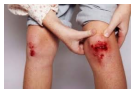
Have We Cured Hemophilia?



George A. Fritsma, MS MLS
The Fritsma Factor; Your Interactive Hemostasis Resource

Click FritsmaFactor for your 6PP handout.

1

Consultations

PrecisionBioLogic
Fritsma Factor Blog Sponsor

BioMedica Diagnostics
A Member of the Scotia Investments Family of Companies
Coag Conversations Series Sponsor

2

ASCLS-OR, ASCLS-WA Joint Spring Seminar
Have We Cured Hemophilia?

ASCLS The American Society for Clinical Laboratory Science OREGON

ASCLS The American Society for Clinical Laboratory Science WASHINGTON

The participant…

- Reviews symptoms, prevalence, pathophysiology.
- Describes factor concentrate Rx from 1980–2014.
- Assays extended half-life (EHL) concentrate Rx.
- Assays factor levels in *bypassing* Rx.
- Assays control levels in *rebalancing* Rx.
- Assays factor levels following gene transfer Rx.

3

Hemophilias: Congenital Single-factor Deficiencies

- Hemophilia A, B, and C prevalence: 1 in 8,000 male births
  - ~300,000 worldwide
  - Equivalent prevalence across people groups
  - 85% Hemophilia A (HA): FVIII deficiency
  - 14% Hemophilia B (HB, "Christmas disease"): FIX deficiency
  - 1% Hemophilia C (HC, Rosenthal syndrome) : FXI deficiency
    - Gene frequency 4.3% in Ashkenazi or Iraqi Jews
- Autosomal recessive single factor deficiencies
  - Composite prevalence ~1 in 1,000,000 prothrombin, V, VII, X, XIII

Bowyer AE, Gosselin RC. Factor VIII and factor IX activity measurements for hemophilia diagnosis and related treatments. Semin Thromb Hemost. 2023;49:609–20. doi: 10.1055/s-0042-1758870. PMCID: PMC10421651.

4

HA & HB Symptoms

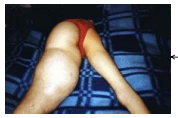
Severe	Moderate	Mild
FVIII or FIX Activity		
<1 U/dL	1–5 U/dL	6–40 U/dL
70% of patients	15%	15%
Spontaneous bleeds	Bleed after minor trauma	Bleed after major trauma

Severe HA or HB

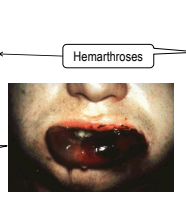
- Birth: umbilical stump and circumcision hemorrhage
- Anatomic bleeds: spontaneous, following injury or invasive procedures
  - Joints, large muscles, body cavities, GI, soft tissue, tongue, kidney, testicles, brain, CNS
  - Chronic joint damage: inflammation, hematomas, hemarthroses
- Annualized bleed rate (ABR) 0–50

5

Hemophilias: Congenital Single-factor Deficiencies



Hemarthroses



Airway Obstruction

6

HA & HB Sex-linked Recessive Inheritance

- Hemophilia A *F8* gene: 186 M bases
  - Deletions, stop codons; missense and nonsense point mutations
  - 25–30% spontaneous mutation rate
- Hemophilia B *F9* gene: 139 M bases
  - >500 gene variants in all regions: missense, nonsense, splice sites
- 90% quantitative deficiencies, 10% qualitative (functional)

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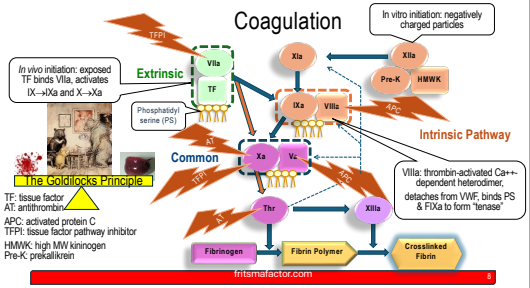
Coagulation



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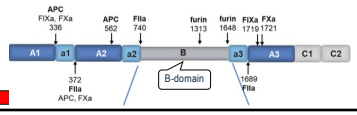
FVIII Structure

FVIII non-covalently binds von Willebrand factor (VWF), prolonging FVIII's plasma half-life from 6 mins to 12 hrs.
In the circulation, the B domain is cleaved. FVIII A1-A2 strands bind FIX while C1-C2 bind phosphatidyl serine, forming the *tenase* complex, tenase binds and activates FX⟶FXa.
Activated protein C, when bound to protein S, cleaves the A1-A2 fragment and mitigates tenase activation.



