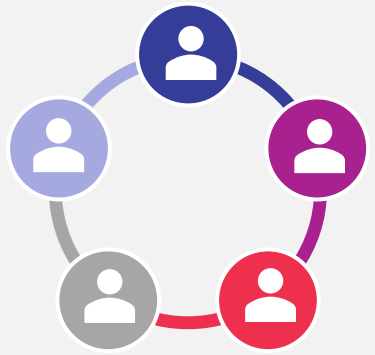
- Evaluations of immunosuppression regimens underway
- Long time horizon of clinical research means answers can’t be obtained rapidly



Consensus building with current evidence is an important interim step

One effort currently underway:
Consensus building with RAND/UCLA appropriateness method

- Developed in the 1980s to assess appropriateness of various therapeutic indications based on available evidence and clinician expertise in the absence of a gold standard
- Appropriateness = the relative weight of the benefits and harms of a medical or surgical intervention
- Group decisions are most robust, as individual decisions are prone to personal biases
- Groups comprising 7-15 experts allow for adequately complex discussion and the collection of differing opinions
- Rating the appropriateness of items without requiring or forcing consensus is the goal



Currently being used to determine expert opinion on the **use of immune suppression** in people with hemophilia receiving gene therapy

RAND, a nonprofit institution that helps improve policy and decision making through research and analysis
UCLA, University of California Los Angeles

Thank You!

