Hemophilia

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Disclosures

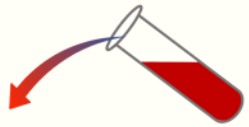
- Research support: (Last 24 Months)
  Biogen/Sanofi, Roche/Genentech, Spark, Pfizer, Takeda/Shire

- Medical Advisory Board (Last 24 months)
  Genentech, CSL, Octapharma

- I will be discussing off-label use of medications

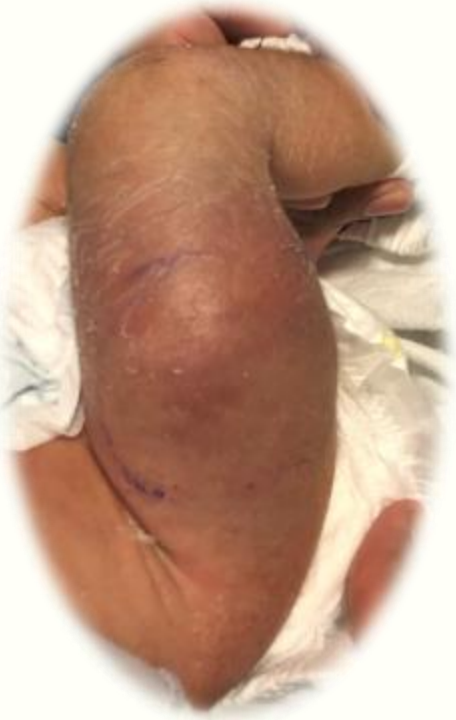
Objectives

- Accurately recognize the inheritance pattern, clinical presentation and laboratory evaluation for Hemophilia
- Understand the risks and benefits of clotting factor administration for the treatment
- Describe 3 approaches to improve the prevention of bleeding events in patients with Hemophilia



Patient 1

- 8 day old male with swollen thigh
  - NSVD Delivery
  - Vitamin K IM
  - NYS Newborn Screen via Heelstick with Oozing
  - Right Thigh Swelling s/p Hep B Vaccination



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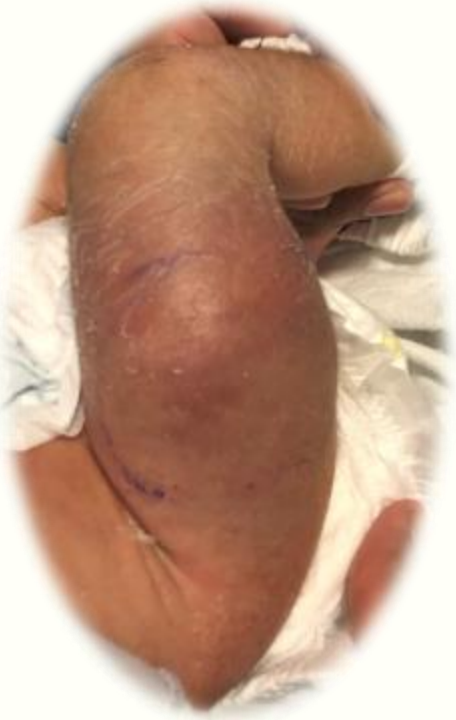

Family History:

Maternal Uncle previously followed at HTC

Deceased @ 30 year of age from HIV Lymphoma

Patient 1

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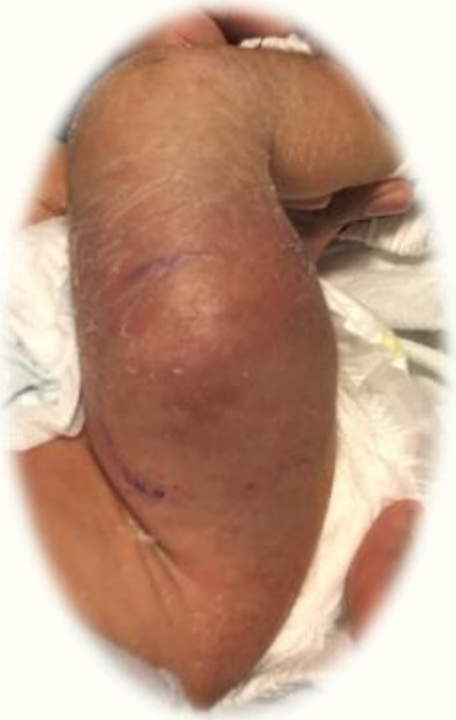
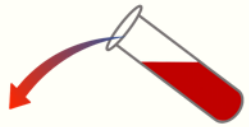


Next Steps ?

- PT: 13.0 secs
- aPTT: 110 secs



Next Steps ?

- FVIII <1%
- FIX 45%
- FXI 58%

Next Steps ?

- FVIII <1%
- FIX 45%
- FXI 58%
- VWF Antigen: 199 %
- VWF Risto Co Factor: 200%

Questions

- What is your threshold for treatment ?
- What medication/dose ?
- What side effects are you concerned about ?
- When would you start prophylaxis ?
- Costs/Risks vs Benefits

History of Hemophilia

2nd Century AD

Babylonian Talmud

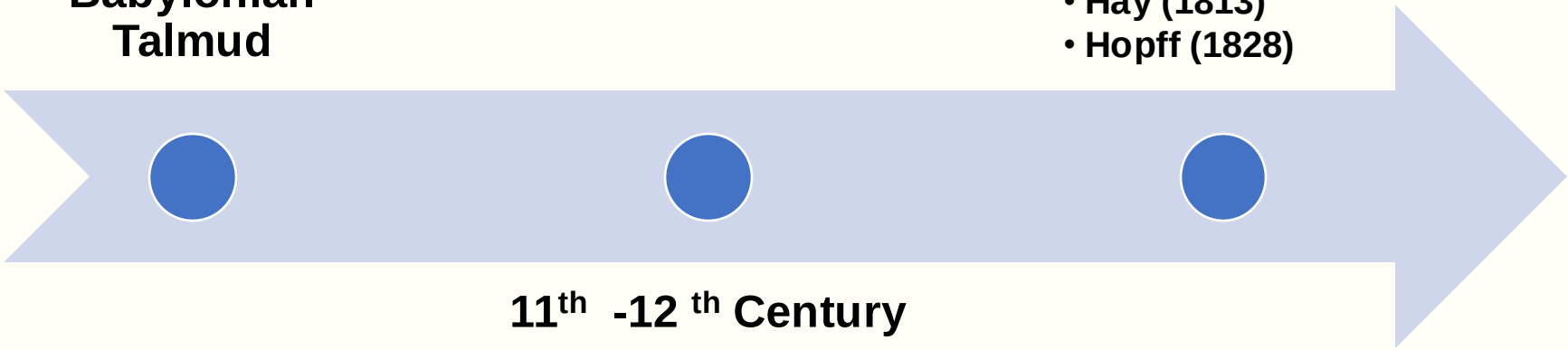
11th -12 th Century

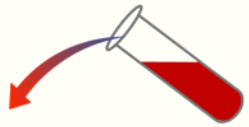
Albucasis

Maimonides

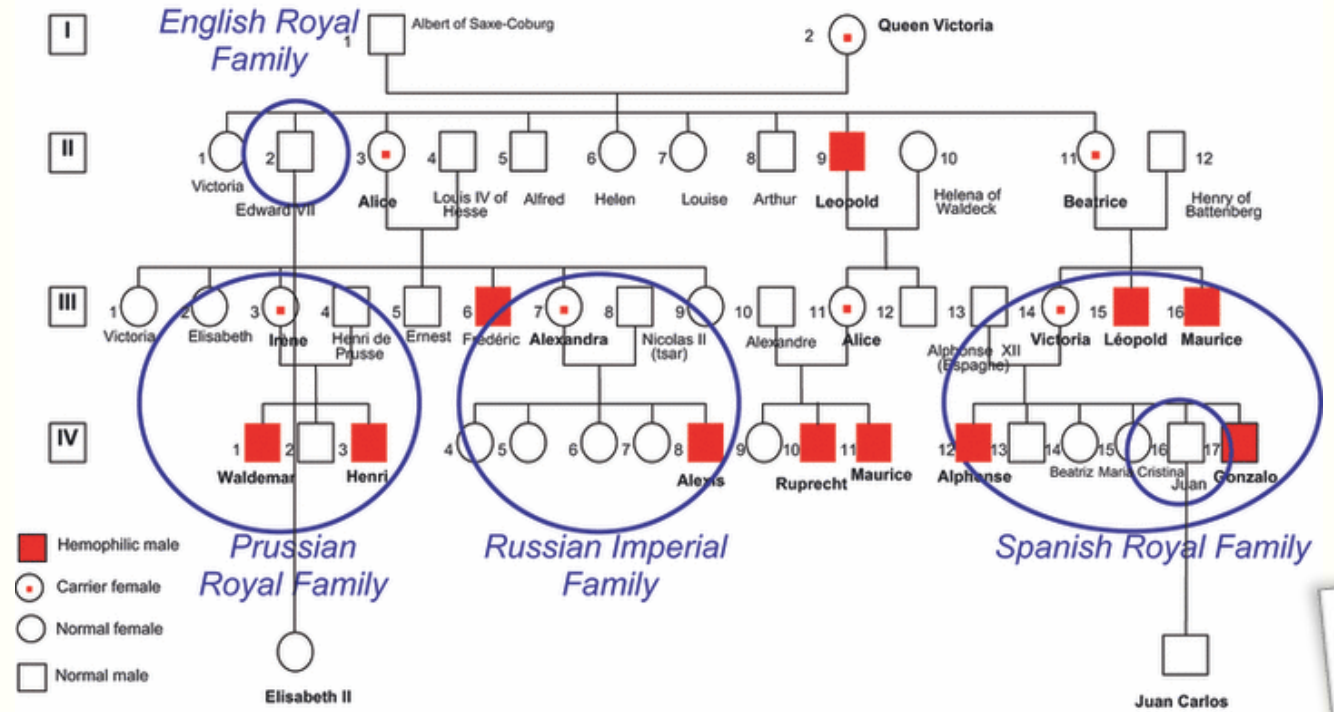
19th Century

- **Otto (1803)**
- **Hay (1813)**
- **Hopff (1828)**



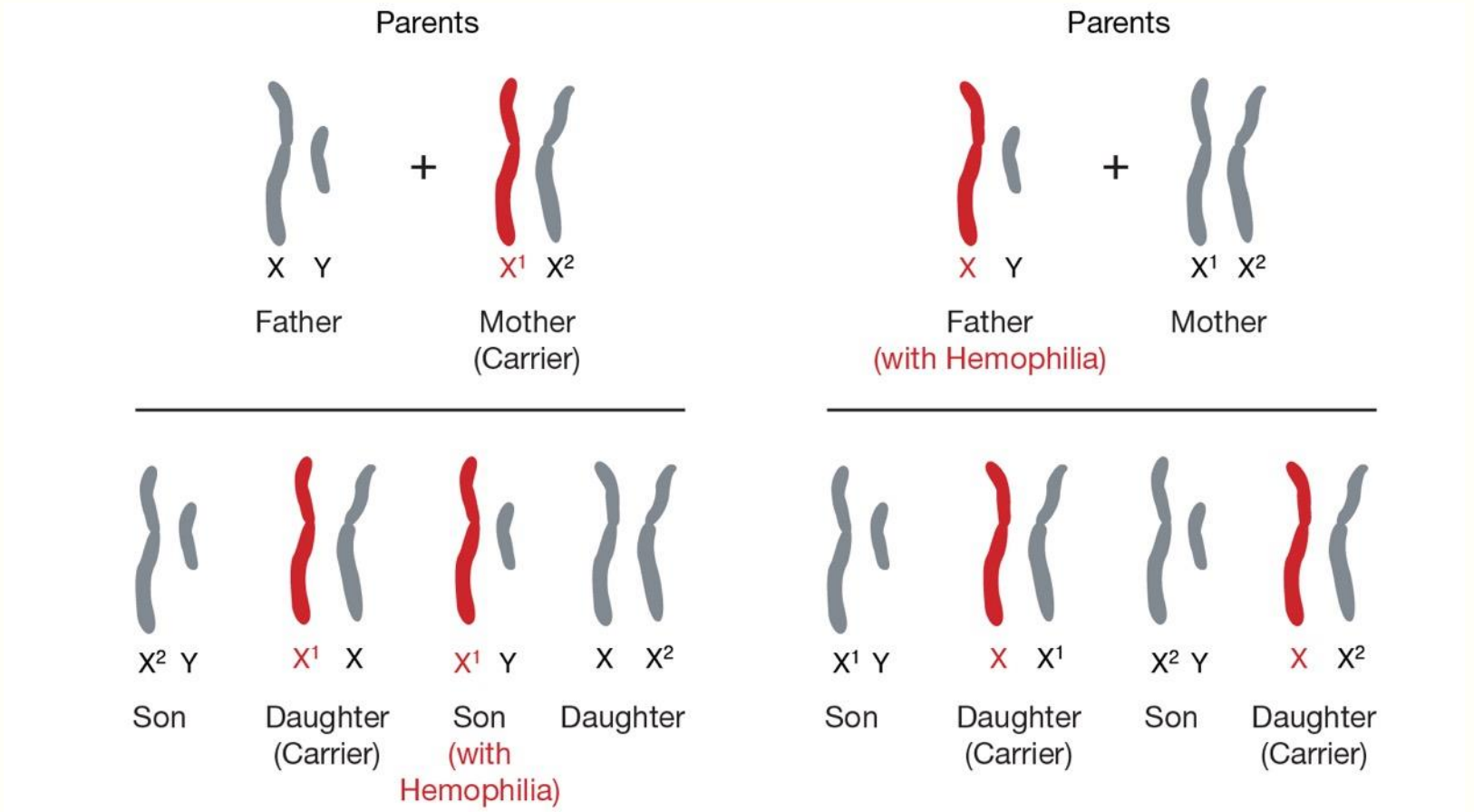


The Royal Disease



Rogaaev et al. Hemophilia (2009)
www.sciencemag.org (State Archived of Russian Federation)
www.scientificanemrica.com

Hemophilia A/B are X-linked disorders

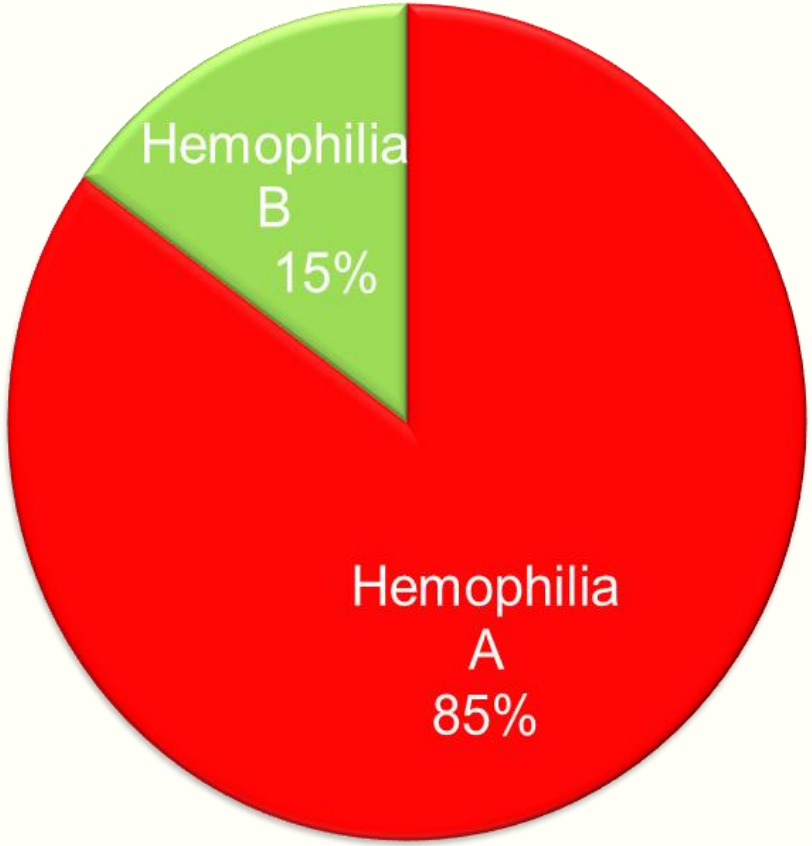


https://www.genome.gov/genetics-glossary/hemophilia

1/3 of patients with hemophilia with no family history

- 1 in 5,000 males (A)
- 1 in 30,000 males (B)

- 30% of cases have NO family history



- *** Advanced Paternal Age Hypothesis**

Rossiter et al. Hum. Mol. Gen. 1994, Carcao, M. Unpublished
Wolf and Lassila, 2019, Haemophilia

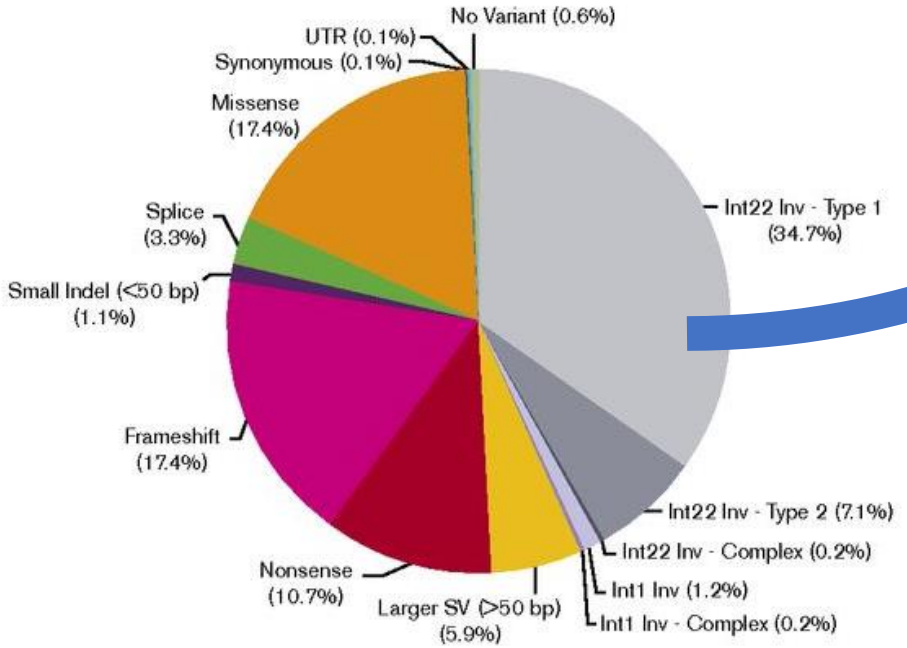
Women Can Have Hemophilia

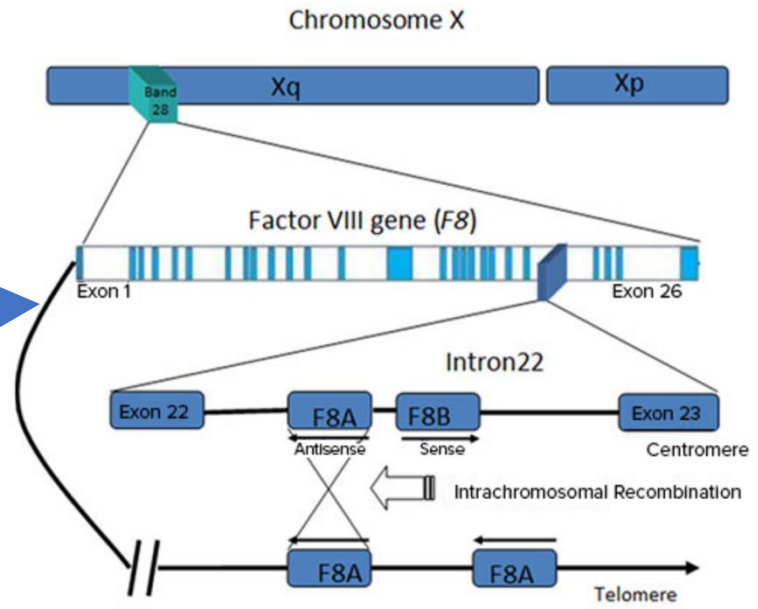
- Lyonization of the normal X chromosome
- Turner syndrome (XO)
- Father with hemophilia / mom as a carrier
- vWD type 2N (Normandy) *

* Von Willebrand Disease

Intron 22 inversion is the most common mutation

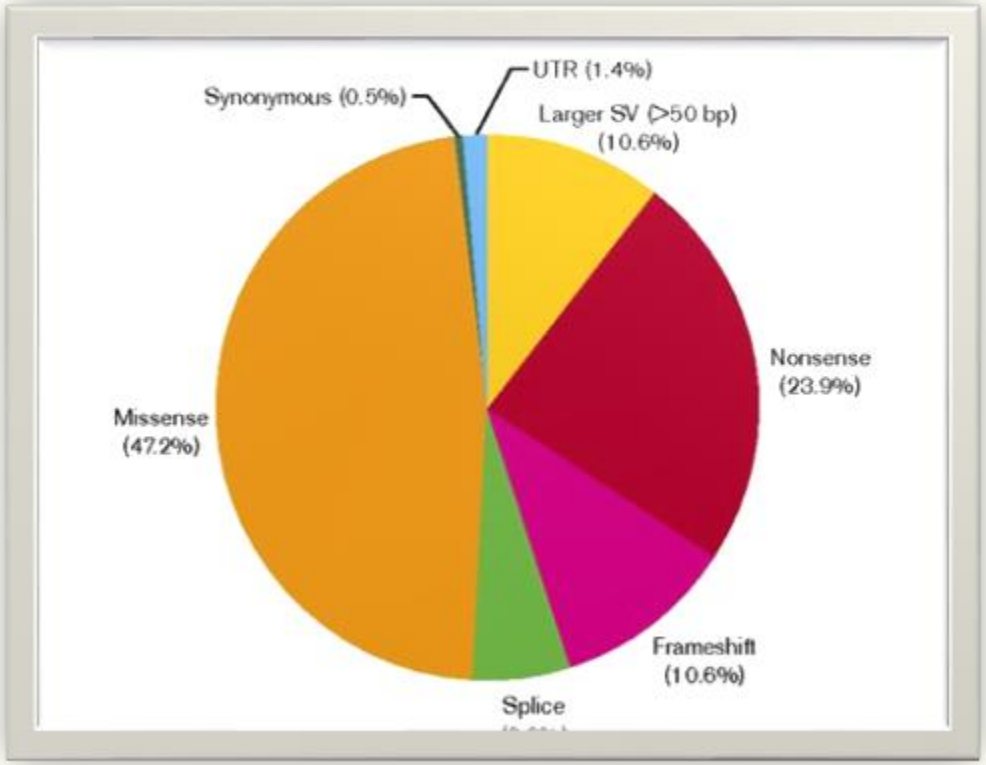
- Exact defect known: ~ 95%
- Mild-moderate hemophilia: Missense 85%
- Severe hemophilia:





Johnsen, J et al. Blood Advances (2017)
https://reference.medscape.com/features/slideshow/hemophilia-a#page=5

F9 Gene Mutations

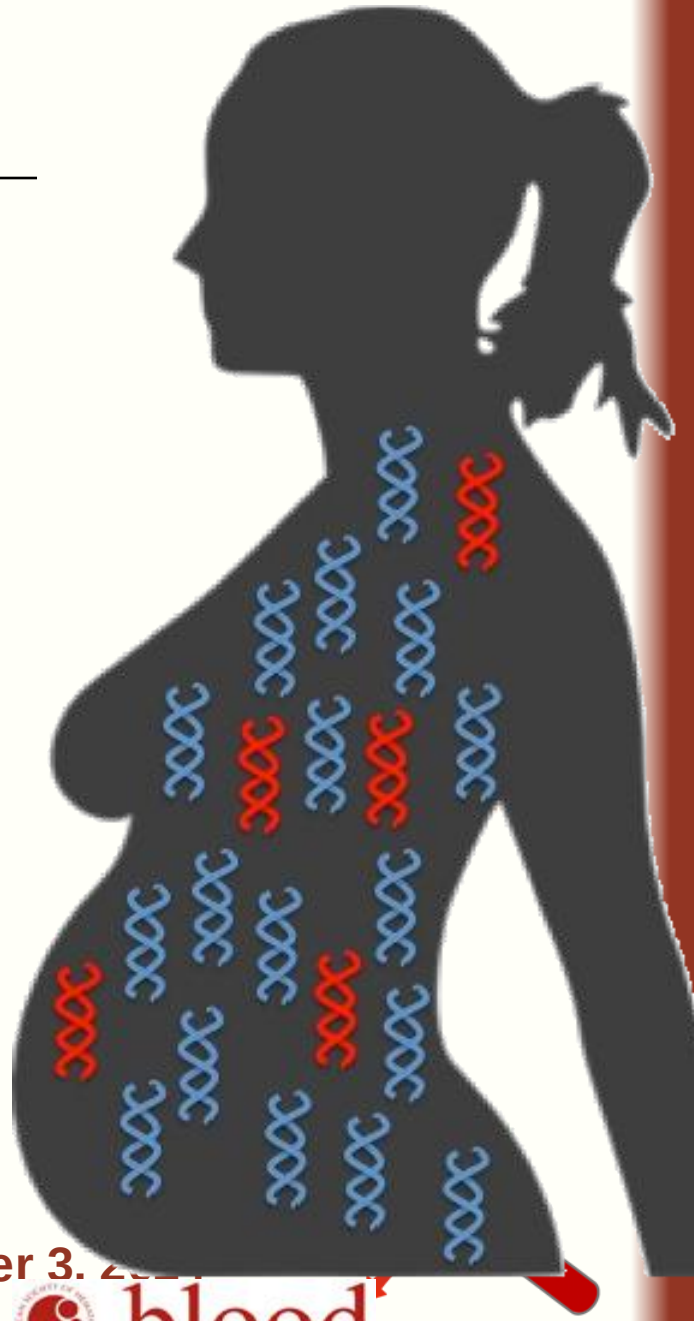


- Missense (47%)
- Nonsense (24%)
- Frameshift (10%)
- Splice Site (6%)

Johnsen, J et al. Blood Advances (2017)