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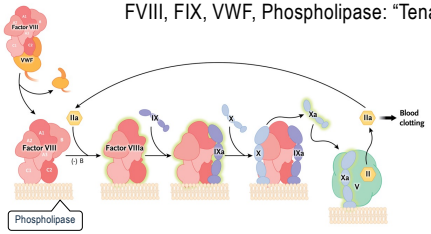
FVIII, FIX, VWF, Phospholipase: "Tenase"



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Early Hemophilia Therapy Attempts

- 1900: Landsteiner defines blood groups
- 1926: Surgeon General published 12 attempts at group-specific WB transfusion
- 1934: McFarlane: topical Russell viper venom
- 1938: Patek and Taylor, Brinkhous: first characterization of anti-hemophilic globulin
- 1938: McFarlane: fresh whole plasma
- 1944: EJ Kohn plasma chemical fractionation

  - Animals: Biggs and Macfarlane, 1954; Bidwell, 1955
  - Kekwick and Wolf, 1957; Soulier, Gobbi, Larrieu, 1957; Blomback, Blomback, Nilsson, 1958

11

Advances in the 1960–70s

- 1960: Kohn fraction prevents bleeds in extractions and minor procedures
  - First appendectomy of hemophilic
  - Stephen Christmas 1954 (HB)
- 1966: CRYO/AHF home therapy (expensive)
  - HEMOFIL M approved in 1966 for on-demand, D/Ced 3-22-25
- 1973: National Hemophilia Foundation, treatment centers
- 1980: prothrombin complex concentrate Rx for HB
  - Also 1980: activated PCC if inhibitors present
- 1980: life expectancy was 60
  - No viral inactivation
  - But high hepatitis incidence, 20,000 donors/pool

Journey, 1973, by Susan and Robert K. Massie

12

Breakthroughs: CRYO, Anti-hemophilic Factor

- 1964, Judith Graham Pool: 1919–75, Stanford
  - Developed cryoprecipitate; first home care opportunity
  - Still used for afibrinogenemia and VWD Rx
  - Died at 56 of brain tumor
  - In 1980 NHF named the Judith Graham Pool research fellowship
- 1968, Kenneth M. Brinkhous: 1908–2000, UNC Chapel Hill
  - First to chemically characterize factor VIII in 1938
  - Developed AHF using hemophilic dog experiments
  - HEMOFIL M AHF released by Baxter-Hyland in 1966
  - In 1972 NHF awarded Brinkhous the Murray Thelin Award

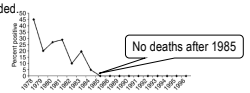
National Hemophilia Foundation (NHF)

13

Human Immunodeficiency Virus Isolation: 1983–84

- July 1983: Pasteur Institute Prof. Luc Montagnier isolates virus from lymphadenopathy patients.
- Feb 1984: Montagnier isolates the virus from CDC AIDS samples, confirmed by Robert Gallo, NIH
- Sep 1984: Alpha and Cutter demonstrate heat treatment is safe and does not raise immunogenicity
- Oct 1984: CDC/PHS publish screening and heat treatment guidelines, adopted by all agencies.
- But by 1984, 63% of 15,500 US hemophilia patients had HIV and all died.
- Since 1/1/1985, following adoption of AIDS antibody immunoassay screening, not a single new factor-transmitted HIV infection has been recorded.

