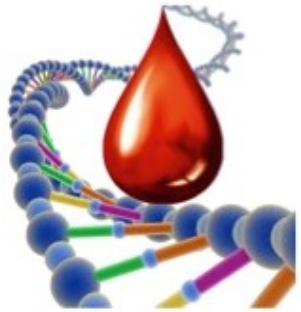
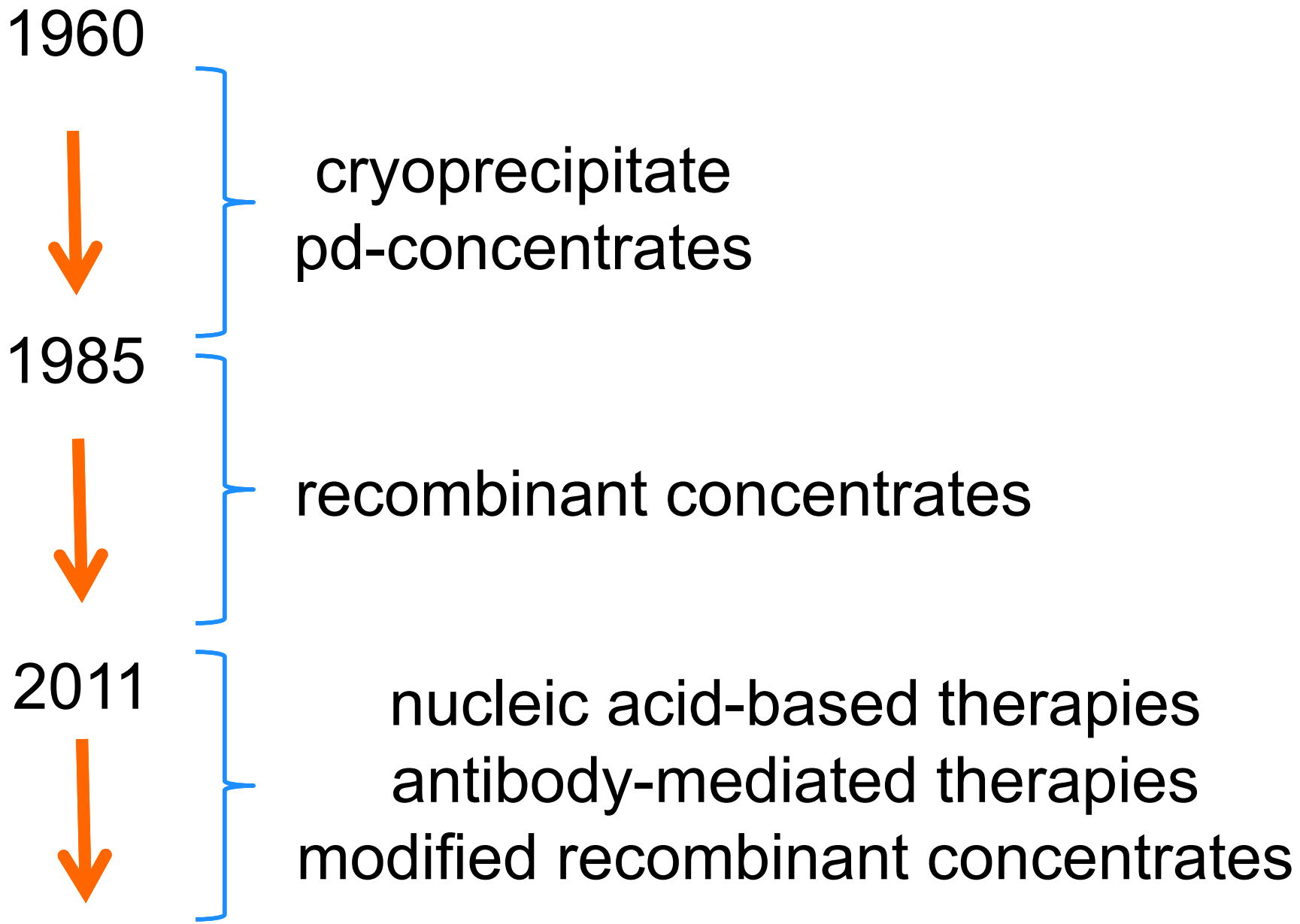
“New developments in Hemophilia Treatment"

David Lillicrap
Department of Pathology and Molecular Medicine
Queen’s University, Kingston, Canada

ECAT Conference, Leiden, November 2018



Clinical and Molecular
Hemostasis Research Group


1960
cryoprecipitate
pd-concentrates
1985
recombinant concentrates
2011
nucleic acid-based therapies
antibody-mediated therapies
modified recombinant concentrates

Proc. Natl. Acad. Sci. USA
Vol. 79, pp. 6461–6464, November 1982
Biochemistry

Isolation and characterization of a cDNA coding for human factor IX

(cDNA hybridization/DNA sequence analysis/blood coagulation)

KOTOKU KURACHI AND EARL W. DAVIE

Department of Biochemistry, University of Washington, Seattle, Washington 98195

Contributed by Earl W. Davie, July 29, 1982

George Brownlee et al. Oxford University, Nature

Factor IX Gene Cloned in Nov 1982

Characterization of the human factor VIII gene

Jane Gitschier, William I. Wood, Therese M. Goralka, Karen L. Wion, Ellson Y. Chen, Dennis H. Eaton, Gordon A. Vehar*, Daniel J. Capon & Richard M. Lawn

Departments of Molecular Biology and * Protein Biochemistry, Genentech, Inc., 460 Point San Bruno Boulevard, South San Francisco, California 94080, USA

The complete 186,000 base-pair (bp) human factor VIII gene has been isolated and consists of 26 exons ranging in size from 69 to 3,106 bp and introns as large as 32.4 kilobases (kb). Nine kb of mRNA and protein-coding DNA has been sequenced and the mRNA termini have been mapped. The relationship between internal duplications in factor VIII and evolution of the gene is discussed.

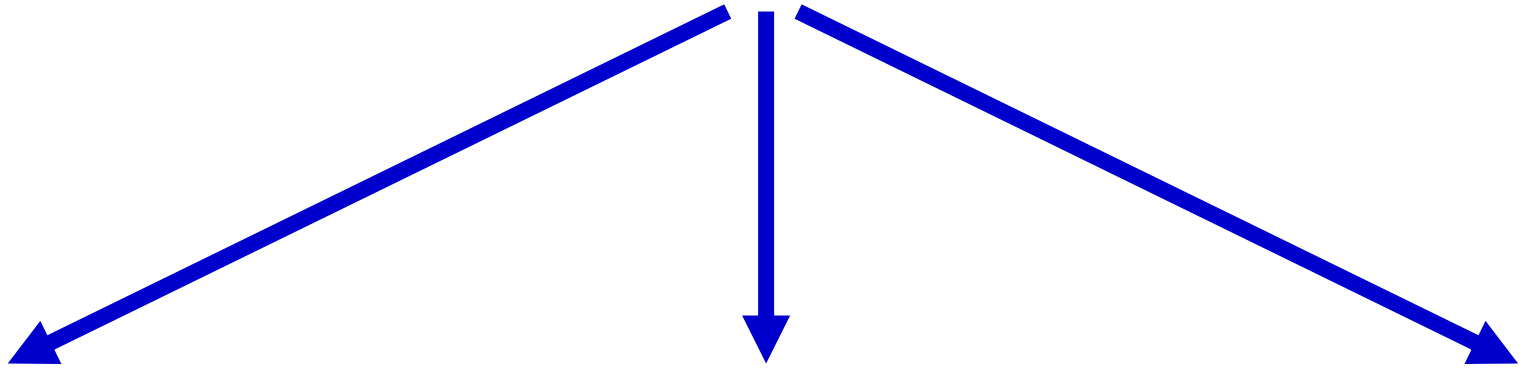
Factor VIII Gene Cloned in Nov. 1984

Reprinted from Nature, Vol. 303, No. 5917, pp. 474-475, 9 June 1983

Haemophilia

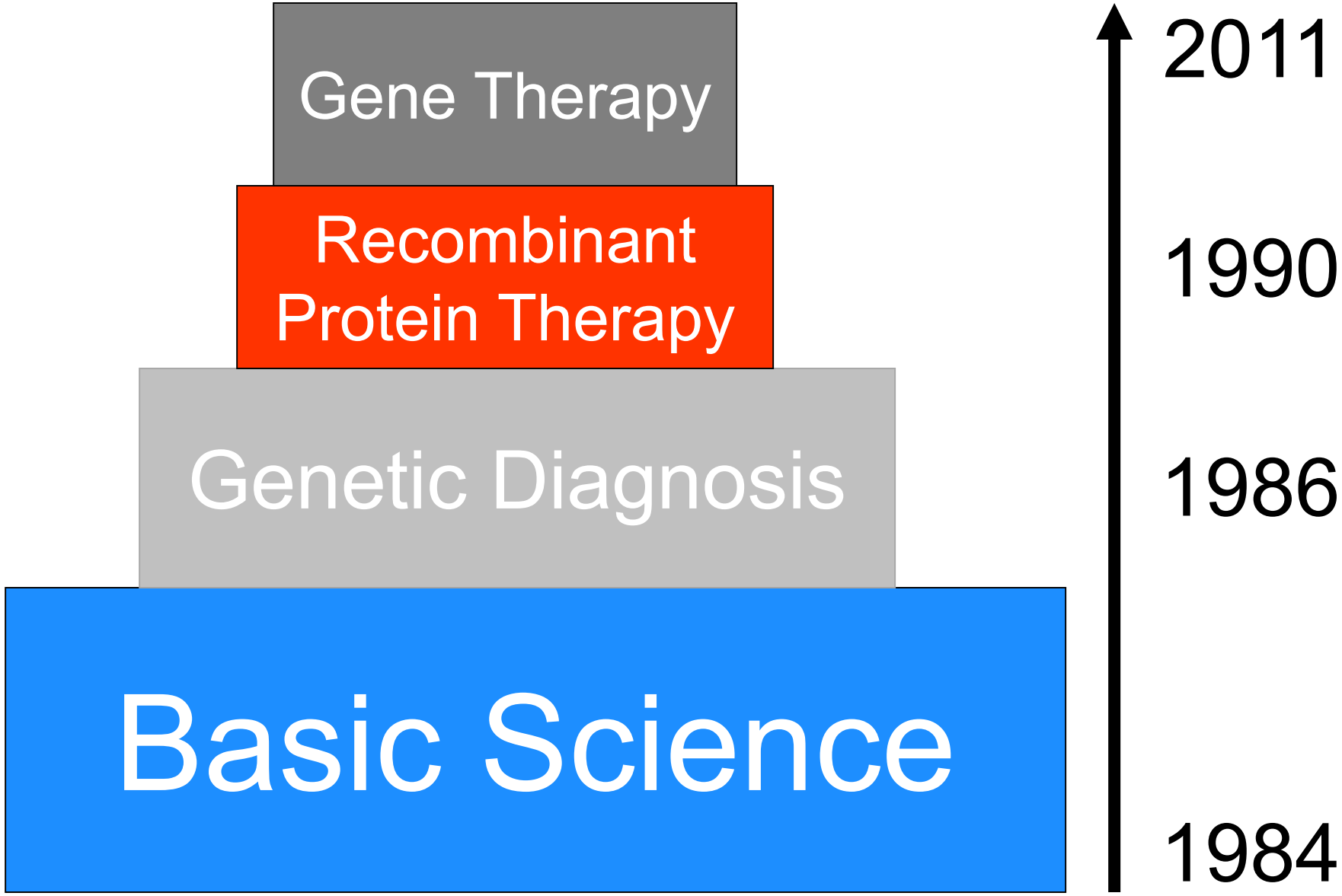
Benefits of cloning genes for clotting factors

from A.L. Bloom



Pathogenesis Diagnosis Therapy

Hemophilia: Translational Medicine



Plasma-derived Clotting Factor Concentrates

From 10,000s of blood donors

Potential for Transmission of Infectious Agents

Red for HIV, yellow for hepatitis, black for death: blood inquiry puts victims first

“Every morning a bell was rung to announce the start of proceedings. But it may just as well have been tolling for the dead. At least 4,689 haemophiliacs were given contaminated blood products in the 1970s and 1980s, and contracted HIV and/or hepatitis C. More than half have since died.”

The Guardian: September 29th 2018

Plasma-derived Clotting Factor Concentrates

From 10,000s of blood donors

Potential for Transmission of Infectious Agents

Since 1990s

- Screening of blood donors - PCR testing
- Treatment of plasma derivatives – heat, detergent, nanofiltration