Prenatal and Genetic Counseling

- Ultrasound
- CVS / Amniocentesis
- Free Fetal DNA (Future State)
- Pre-Implantation Genetic Diagnosis
- Mode of Delivery



Tsui N B Y et al. Blood 2011;117:3684-3691

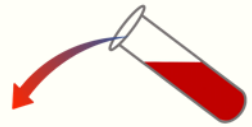
blood

Mode of Delivery

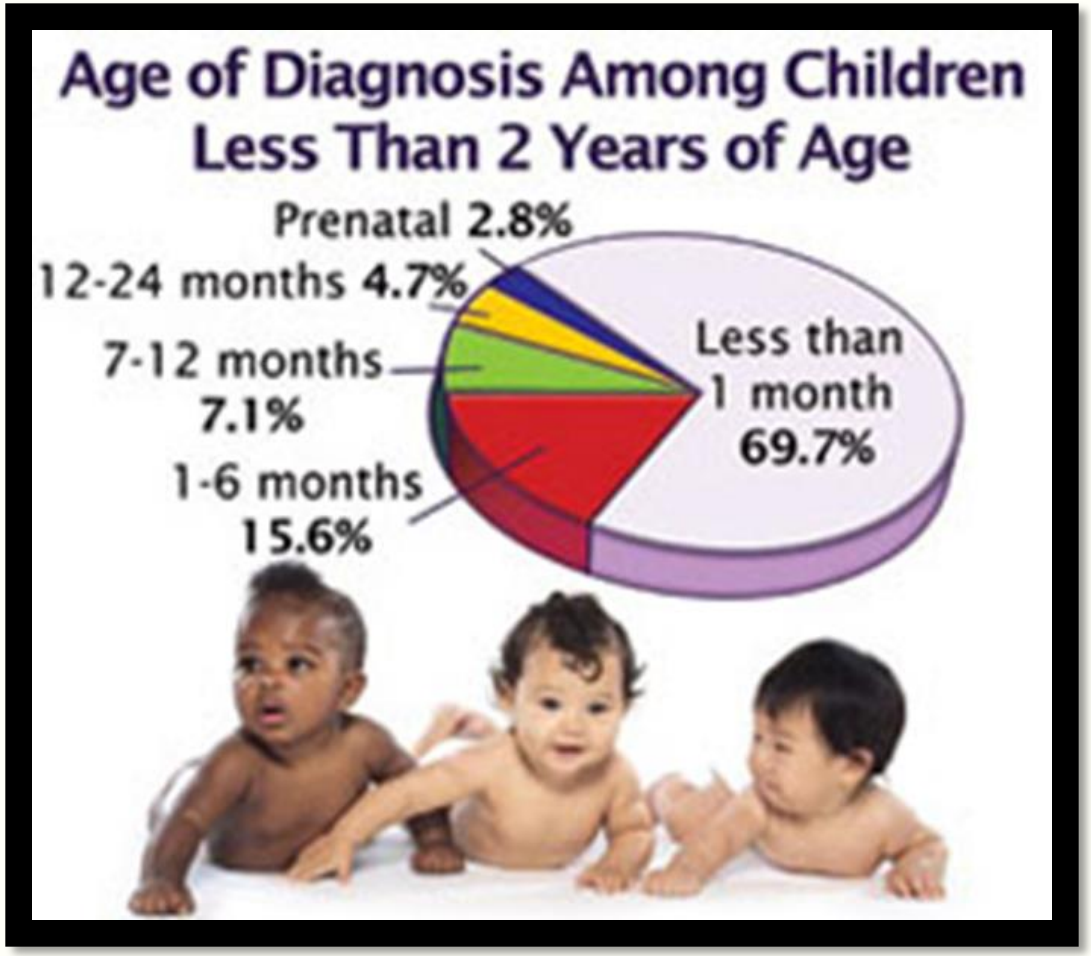
Planned Mode of Delivery	ICH	Risk
Vaginal	17/688	2.5%
- Spontaneous	8/541	1.5%
- Instrumented	7/68	10.2%
- C/S after labor	2/79	2.5%
Cesarean	2/125	1.6%

- No fetal electrodes
- No FORCEPS
- No VACCUM
- Avoid HEELSTICK
- No IM Injection
- Cord Blood Sample

Anderson et al. Hematologica (2019)

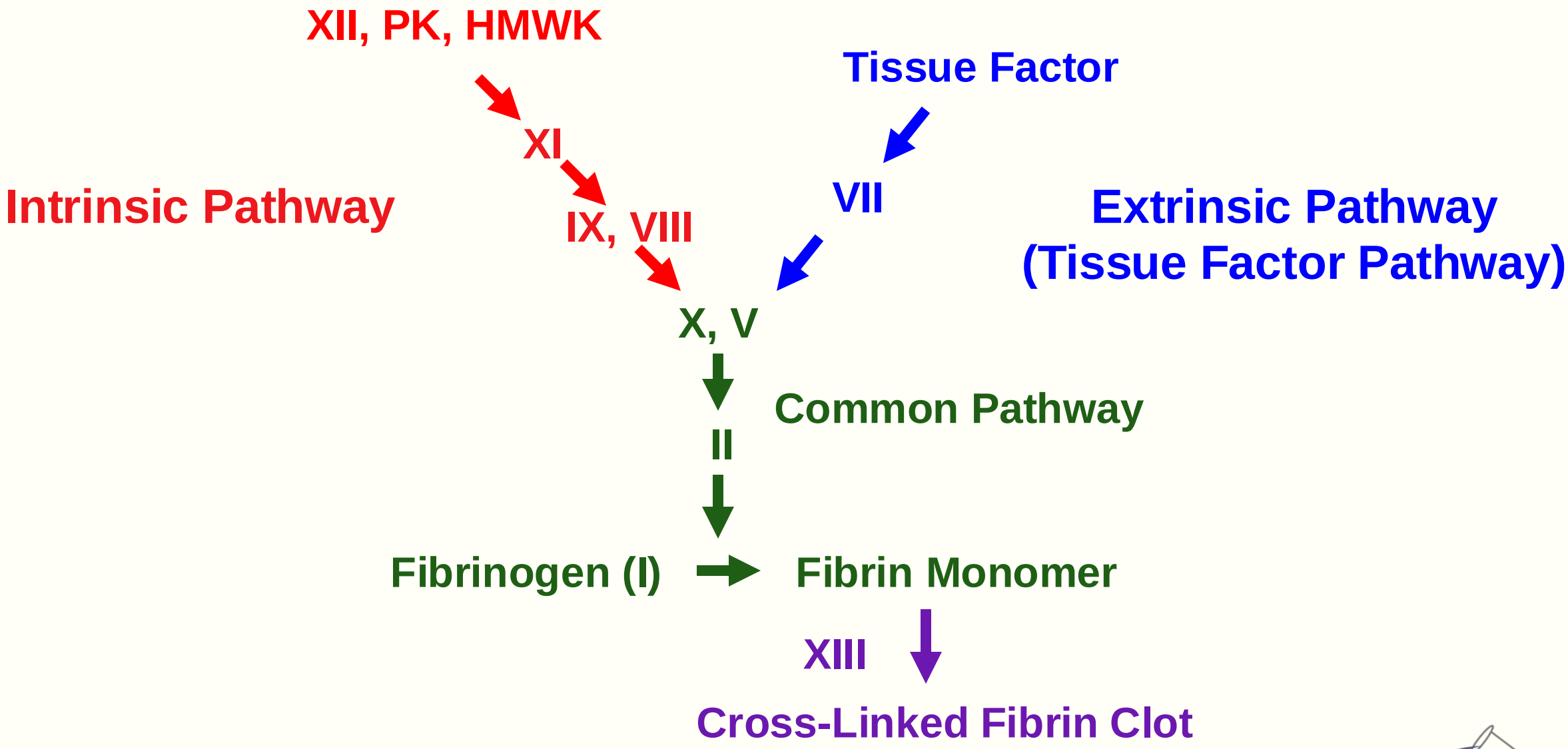


Hemophilia Presentation

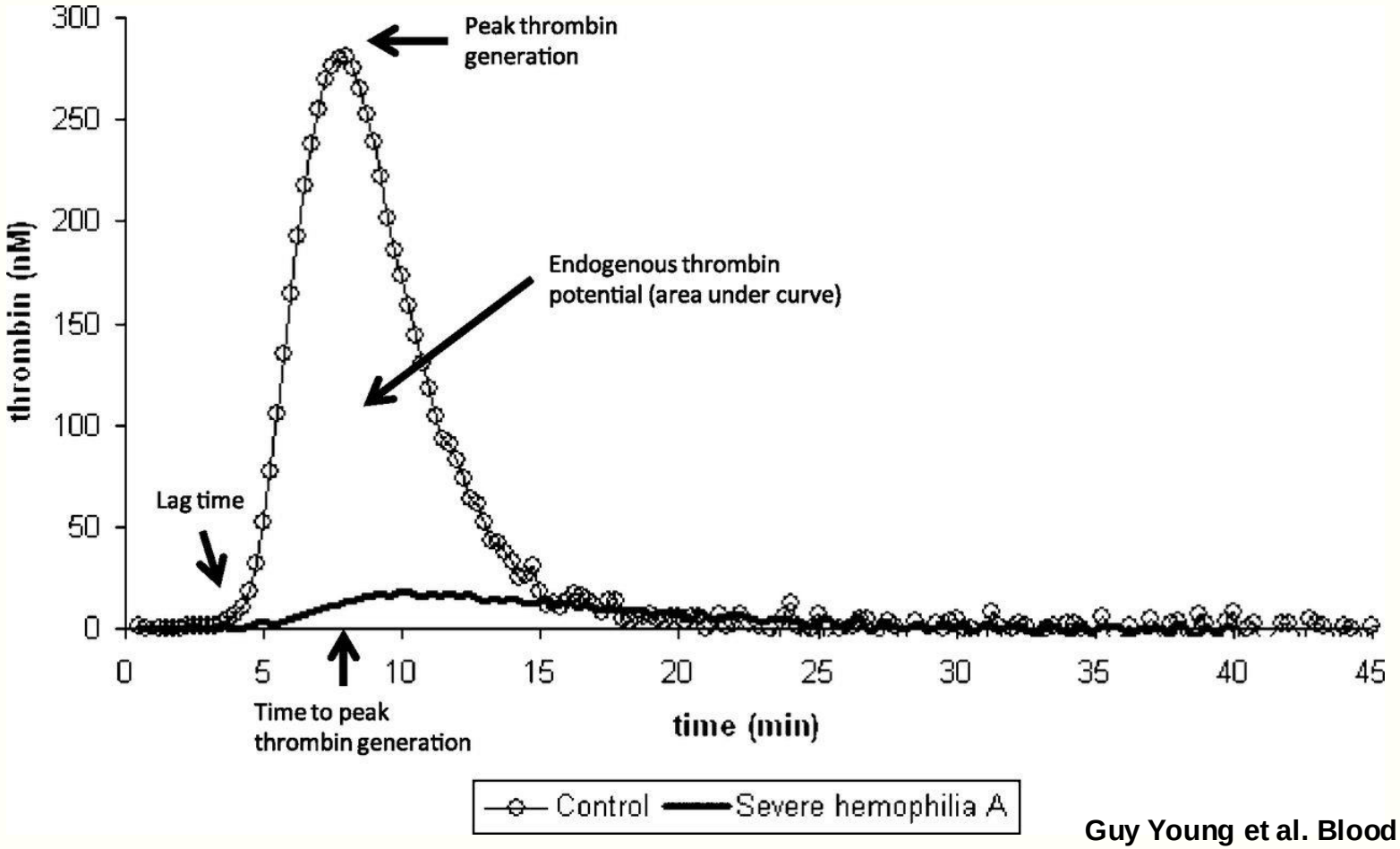


http://www.cdc.gov/ncbddd/hemophilia/data.html

Coagulation Cascade

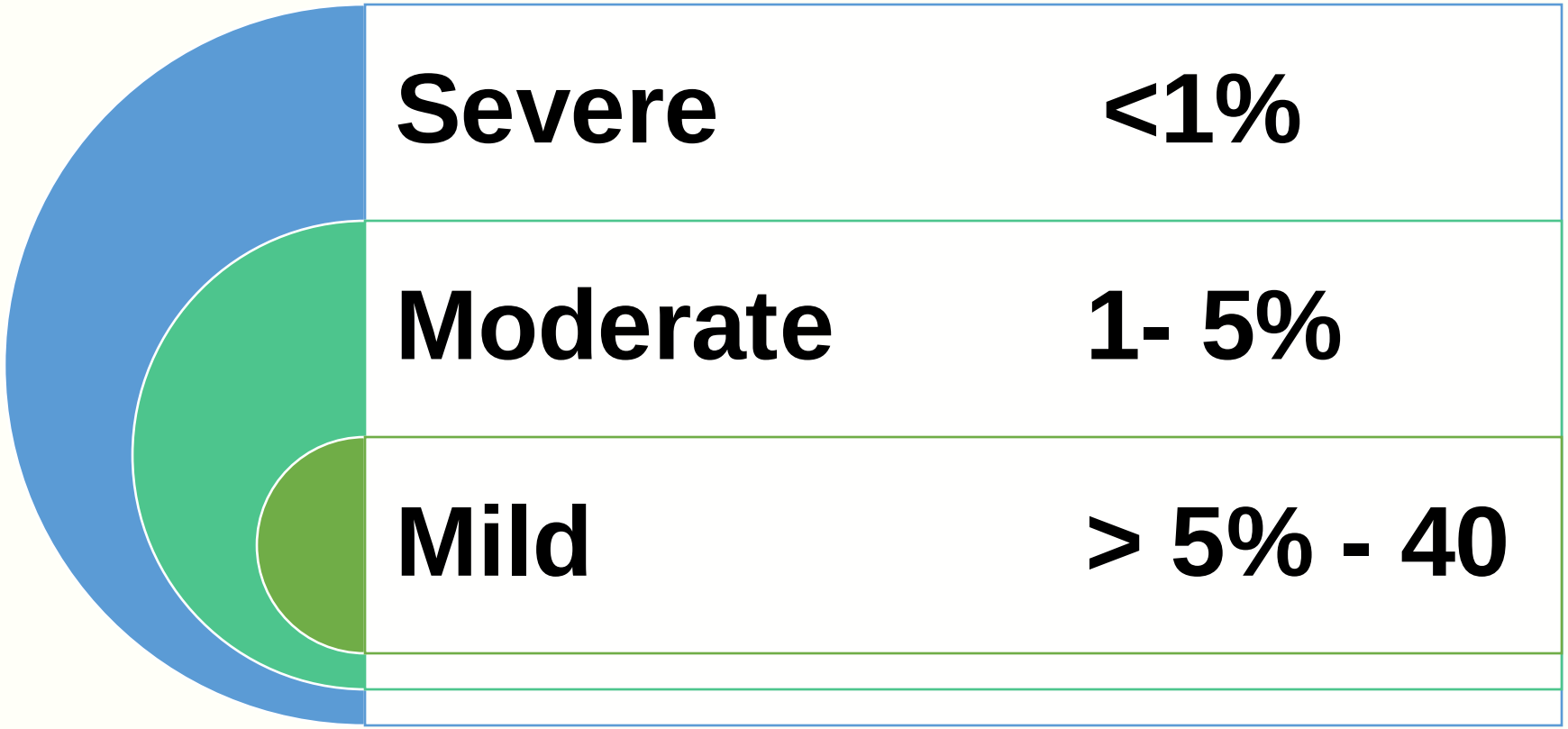


Hemophilia patients have poor thrombin generation



Guy Young et al. Blood (2013)

Laboratory classification of severity

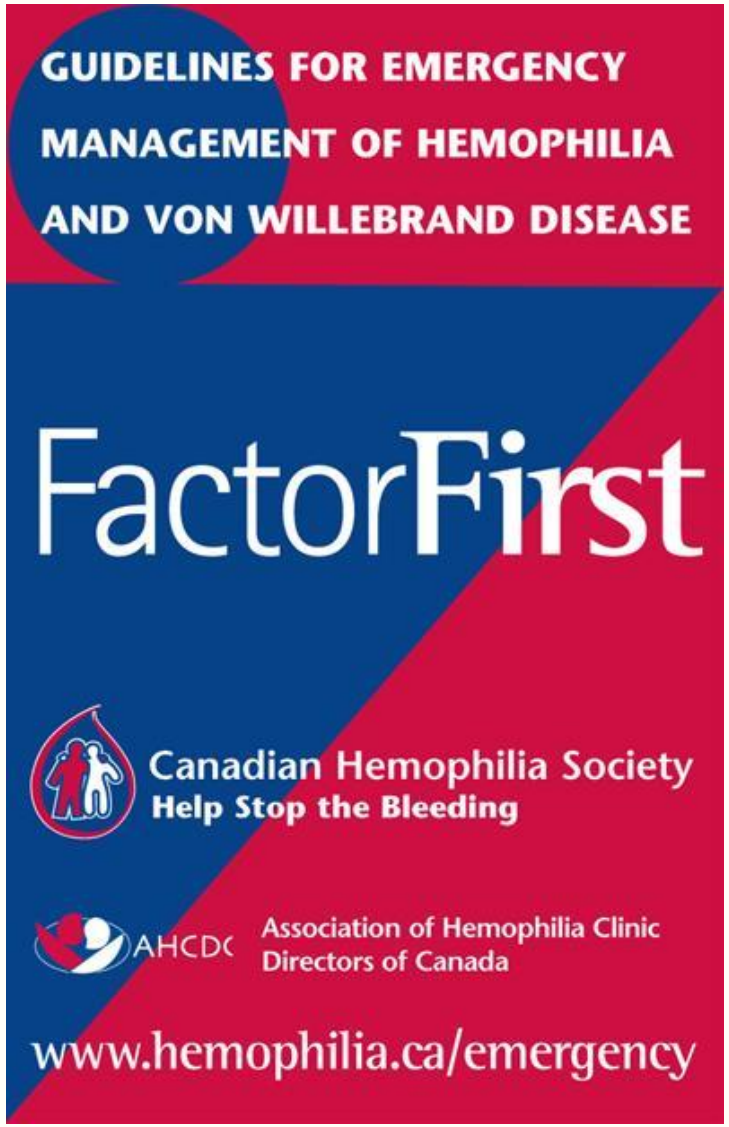


Joint disease progression in hemophilia

http://www.hemophilia.in/

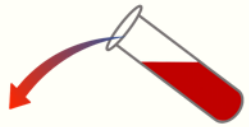
Stop the bleeding!!

- High Priority @ Triage
- Treat first →
  Diagnostic testing later
- Treat based on history even in the absence of physical signs
- Patients often bring their clotting factor with them

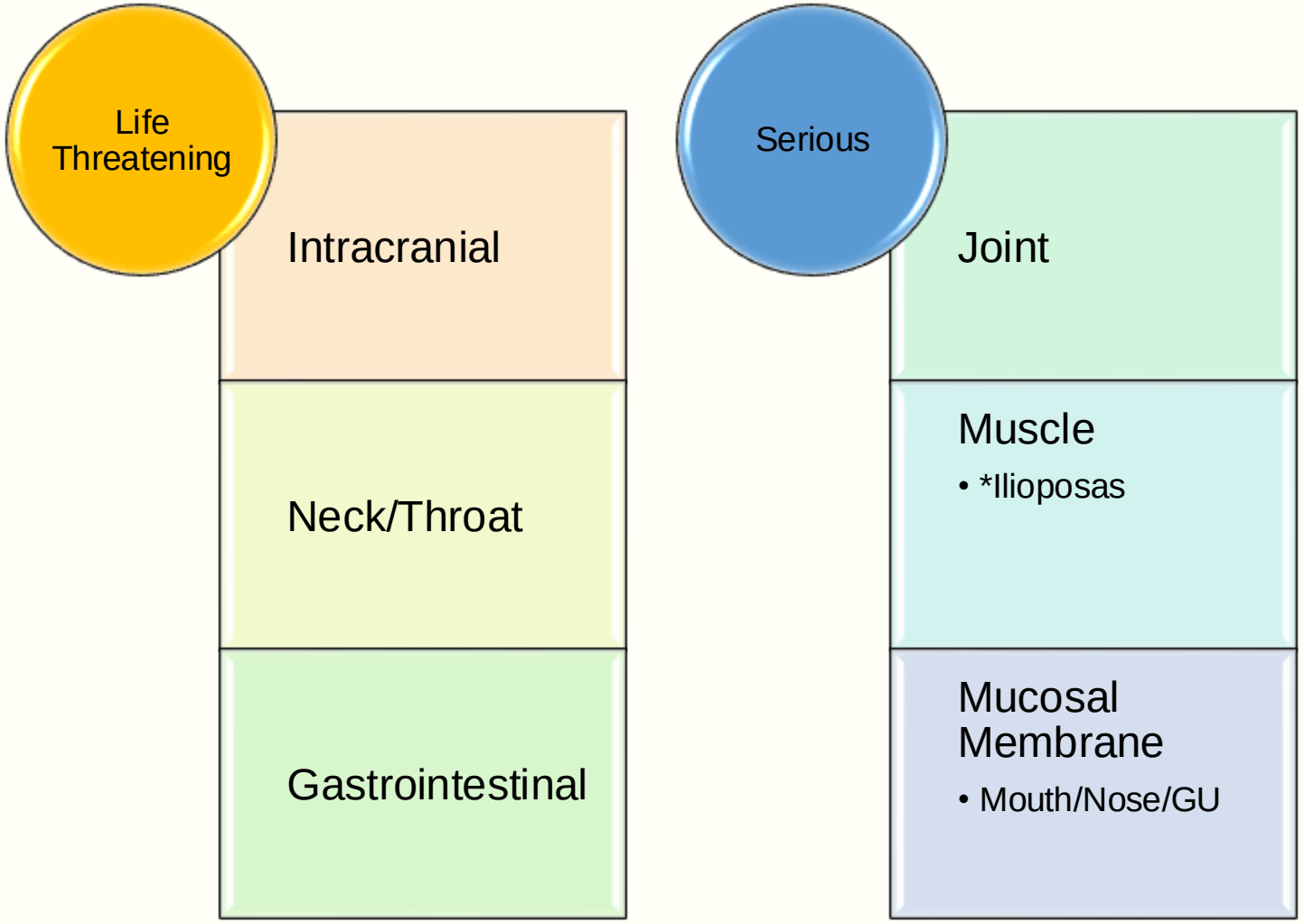


Factor Replacement

Factor VIII	1u/kg raises FVIII levels by 2%	1/2 life: 12 hrs
Factor IX	1u/kg raises FIX levels by 1%	1/2 life: 20-24 hrs • rFIX dosing = 1.3 x pFIX