Backup Slides

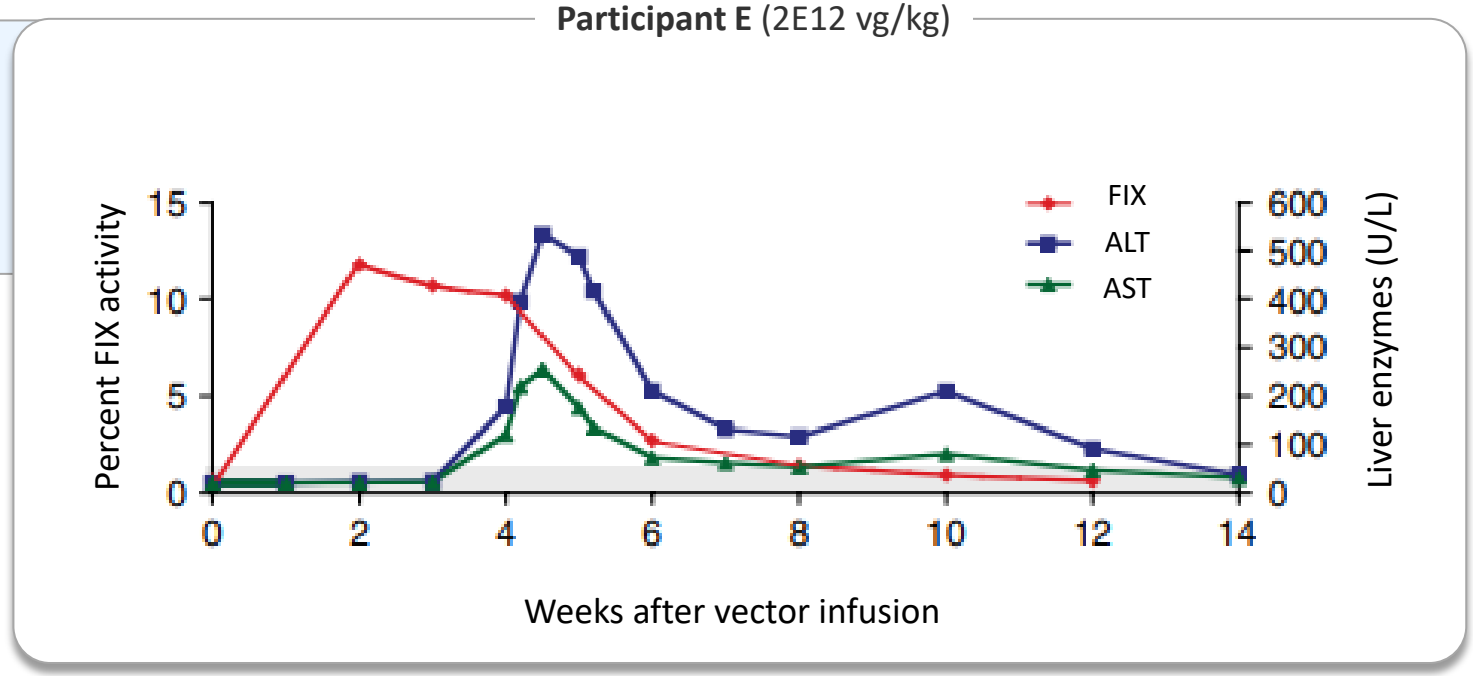
rAAV-hAAT-F.IX: FIX in AAV2 capsid

rAAV-2 vector expressing human factor IX infused through the hepatic artery

7 participants with severe hemophilia B

3 doses: 8E10 vg/kg, 4E11 vg/kg, or 2E12 vg/kg

- Therapeutic levels of FIX achieved at the highest dose tested
- Duration of expression at therapeutic levels was limited to a period of ~8 weeks
- Gradual decline in FIX accompanied by a transient asymptomatic elevation of liver transaminases that resolved without treatment



Loss of FIX transgene expression and transient mild elevations of serum liver enzymes were correlated with a CD8+ T cell response against the AAV2 capsid, directed against the transduced hepatocytes

ALT, Alanine aminotransferase; AST; aspartate transaminase; FIX, factor IX; P, prednisolone; vg, vector genomes

Manno CS, et al. Nat Med 2006;12:342-47

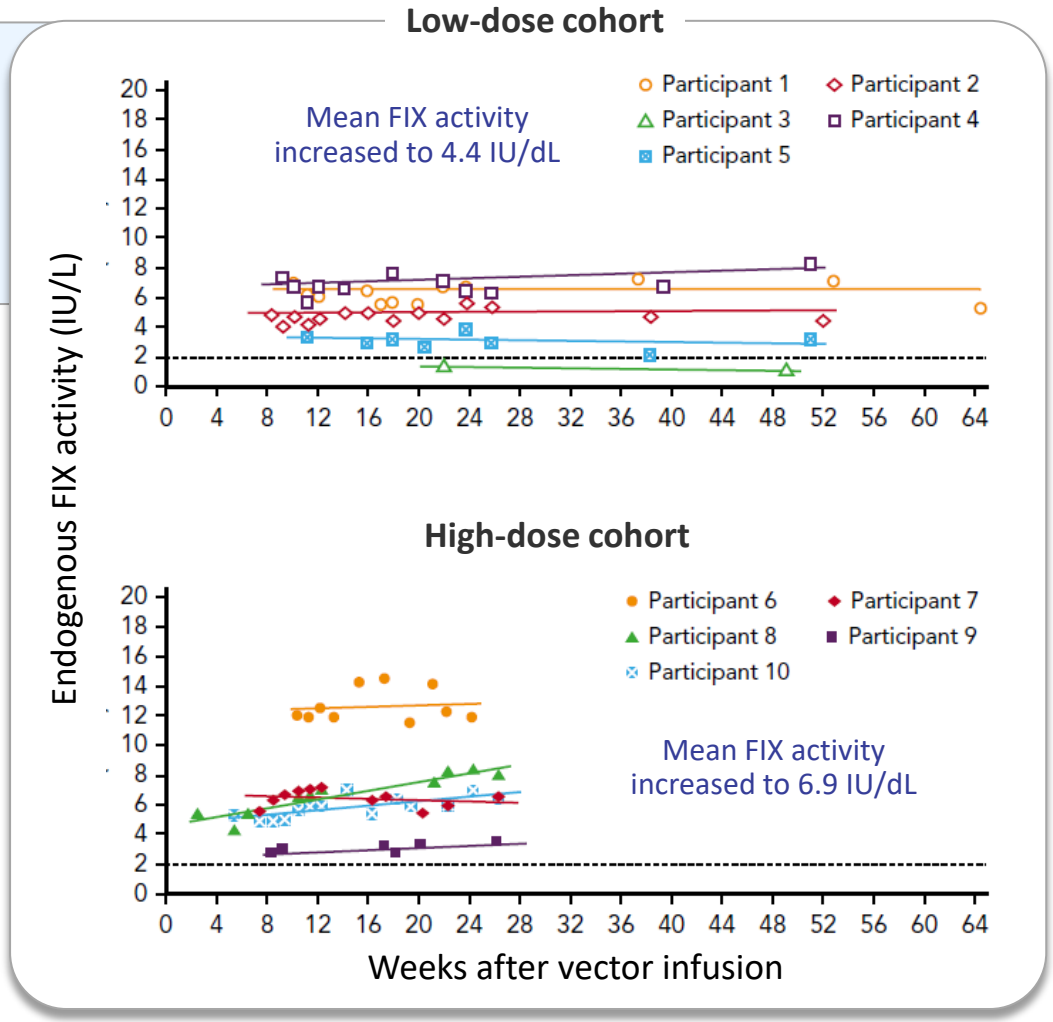
AMT-060: hFIXco in AAV5 capsid[1]