Donor blood is now screened for HIV, HBV, HCV, CMV, RSV, syphilis, other viruses in endemic areas



US HIV deaths in hemophilia cohorts

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Recombinant Clotting Factors

Genentech

THE GENETICS INSTITUTE

- 1976: *Genentech* incorporated
- 1981: *Genetics Institute* incorporated
- Regulatory uncertainty: sterility, consistency questions generate 600 tests/lot
- 1982: both cloned *f9* gene and produced rFIX, a small molecule
  - r = recombinant versus plasma-derived = pdFIX
  - Poor yields, searching for a way to provide gamma-carboxylation
- 1984: Both cloned *f8* gene and produced rFVIII, but yields were small
  - 1985: rVWF coexpressed, improved rFVIII yields

Pipe SW. Recombinant clotting factors. Thromb Haemost 2008; 99: 840–50.

15

Standard Half-life (SHL) Recombinant FVIII (rFVIII) to Market

- 3/27/1987: UNC Chapel Hill, successful phase II infusion into a 39 YOA hemophilic
- Cultured in human serum albumin (HSA) matrix, theoretical viral transmission risk
- Genetics Institute with Baxter Hyland produced the first FDA-approved rFVIII
  - Recombinate® Bought by Takeda—approved 1992, D/Ced 3-22-25
- Genentech and Bayer produced Kogenate® approved 1993, D/Ced 2022

Takeda

BAYER

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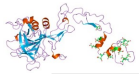
SHL pdFIX, rFIX

- Mononine® (Behring) D/Ced 2020
- Immunine® (Baxter) only remaining pdFIX, 2012
- Genetics Institute BeneFix® rFIX (no HSA, now Pfizer) 1998
  - Barrier: γ-carboxylation of 12–20 glutamic acids at amino terminus requires vitamin K, coexpressed γ-glutamyl carboxylase & furin activation enzyme
  - Prophylaxis: 90% reduced annual bleed rate



17

2003–now: SHL rVIII or IX Adoption

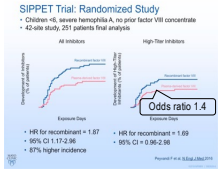
- Hemophilics migrate from pdFVIII or IX to abundant rFVIII or IX
  - ReFacto® (SOBI-Pfizer) 2000, cultured in HSA D/Ced 2024
- Rx migrated from on-demand to 2 or 3X/week infusion prophylaxis
  - 90% annual bleed rate (ABR) reduction
- For complete list of rFVIII and IX Rx see Clot Club in DiaPharma website
- BUT: recombinants doubled inhibitor frequency over plasma-derived

ADVATE (Antihemophilic Factor (Recombinant))

18

Hemophilia A Inhibitor: pdFVIII Versus rFVIII

- Inhibitor: IgG4 alloantibody to FVIII concentrate
- Meta-analysis of 20 trials; pdFVIII Vs. rFVIII
- 1248 pts, 798 severe, median age 12
- 632 treated w/ pdFVIII and 616 w/ rFVIII
- 14% inhibitors in pdFVIII arm, 25% in rFVIII
- BUT: high titer: 8.8% in pdFVIII, 12.3% in rFVIII

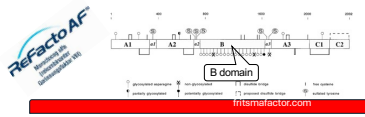


- Iorio A, Marcucci M, Marchesini E, Mannucci P. Plasma derived, and recombinant factor VIII concentrates include a different rate of inhibitor development in hemophilia A patients. A systematic review of the literature. J Thromb Haemost 2009;7
- Prezotti ANL, Frade-Guanaes JO, Yamaguti-Hayakawa GG, Ozelo MC. Immunogenicity of current and new therapies for hemophilia A. Pharmaceuticals (Basel). 2022;15:911. doi: 10.3390/ph15080911.

19

SHL B-domain Deleted (BDD) rFVIII

- Hemostatic efficacy: 90% ABR reduction
- Available for prophylaxis in severe hemophilia
- Elevated inhibitor risk remains
- Advate® (Takeda) 2003: no HSA
  - Xyntha® (Wyeth, SOBI-Pfizer) 2008: no HSA



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Extended Half-Life (EHL) rFVIII BDD Concentrates