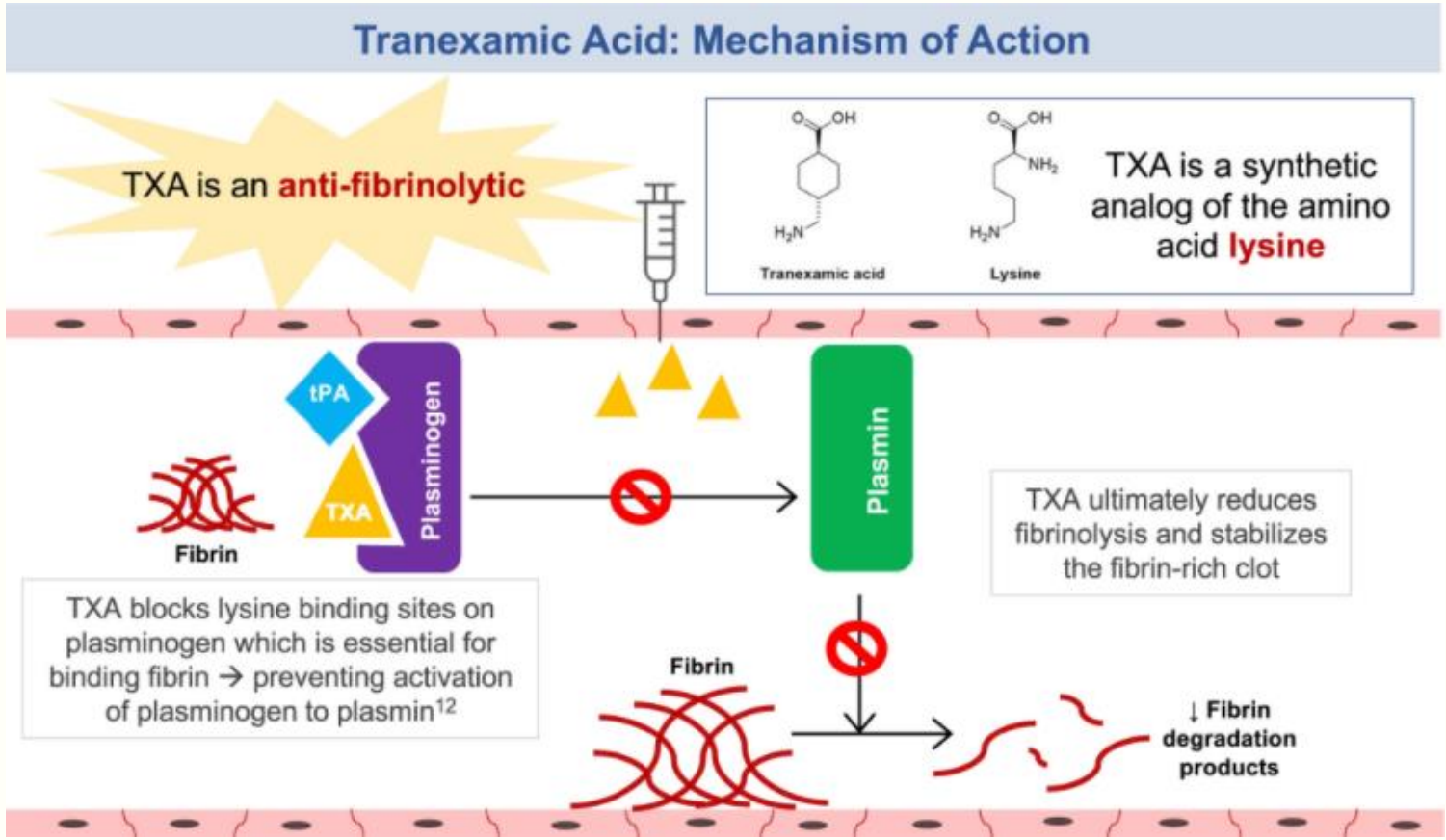


High Risk Hemorrhage



Srivastava et al. WHF Guidelines for the Management of Hemophilia 3rd Ed. , 2020

Anti-Fibrinolytic Therapy

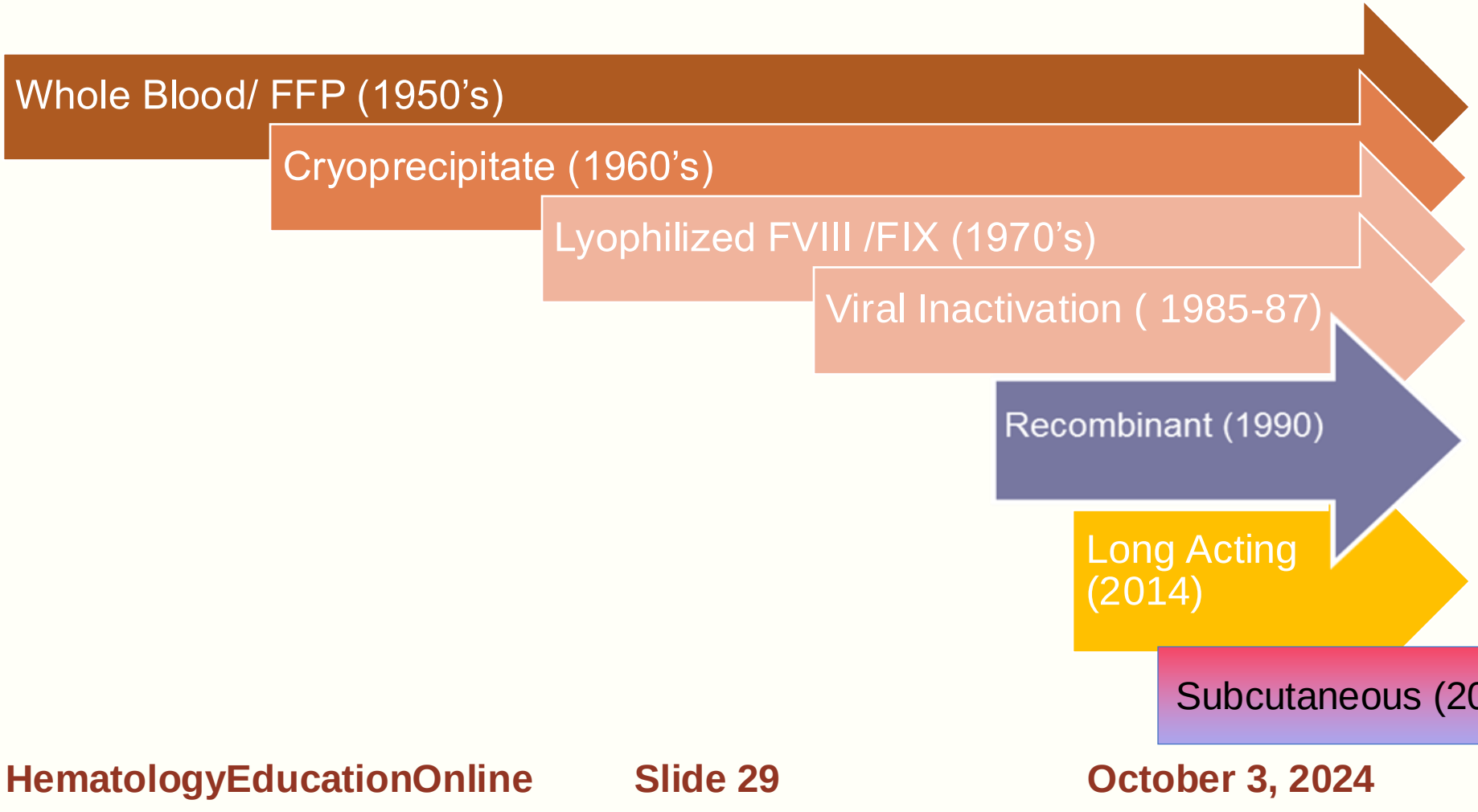


- Aminocaproic Acid
  50- 100mg/kg q6
- Tranexamic Acid
  10-20mg/kg q 8 IV
  1300mg po q8 PO

- Mucosal Bleeding
- Adjunctive Therapy

Relker, N. et al. RPTH (2021)

Advance in safe, effective, home based therapy for hemophilia

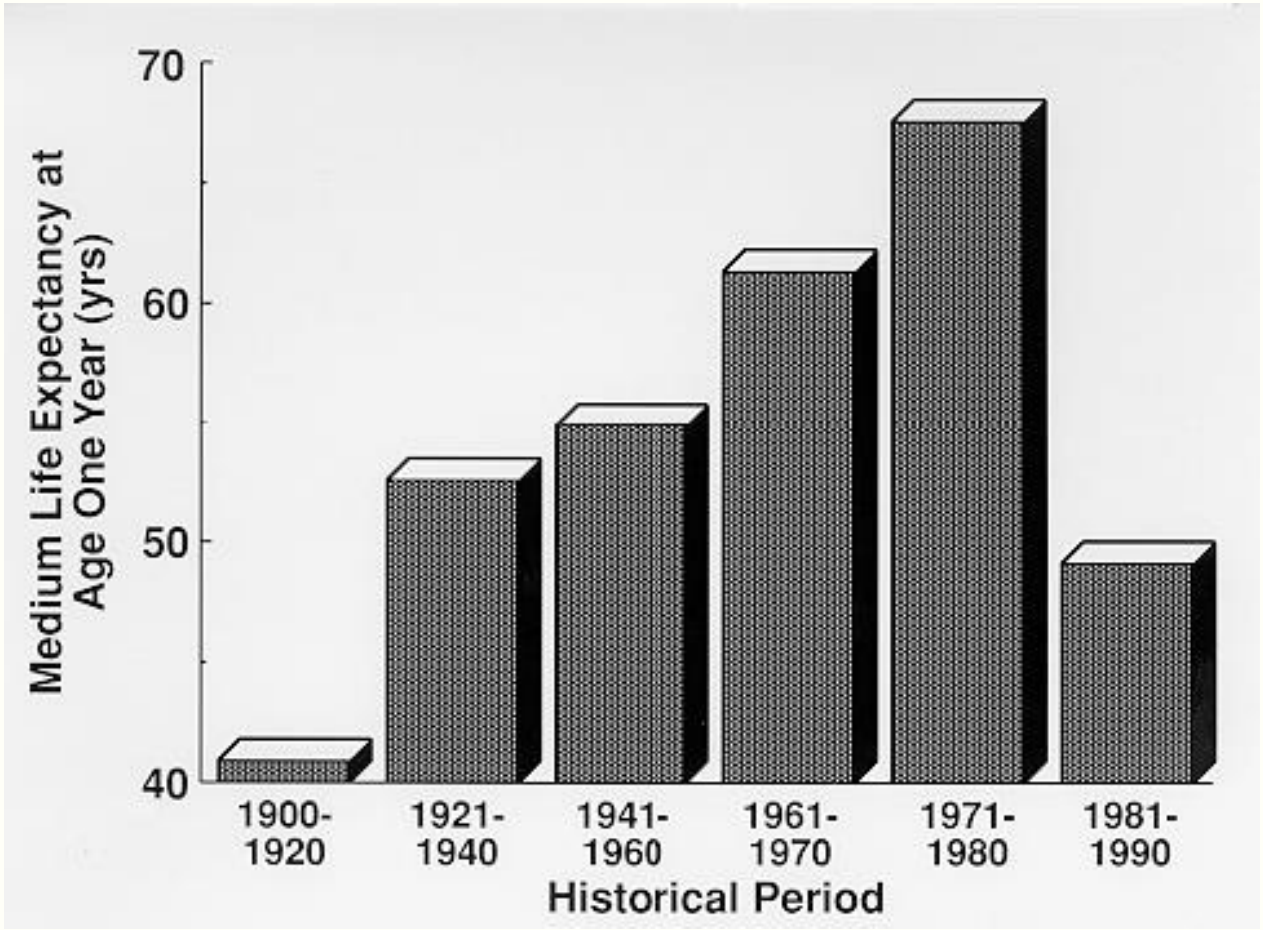


Infectious Complications

- Hepatitis A
- Hepatitis B
- Hepatitis C
- HIV

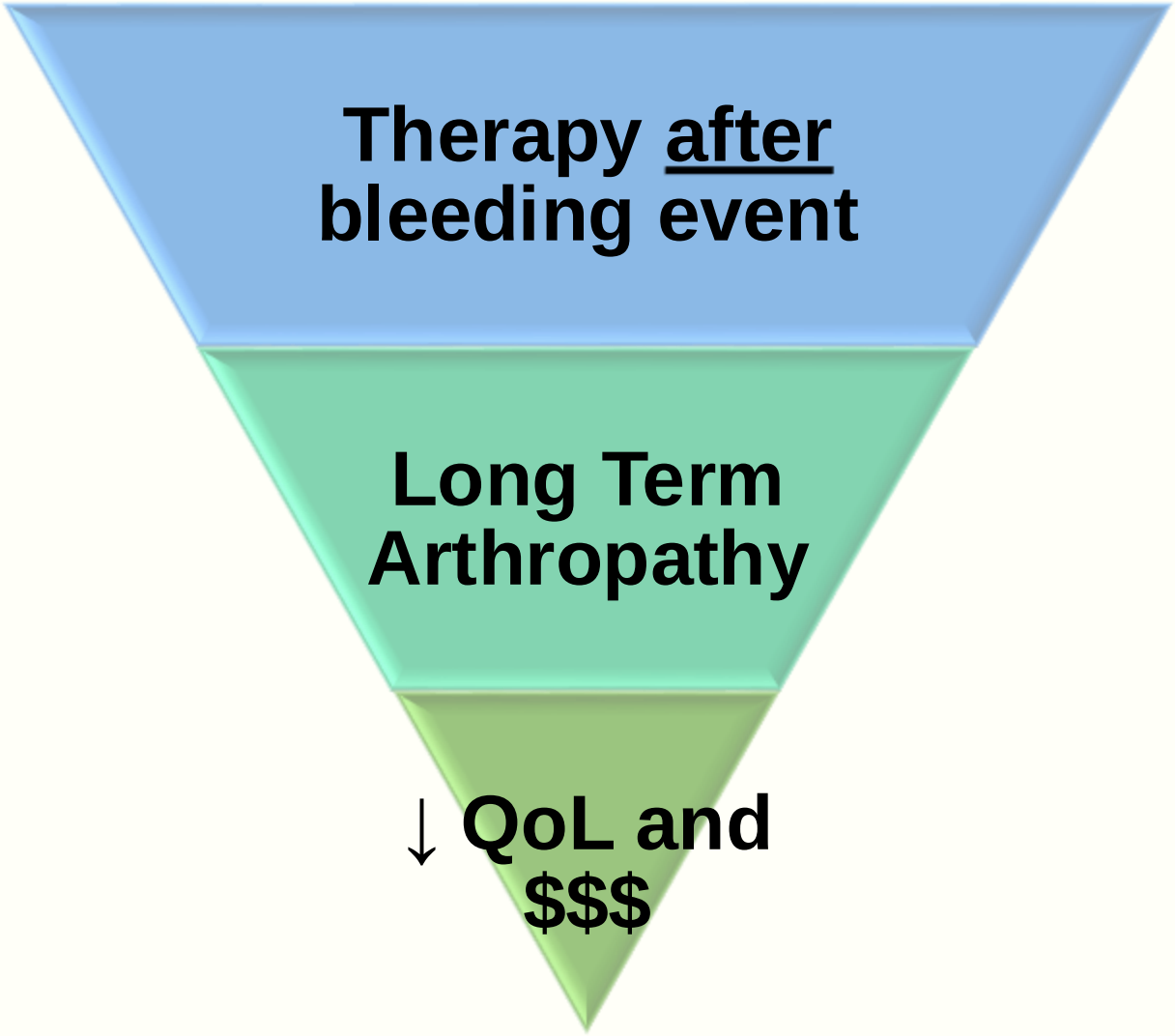
https://www.hemophiliafed.org/news-stories/2014/03/1980s-hemophilia-hivaids-hepatitis-c/

HIV Infection impact of hemophilia population



Jones and Ratnoff, 1991
http://www.niaid.nih.gov/topics/hivaids.

Treatment- On Demand



Joint Outcome Study: Prophylaxis Randomized Control Trial

The NEW ENGLAND JOURNAL of MEDICINE

ESTABLISHED IN 1812 AUGUST 9, 2007 VOL. 357 NO. 6

Prophylaxis versus Episodic Treatment to Prevent Joint Disease in Boys with Severe Hemophilia

Marilyn J. Manco-Johnson, M.D., Thomas C. Abshire, M.D., Amy D. Shapiro, M.D., Brenda Riske, M.S., M.B.A., M.P.A., Michele R. Hacker, Sc.D., Ray Kilcoyne, M.D., J. David Ingram, M.D., Michael L. Manco-Johnson, M.D., Sharon Funk, B.Sc., P.T., Linda Jacobson, B.S., Leonard A. Valentino, M.D., W. Keith Hoots, M.D., George R. Buchanan, M.D., Donna DiMichele, M.D., Michael Recht, M.D., Ph.D., Deborah Brown, M.D., Cindy Leissinger, M.D., Shirley Bleak, M.S.N., Alan Cohen, M.D., Prasad Mathew, M.D., Alison Matsunaga, M.D., Desiree Medeiros, M.D., Diane Nugent, M.D., Gregory A. Thomas, M.D., Alexis A. Thompson, M.D., Kevin McRedmond, M.D., J. Michael Soucie, Ph.D., Harlan Austin, Ph.D., and Bruce L. Evatt, M.D.

Manco-Johnson et al. NEJM (2007)

Prophylaxis prevents hemarthrosis

Table 2. Outcome Data.*

Variable	Prophylaxis (N = 32)	Enhanced Episodic Therapy (N = 33)	P Value
Mean	653±246	187±100	<0.001
Total	20,896	6,176	
Reported no. of factor VIII units infused			
Mean	352,793±150,454	113,237±65,494	<0.001
Total	11,289,372	3,736,807	
Joint hemorrhages (no./participant/yr)			
Mean	0.63±1.35	4.89±3.57	<0.001
Median	0.20	4.35	
Total hemorrhages (no./participant/yr)			
Mean	3.27±6.24	17.69±9.25	<0.001
Median	1.15	17.13	

* Plus–minus values are means ±SD. The data on MRI and radiographic findings include interim-analysis data for children who were removed from the study because of early joint failure.

Manco-Johnson et al. NEJM (2007)

Weak correlation of clinical bleeding with MRI joint damage

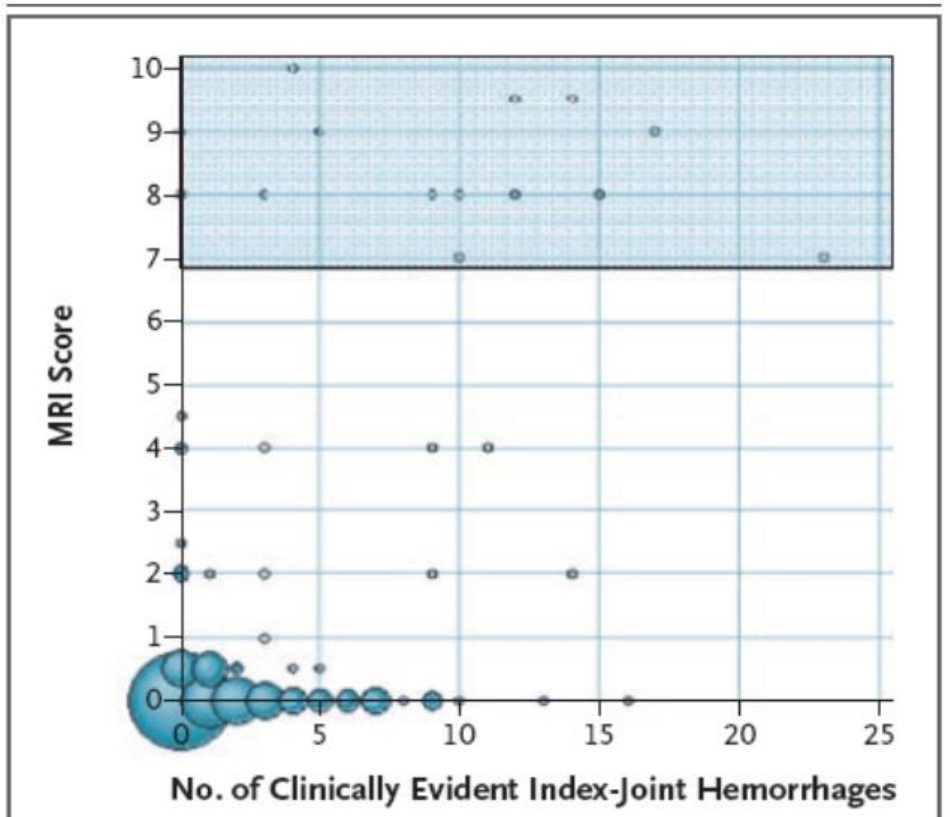
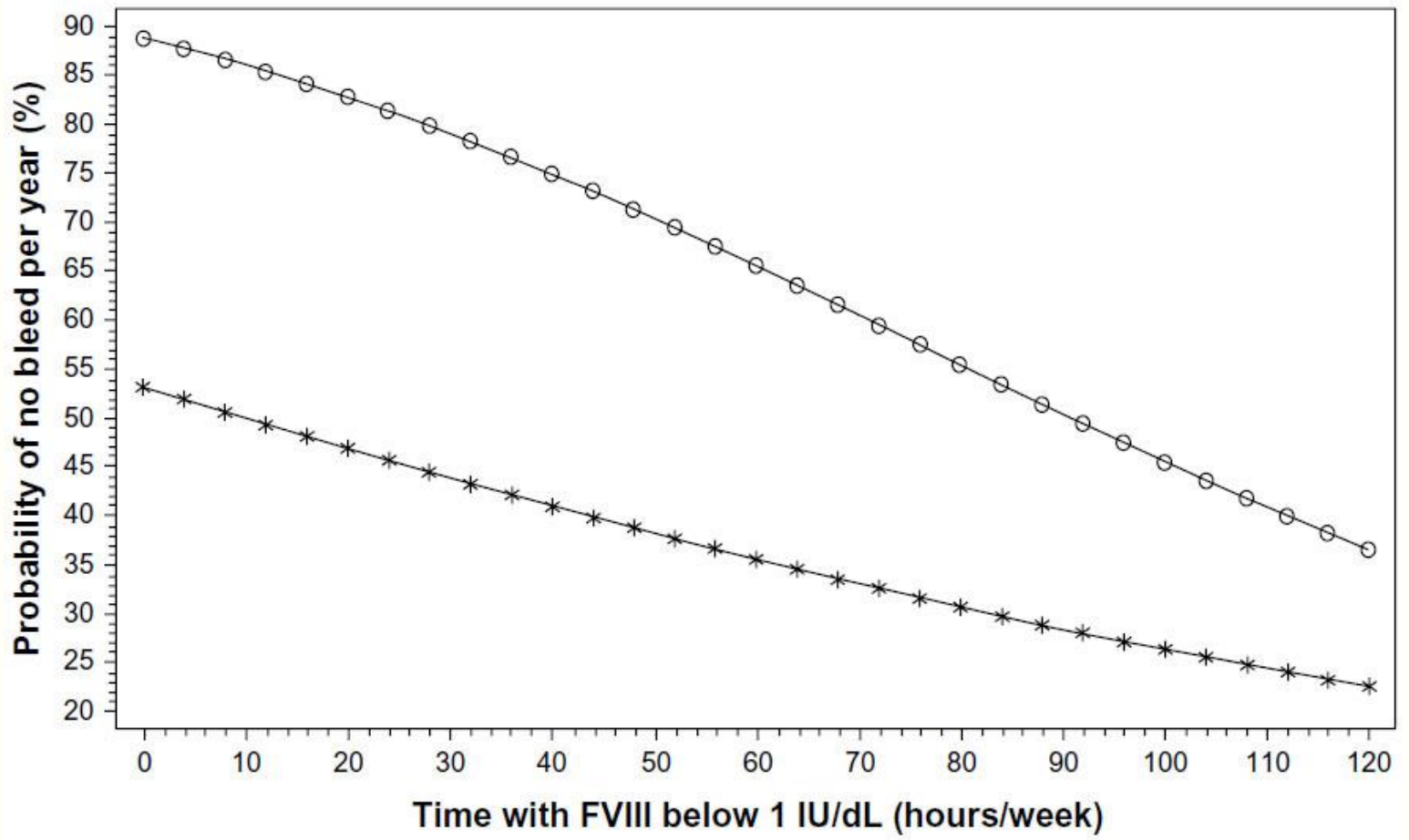


Figure 2. MRI Score for Index Joint According to the Number of Hemorrhages in That Joint for Both Treatment Groups.

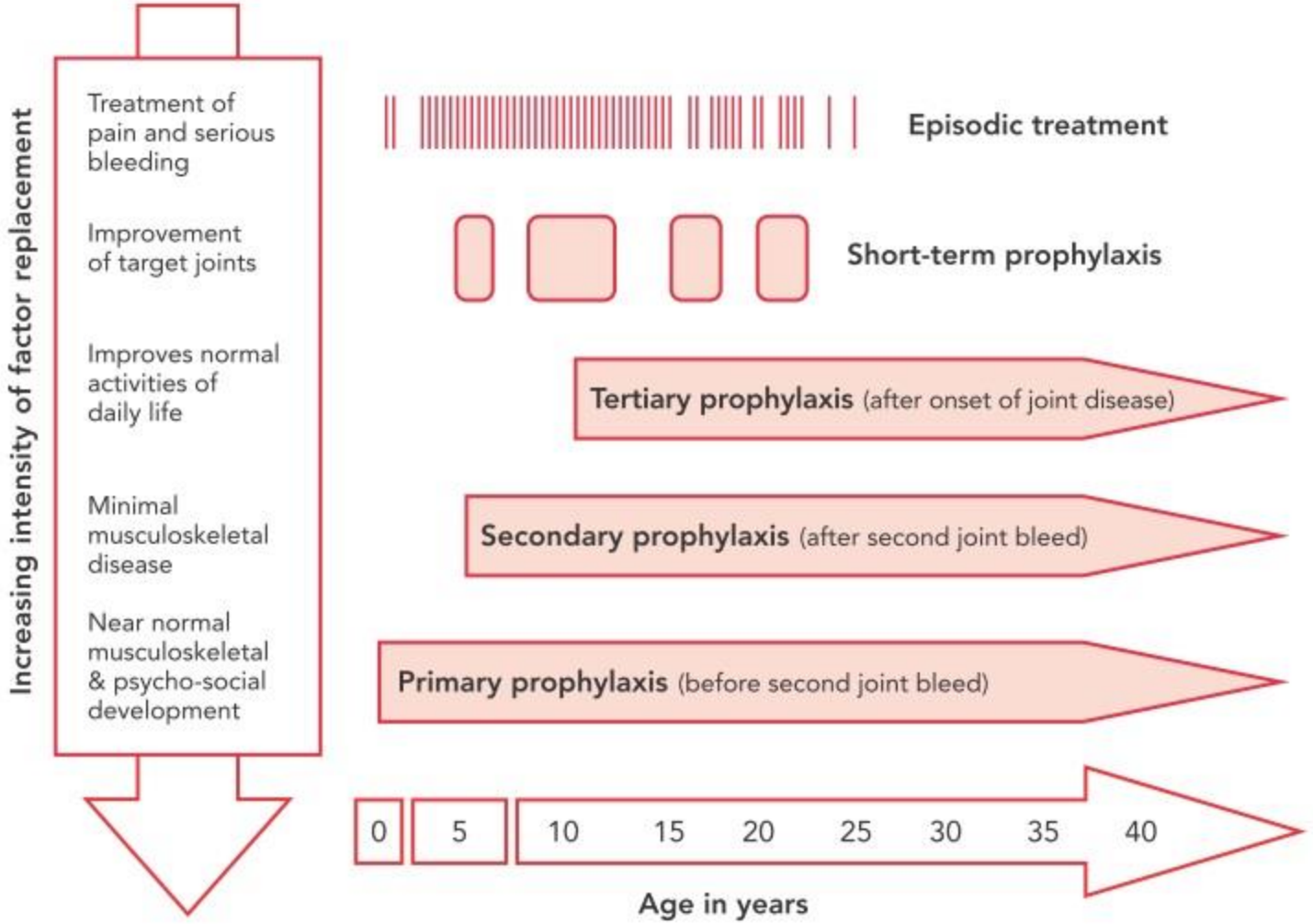
Manco-Johnson et al. NEJM (2007)