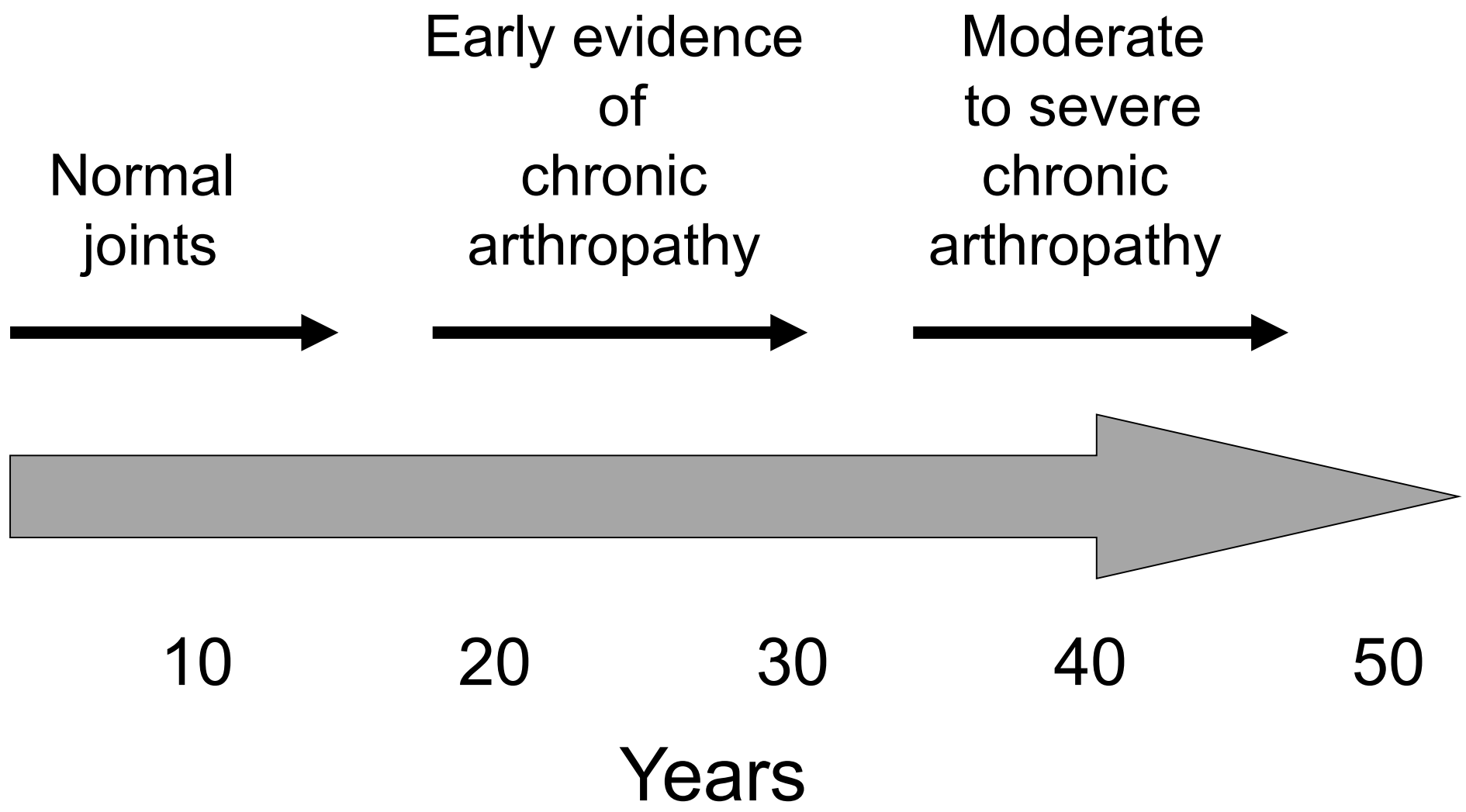
Current Hemophilia Therapy

Safe and Effective

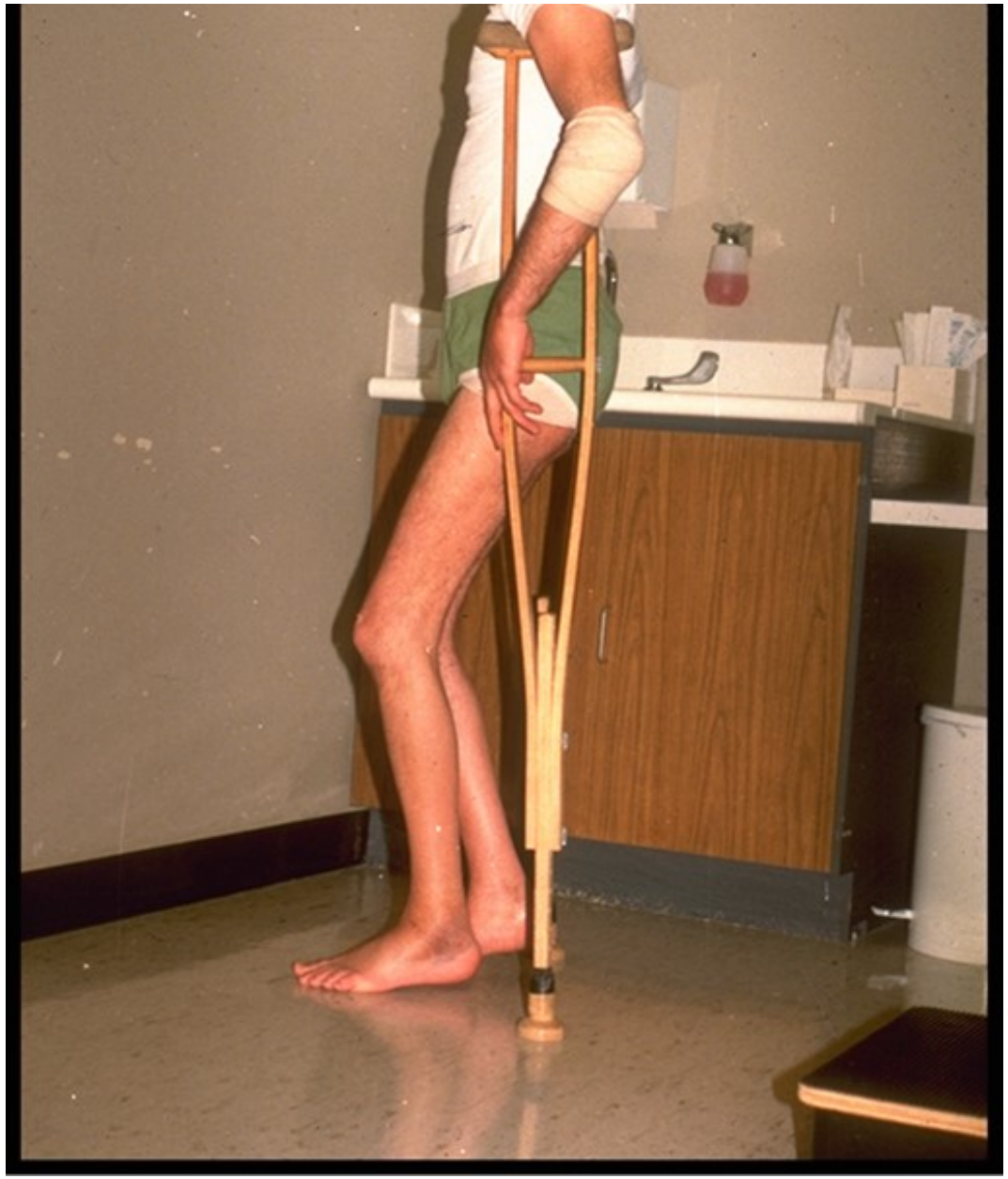
but….

Limitations to Current Hemophilia Treatment

- Inconvenient
  - repeated intravenous infusions
- Immunogenic
  - 30% inhibitor incidence in hemophilia A
- Costly
- Only available to ~30% of all hemophiliacs globally