AMT-060 combined the previously tested codon-optimized *FIX* gene cassette and LP1 promoter[2] with an **AAV5** capsid[1]

10 adults with hemophilia B (FIX ≤2% of normal) and severe-bleeding phenotype, and absence of antibodies against AAV5

AMT-060 5E12 vg/kg (low dose) or 2E13 vg/kg (high dose)

- FIX expression levels were stable throughout the observation period; 8 of 9 participants receiving FIX at study entry stopped prophylaxis
- Limited, asymptomatic, and transient ALT elevations in the low-dose (n = 1) and higher-dose (n = 2) cohorts were treated with a short tapering regimen of prednisolone
- Unlike studies using AAV8[2], **no decrease in FIX activity or capsid-specific T cell responses were detected during transaminase elevations**



ALT, Alanine aminotransferase; FIX, factor IX; hFIXco, codon-optimized human FIX transgene; vg, vector genomes

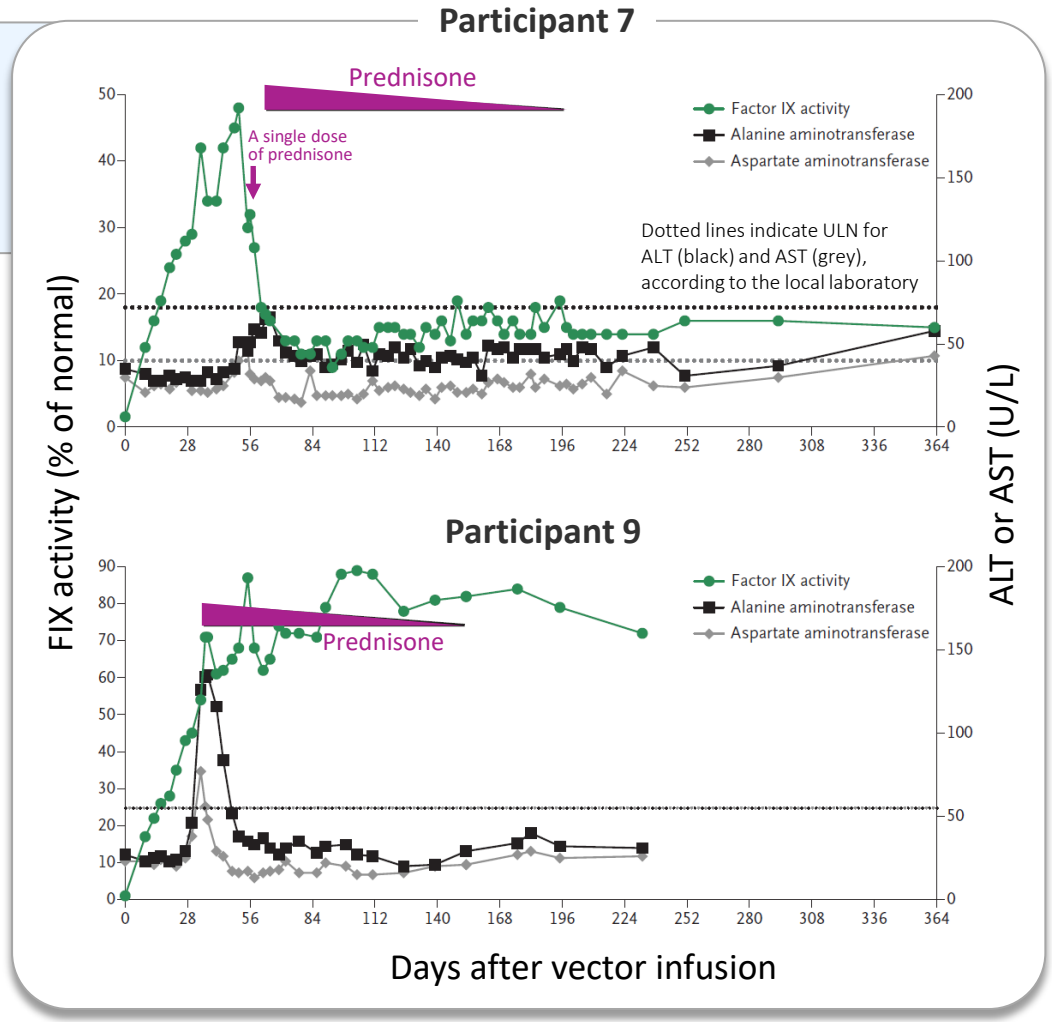
1. Miesbach W, et al. Blood 2018;131:1022-31;
2. Nathwani AC, et al. N Engl J Med 2014;371:1994-2004; 3. George LA, et al. N Engl J Med 2021;385:1961-73