Time Below 1% → ↑ Risk of Bleeding



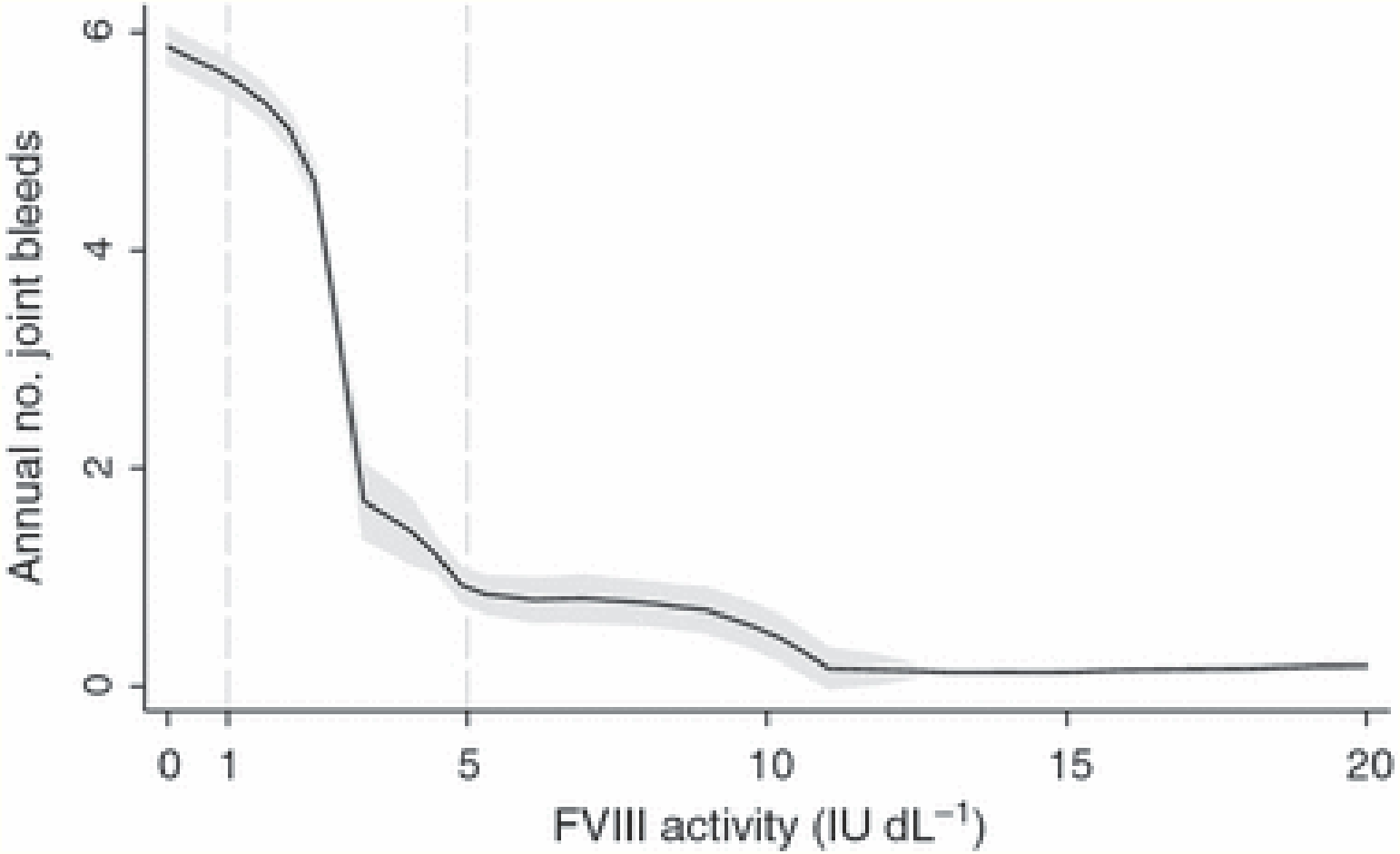
- Collins PW, et al *J Thromb Haemost*. 2009;7(3):413–420.[33] Copyright © International Society on Thrombosis and Haemostasis

Treatment- Prophylaxis



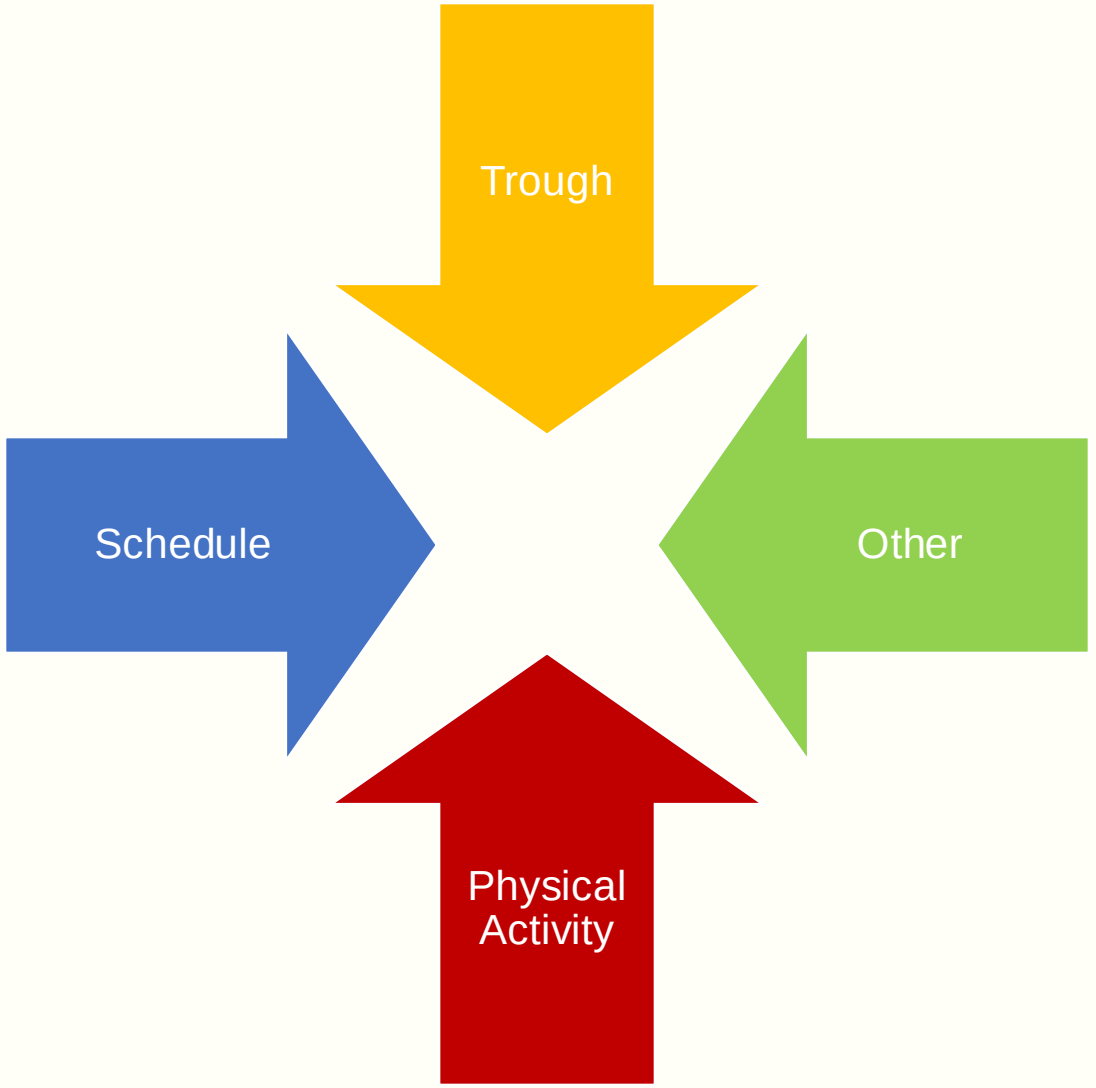
Adapted from Blood Transfus 2008 Sep;6 Suppl 2:s4-11

What is the ideal Target for Prophylaxis ?



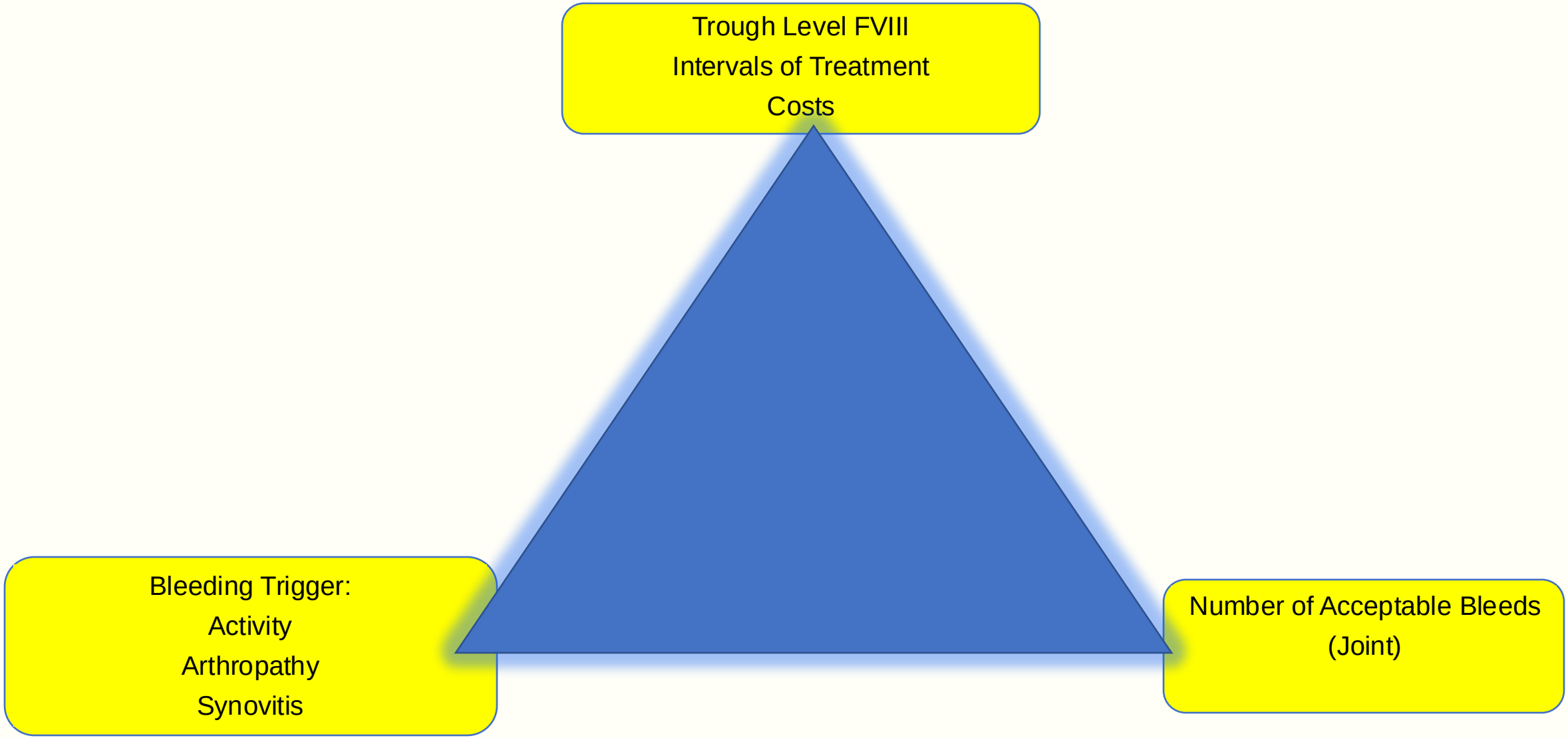
Haemophilia
Volume 17, Issue 6, pages 849-853, 5 MAY 2011 DOI: 10.1111/j.1365-2516.2011.02539.x
http://onlinelibrary.wiley.com/doi/10.1111/j.1365-2516.2011.02539.x/full#f2

Decision Making



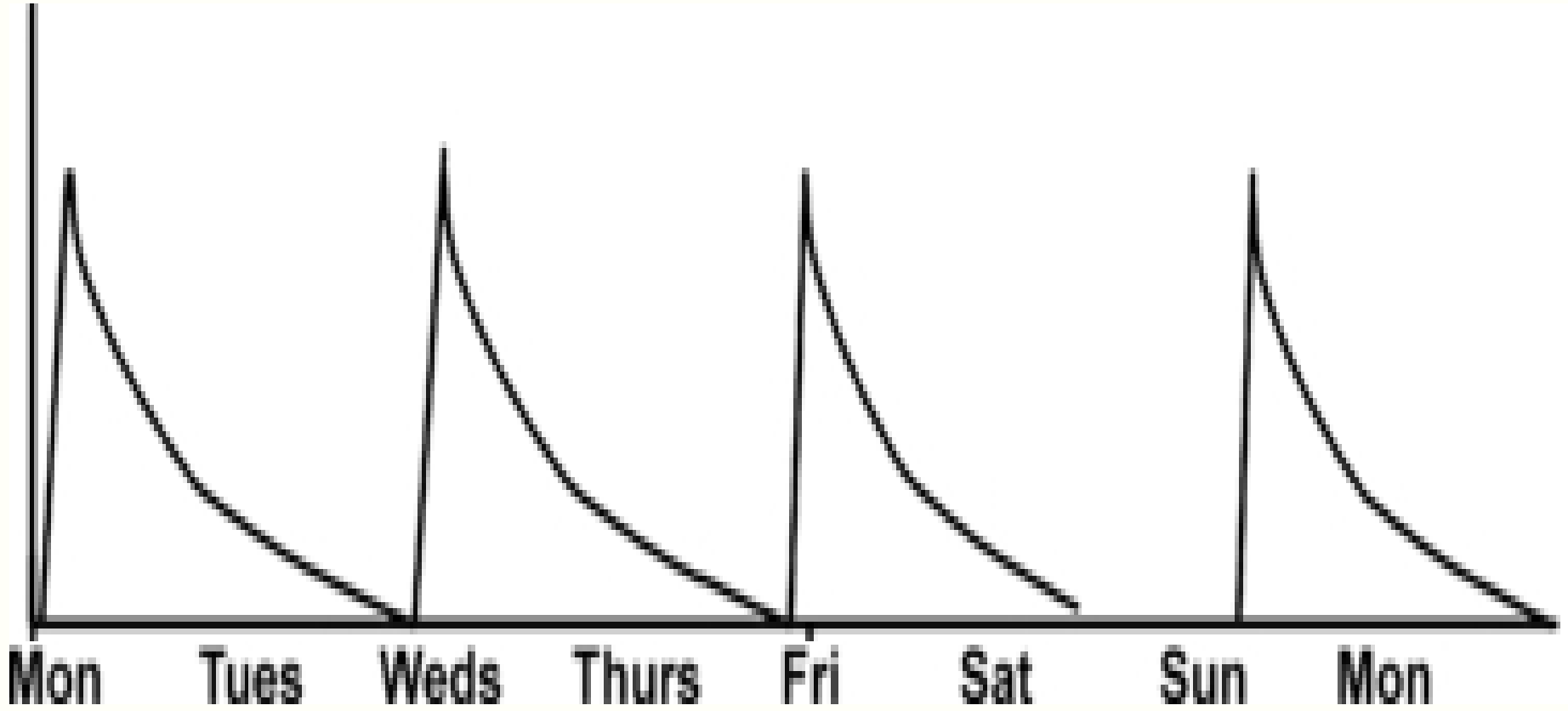
Cost
Delivery
Personal PK
Immunogenicity
Infectious Risk

Adherence



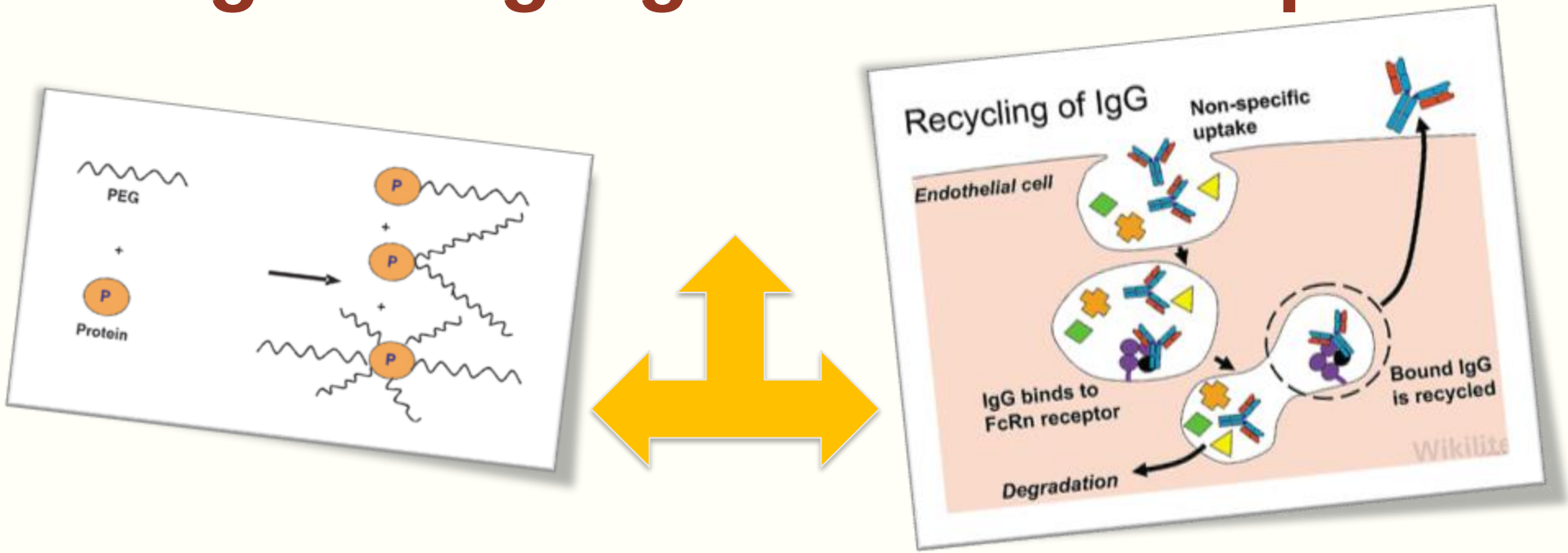
Oldenberg, J. Blood 2015

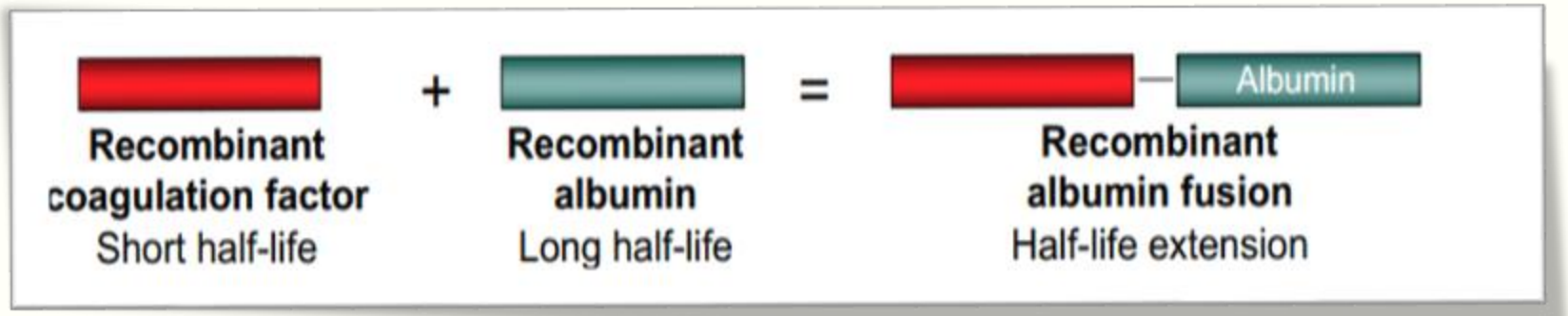
Personalized prophylaxis



Haemophilia
pages 131-135, 25 JUN 2012 DOI: 10.1111/j.1365-2516.2012.02838.x
http://onlinelibrary.wiley.com/doi/10.1111/j.1365-2516.2012.02838.x/full#f2

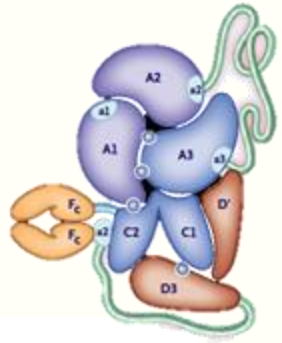
Long Acting Agents for Hemophilia

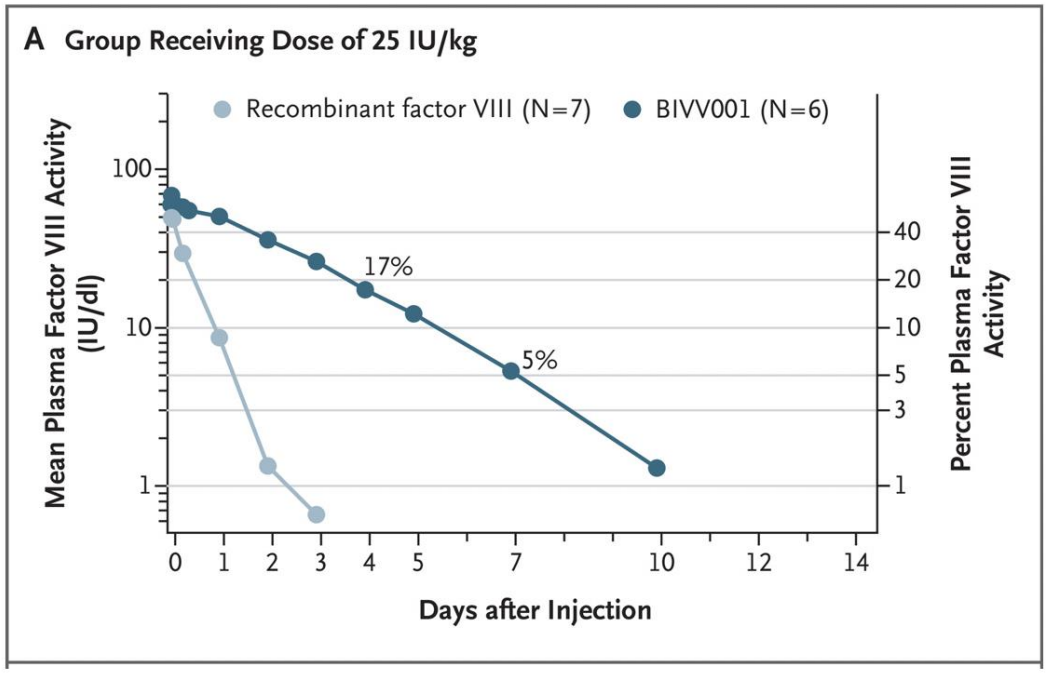


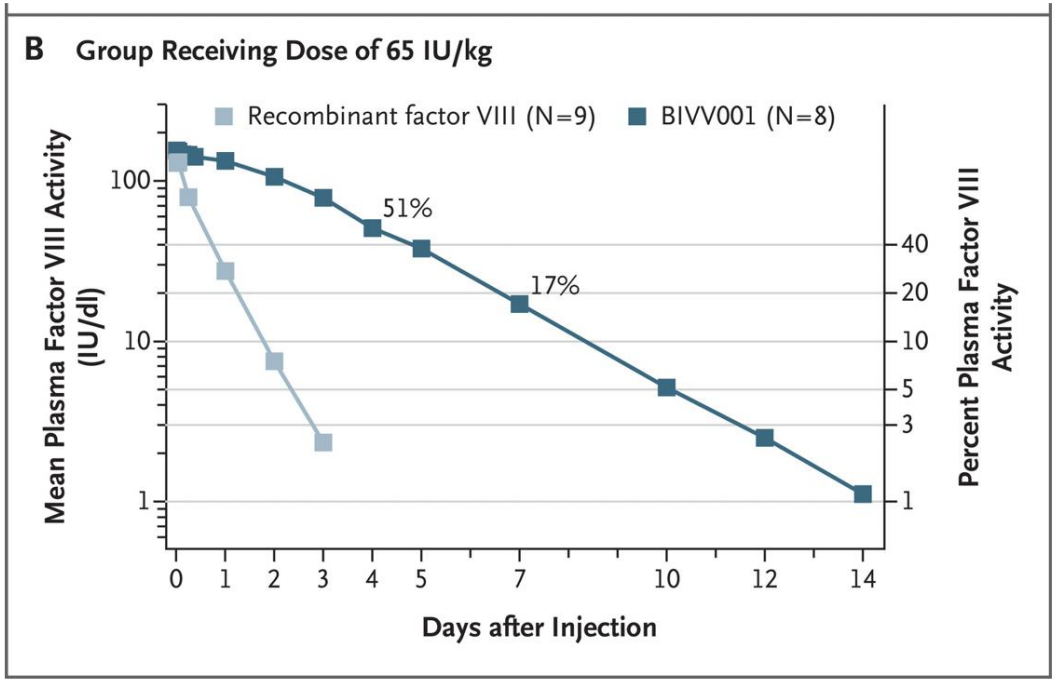


http://www.biopharminternational.com/biopharm/article/articleDetail.jsp?id=317577&sk=&date=&pageID=3
Hobbs, J. http://www.wikilite.com/wiki/index.php/File:Recycling_of_IgG_by_FcRn.jpg / http://www.transfusion.com.au