Development of long-term musculoskeletal disability in hemophilia

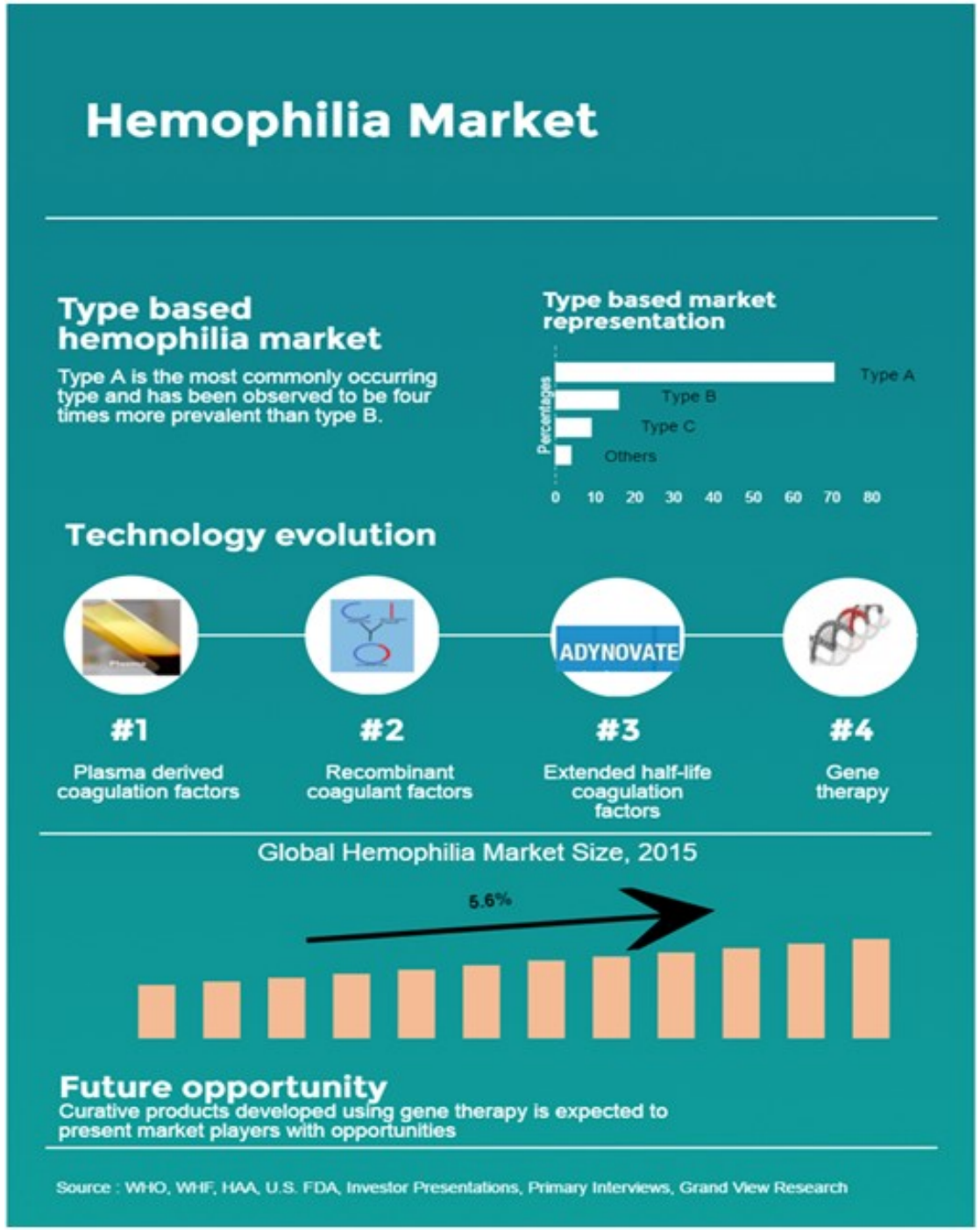


Chronic Hemophilic Arthropathy

Enhanced Commercial Opportunity

a) Intellectual property/Patent expiration

b) Opening of a global market



Global Hemophilia Market Value

€ 8.5 billion

5.6% annual growth

Hemophilia Therapy in 2018

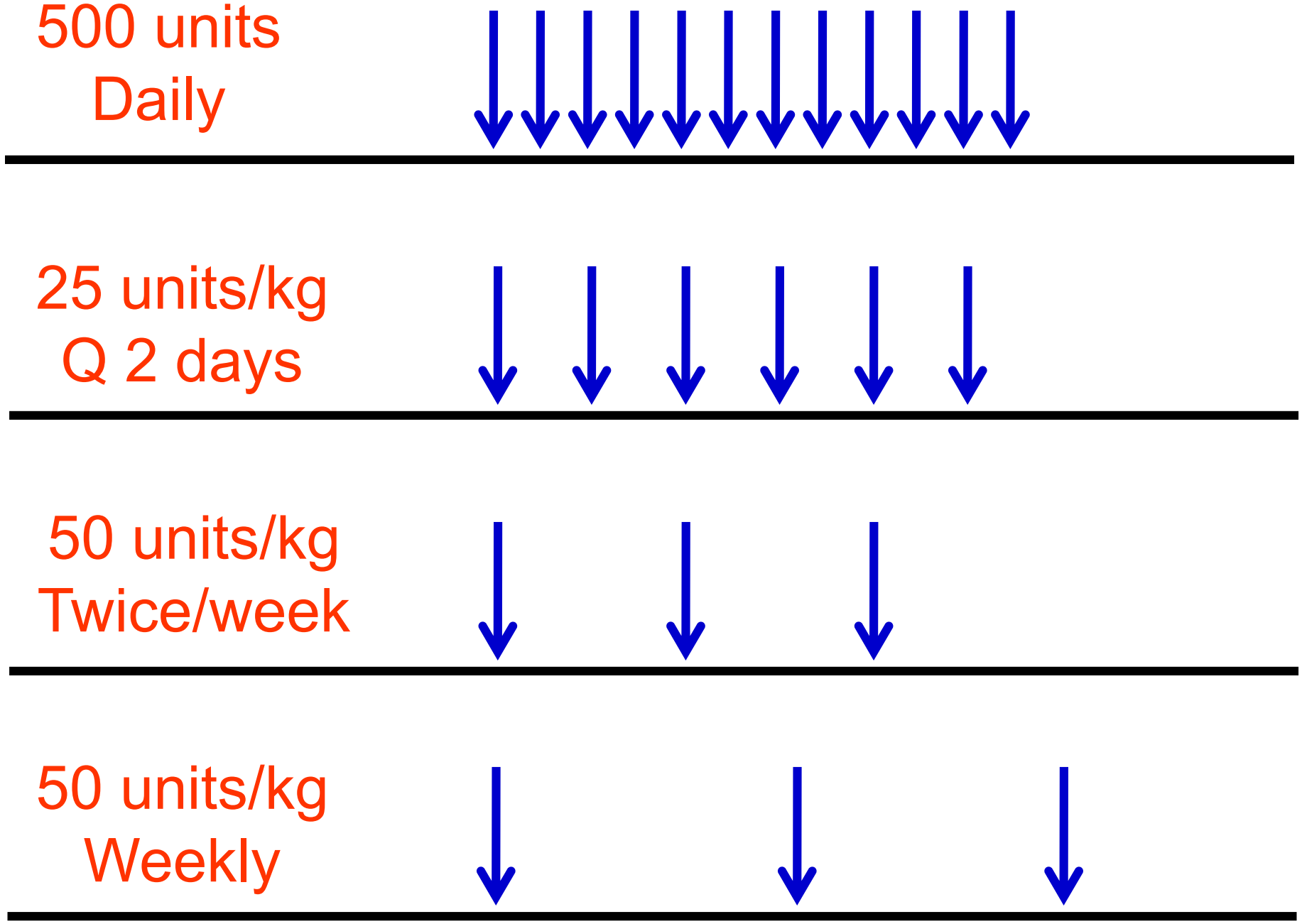
For Severe Hemophiliacs <1% FVIII/FIX

On-demand therapy

~2-6 infusions/month - Chronic hemophilic arthropathy

Prophylactic therapy

1-3 infusions/week - Long-term musculoskeletal benefit


500 units
Daily
25 units/kg
Q 2 days
50 units/kg
Twice/week
50 units/kg
Weekly

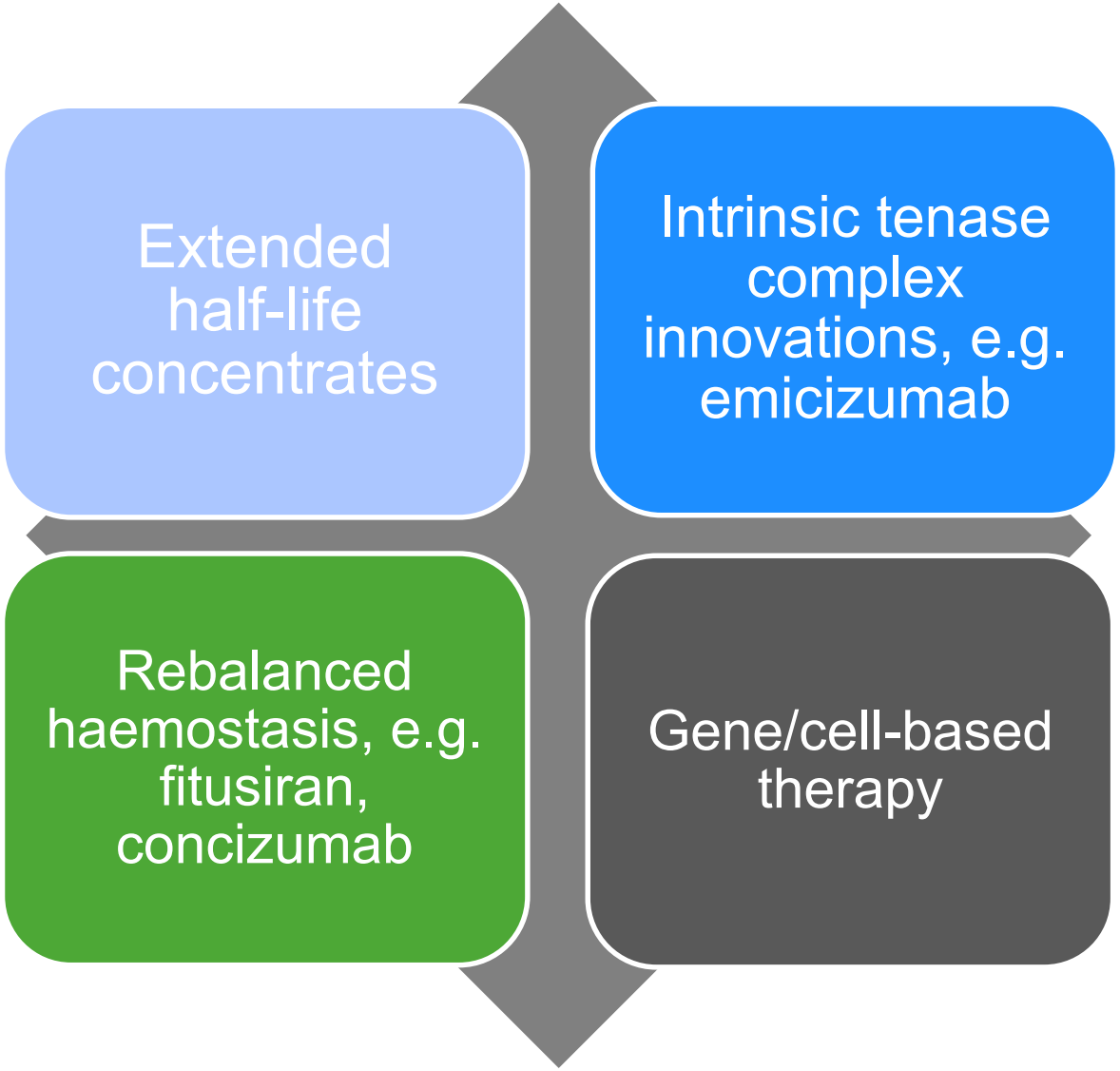
Coagulation Factor Half-lives

Factor VIII ~12 hrs (x3/week)

Factor IX ~24 hrs (x2/week)

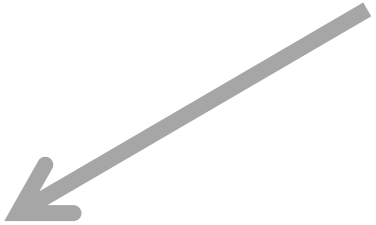
Therapeutic Goal - Weekly Prophylactic Infusions

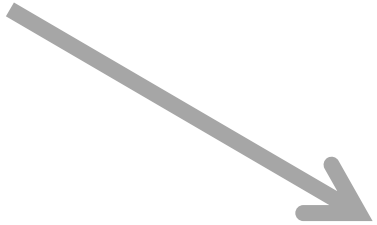
Range of novel hemophilia therapies



1. Franchini M, Mannucci PM. *Blood Transfus* 2018;14:1–5; 2. Bauer KA. *Am J Manag Care* 2015;21(Suppl 6):S112–S122;
3. Peterson JA, *et al. Thromb Res* 2016;141(Suppl 2):S28–S30; 4. Ohmori T, *et al. J Thromb Haemost* 2015;13(Suppl 1):S133–S142.

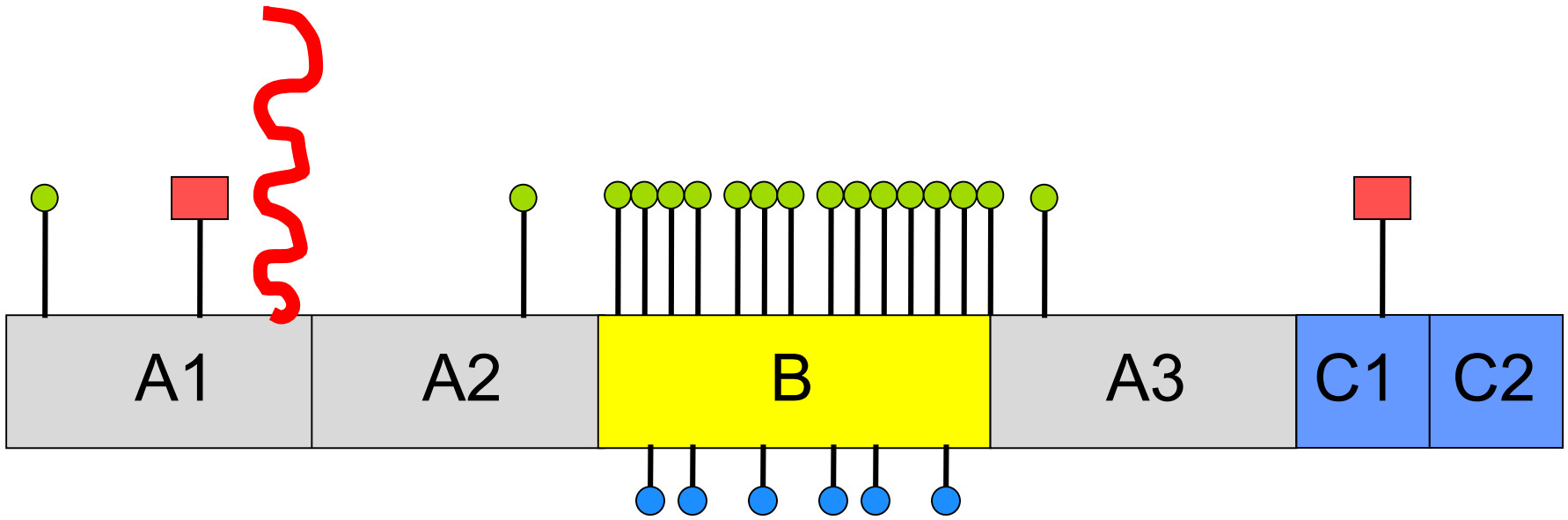
Prolonged Protein Half-life





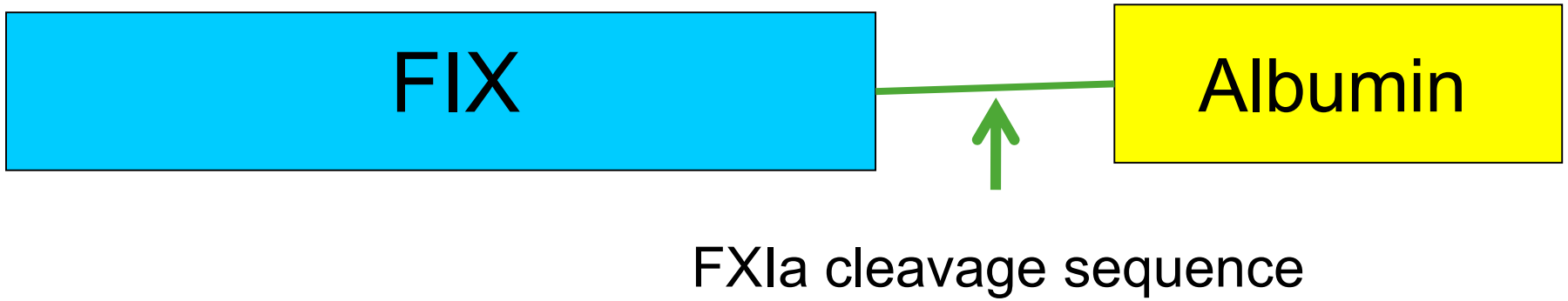
Hydrophilic Polymer Conjugation
(eg PEGylation)

Variant Protein Generation
(eg fusion factors)



Site-Specific or Random Chemical Modification

PEG additions interfere with clotting factor clearance mechanisms



Factor IX/FVIII Fusion cDNAs - Proteins

Albumin and IgFc as Fusion Partners

- Present in plasma at high concentrations

  IgG 12 g/L Albumin 42 g/L

- Half-life ~25 days

- Same mechanism of rescue (FcRn receptor)

  - present in endothelial endosomes

Clotting Factor Half-Life Extensions

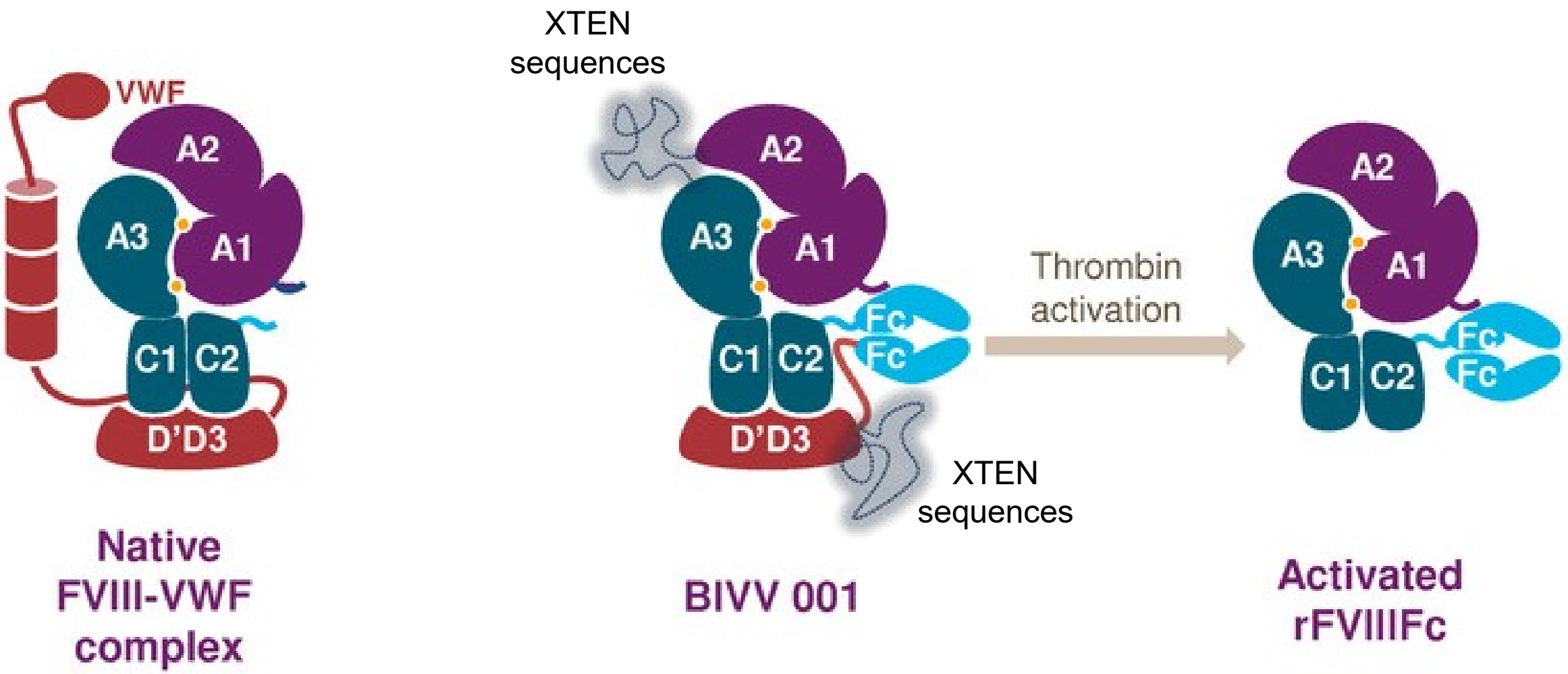
Factor IX 3 to 5-fold → 1 infusion Q7-14 days

(Santagostino et al Blood 2012)
(Negrier et al Blood 2011)
(Shapiro et al Blood 2012)

Factor VIII 1.5 to 1.8-fold → 1 infusion Q3-4 days

(Powell et al Blood 2012)
(Coyle et al JTH 2014)
(Mahlangu et al Blood 2014)