SPK-9001: FIX Padua variant in bioengineered capsid

SPK-9001: A bioengineered capsid, liver-specific promoter and **factor IX Padua (factor IX–R338L)** transgene, a naturally occurring gain-of-function mutation that results in **specific activity 8 to 12 times higher than nonmutant FIX**

10 men with hemophilia B (FIX ≤2% of normal) were given **5E11 vg/kg** dose

- Sustained levels of FIX coagulant activity ~30% of normal
- Two of 10 participants had an asymptomatic, transient increase in ALT that was managed with a course of prednisone
  - ALT/AST returned to baseline levels after prednisone initiation
  - Transgene expression was maintained after prednisone was tapered and stopped
  - Positive ELISPOT assays in these 2 participants confirmed an immune response to AAV-Spark100 capsid peptides



ALT, alanine aminotransferase; AST, aspartate aminotransferase; ELISPOT, enzyme-linked immunosorbent spot; FIX, factor IX; PBMC, peripheral blood mononuclear cell; ULN, upper limit of normal range; vg, vector genomes

George LA, et al. N Engl J Med 2021;385:1961-73

AMT-061 (etranacogene dezaparvovec): FIX Padua variant in AAV5 capsid[1]

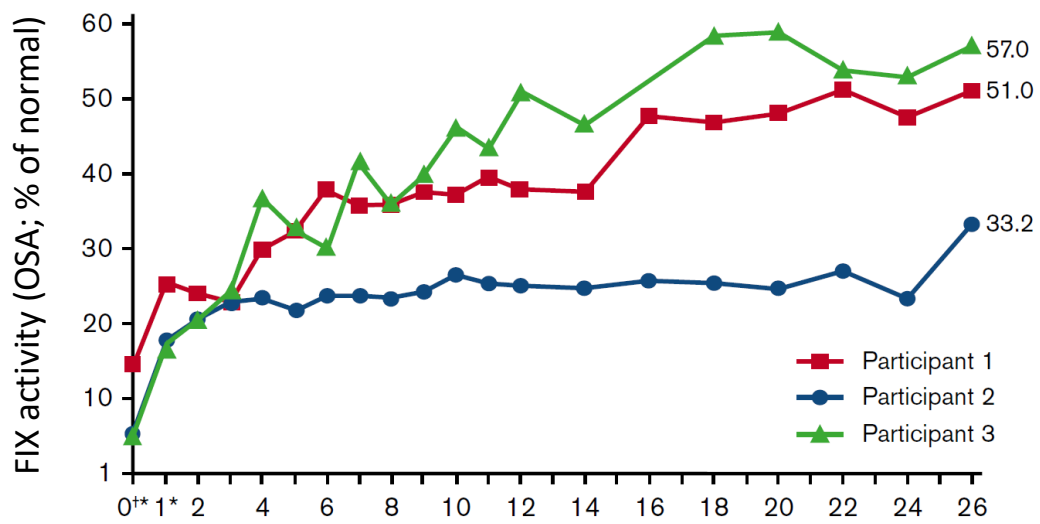
AMT-061 has a similar AAV5-based construct to AMT-060, with the substitution of hFIXco for the more active **factor IX Padua (factor IX–R338L)** transgene[1]