Efaefanesoctocog (rFVII Fc-VWF-XTEN) Extended Half Life $T_{1/2}$ = 42.5 hours

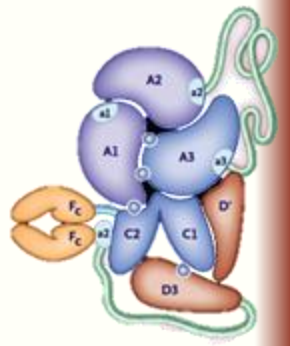


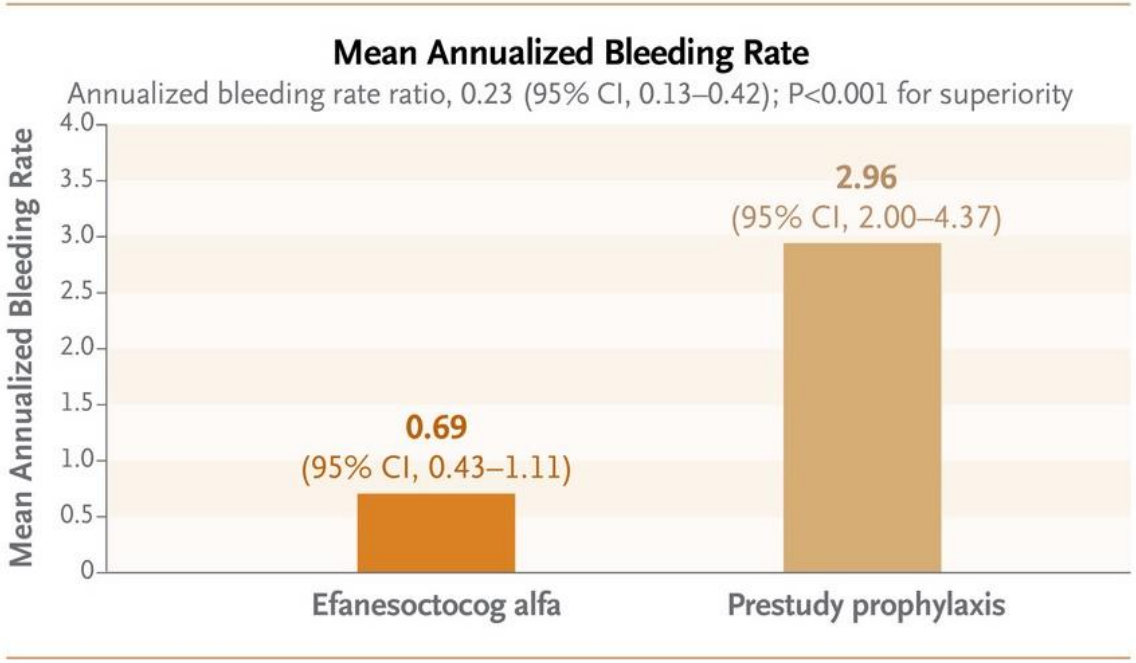


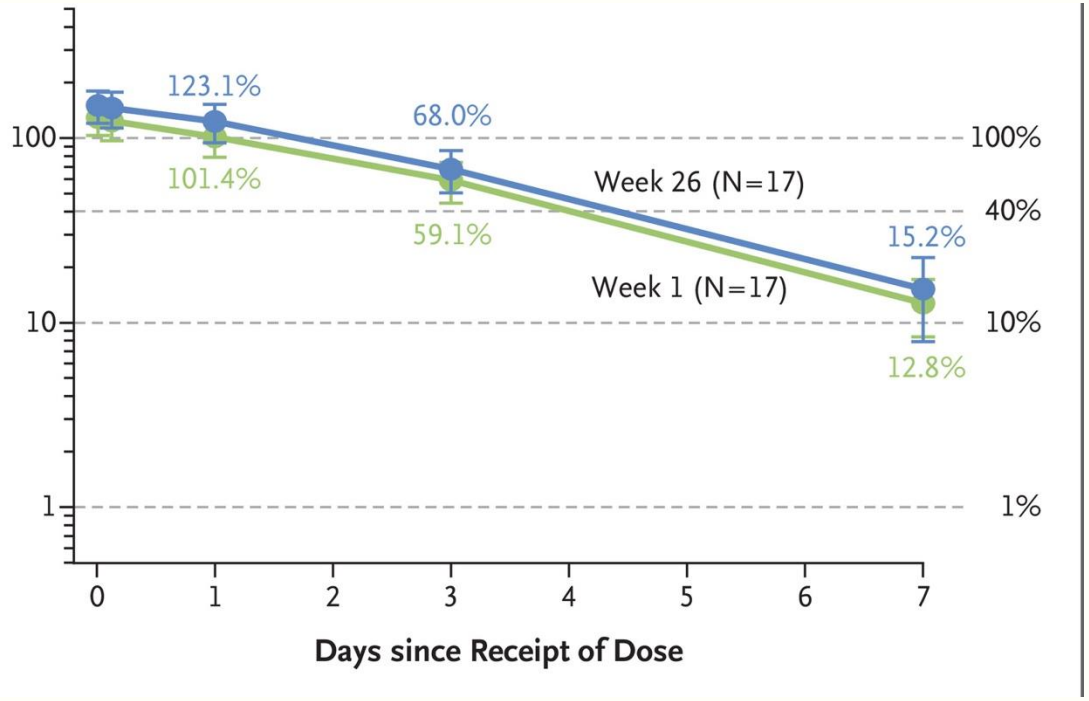


Konkle et al. NEJM 2020

Efanesoctocog Alpha



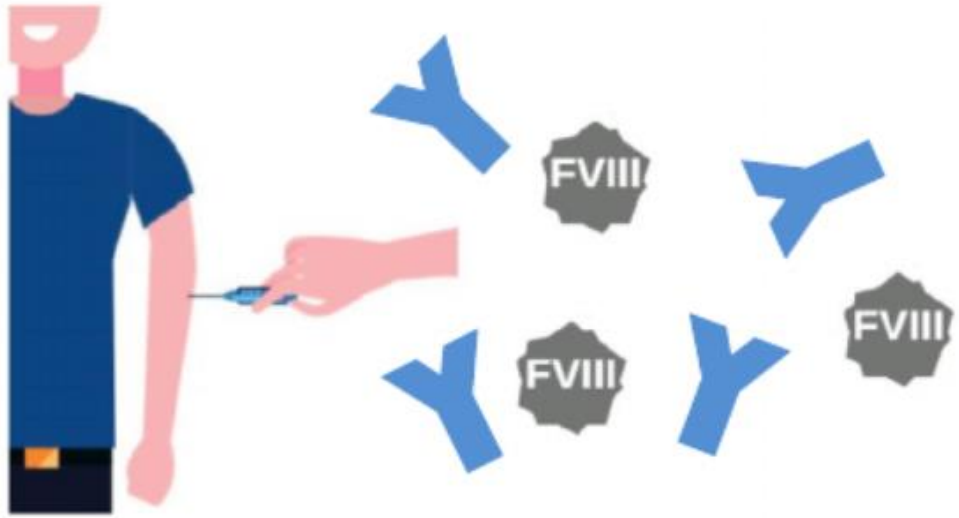




von Drygalski A et al. NJEM 2023

Inhibitors – Alloantibody

- 25 - 30% in severe Hemophilia A
- 3%-10% in Hemophilia B FVIII
  - *~ 25% with allergic reaction phenotype
- Poor Control of Bleeding
- High Cost, Morbidity and Mortality



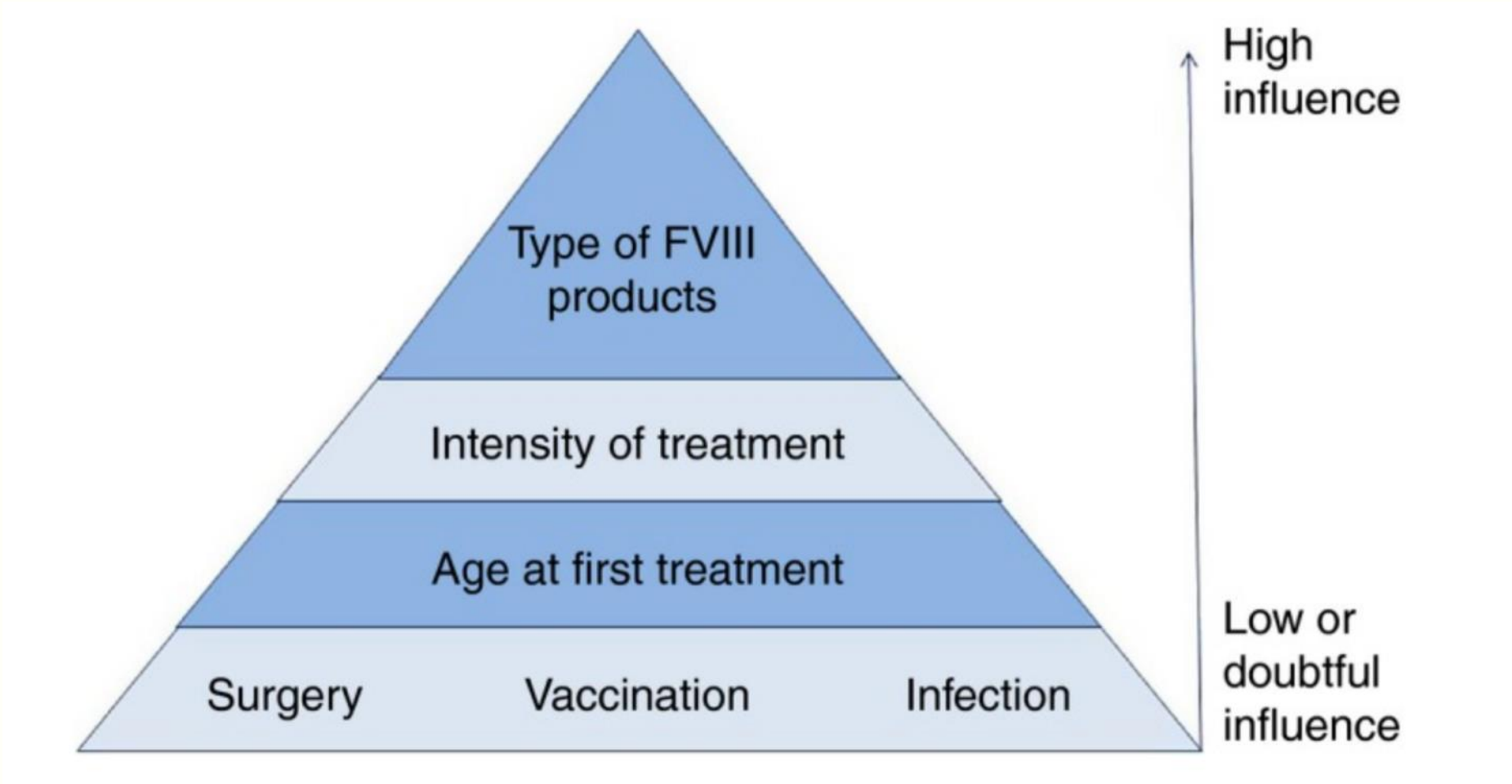
Jardim LL, et al, *Res Pract Thromb Haemost (2020)*
Katz et al. *Haemophilia* 1996;2:28–31.
Male et al Haematologica (2020)

Inhibitors

- High-titer inhibitor: >5 BU
- Low-titer inhibitor: <5 BU
- Transient inhibitor:
  - Persists for 6-8 months or less
  - Usually low titer

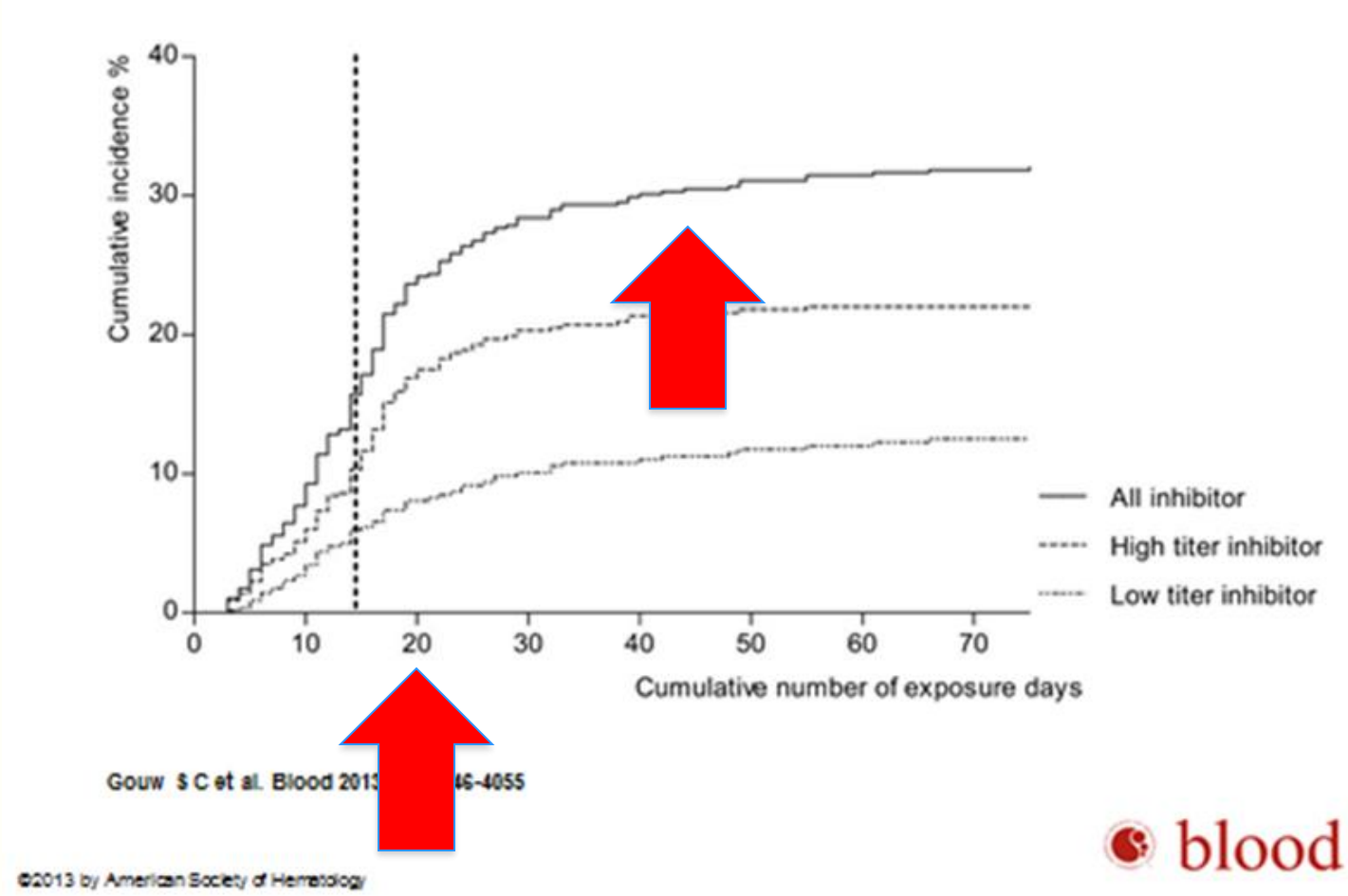
Jardim LL, et al, *Res Pract Thromb Haemost (2020)*

Risk Factors for Inhibitors Development



Peyvandi and Garagiola, Res Pract Thromb Haemost (2018)

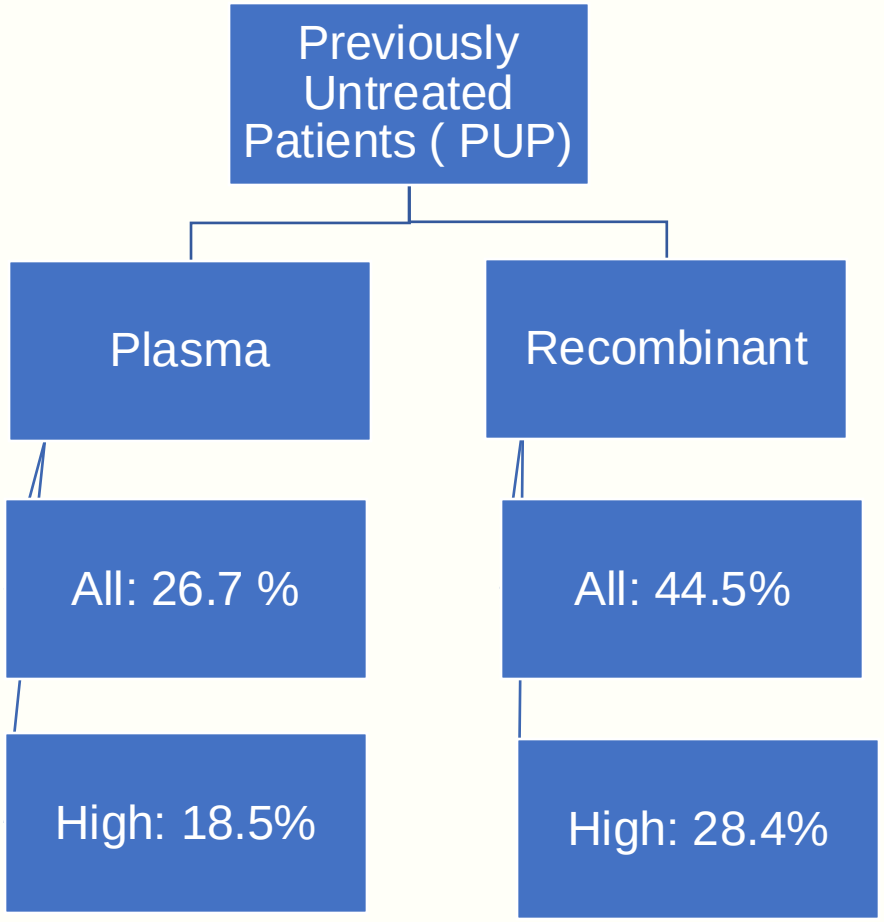
Inhibitors develop with median of 14.5 exposure days.

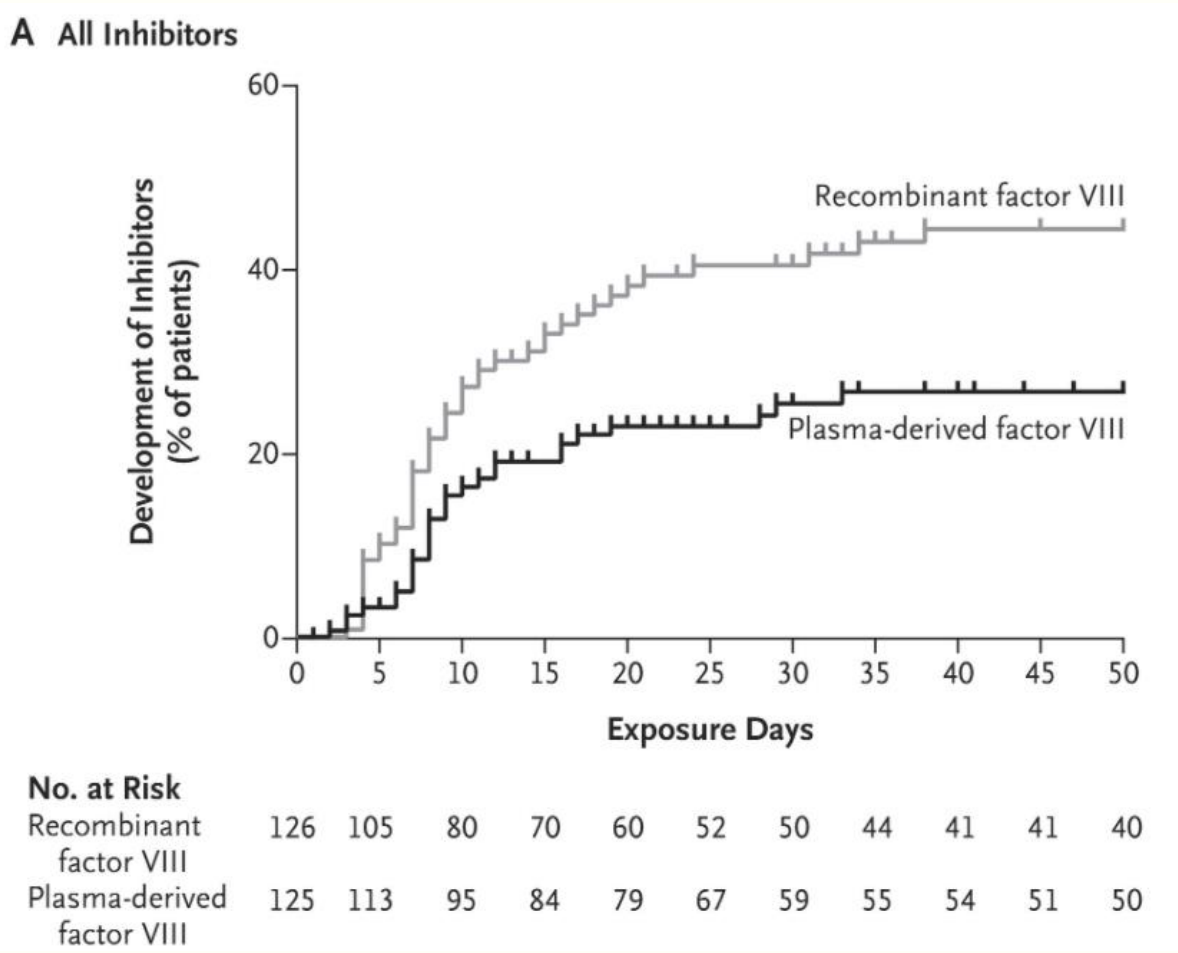


Gouw S C et al. Blood 2013 46-4055

blood

SIPPET STUDY
(Survey of Inhibitors in Plasma-Product Exposed Toddlers)





Peyvandi, F. et al NEJM (2016)

Immunogenicity of Inhibitors

Table 2. Characteristics of standard half-life (SHL) recombinant factor VIII products currently used for hemophilia A treatment.

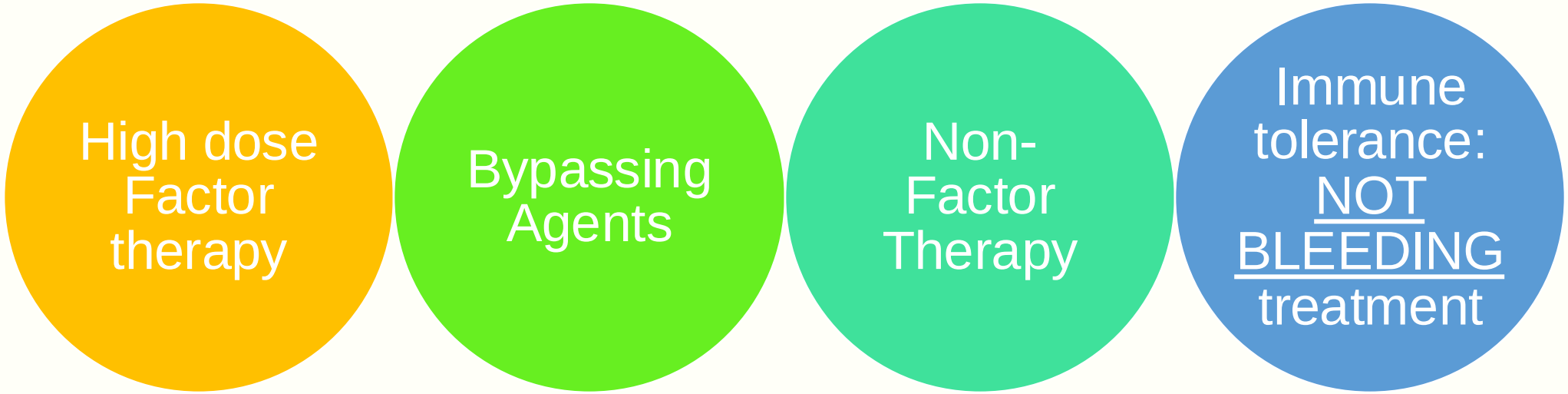
Product (Brand)	Company	Year of First Licensing	rFVIII Generation	Cell Line	Stabilizer	FVIII	Half-Life (Hours)	Immunogenicity PTPs (%)	Immunogenicity PUPs (%)	Ref.
Octocog alfa (Recombinate)	Takeda	1992	First	CHO	Human albumin	full-length	15	0.12 All inhibitors 0.06 HT inhibitors	23.9 All inhibitors 11.3 HT Inhibitors	[44–46]
Octocog alfa (Kogenate FS)	Bayer	1993	Second	BHK	Sucrose	full-length	11	No inhibitors	15–50.1 All inhibitors 9.8–31.6 HT inhibitor	[9,23,47]
Octocog alfa (Advate)	Takeda	2003	Third	CHO	Trehalose	full-length	9–12	0.92 All inhibitors	29.1–38 All inhibitors 12.7–26 HT inhibitors	[48–50]
Moroctocog alfa (Xyntha/ ReFacto AF)	Pfizer	2008	Third	CHO	Sucrose	B-domain deleted	8–11	1.47 All inhibitors	33 All inhibitors 14.5 HT inhibitors	[51,52]
Turoctocog alfa (Novoeight)	Novo Nordisk	2013	Third	CHO	Sucrose	B-domain truncated	11	No inhibitors	43.1 All inhibitors 27.6 HT inhibitors	[53,54]
Simoctocog alfa (Nuwiq)	Octapharma	2015	Fourth	HEK	Sucrose/ arginine	full-length	12–17	No inhibitors	26.7 All inhibitors 16.2 HT inhibitors	[36,55]
Octogog alfa (Kovaltry)	Bayer	2016	Third	BHK	Sucrose	full-length	12.2–14.2	0.93 All inhibitors	54.8 All inhibitors 40.5 HT inhibitors *	[56,57]
Lonoctocog alfa (Afstyla)	CSL Behring	2016	Third	CHO	Sucrose/ L-histidine,	B-domain truncated single chain	14.5	No inhibitors	52 All inhibitors 26 HT inhibitors **	[58]

Product (Brand)	Company	Year of First Licensing	Technology	Cell Line	FVIII	Half-Life (Hours)	Immunogenicity PTPs (%)	Immunogenicity PUPs (%)	Ref.
Efmoroctocog alfa (Elocta, Eloctate)	Sanofi	2014	IgG1-Fc-fusion	HEK	B-domain deleted	19 (OSA) 20.9 (CSA)	No inhibitor No anaphylaxis	31.1 All inhibitors 15.6 HT inhibitors No anaphylaxis	[66,67,77,78]
Rurioctocog alfa pegol (Adynovi, Adynovate)	Takeda	2015	Random PEGylation	CHO	full-length	14.3–16 (OSA)	No inhibitor No anaphylaxis	19.2 All inhibitors	[63,73,79]
Damoctocog alfa pegol (JIVI)	Bayer	2018	Site-specific PEGylation	BHK	B-domain deleted	19 (OSA) (>12 yo) 15–16 (OSA) (<12 yo)	No inhibitor 1.5 hypersensibility 3.7 anti-PEG Ab	NA	[64,72]
Turoctocog alfa pegol (N8-GP, Esperoct)	Novo Nordisk	2019	Site-specific glycoPEGylation	CHO	B-domain truncated	15.8–19.9 (CSA) (>12 yo) 13.2–14.2 (CSA) (<12 yo)	0.6 All inhibitors 12.3 anti-PEG Ab (>12 yo) 29.4 anti-PEG Ab (<12 yo)	29.9 All inhibitors 14.9 HT inhibitors No anaphylaxis	[65,71,80]

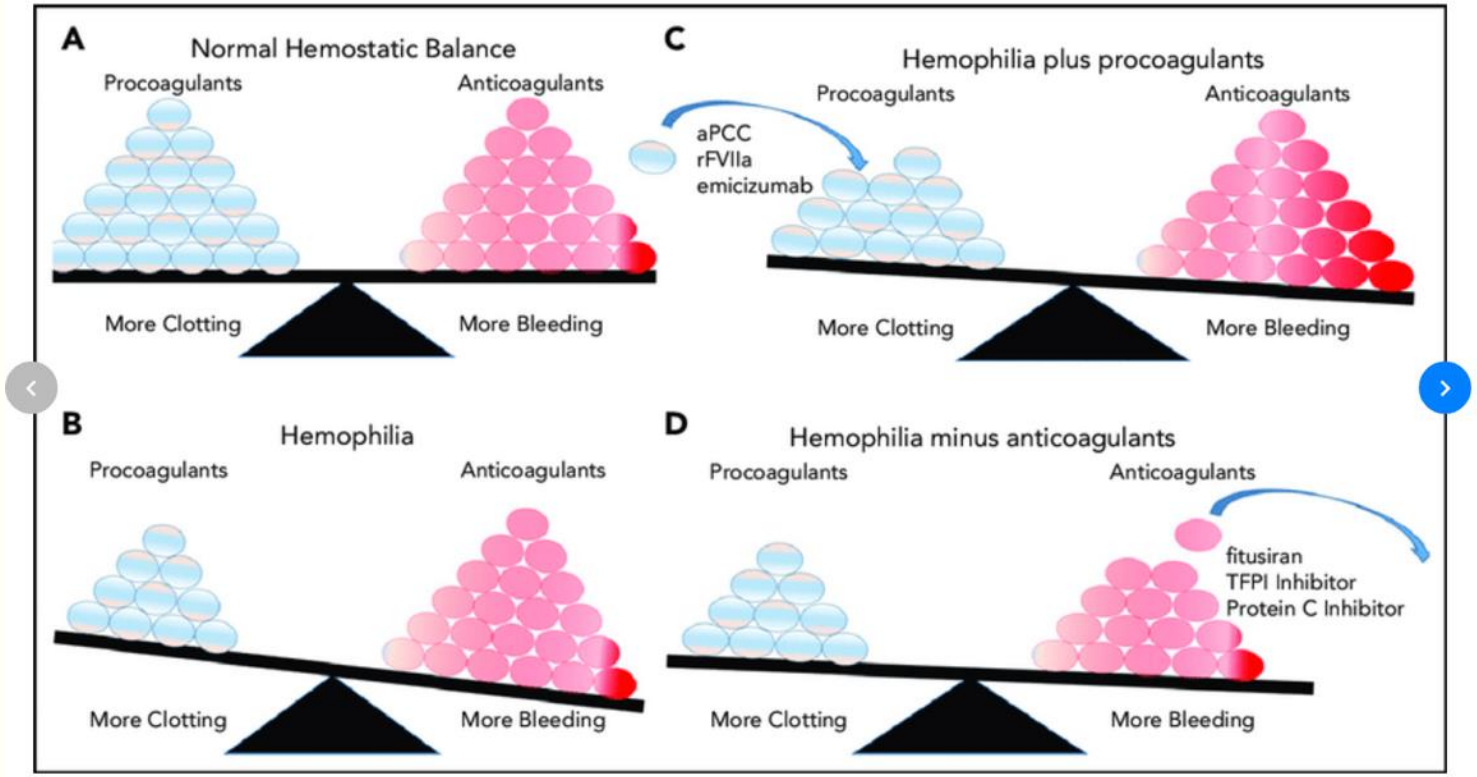
PTPs, previously treated patients; PUPs, previously untreated patients; FVIII, factor VIII; CHO, Chinese hamster ovary cell line, BHK, [illegible]; HEK, human embryonic kidney; OSA, one-stage clotting assay; CSA, chromogenic substrate assay; Ab, antibody; NA, not available; Ref., references.