FVIII half-life limited by dominance of VWF



BIVV001 Novel FVIII.Fc-VWF D'D3 Molecule

Late Breaking Abstract WFH Congress Glasgow 2018 – Bioverativ/Sanofi

BIVV001 Preliminary Results

EXTEN-A Phase 1/2A Clinical Study

4 adult severe hemophilia A pts - single infusion of BIVV001 at 25 IU/kg

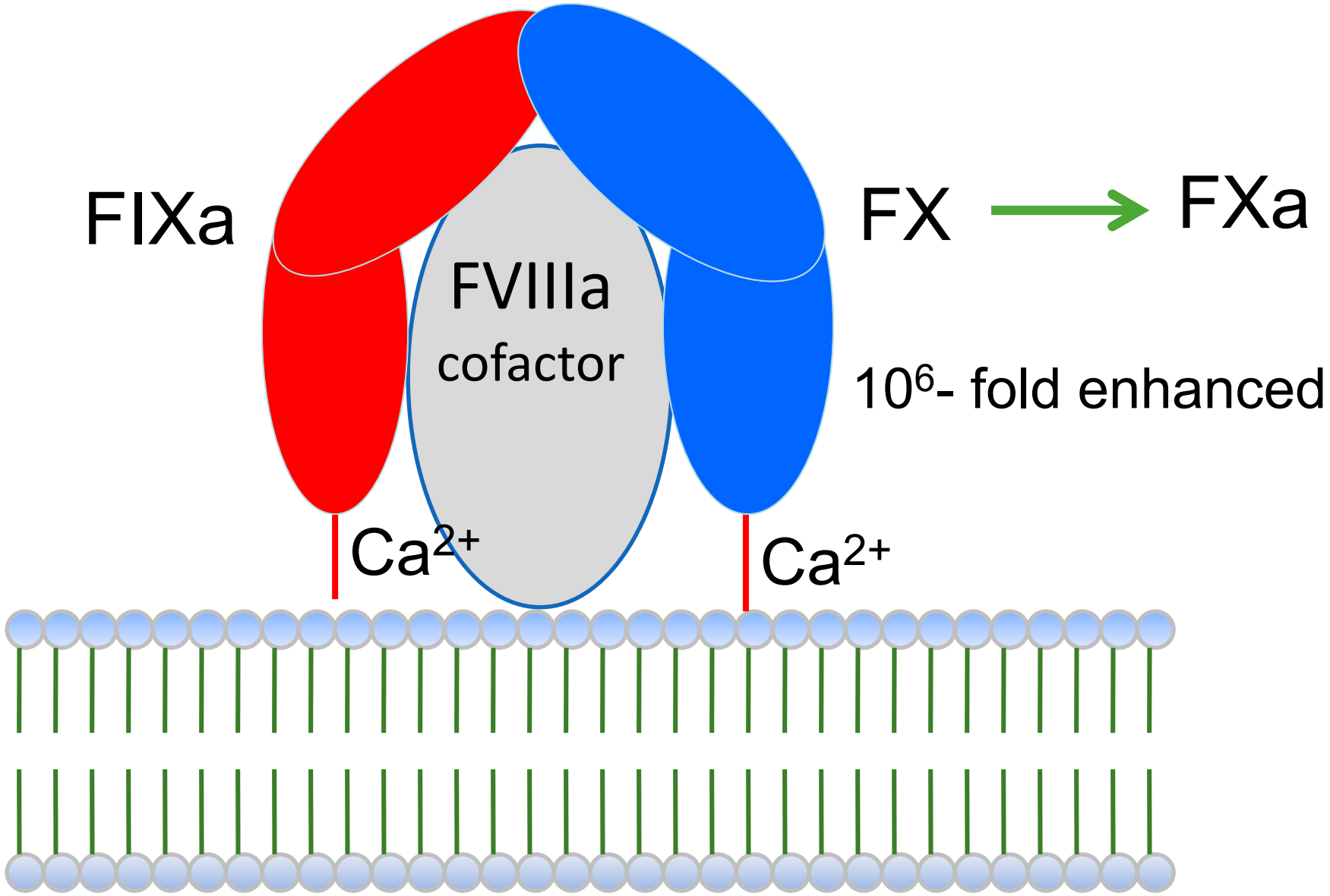
FVIII half life of 37 hrs

Mean residual FVIII levels

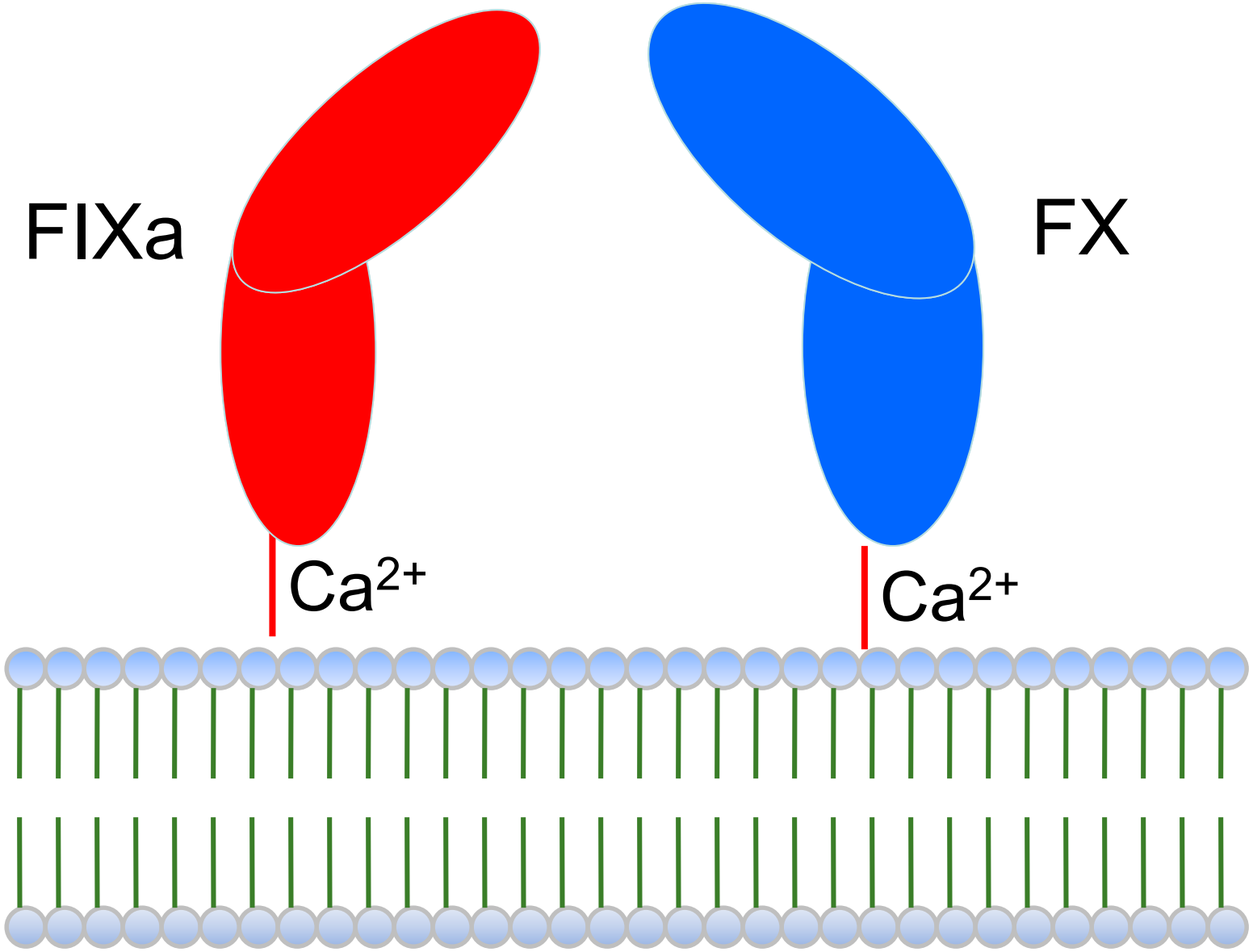
13% at 5 days

5.6% at 7 days

Late Breaking Abstract WFH Congress Glasgow 2018

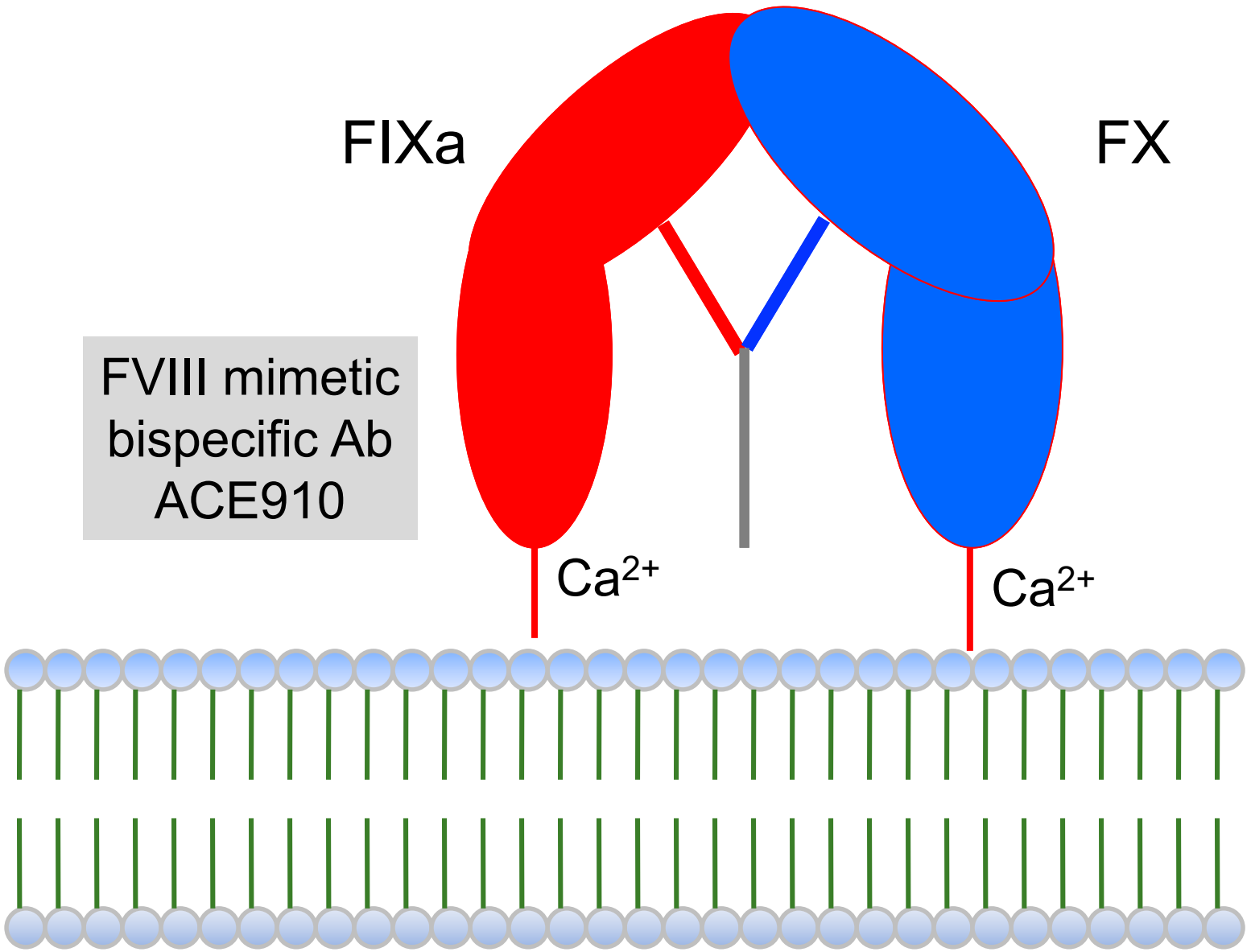


Intrinsic Tenase Complex



Intrinsic Tenase Complex in Hemophilia A

ACE910 bispecific antibody: (Emicizumab)



Chugai
Genentech
Roche

The NEW ENGLAND JOURNAL of MEDICINE

ORIGINAL ARTICLE

Emicizumab Prophylaxis in Hemophilia A with Inhibitors

Johannes Oldenburg, M.D., Ph.D., Johnny N. Mahlangu, M.D., Benjamin Kim, M.D., Christophe Schmitt, Pharm.D., Michael U. Callaghan, M.D., Guy Young, M.D., Elena Santagostino, M.D., Ph.D., Rebecca Kruse-Jarres, M.D., M.P.H., Claude Negrier, M.D., Ph.D., Craig Kessler, M.D., Nancy Valente, M.D., Elina Asikanius, M.Sc., Gallia G. Levy, M.D., Ph.D., Jerzy Windyga, M.D., and Midori Shima, M.D., Ph.D.

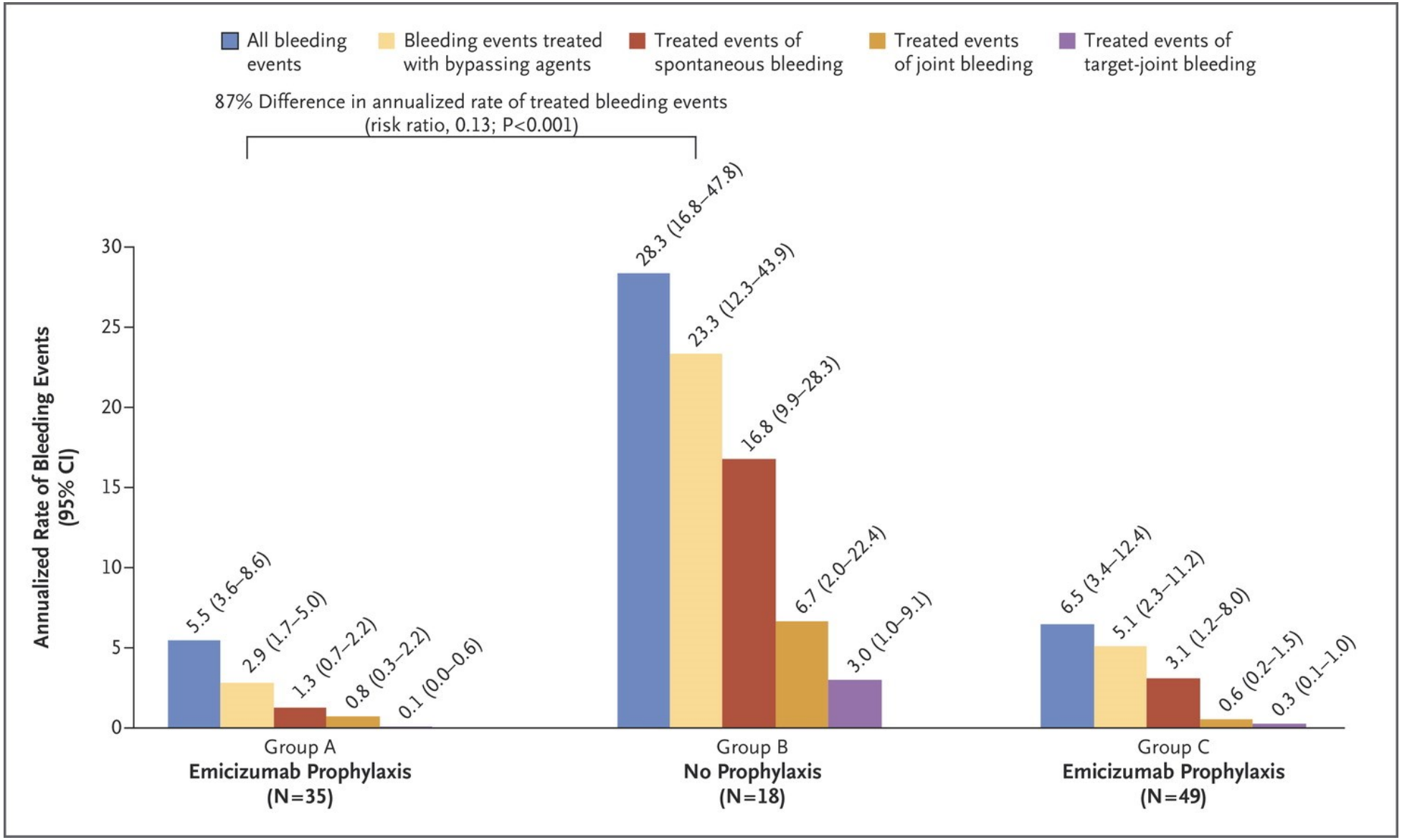
N Engl J Med 2017; 377:809-818 | August 31, 2017 | DOI: 10.1056/NEJMoa1703068