Name	Distributor	Mechanism	Half-life (h)
Eloctate	Bioverative, SOBI	IgG Fc fragment fusion, 2014	19.7 ± 2.3
Adynovate	Takeda	Branched chain PEGylated, 2016	14.7 ± 3.8
Afstyla	CSL Behring	Single chain PEGylated. 2016	10–14
Jivi	Bayer	60-kDa PEGylated, 2018	14.3 ± 3.7
Esperoct	Novo Nordisk	60-kDa glycoPEGylated, 2019	10–14
Altuviiio	Sanofi	Fc and VWF fragment fusion, 2023	38–48
EHL rFIX Concentrates			
Alprolix	Bioverative/SOBI	IgG1 Fc fragment fusion, 2014	86.5 ± 32.2
Idelveon	CSL Behring	Albumin fusion, 2016	104 ± 18.7
REBININE/Refixia	Novo Nordisk	40-kDa glycoPEGylated, 2017	70–89

But how to measure?

21

One-stage Clot-based FVIII Assay (OSA)

Same procedure for FIX or FXI

- Prepare platelet-poor patient plasma
- Dilute plasma 1:10 with Owren-Kohler buffer
- Mix dilution 1:1 with commercial FVIII-depleted reagent plasma
  - Ensures all factors present except the one being measured
- Add an equal volume of PTT reagent (1:1), Incubate 3"
- Add an equal volume of $CaCl_2$, time to clot formation
- Compare time interval to calibrator curve, convert to U/dL of factor

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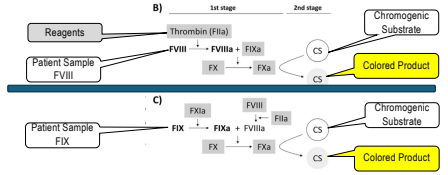
OSA Considerations

- Platelet-poor patient plasma
- Plasma diluent: Owren's, Owren-Koller, Owren-veronal, HEPES, imidazole buffer
- PTT reagent particulate activator: silica, kaolin, ellagic acid, polyphenols, Celite
- PTT phospholipid (PL) source: animal, plant, synthetic
  - PLs: phosphatidylserine, phosphatidylcholine, phosphatidylinositol, sphingomyelin, phosphatidylethanolamine
- Lower limit of quantification (LLOQ) <1 U/dL
- Coagulometer: optical or electromechanical, sole-source reagents
- FVIII-immunodepleted frozen or lyophilized plasma
  - <1 U/dL factor, with or without VWF
  - Congenital deficient becoming rare
  - All other factors present at ~100 U/dL
- Calibrator: traceable, 3–7 dilutions, must match EHL concentrate
- Calibration curve: linear, log–log; linear–log, polynomial

23

Two-stage Chromogenic Substrate (CSA) VIII & IX Assays



Müller J, Miesbach W, Prüller F, et al. Standing Commission Labor (STAEKOLA) of the Society of Thrombosis and Haemostasis Research (GTH). An update on laboratory diagnostics in haemophilia A and B. Hamostaseologie. 2022 ;42:248–260. doi: 10.1055/a-1665-6232.

24

CSA Considerations

- More complex and expensive?
- The OSA relies on patient plasma to provide normal procoagulant activities required for clot formation.
- The chromogenic assay only requires FXa generation from the FVIIIa/FIXa complex, thereby isolating the two factors that may be deficient in HA or HB.
  - The **FVIII** CSA supplies excess reagent **FIX** so the amount of plasma FIX is irrelevant.
  - The **FIX** CSA supplies excess reagent **FVIII** so the amount of plasma FVIII is irrelevant.
- CSAs that use bovine FX and/or FII provide superior accuracy and precision over human reagents.
- Only CSAs with bovine FX reagent provide accurate FVIII measurement in emicizumab (HemLibra) Rx.
- CSAs and OSAs underestimate Humate-P (pdFVIII), Wilate (pdFVIII/VWF), Obizur (porcine FVIIIa)
- CSAs underestimate gene transfer FVIII and FIX.

- Quinton T, Fraser J, Black KM, Sadeghi-Khomami A. Performance of a chromogenic factor IX activity assay in the recovery of factor IX replacement therapies. Poster presented at ISTH 2022.
- Douglas C, Quinton T, Cabanban E, Black K, Sadeghi-Khomami A. A standardized kit for a chromogenic modified Nijmegen-Bethesda assay: repeatability, reproducibility, and analytical sensitivity. Blood 2018; 132 Supplement 1: 1201.