Prezotti ANL, et al Pharmaceuticals (Basel). 2022
PMCID: PMC9331070.

Inhibitor Treatment Options



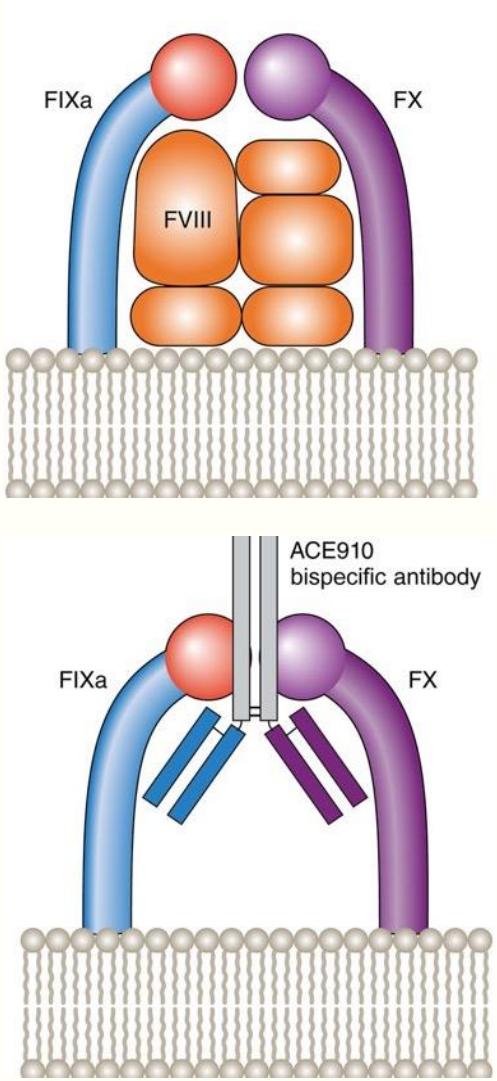
Rethink the approach



Mechanisms of novel hemophilia therapies. (A) Normal hemostatic balance tipped in favor of bleeding, for example, (B) in hemophilia A from lack of coagulation FVIII. (C) One approach to improve hemostatic balance in hemophilia is to add additional procoagulants; (D) another approach is to remove or inhibit anticoagulants. Adapted from Willyard. 64

Callaghan et al. Blood Advances (2018)

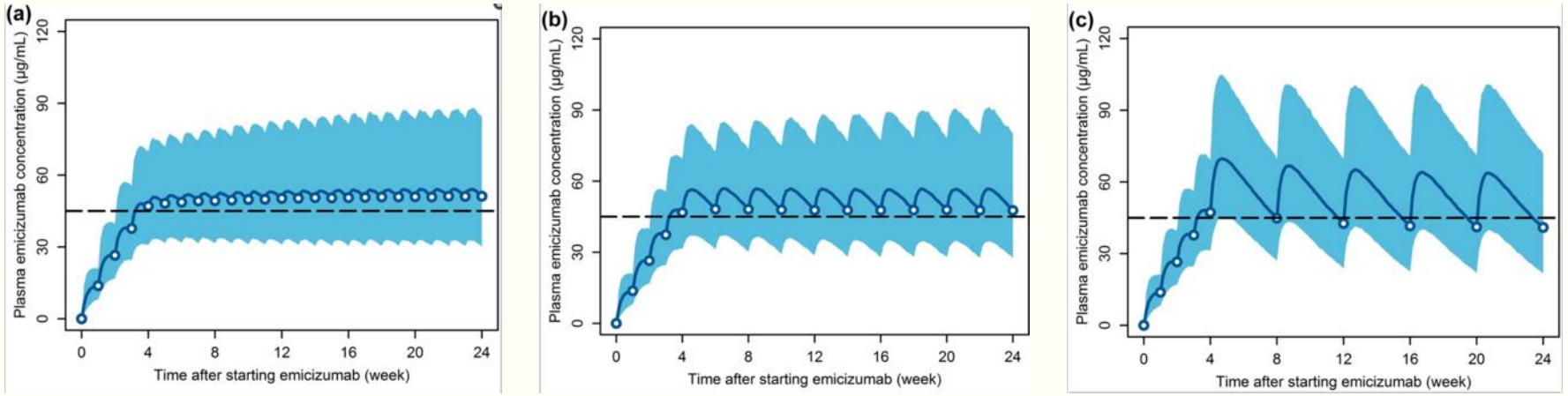
Non Factor Therapy



- Emicizumab (ACE-910)
- Humanized Bispecific Antibody
- Half Life ~ 3 weeks
- No structural homology to FVIII
- Hemophilia A with and without inhibitors
- Subcuatneous

Makris, Blood (2016)

Steady State Prevention of Bleeding

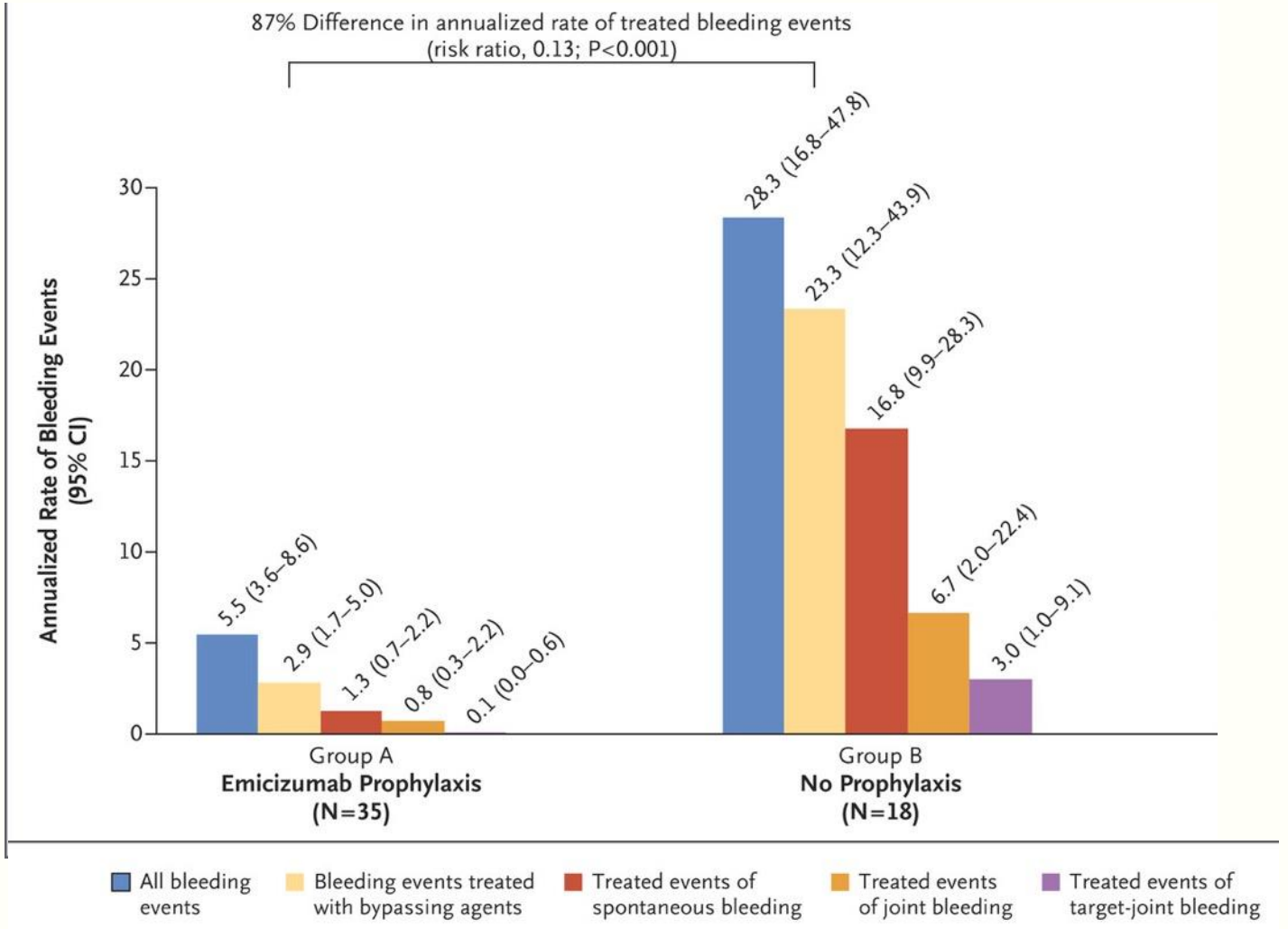


Weekly **q 2 weeks** **Monthly**

Yoneyama et al. Clinical Pharmacokinetics (2018)

HAVEN-1 :
Adult Inhibitor Patients

BLEEDING. ↓



Oldenburg J et al. N Engl J Med 2017;377:809-818.

Decrease Bleeding vs Prophylaxis HAVEN 3 (Non- Inhibitor Patients)

Table 2. Treated Bleeding Events in Participants Receiving Emicizumab Prophylaxis (Group D), as Compared with Events in the Same Participants during Prophylactic Factor VIII Treatment Previously in the Noninterventional Study.*

Variable	Group D in Current Trial: Emicizumab Prophylaxis (N=48)	Noninterventional Study: Factor VIII Prophylaxis (N=48)
Median duration of efficacy period (range) — wk†	33.7 (20.1–48.6)	30.1 (5.0–45.1)
Annualized rate of bleeding events, model-based (95% CI)‡	1.5 (1.0–2.3)	4.8 (3.2–7.1)
Rate ratio vs. control (95% CI)	0.32 (0.20–0.51)	—
Percent difference vs. control	−68§	—
Median annualized rate of bleeding events (IQR)	0.0 (0.0–2.1)	1.8 (0.0–7.6)
Percent of participants with 0 bleeding events (95% CI)	54 (39–69)	40 (26–55)
Percent of participants with 0–3 bleeding events (95% CI)	92 (80–98)	73 (58–85)

Emicizumab Clinical Data

Study, year[ref]	Study design	Study population	Dosing	Main results	
				Efficacy	*Safety*
HAVEN 1, 2017[26]	Phase III randomised open-label	109 (adolescent and adult haemophilia A with inhibitors)	Loading dose: 3 mg/kg/week for 4 weeks Maintenance dose: 1.5 mg/kg/week	Emicizumab prophylaxis *vs* no prophylaxis resulted in an 87% reduction of ABR	5 SAEs (3 thrombotic microangiopathies and 2 thromboses)
HAVEN 2, 2017[27]	Phase III non-randomised open-label	60 (paediatric haemophilia A with inhibitors)	Loading dose: 3 mg/kg/week for 4 weeks Maintenance dose: 1.5 mg/kg/week, or 3 mg/kg every 2 weeks, or 6 mg/kg every 4 weeks	Emicizumab prophylaxis *vs* no prophylaxis resulted in a 99% reduction of ABR	No thrombotic events
HAVEN 3, 2018[28]	Phase III randomised open-label	152 (adolescent and adult haemophilia A without inhibitors)	Loading dose: 3 mg/kg/week for 4 weeks Maintenance dose: 1.5 mg/kg/week, or 3 mg/kg every 2 weeks	96% and 97% reduction in ABR in the two emicizumab arms, respectively, compared to episodic FVIII therapy	No major safety issues
HAVEN 4, 2017[29]	Phase III non-randomised open-label	48 (adolescent and adult haemophilia A with or without inhibitors)	Loading dose: 3 mg/kg/week for 4 weeks Maintenance dose: 6 mg/kg every 4 weeks	Efficacy results similar to HAVEN 1, 2, and 3	No major safety issues

ABR: annualised bleeding rate; SAEs: serious adverse events; FVIII: exogenous factor VIII.

Franchini M, Marano G, Pati I, et al. *Blood Transfus*. 201

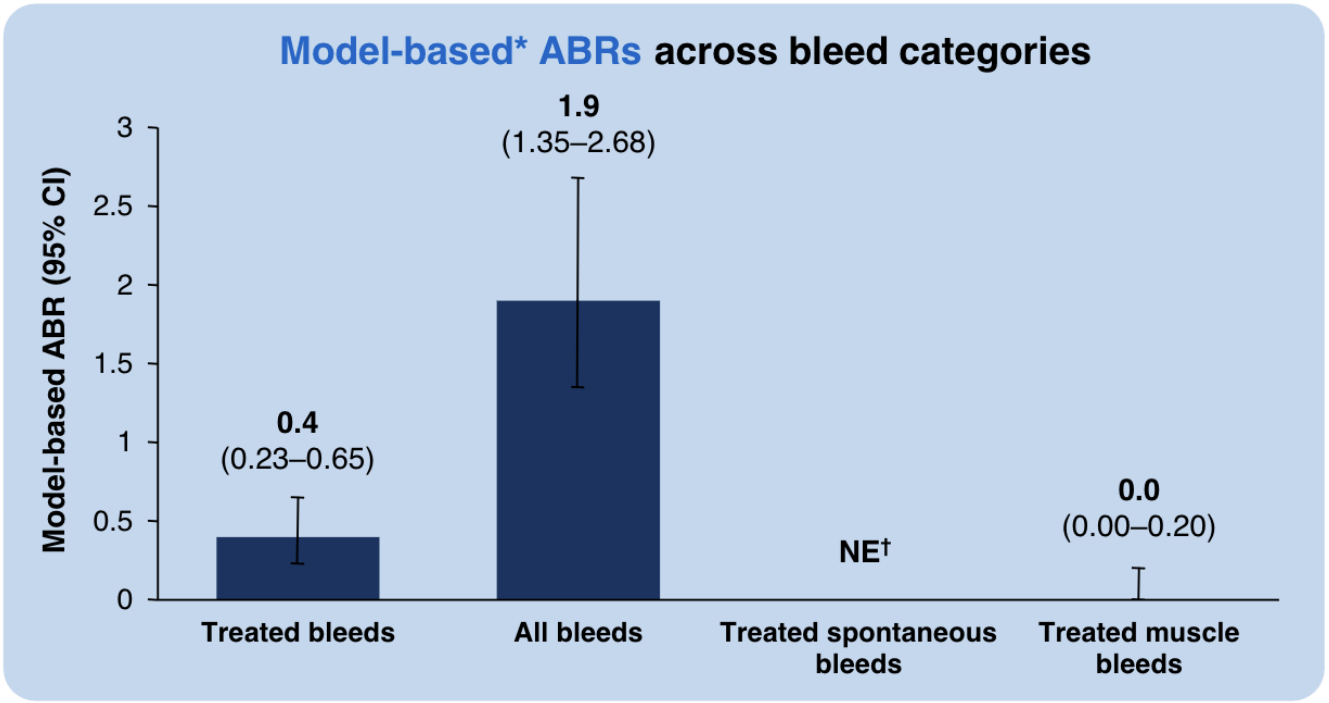
Pediatric Emicizumab Clinical Data

	HAVEN2[32]	HAVEN2[32]	HAVEN2[32]	Barg et al.[37]	Catarino et al.[38]	Batsuli et al.[47]	HOHOEMI[33]	HOHOEMI[33]
	QW	Q2W	Q4W	QW	QW	QW/ Q2W	Q2W	Q4W
Patients, n	68	10	10	11	7		6	7
FVIII-inhibitors	Yes	Yes	Yes	Yes	Yes	Yes	No	No
Current ITI	No	No	No	No	No	Yes	No	No
Median age (range)	6 years (1–15)	8 years (2–10)	9 years (2–11)	26 months (2–80)	(3 months - 27 years)	2 years (1.7–12)	6.6 years (1.5–10.7)	4.1 years (0.3–8.1)
Median follow-up (range)	57.6 weeks (17.9–92.6)	21.3 weeks (18.6–24.1)	19.9 weeks (8.9–24.1)	36 weeks (22–58)	(3–13 months)	35 weeks (21–40)	39.9 weeks (37.9–41.4)	34.1 weeks (24.1–37.1)
Treated ABR* (95% CI)	0.3 (0.17; 0.5)	0.3 (0.0; 1.7)	2.2 (0.7; 6.8)	NA	NA	NA	1.3 (0.6; 2.9)	0.7 (0.2; 2.6)
% of zero treated bleeds	77	90	60	63	86	43	33	71

Notes: QW: 3 mg/kg/week loading dose and 1.5 mg/kg/week maintenance dose; Q2W: 3 mg/kg/week loading dose and 3 mg/kg every 2 weeks; Q4W: 3 mg/kg/week loading dose and 6 mg/kg every 4 weeks; *model-based ABR estimated by use of binomial regression model.
Abbreviations: CI, confidence interval; NA, non applicable.

Le Quellec, S., 2020. Clinical Evidence and Safety Profile of Emicizumab for the Management of Children with Hemophilia A. Drug Design Development and Therapy.. doi:10.2147/dddt.s167731

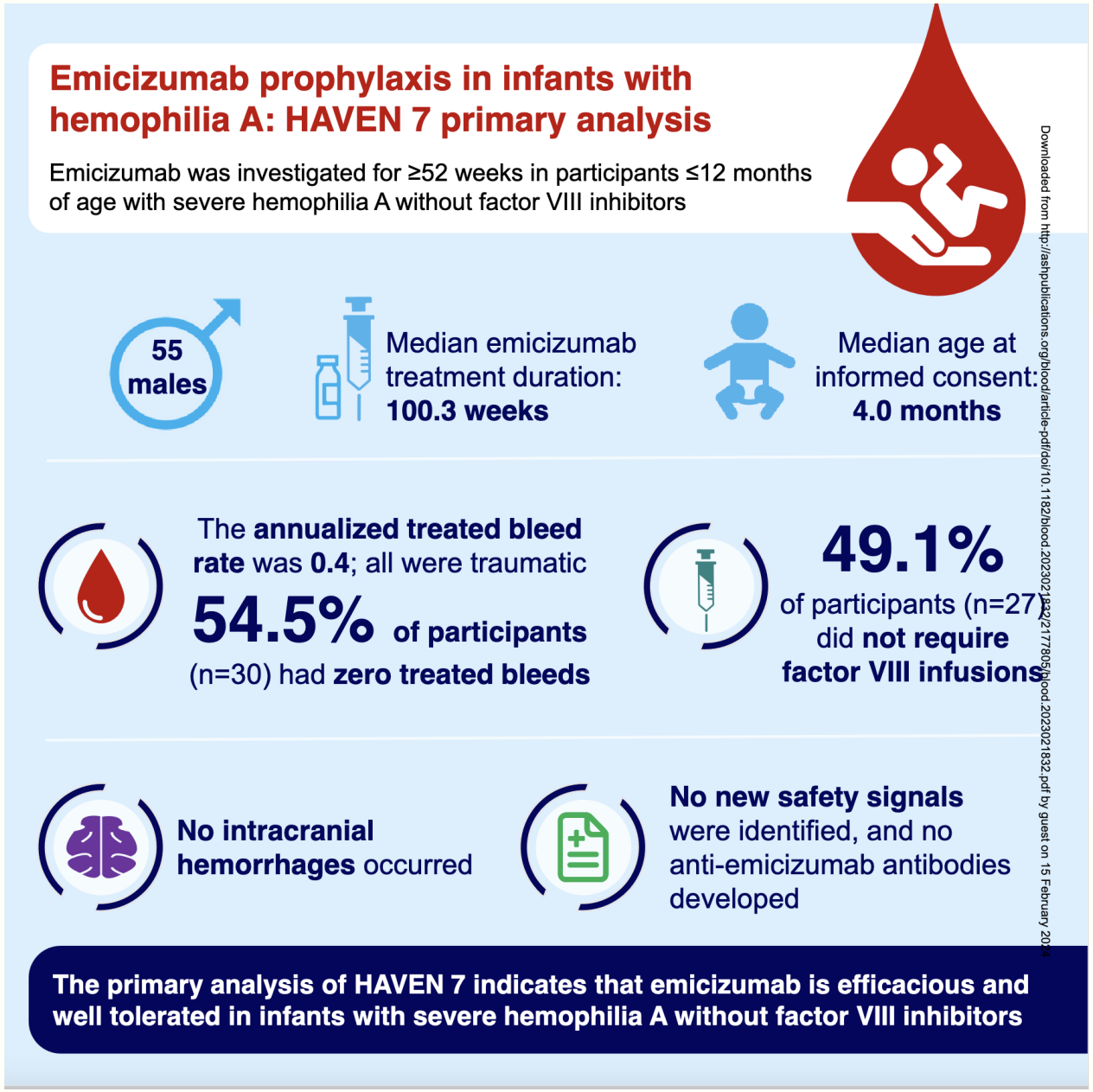
Pediatric Data – HAVEN 7



	Emicizumab (N=54)
Participants with ≥1 AE, n (%)	50 (92.6)
Total number of AEs, n	314
Total number of deaths, n	0
Withdrawal due to AE, n	0
Total number of participants with ≥1	
AE with fatal outcome, n	0
SAE*, n (%) [events]	8 (14.8) [12]
Related AE, n (%) [events]	9 (16.7) [23]
Grade ≥3 AE, n (%) [events]	12 (22.2) [16]
AEs of special interest, n	
Systemic hypersensitivity reactions and anaphylactic / anaphylactoid reactions	0
TEs and hypercoagulation	0
Microangiopathic haemolytic anaemia or TMA	0

Pipe et al. EHAD 2023
Pipe at al. ASH 2022

HAVEN -7

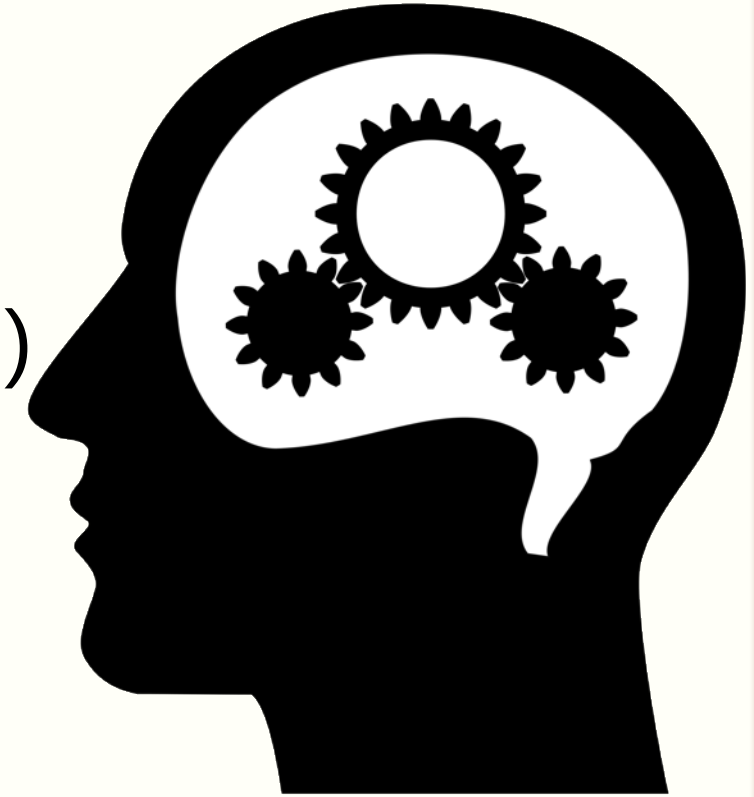


No participant in HAVEN 7 had tested positive for ADAs at CCOD. This reflects the low immunogenicity rate for emicizumab reported in a pooled analysis of the phase 3 clinical trials HAVEN 1–5, HOHOEMI, and STASEY, across which 5.1% of participants developed ADAs, including 0.6% for whom ADAs were associated with a decrease in emicizumab exposure.[35] In HAVEN 7, 24 participants were tested for FVIII inhibitors following at least three EDs or two consecutive doses of FVIII; two participants (3.6% of the trial population; 8.3% of those tested), both PUPs, tested positive for confirmed *de novo* FVIII inhibitors. As approximately half of the trial population (28/55) received FVIII treatment on study (with a median of one ED), and only 24/55 were tested for FVIII inhibitors, many participants are still in the ED risk period for inhibitor development. The long-term follow-up will provide further data on the impact of emicizumab on rate and timing of FVIII inhibitor development.