Comments open through September 6, 2017

FVIII Mimetic Bispecific Antibody (Emicizumab) Chugai-Genentech-Roche

- 109 hemophilia A pts with FVIII inhibitors enrolled
- Weekly subcutaneous Emicizumab
- 87% reduction in annualized bleeding rate with Emicizumab
- 63% of pts on Emicizumab prophylaxis had zero bleeds

FVIII Mimetic Bispecific Antibody (Emicizumab/Hemlibra: Chugai-Genentech-Roche)



FVIII Mimetic Bispecific Antibody (Emicizumab/Hemlibra: Chugai-Genentech-Roche)

Serious Adverse Events

5 FVIII inhibitor pts being treated for breakthrough bleeding with ACE910 and high, repeat doses of a bypassing agent (FEIBA) experienced SAEs

2 pts - venous thromboembolic events

3 pts - thrombotic microangiopathies

All events had unusual clinical features

The NEW ENGLAND JOURNAL of MEDICINE

ESTABLISHED IN 1812 AUGUST 30, 2018 VOL. 379 NO. 9

Emicizumab Prophylaxis in Patients Who Have Hemophilia A without Inhibitors

J. Mahlangu, J. Oldenburg, I. Paz-Priel, C. Negrier, M. Niggli, M.E. Mancuso, C. Schmitt, V. Jiménez-Yuste, C. Kempton, C. Dhalluin, M.U. Callaghan, W. Bujan, M. Shima, J.I. Adamkewicz, E. Asikanius, G.G. Levy, and R. Kruse-Jarres

Emicizumab in Hemophilia A patients without inhibitors

Enrolled 152 severe hemophilia A pts > 12 yrs

Group A - 1.5 mg/kg SC weekly
Group B - 3.0 mg/kg SC every 2 weeks
Group C - No prophylaxis

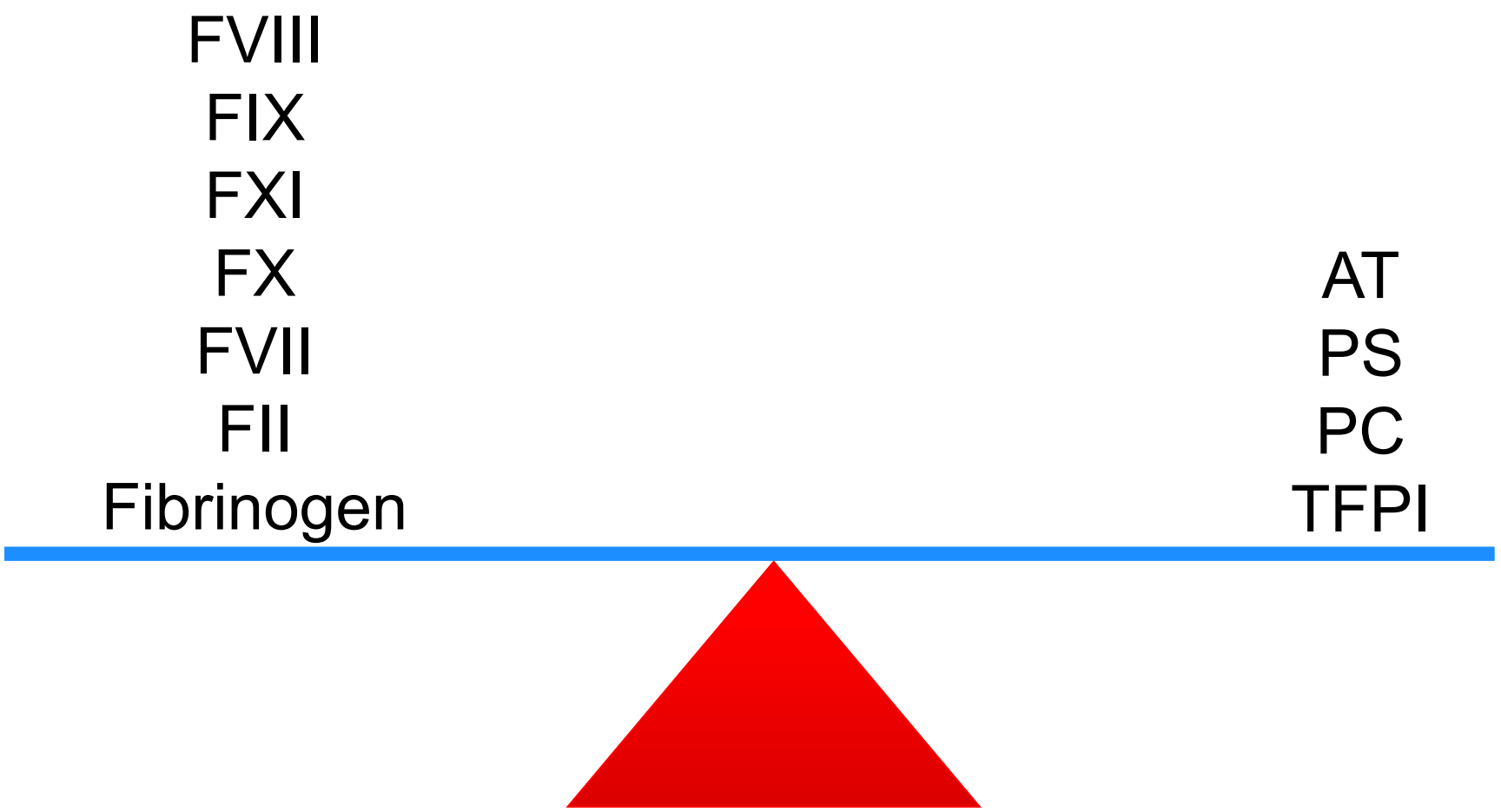
Annualized Bleed Rates:

Group A – 1.5
Group B – 1.3
Group C – 38.2

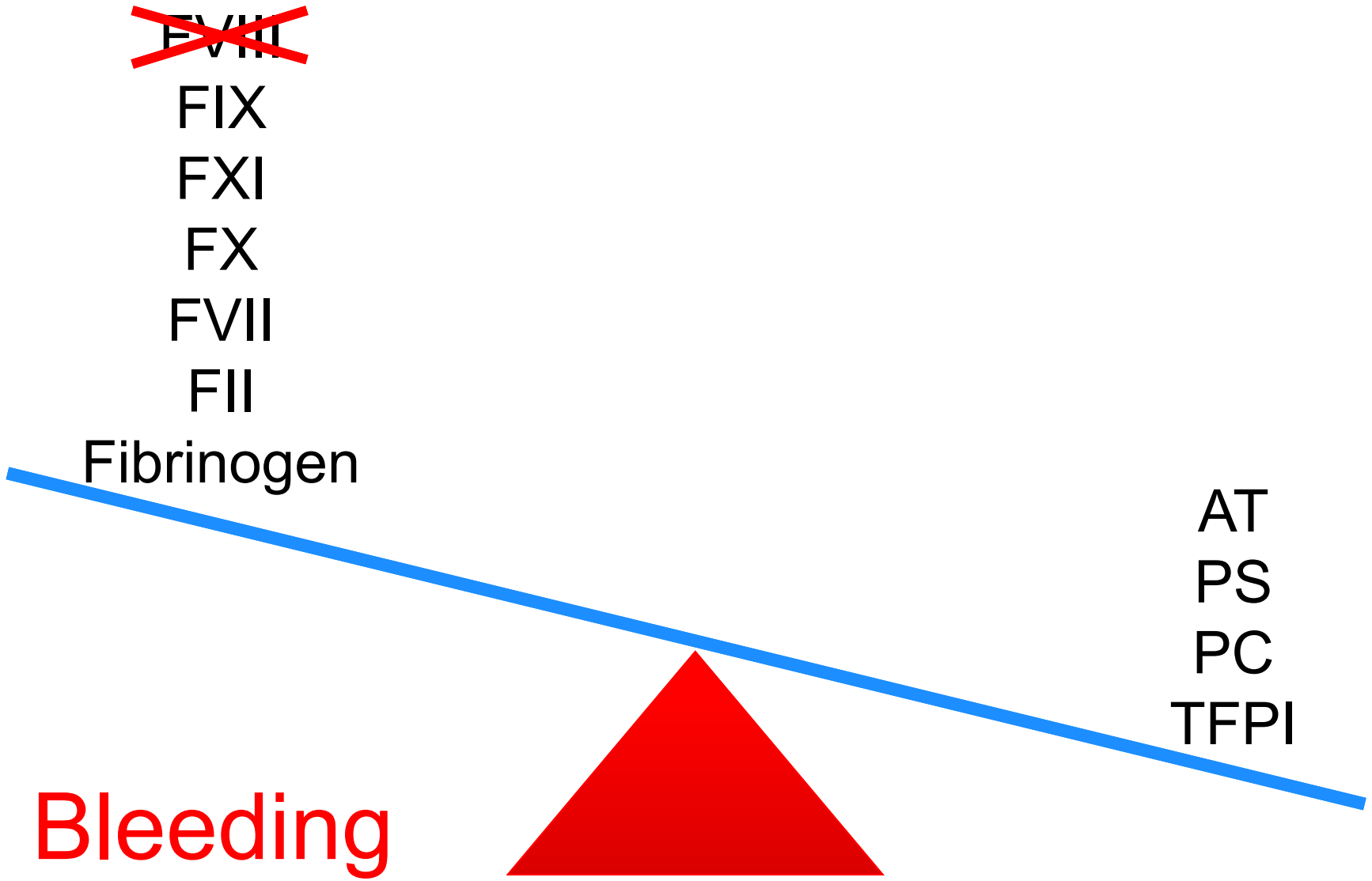
Zero Bleed Rates:

Group A – 56%
Group B – 60%
Group C – 0%

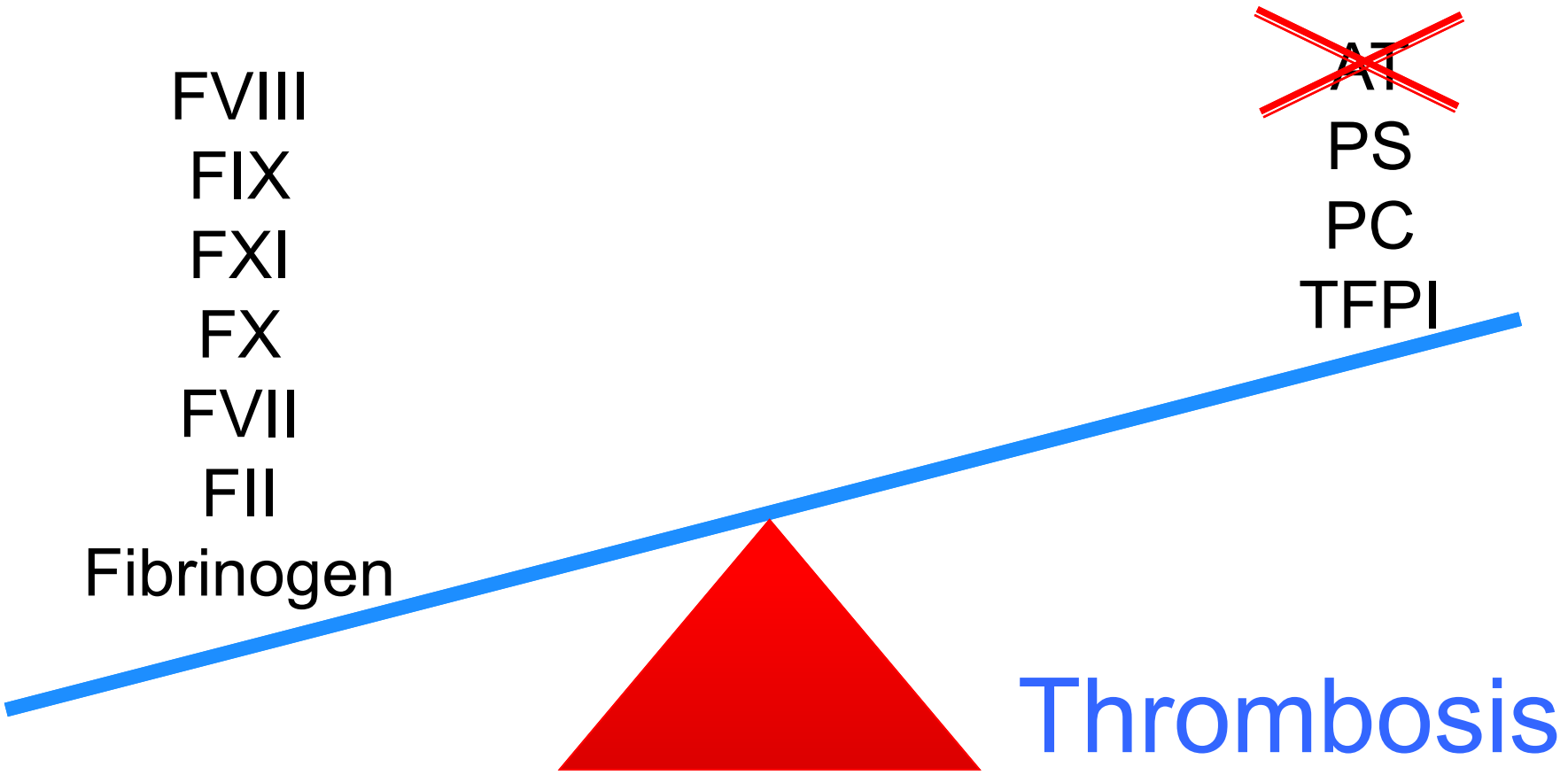
“Balanced Hemostasis”