25

One-stage clot-based assay (OSA) v. Two-stage Chromogenic Assay (CSA)

- When diagnosing FVIII or FIX deficiency; OSA and CSA results usually match.
  - If discordant but both are within the RI or in the 10–40 U/dL range, establish Dx.
  - Result of one is within RI but the other falls in the 10–40 U/dL range…
    - Bleeding phenotype coincides with the lower value.
    - For a firm diagnosis, follow up with genetic sequencing.
- OSA or CSA may underestimate or overestimate concentrate plasma concentration…
  - Use dedicated calibrators and controls.
  - Document methodology, identify reagents.
  - Match and validate with computed Rx concentrate.
  - Check published data, document correction factors.
  - Participate in external quality assessments.
- Communicate limitations with hemophilia center

Most assays are employed to follow Rx, not to diagnose.

Sommer JM, Sadeghi-Khomami A, Barnowski C, Wikén M, Willemze AJ. Real-world assay variability between laboratories in monitoring of recombinant factor IX Fc fusion protein activity in plasma samples. Int J Lab Hematol. 2020 Jun;42(3):350-358. doi: 10.1111/ijlh.13189.

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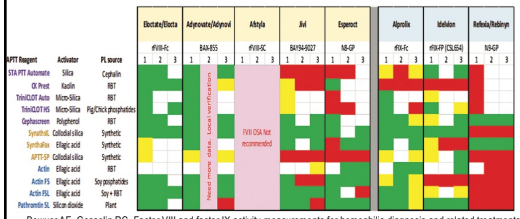
EHL FVIII & FIX Concentrates Assayed by OSA



Bowyer AE, Gosselin RC. Factor VIII and factor IX activity measurements for hemophilia diagnosis and related treatments. Semin Thromb Hemost. 2023;49:609–20. doi: 10.1055/s-0042-1758870. PMID: 36473488; PMCID: PMC10421651..

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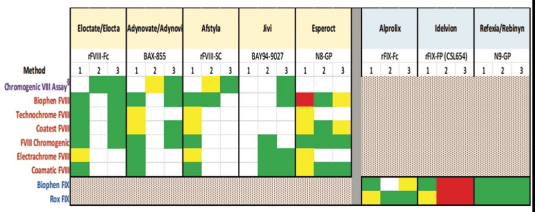
EHL FVIII & FIX Concentrates Assayed by CSA



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Current HA and HB Rx Regimens

- On-demand with SHL porcine (pFVIII), pdFVIII, rFVIII; pdFIX, rFIX
  - Moderate or mild HA, HB w/ occasional bleeds, Rx before procedures
  - Breached factor bypass or gene Rx
  - Underresourced communities
- Prophylaxis with SHL rFVIII or rFIX
  - Reduce annual bleed rate (ABR)
  - HA 2–3 infusions per week
  - HB 2 infusions per week
- EHL concentrates
  - HA 1–2 infusions per week
  - HB <1 infusion per week
- Factor bypassing Rx
  - Emicizumab HemLibra® 2017
  - Mim8 at FDA, approval December 2025?
- Rebalancing Rx in trials
  - Fitusiran AT mRNA suppression, FDA Approval March 2025?
  - TFPI and APC MoAbs approved in 2023
- Genetic transfer Rx approved 2021
  - HB: 2 therapeutics (not anymore!)
  - HA: 1 therapeutic

Østergaard H, Lund J, Greisen PJ, et al. A factor VIIIa-mimetic bispecific antibody, Mim8, ameliorates bleeding upon severe vascular challenge in hemophilia A mice. Blood. 2021;138:1258–68. doi: 10.1182/blood.2020010331. PMCID: PMC8499050.