Pipe et al. Blood Advances 2023

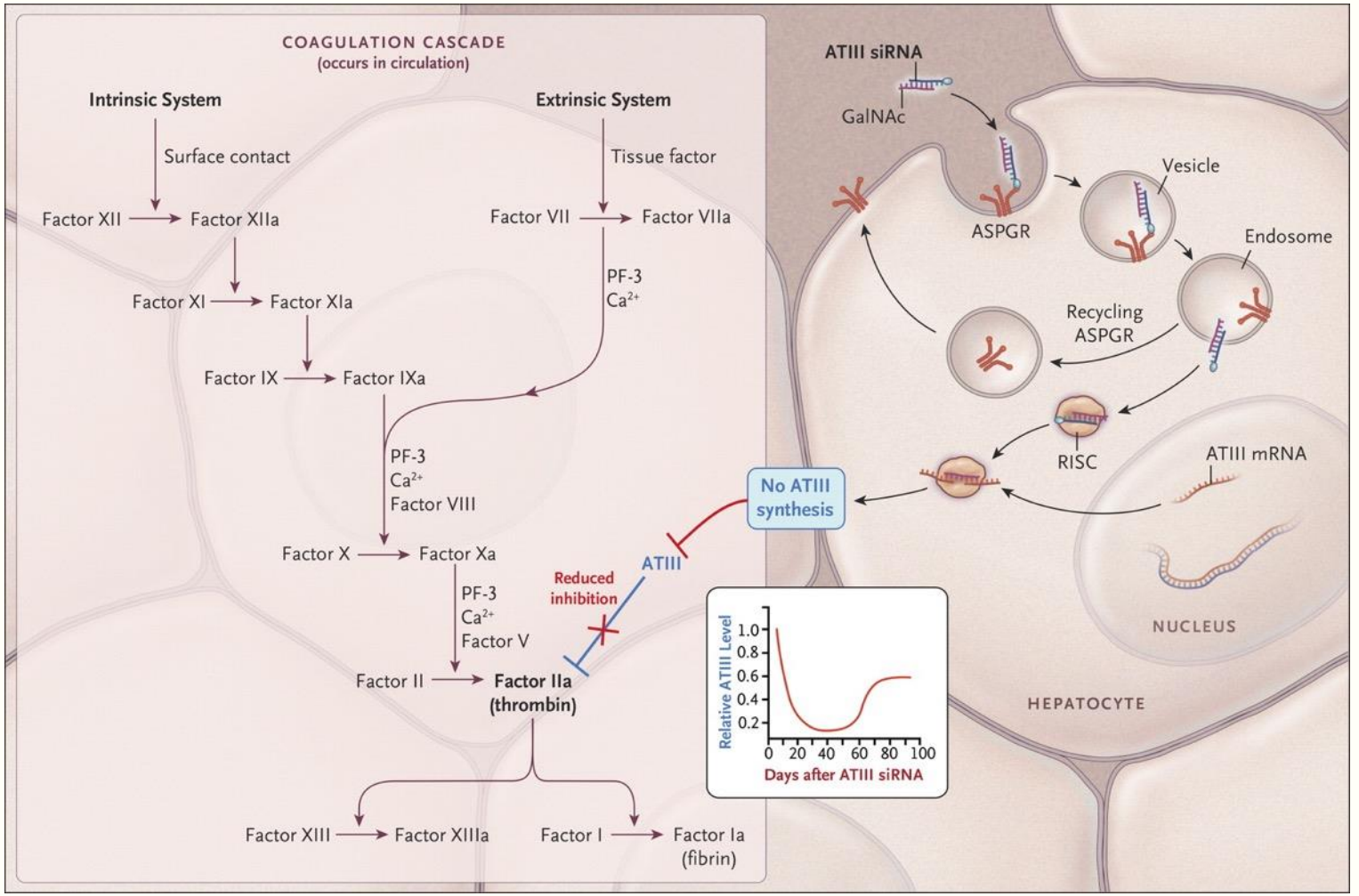
New Challenges

- Treatment of Acute Bleeding Events
- Surgical Procedures
- Risks of Inhibitor Development
- Role of Immune Tolerance Induction (ITI)



https://www.goodfreephotos.com/

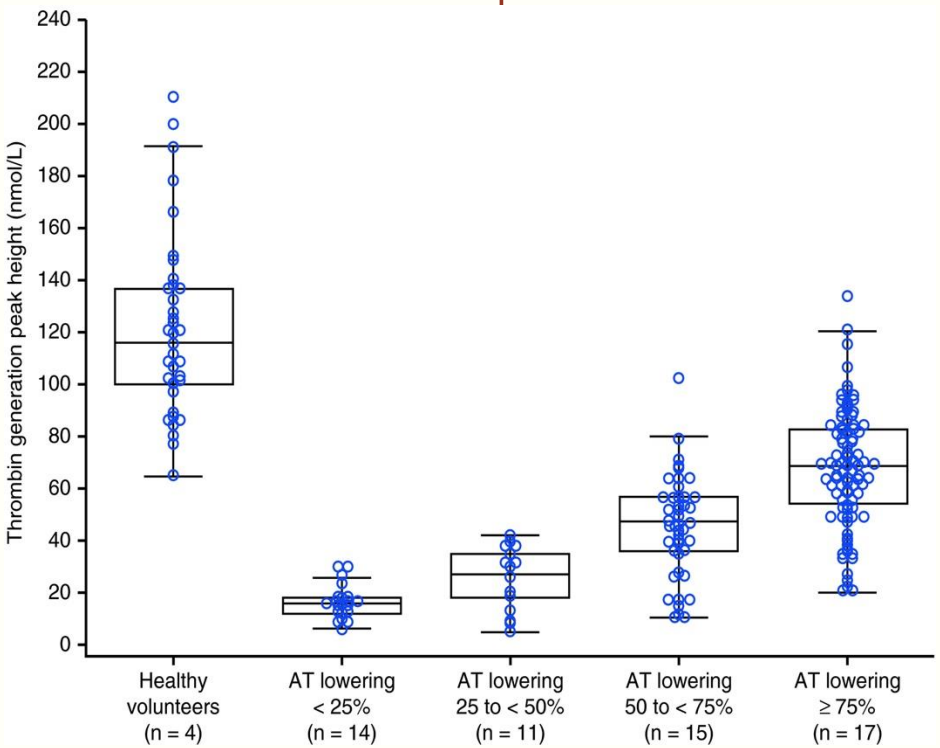
Antithrombin Modulation



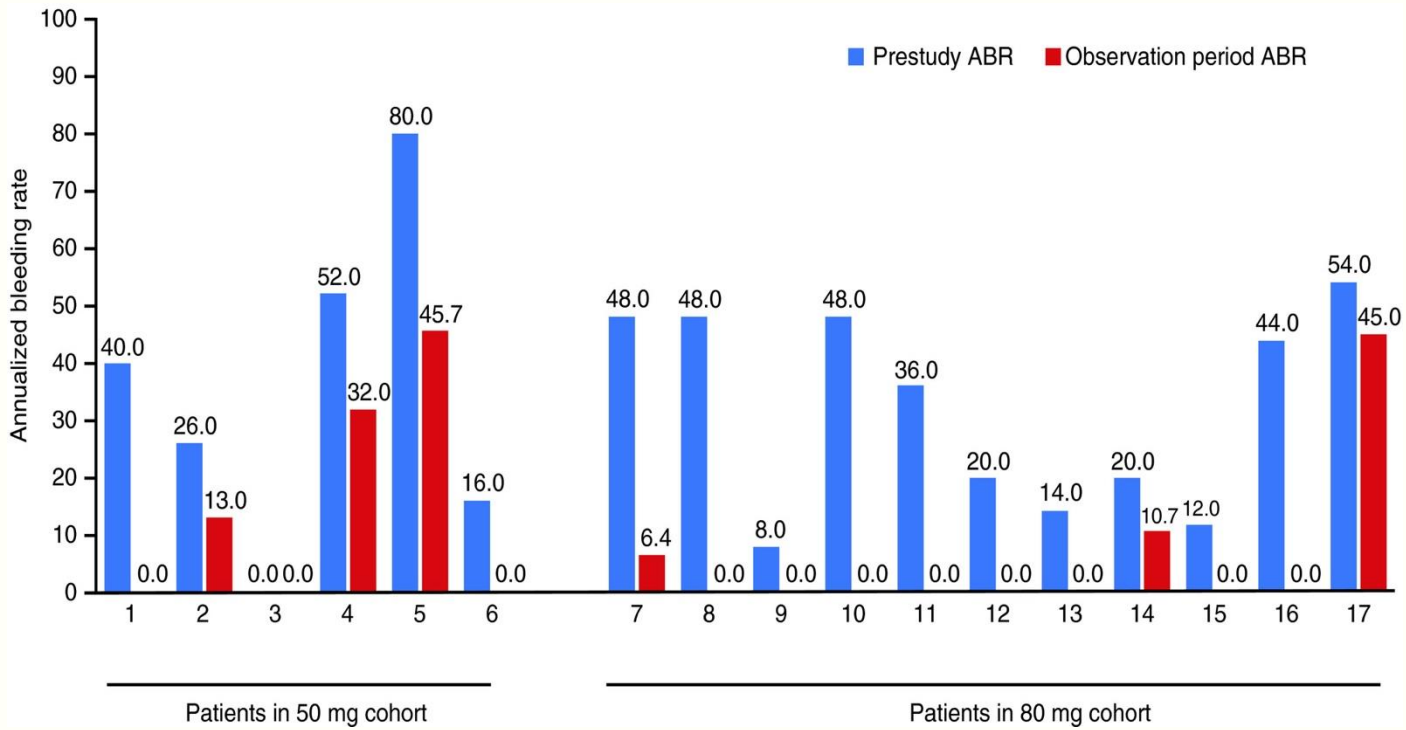
Ragni, NEJM (2015)

Antithrombin ATLAS Trials - Fitsuran

Thrombin Generation with AT



Annual Bleeding Rate



Pasi et al , JTH (2021) - Phase I Inhibitor Cohort (ATLAS)
Sponsor: Sanofi

Concizumab: Explorer 7 trial

Median ABR (P25–P75)	HAwI Concizumab PPX (n=76)*	HBwI Concizumab PPX (n=51)*
Treated spontaneous and traumatic bleeding episodes	0.0 (0.0–3.7)	0.0 (0.0–3.3)

Estimated Mean ABR (95% CI)	HAwI No PPX (arm 1)	HAwI Concizumab PPX (arm 2)	HAwI ABR ratio (95% CI)
Treated spontaneous and traumatic bleeding episodes	18.3 (10.18–32.87)	**1.6 (0.89–2.83)**	0.09 (0.04–0.18) 91% reduction

Estimated Mean ABR (95% CI)	HBwI No PPX (arm 1)	HBwI Concizumab PPX (arm 2)	HBwI ABR ratio (95% CI)
Treated spontaneous and traumatic bleeding episodes	7.2 (2.61–20.06)	**2.2 (0.76–6.52)**	0.31 (0.07–1.36) 69% reduction

N=133

32 week follow up

SC daily

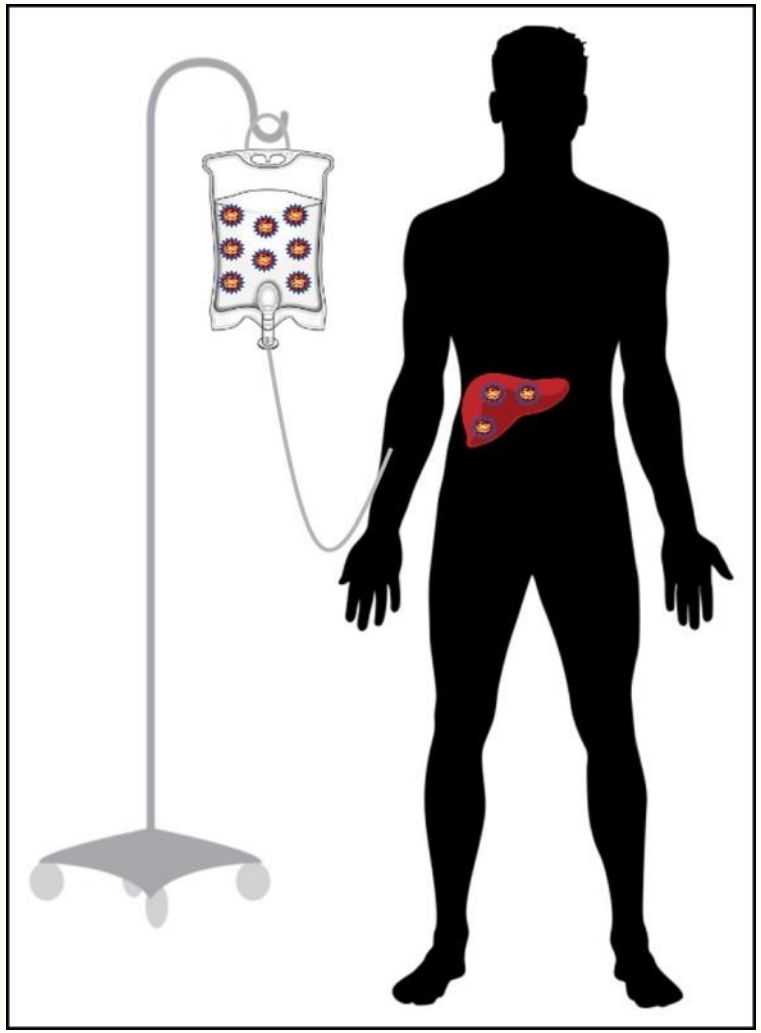
Study paused and restarted after thrombotic events

Dose adjustment at week 4, no further thrombotic events recorded

ABR, annualised bleeding rate; CI, confidence interval; HAwI, haemophilia A with inhibitors; HBwI, haemophilia B with inhibitors; PPX, prophylaxis; SC, subcutaneous

1. Mathias et al. Oral presentation OR06 Presented at 16th Annual Congress of European Association for Haemophilia and Allied Disorders 2023, 7–10 February 2023, Manchester.
2. Frei-Jones et al. *Blood* 2022;140(Supplement 1):466–468.

Gene Addition Therapy - Hemophilia

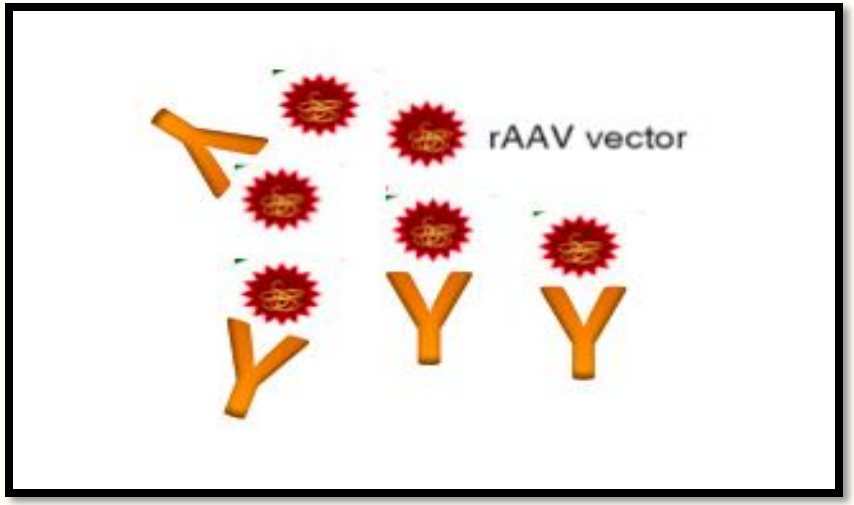


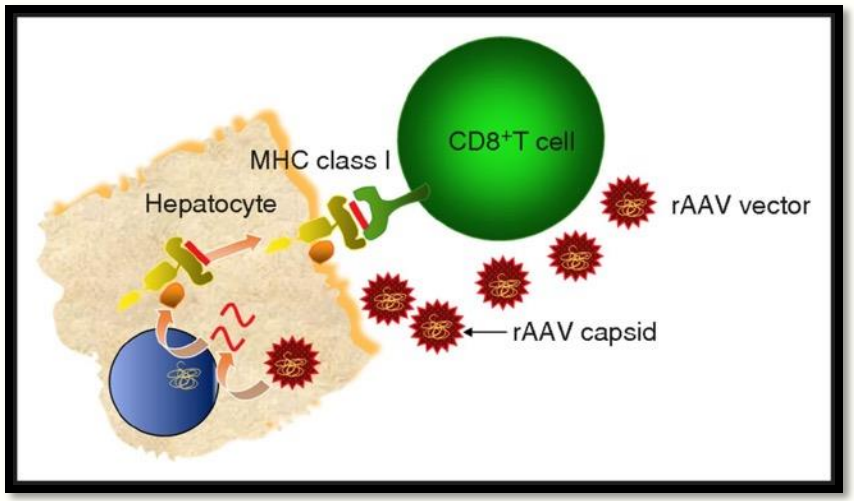
- Not Dominant Negative
- Molecular Characterization
- Animal Model
- Measurable biomarker
- Phenotype/Genotype Correlation
- Progressive Disease

Rogers and Herzog, Frontier in Bioscience, (2015), George, L. Blood advances (2017)

Challenges with Gene Therapy

- Neutralizing Antibodies
- Immune Response
- Dose Response
- Durable Response
- Genotoxicity
  - Integration ?
  - Dividing Hepatocytes ?
  - Long Term Risks ?

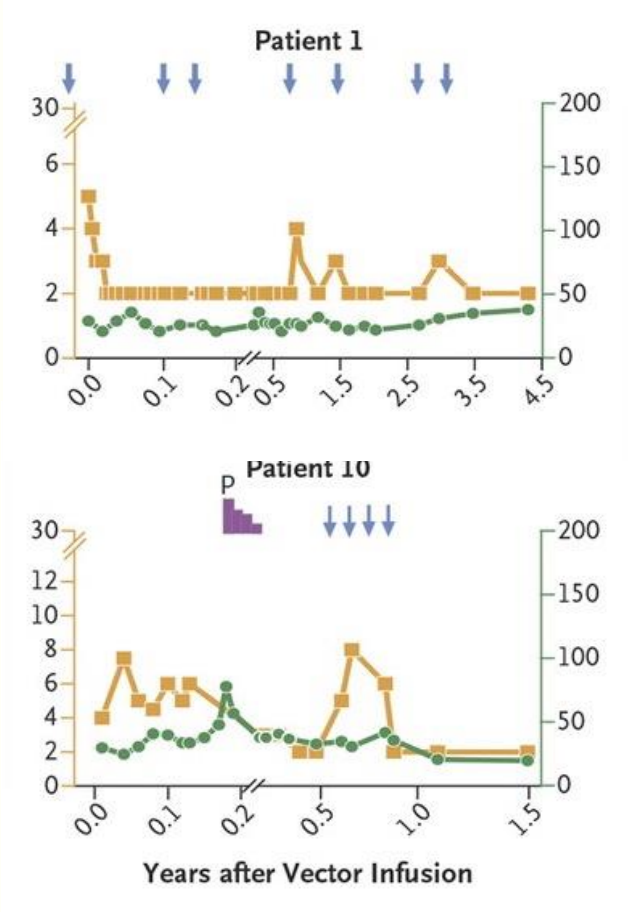




Adapted from Lindsey A. George Blood Adv 2017;1:2591-2599

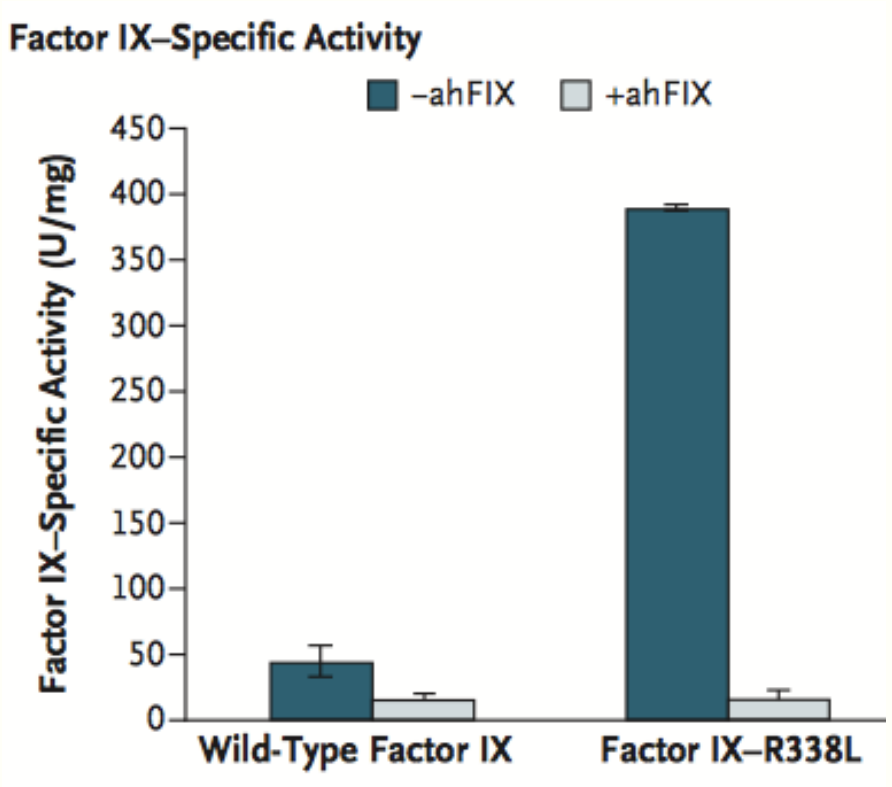
Hemophilia B Gene Therapy

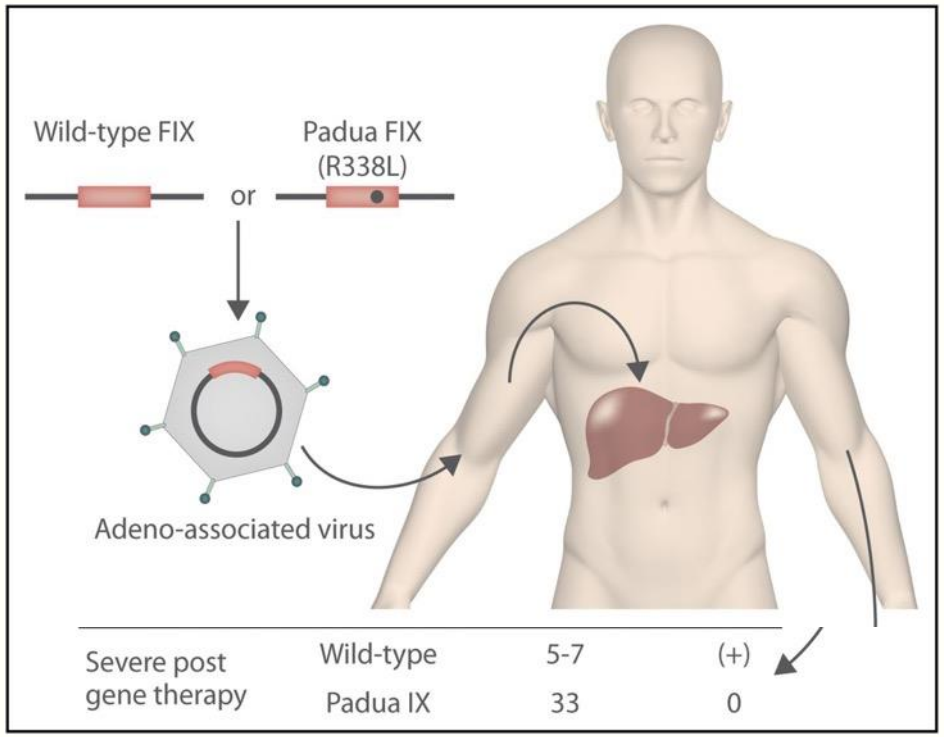
- 10 patients
- Single AAV Vector Infusion
- Peripheral Vein
- Factor IX 1-6% expression
- 8 + years of follow up
- No late toxic effects
- Stable Expression