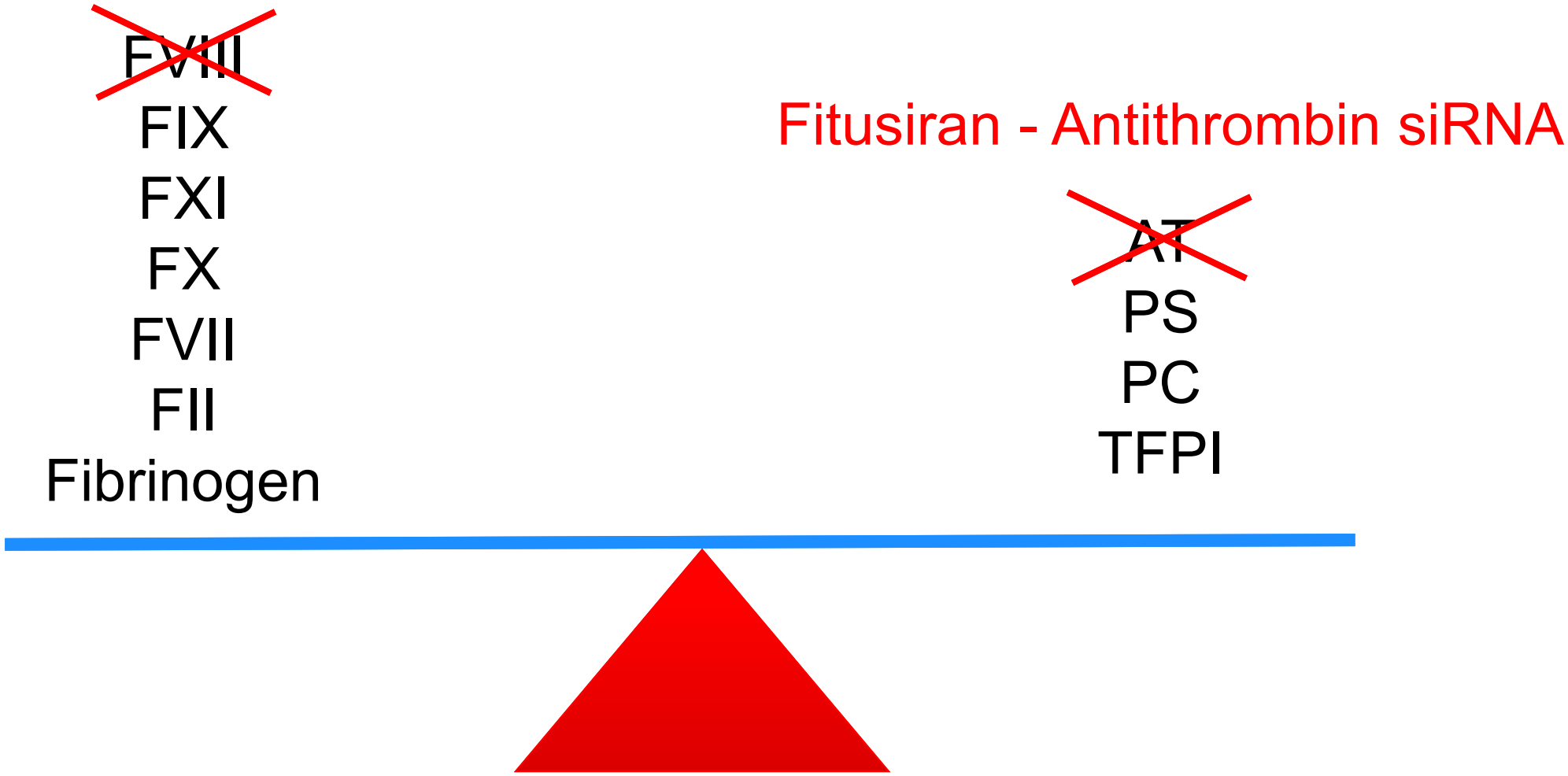
Unbalanced Hemostasis - Hemophilia A



Unbalanced Hemostasis - Antithrombin Deficiency

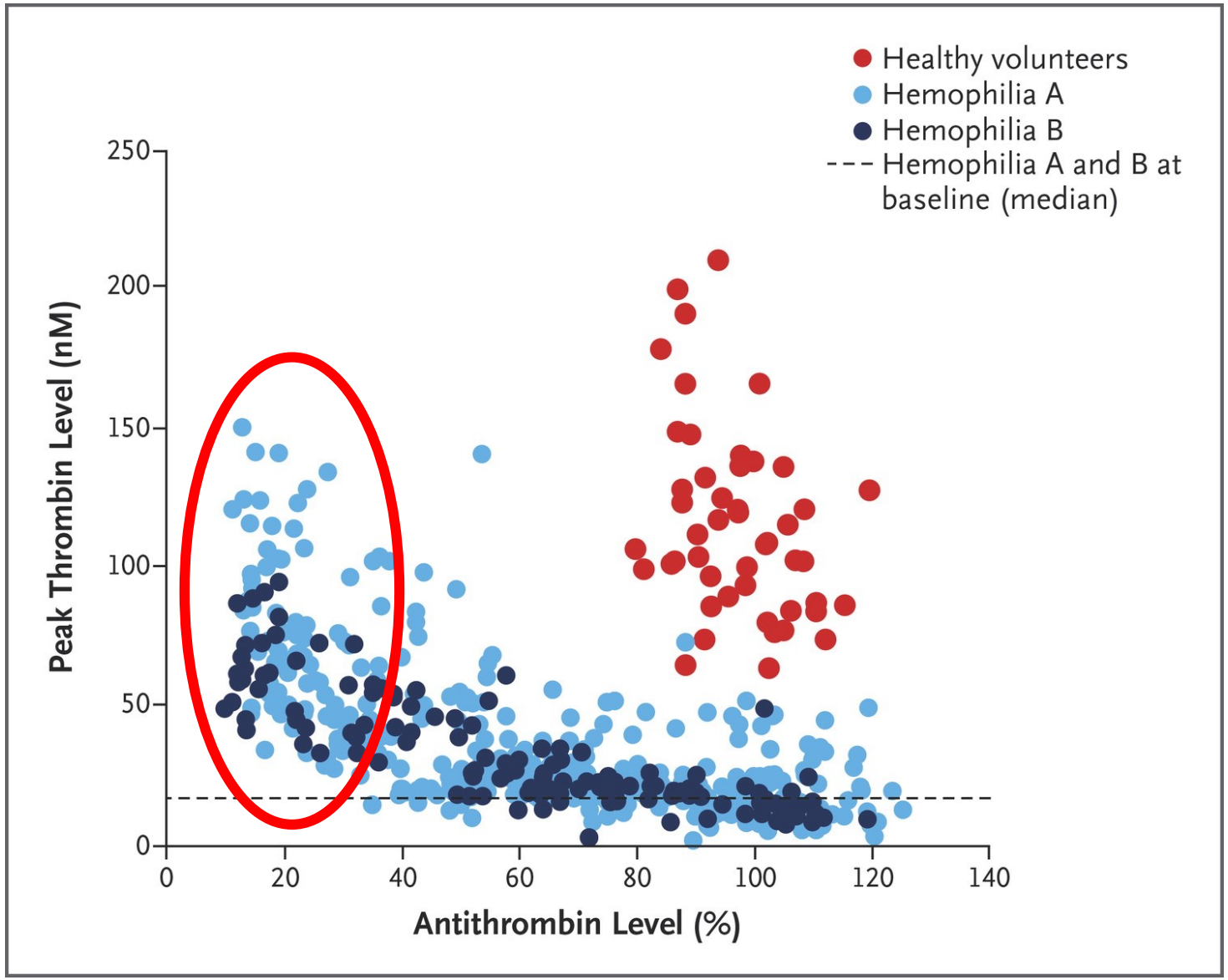


“Re-Balanced Hemostasis”



Ragni M. NEJM 2015; 373

Peak Thrombin Levels Achieved in Hemophilia A and B Patients Treated with Fitusiran

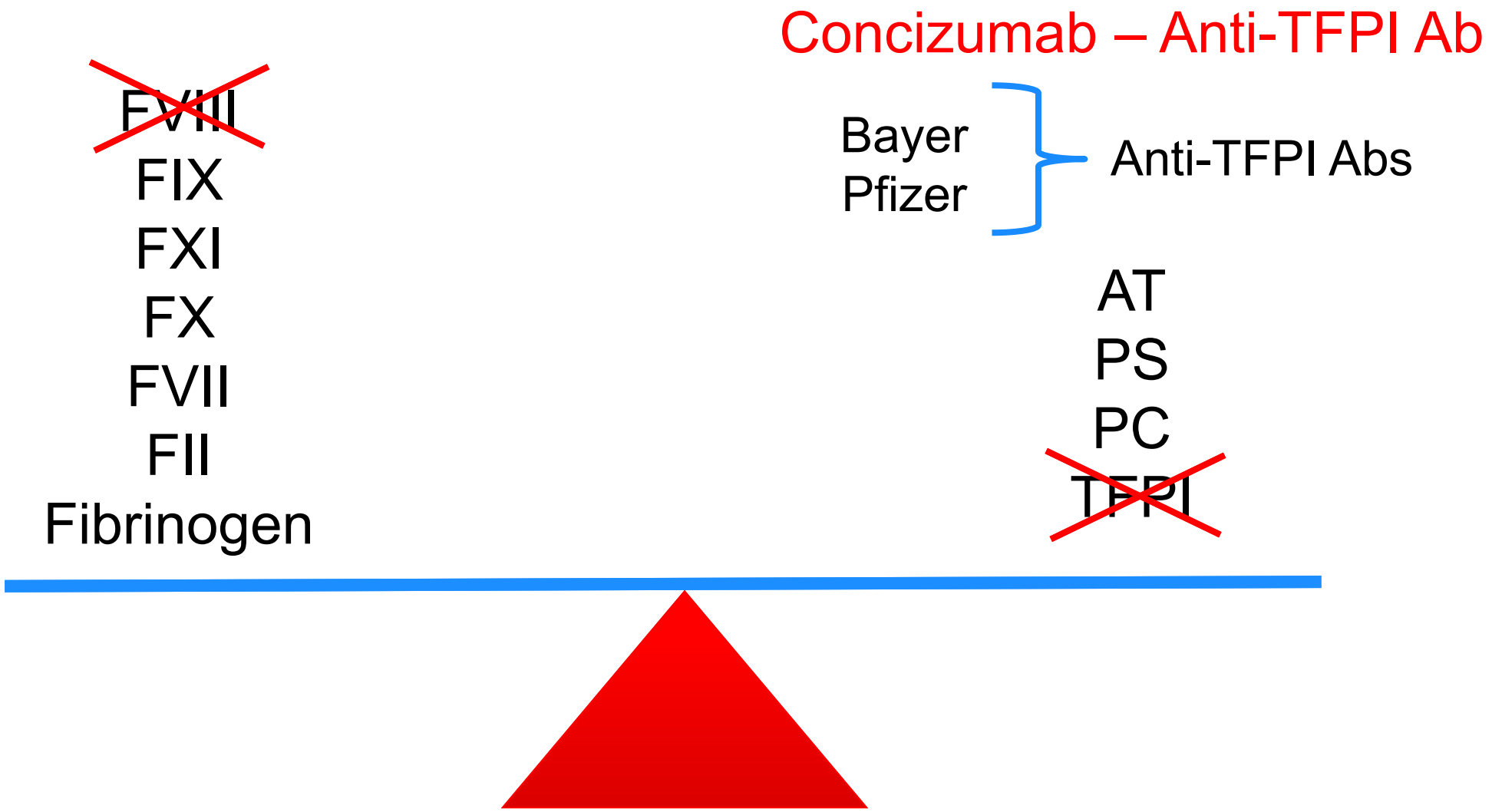


? Ability to regulate thrombin generation under non-steady state conditions -

- Inflammation
- Trauma
- Co-administration of other pro-coagulant therapies

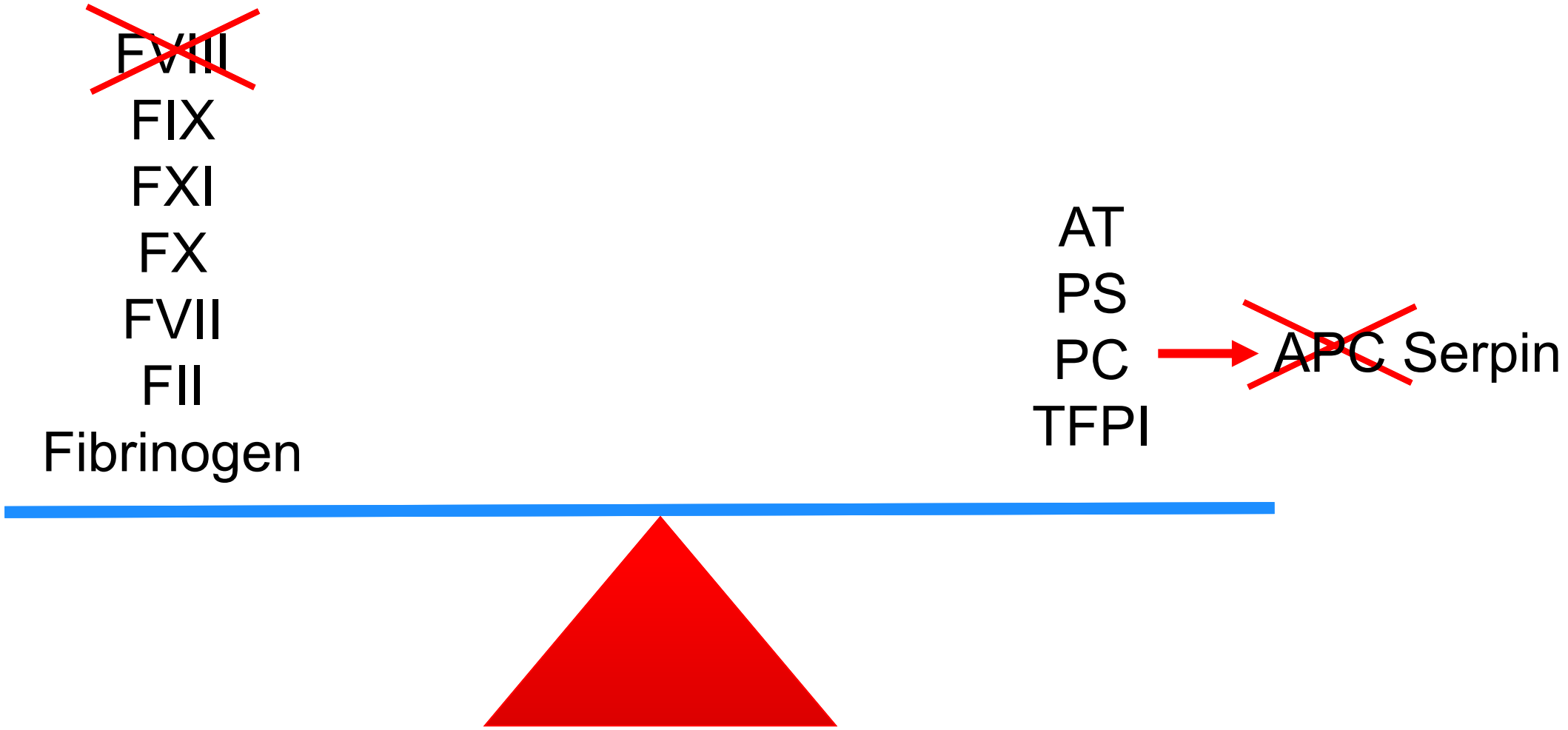
Pasi et al NEJM 2017

“Re-Balanced Hemostasis”