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FVIII Bypassing Therapeutics

FEIBA: 50 U/kg/12 h prophylactic, 70 U/kg/8 h in hemorrhage	• Approved 1986 for on-demand Rx, 2013 for prophylaxis • Limit 200 U/kg/24 h to avoid DIC • Use chromogenic substrate FVIII assay when needed
rFVIIa: NovoSeven® 90 μg/kg/6 h (FVII half-life)	• Approved 1999 for on-demand Rx • Binds cell-bound tissue factor, no DIC risk
Emicizumab (HemLibra®): prophylaxis w/ or w/o inhibitors	• Approved 2017 for prophylaxis • 70% of hemophilics in industrialized countries • Use bovine-based chromogenic substrate FVIII assay

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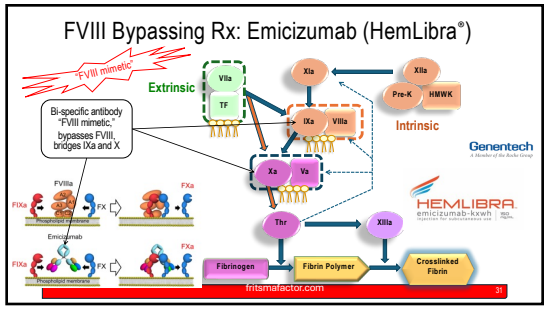

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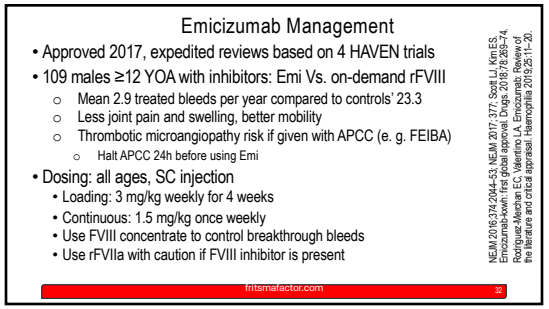

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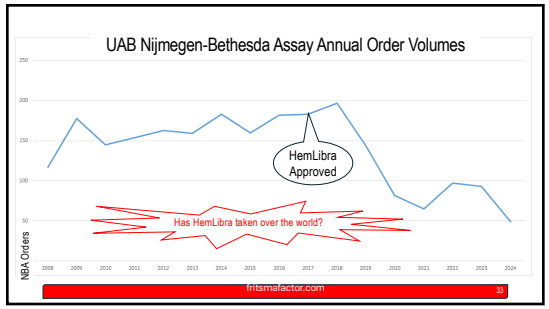

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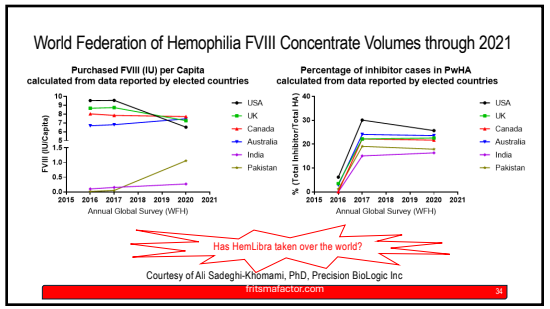

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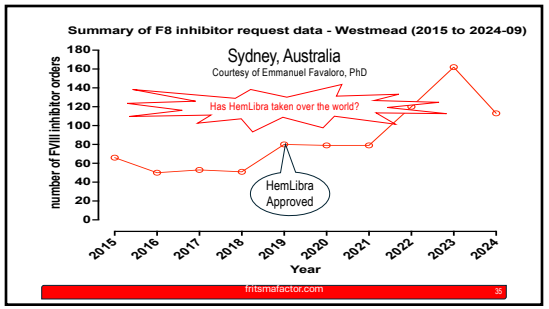

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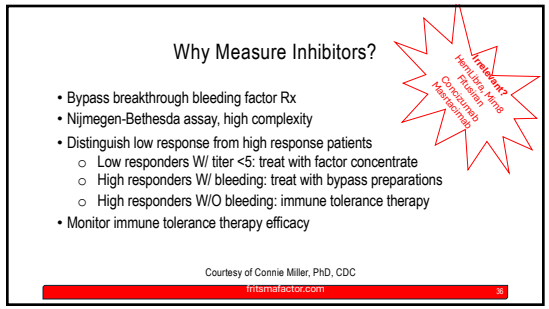