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

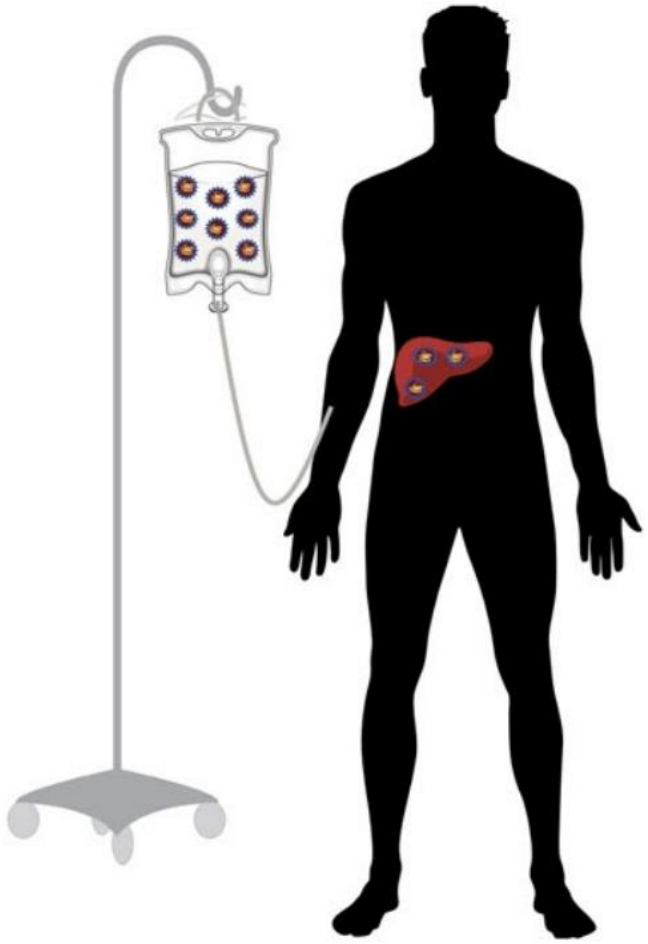
Padua FIX B Gene Therapy

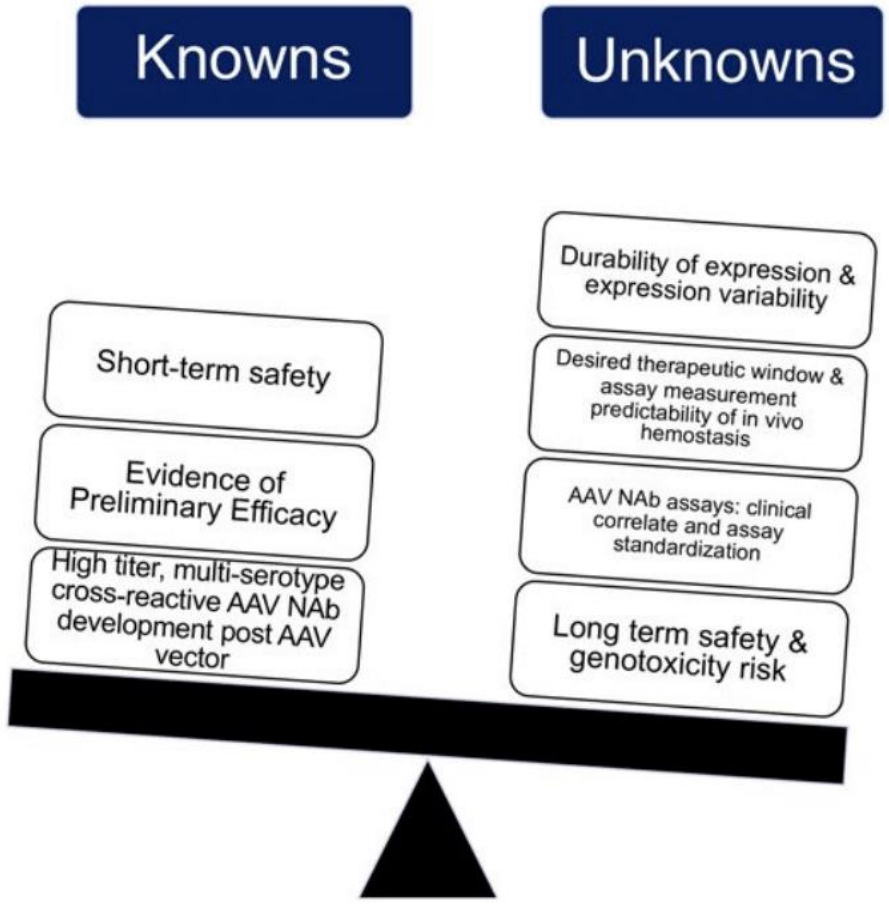




Makris M, Blood 2018;131:952-953
Simioni et al. NEJM, 2009

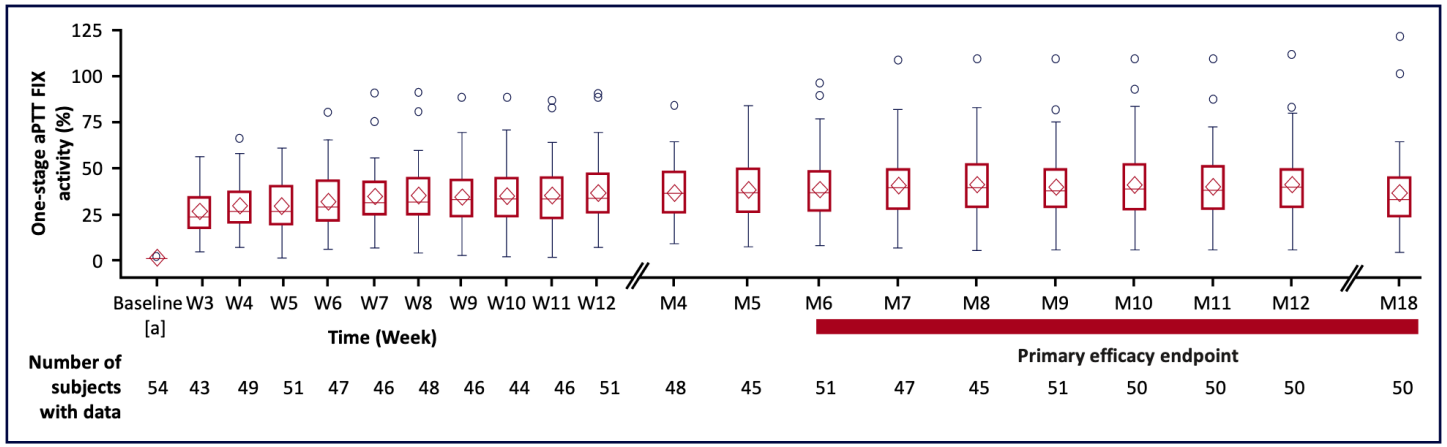
Challenges with Gene Therapy Trials





George LA. *Hematology Am Soc Hematol Educ Program* (2021) ;

HOPE- B (Phase III – AMT-061)



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.

- Dose: 2 x 10 ^13
- No planned immunosuppressio
- Follow up:
  - 6 month : FIX Activity:: 39 %
  - 18 month: FXI Activity: 36.9 %

- N=1 with HCC
- History of HCV
- No clear integration event as causality
- Increased liver US screening

Miesbach et al. EHAD 2022
Schmidt et al. ISTH 2021
Sponsor: Uniqure/CSL

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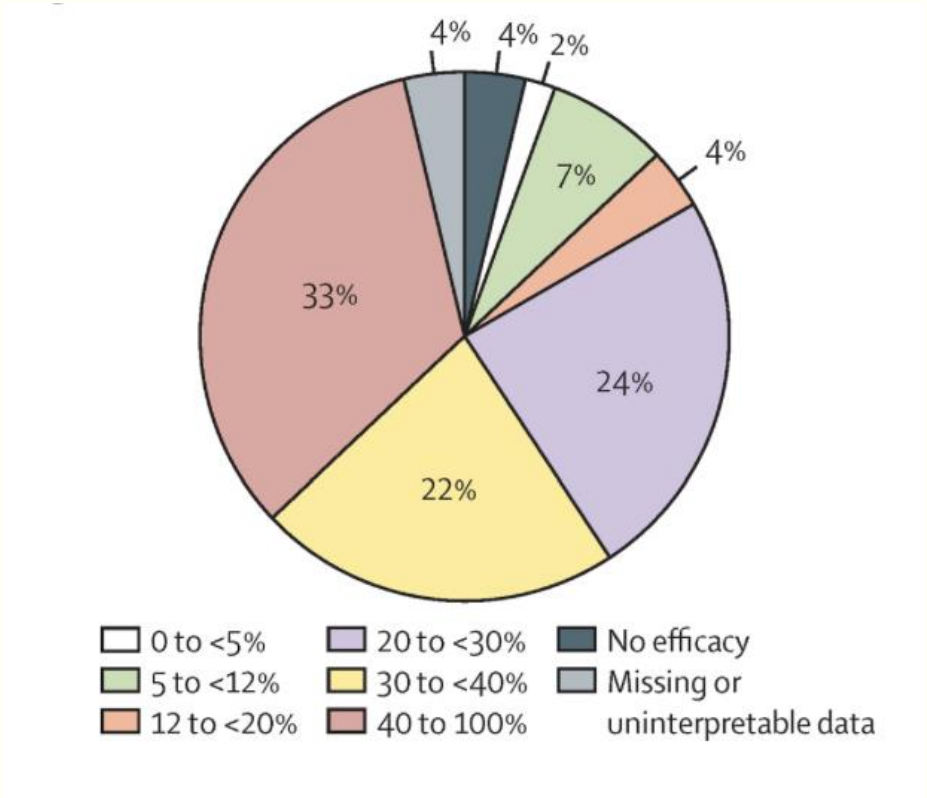
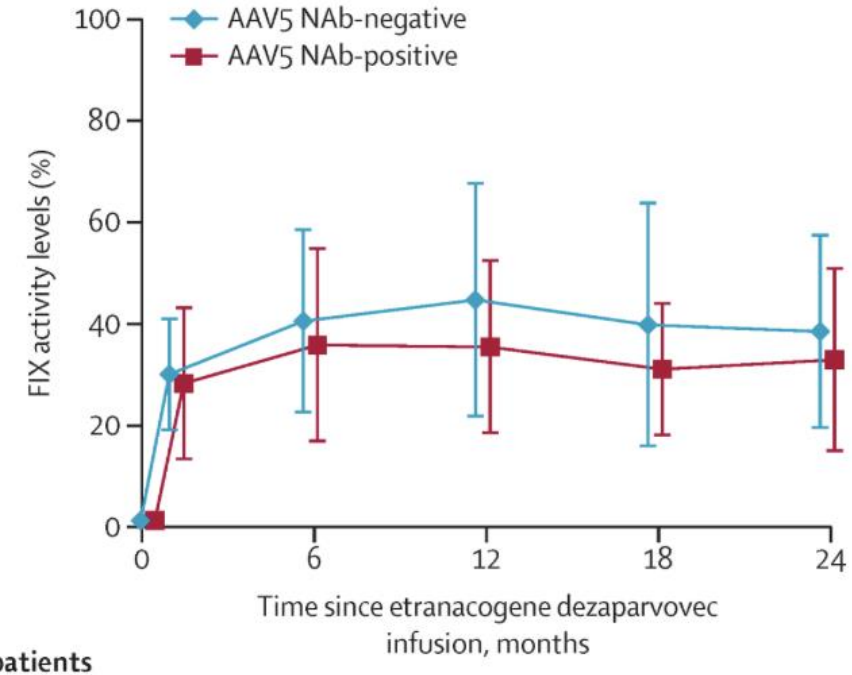
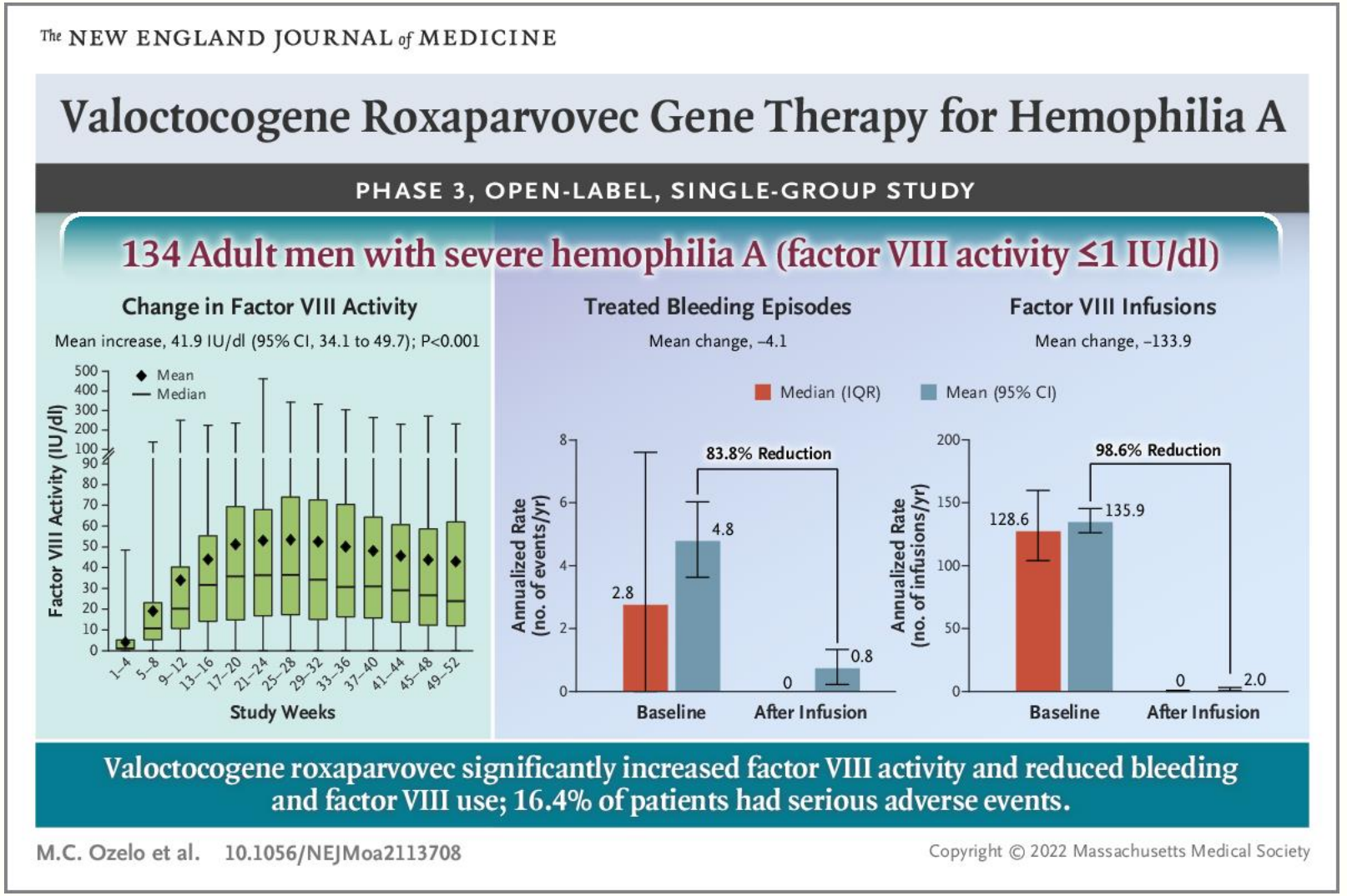




Number of patients						
AAV5 NAb-negative	33	32	33	32	33	33
AAV5 NAb-positive	21	17	18	18	17	17

Coppens, Lancet 2024
Sponsor: Uniqure/CSL

Hemophilia A Gene Therapy – Durability

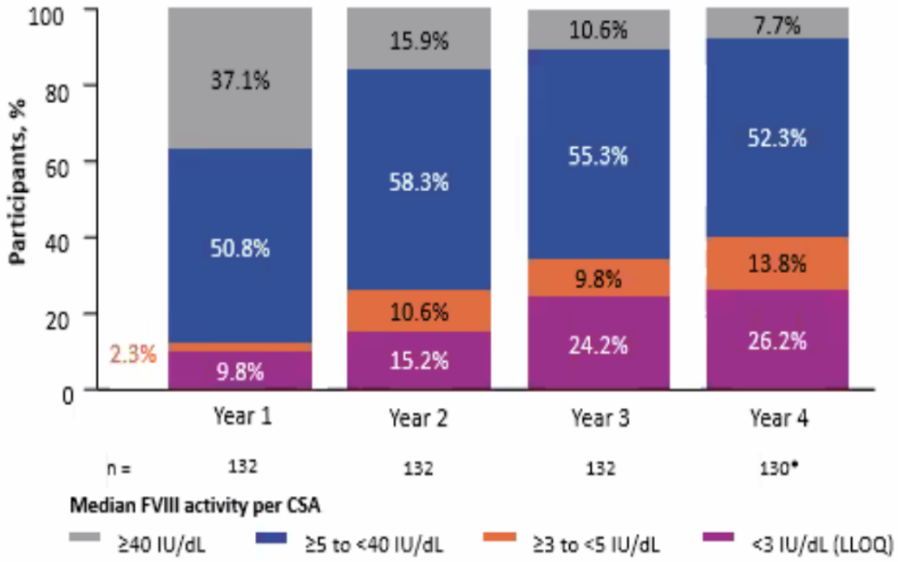


Pasi KJ, et al. NEJM 2020
Sponsor: Biomarin

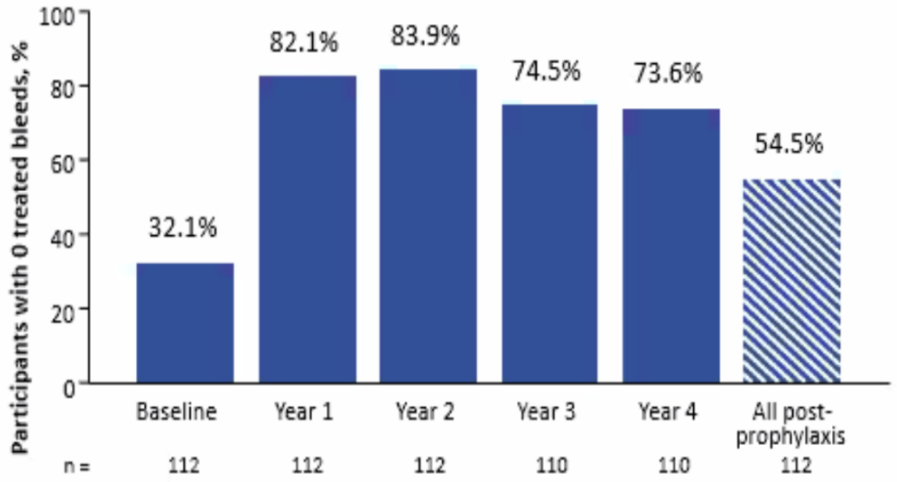
Hemophilia A Gene Therapy – Durability

- GENEr8-1: phase 3 GT for HA with 4 years follow-up
  - AAV5-hFVIII-SQ (valoctocogene roxaparvovec) $6x10^{13}$ vg/kg

Median FVIII activity over time



In year 4, >70% of participants had no treated bleeds

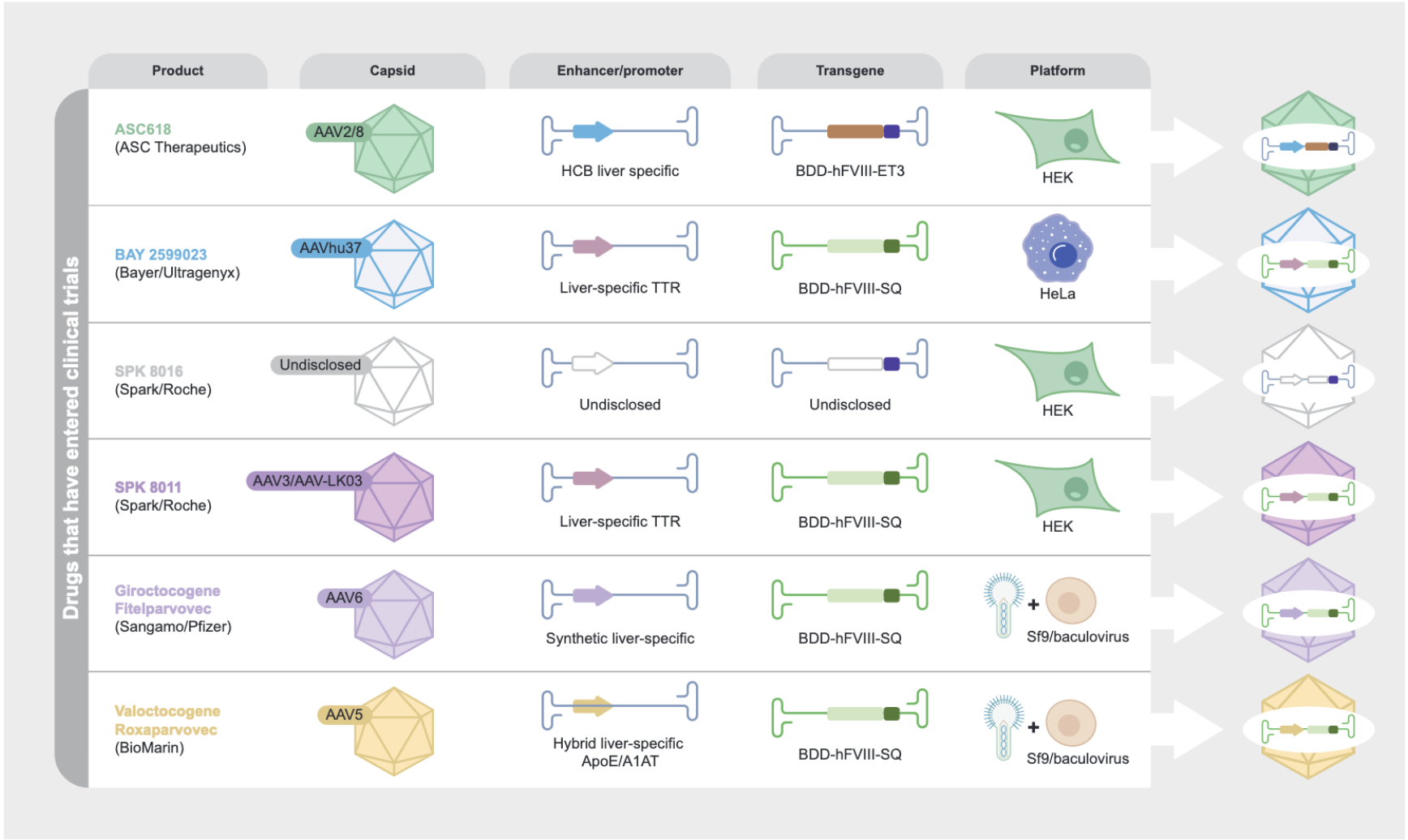


*2 participants did not reach year 4 follow-up, Week 208 data are based on 130 participants. For participants who discontinued the study, missing FVIII values post-discontinuation were imputed as 0 IU/dL through the data cutoff date.

CSA, chromogenic substrate assay; FVIII, factor VIII; GT, gene therapy; HA, haemophilia A; LLOQ, lower limit of quantification; mITT, modified intention-to-treat.

Leavitt AD (ISTH 2024)
Sponsor: Biomarin

Active Gene Therapy Trials:



Pipe SW, et al. Curr Gene Ther. (2023); PMID: 36111754

Active Gene Therapy Trials:

Table 1. Recent hemophilia B gene therapy trial using AAV

Sponsor	Transgene	No. of CpG motifs in transgene	Serotype	Genome format	Method of vector delivery	Dose range (vg/kg)	Mean stable FIX activity levels	No. of patients with transaminitis	Current status
Avigen/CHOP	Wild-type *FIX*	19	AAV2	ssAAV	IM	2e11 to 1.8e12	Transient at a maximum level of 1.6%	0	Closed
Avigen/CHOP Coagulin-B	Wild-type *FIX*	19	AAV2	ssAAV	Intrahepatic artery	2e11 to 2e12	Transient with a maximum of 12%	1/2 at highest dose	Closed
St Jude/UCL	Codon-optimized *FIX*	0	AAV8	scAAV	Systemic	2e11 to 2e12	5.1%	4/6 patient at highest dose	Closed
Takeda (Baxalta; BAX 335)	Codon-optimized *FIX* + Padua mutation	99	AAV8	scAAV	Systemic	2e11 to 3e12	Transient except in 1 patient who had expression of ~20% at last report	2/6 patients treated at or above 1e12 received steroids in response to ALT elevations and 1 patient received prophylactic steroids.	Closed
Spark Therapeutics (SPK-9001, fidanacogene elaparvovec)	Codon-optimized *FIX* containing the Padua mutation	0	AAV- Spark100	ssAAV	Systemic	5e11	19.8% at 5 years	3/15 patients	Transitioned to phase 3 with Pfizer
uniQure (AMT-060)	Codon-optimized *FIX*	0	AAV5	ssAAV	Systemic	5e12–2e13	6.9% 5.2% at 5e12-vg/kg dose and 7.2% at 2e13-vg/kg dose at 5 years	2/5 at highest dose	A new program, AMT-061, that contains the FIX Padua in development with CSL Behring
CSL Behring (AMT-061, etranacogene dezaparvovec)	Codon-optimized *FIX* containing the Padua mutation	?0	AAV5	?ssAAV	Systemic	2e13	36.9%	9/54	Under regulatory review
Dimension Therapeutics (DTX101)	Codon-optimized *FIX*	96	AAVrh10	ssAAV	Systemic	1.6e12–5e12	6.7%	3/3 at highest dose	Closed
Freeline Therapeutics (FLT-180a, verbrinacogene setparvovec)	Codon-optimized *FIX* containing the Padua mutation	5	AAV-S3 synthetic capsid	ssAAV	Systemic	3.84e11–1.28e12	30%-279%	8/10	Clinical trials ongoing
Belief BioMed (BBM-H901)	Codon-optimized *FIX* containing the Padua mutation	0	AAV843 synthetic capsid	scAAV	Systemic	5e12	36.9%	2/10	Clinical trials ongoing
Sangamo Bioscience (SB-FIX)	Codon-optimized *FIX*	Not known	AAV6/zinc finger–mediated targeted integration into the albumin locus in hepatocytes	ssAAV	Systemic		Unknown	Unknown	Closed

IM, intramuscular; scAAV, self-complementary AAV; ssAAV, single stranded AAV.

Pipe SW, et al. Curr Gene Ther. (2023); PMID: 36111754