Ragni M. NEJM 2015; 373

“Re-Balanced Hemostasis”



a) Inhibiting Antithrombin

- ❖ Reducing its production
  - siRNA
- ❖ Inhibiting its function
  - Antibody/Aptamer/Peptide

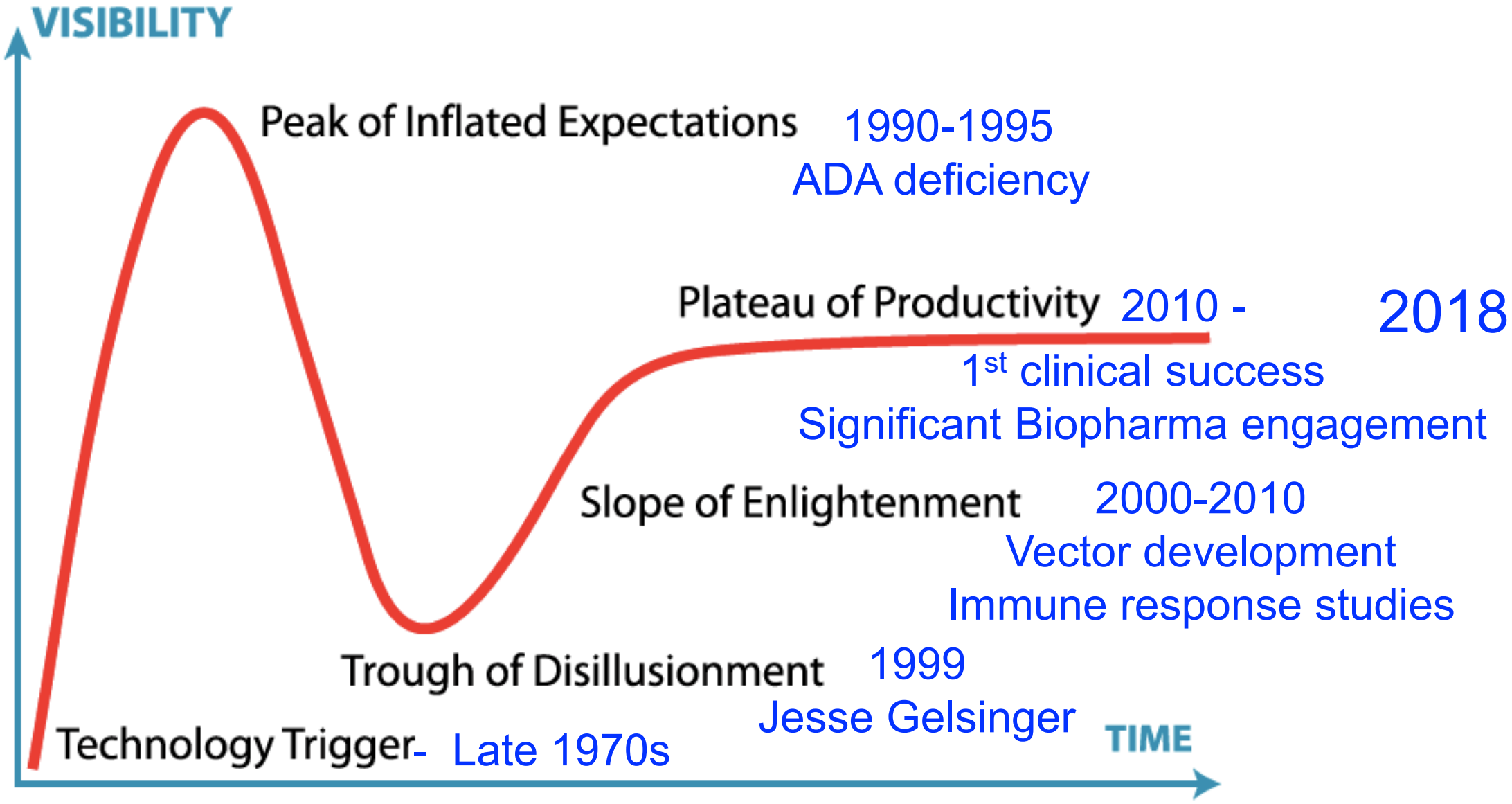
b) Inhibiting TFPI

- ❖ Anti-TFPI antibodies

c) Inhibiting Activated Protein C

- ❖ Novel bioengineered Anti-APC Serpin

Hemophilia Gene Therapy

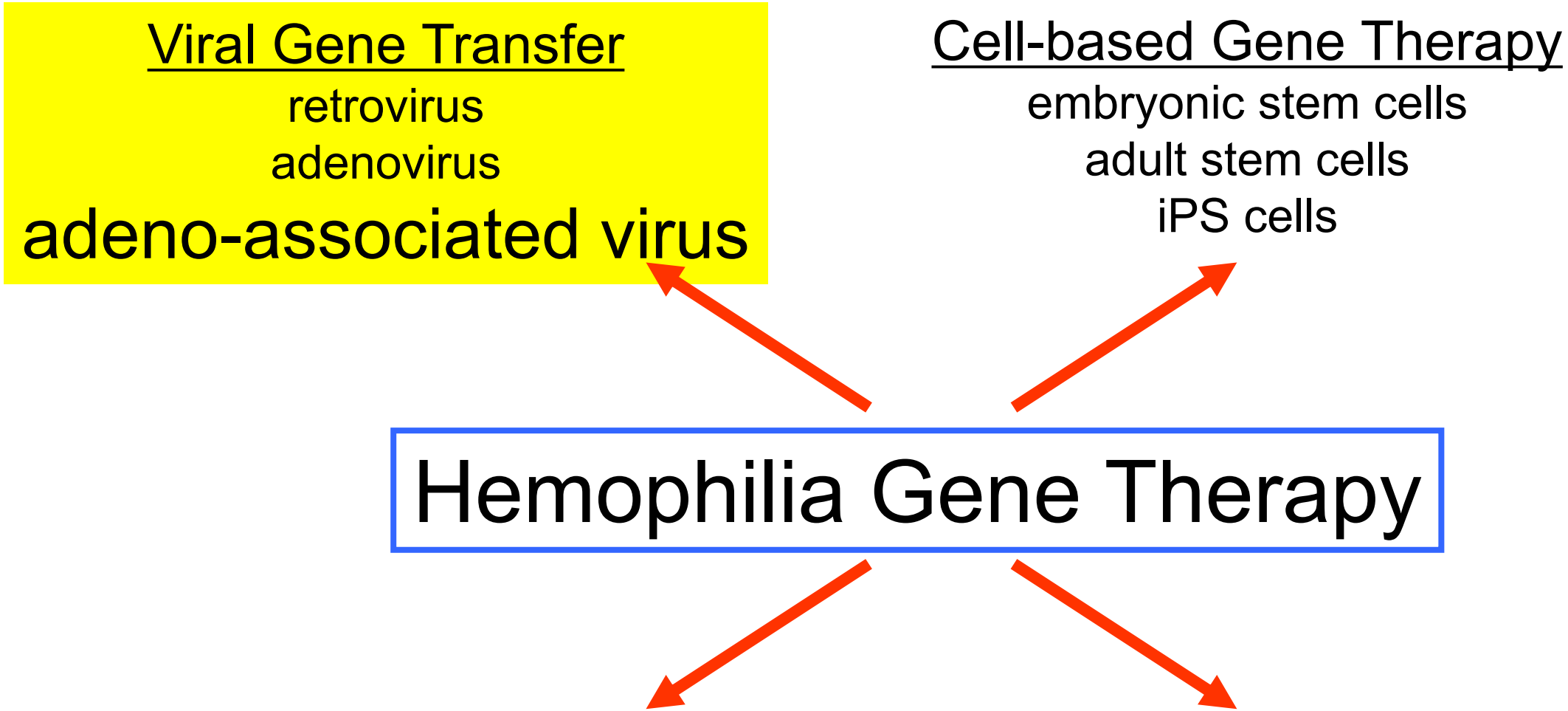


The Gartner Hype Cycle for Hemophilia Gene Therapy

Hemophilia Gene Therapy - Initial Clinical Trials

Small Phase I/II Clinical Trials Involving a total of ~50 patients
(2000 – 2006)

- Ex vivo electroporation into autologous fibroblasts (FVIII)
- IV retroviral vector (FVIII)
- IV adenoviral vector (FVIII)
- Ex vivo retroviral vector into autologous fibroblasts (FIX)
- IM AAV vector (FIX)
- Hepatic artery AAV vector (FIX)

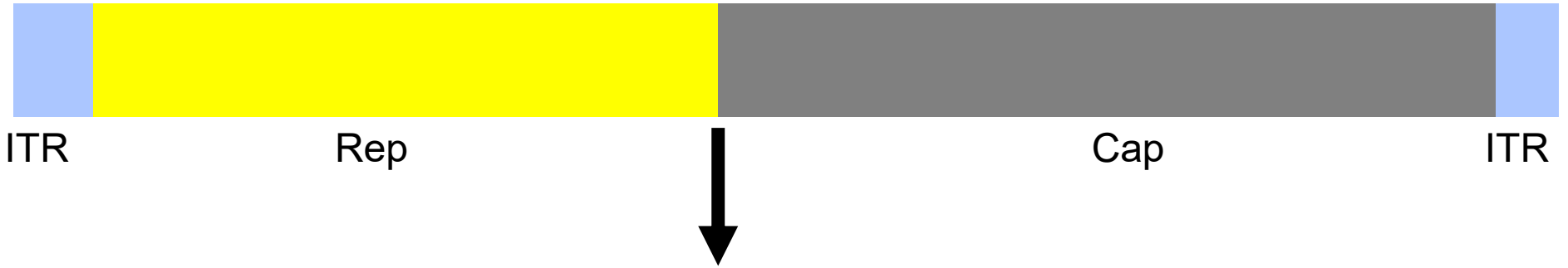

Viral Gene Transfer
retrovirus
adenovirus
adeno-associated virus
Cell-based Gene Therapy
embryonic stem cells
adult stem cells
iPS cells
Hemophilia Gene Therapy
Mutation Repair
zinc finger nucleases
TALENs
Crisp nuclease system
Non-Viral Gene Transfer
hydrodynamic delivery
oral chitosan nanoparticles
targeted nanoparticle

AAV Advantages as a Gene Therapy Vehicle

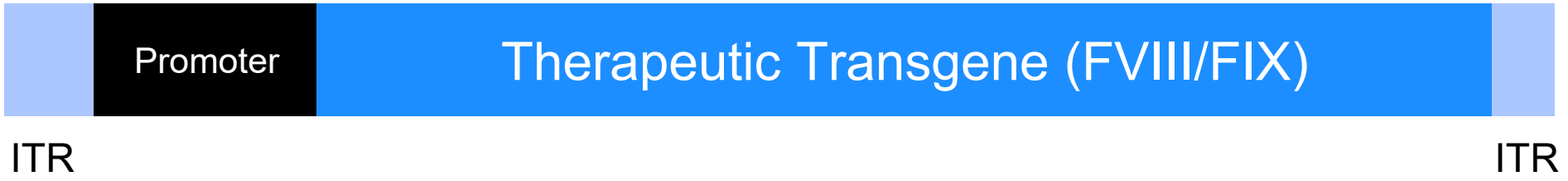
- WT AAV non-pathogenic in humans
- Different serotypes - different tissue tropisms
- Majority of vector exists as extrachromosomal/episomal concatemers
  - circular AAV genome polymers

Wild Type AAV: 4.7 kb Single Stranded DNA Genome

- AAV Virus genome



RECOMBINANT AAV VECTOR GENOME



AAV TRANSGENE CONSTRUCT

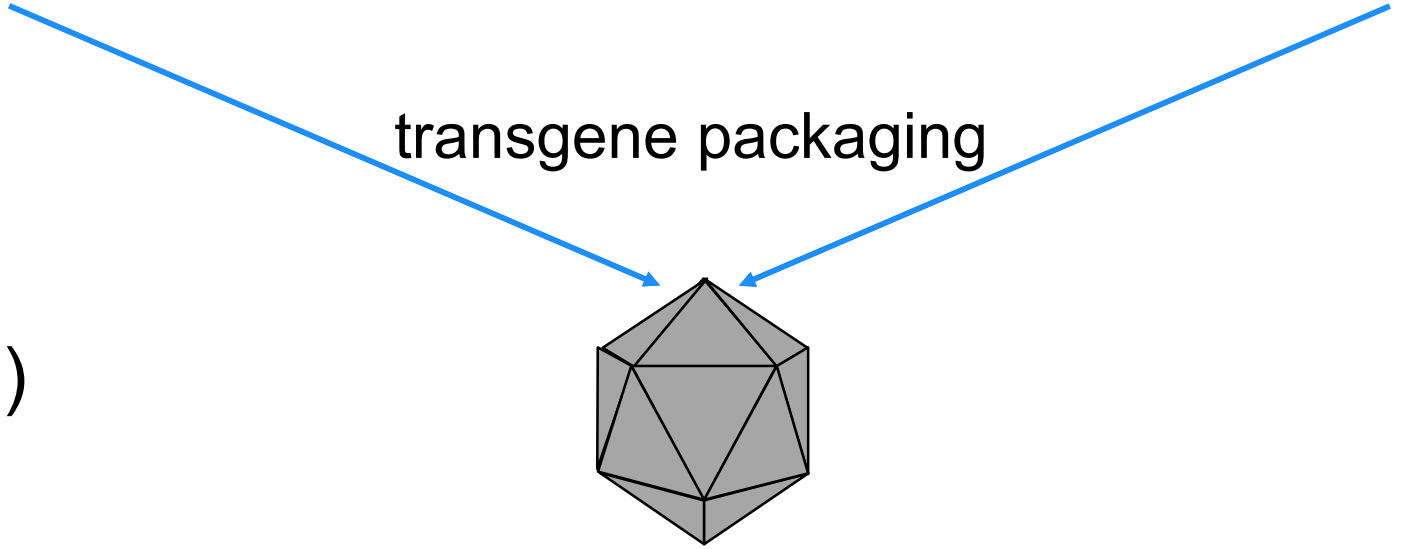
- FIX cDNA 1.3 kb
- B domain-deleted (BDD) FVIII cDNA ~4.7 kb