Three adults with hemophilia B (FIX ≤2% of normal) and **positive** for low levels of AAV5 neutralizing antibodies were administered a dose of 2E13 vg/kg

- Clinically relevant increases in FIX activity over 6 months
- No clinically significant elevations in liver enzymes or inflammatory markers

No use of corticosteroids related to treatment required

FIX activity by OSA[1]



†Week 0 reflects FIX activity before AMT-061 treatment.
*Early samples may include activity from exogenous FIX replacement.

FIX, factor IX; hFIXco, codon-optimized human FIX transgene; OSA, one-stage assay; vg, vector genomes

1. Von Drygalski A, et al. Blood Adv 2019;3:3241-47

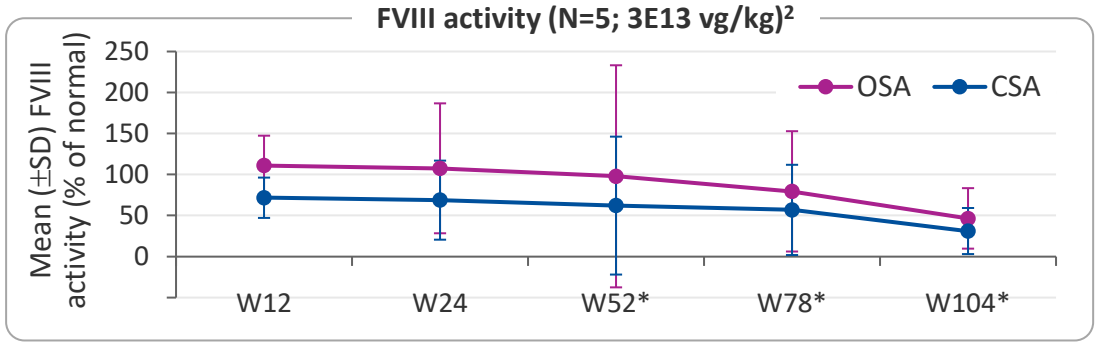
Giroctocogene fitelparvovec: B-domain deleted FVIII in AAV6 capsid

PF-07055480 (SB-525; giroctocogene fitelparvovec) contains a **modified B-domain-deleted FVIII coding sequence** in an expression cassette designed for production of high yields of the vector and optimal liver-specific expression of FVIII protein[1]

Clinical studies in adult male participants with severe hemophilia A (FVIII ≤1% of normal) and no anti-AAV6 NAbs

Phase 1/2 (N=11)[2]

- 9E11—1E13 (N=6) and 3E13 vg/kg (N=5)
- 4/5 high-dose participants received corticosteroids for ALT/AST elevations
- FVIII activity 25.4% via CSA at week 104 in highest dose cohort[1]



Phase 3 AFFINE study (N~50)

- ~50% enrolled; no interim analyses planned
- **FVIII levels >150% in some treated participants led to clinical hold** due to concern for risk of thrombosis[1]
  - Some being treated with oral anticoagulants to reduce risk of thrombosis
  - 1 participant experienced DVT below the knee[3]

May 2022: FDA clinical hold has been lifted[3]

Voluntary pause in dosing new patients continues until protocol amendment for managing elevated FVIII levels has been agreed

ALT, Alanine aminotransferase; AST, aspartate aminotransferase; CSA, chromogenic clotting assay; DVT, deep vein thrombosis; FVIII, factor FVIII; NAbs, neutralizing antibodies; OSA, one-stage assay; SD, standard deviation; SAE, serious adverse event; W, week

1. https://wfh.org/fda-places-the-pfizer-sangamo-therapeutics-phase-3-affine-haemophilia-a-gene-therapy-study-on-clinical-hold/; 2. Visweshwar N, et al. Blood 2021;138:564–66; 3. https://endpts.com/fda-releases-second-pfizer-gene-therapy-from-clinical-hold-as-hemophilia-program-gets-ok-to-restart/