AAV Vector Particle Packaging & Structure

AAV Vector Genome (DNA)

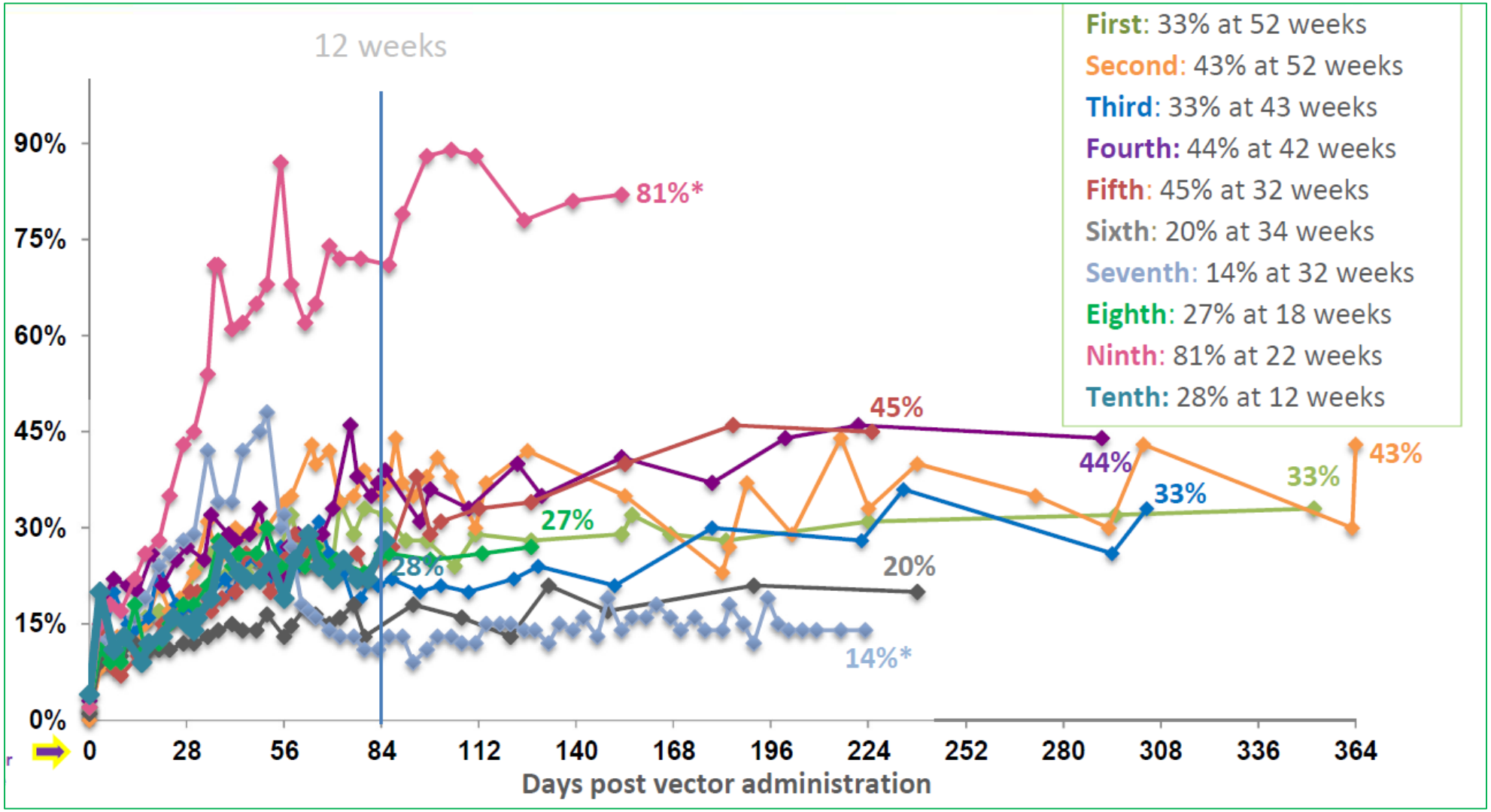
+

Protein coating (particle capsid)



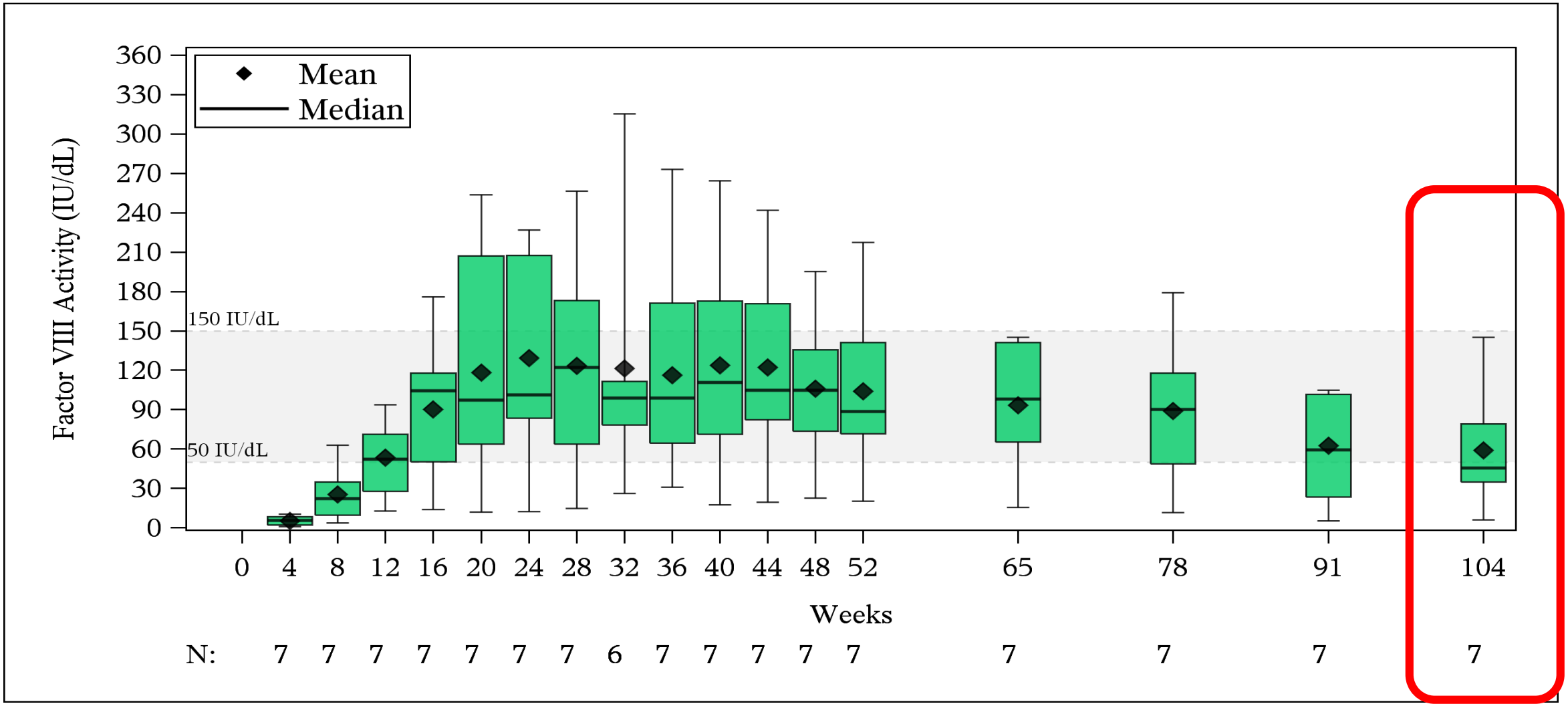
Different particle capsids (serotypes) = Different tissue tropisms

Results with SPK-9001 Factor IX Gene Therapy Trial
5 x 10^{11} vg/kg (no prophylactic steroids)



George et al. NEJM 2017

BMN 270 - Mean FVIII Activity Levels at 2 years (6e13 vg/kg)

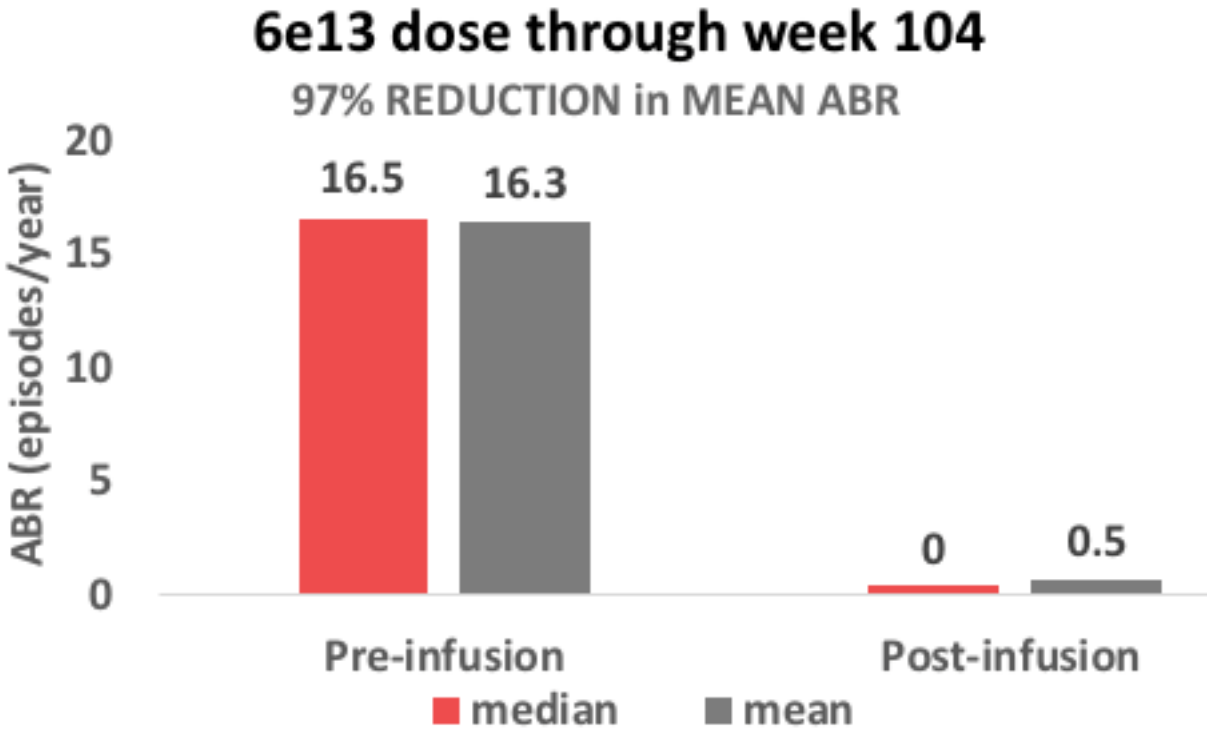


The upper and lower box bounds represent 25th and 75th percentiles. The whisker lines represent the minimum and maximum values.

Biomarin Abstract - WFH Congress 2018

Substantial Reduction in Treated Annualized Bleed Rates with BMN 270

(starting 4 weeks post-vector infusion)



Patients Bleed Free

Baseline	Year 1	Year 2
15%	71%	86%

All patients off prophylaxis

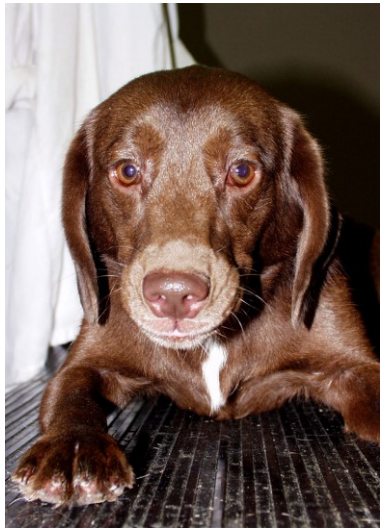
Consideration of AAV Vector Doses (vg/kg)

Nathwani 2011:	AAV8 Low dose	2×10^{11}	
Nathwani 2011:	AAV8 High dose	2×10^{12}	(10-fold)
Spark 2016:	AAV8-ish Starting dose	5×10^{11}	(2.5-fold)
Biomarin 2016:	AAV5 Low dose	6×10^{12}	(30-fold)
Biomarin 2016:	AAV5 High dose	6×10^{13}	(300-fold)

Hemophilia Gene Therapy Challenges: July 2018

- Pre-existing anti-AAV immunity - (~50%)
- Transient AAV-associated transaminitis
- Questions of long-term genotoxicity
- Capacity for vector production

Is AAV-Mediated Liver Gene Transfer Curative for Hemophilia?



9 severe hemophilia A dogs - mean follow up 10.5 years
Single AAV portal vein infusions

Pre-AAV FVIII	0%	Post-AAV FVIII	12%
Pre-AAV bleeds	4.3/yr	Post-AAV bleeds	0.2/yr

Nathwani FIX AAV8 human hemophilia B cohort > 8yrs

Summary & Conclusions

- We are witnessing a remarkable time for innovation in hemophilia therapy.
- These advances have followed 30 years of basic and pre-clinical molecular science studies.
- These new therapies will enhance the quality of life of persons with hemophilia and will significantly benefit their long-term musculoskeletal health.
- Infrequent adverse events have been documented with most of these therapies and long-term surveillance will be needed to ensure safety.
- Gene therapy for hemophilia may provide the opportunity for disease cure.

Queen’s University, Kingston, Canada