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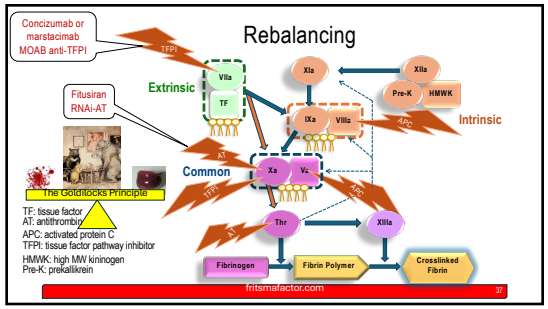

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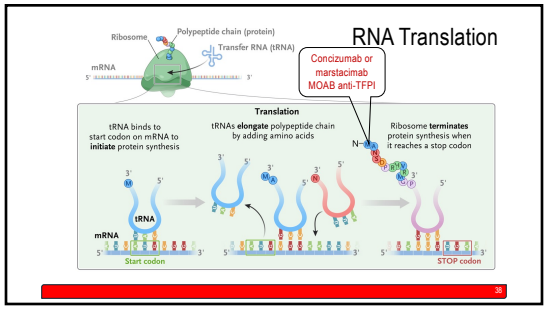

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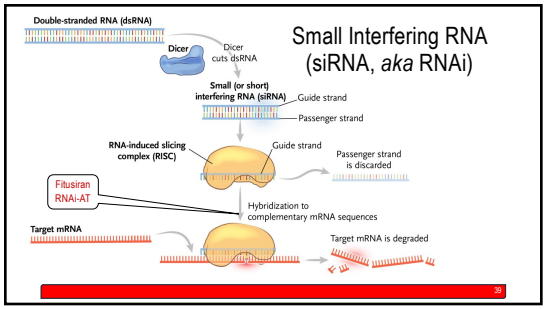

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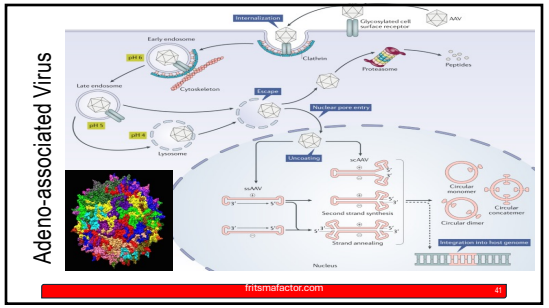
Rebalancing Therapy: Suppress TFPI or AT

- MOABs suppress TFPI translation, both FDA approved December 2024
  - Concizumab (Alhemo® Novo Nordisk) daily injection, annual cost ~$393,000
  - Marstacimab (Hympvazi® Pfizer) weekly injection, annual cost ~$795,600
  - Both shipped Feb 2025
- Synthetic interfering small RNA (RNAi) may complement any mRNA sequence
  - Suppresses mRNA translation: first used in 1990 for petunia modification
  - Fitusiran® (Sanofi) RNAi-AT targets antithrombin, FDA approval March 2025(?)
- Same Rx works for HA or HB
  - No need to assay FVIII or FIX; will monitor TFPI or AT

- Napoli C et al, Plant Cell , 1990; Novina CD, Sharp PA, Nature 2004
- Margaret Ragni, MD, MPH, University of Pittsburgh, THSNA, Chicago 4/14/16

fritsmafactor.com 40

40



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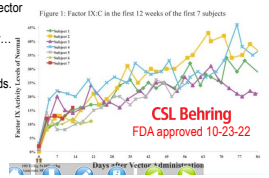
HEMGENIX® Etranacogene Dezaparvovec-dnb Hemophilia B (FIX) Gene Transfer *Cure*

- Adeno-associated virus (AAV) liver tropism vector
- For hemophilia B patients who…
  - Currently use FIX prophylaxis therapy, or…
  - Have current or historical life-threatening hemorrhage, or…
  - Have repeated, major spontaneous bleeds.
- *Factor IX Padua* gene natural variant
  - Activity 8X wild-type
- Transient liver enzymes, treat with prednisolone, no inhibitors
- Rack-rate charge is $3.5 m/dose



Shah J, Kim H, Sivamurthy K, et al. Comprehensive analysis and prediction of long-term durability of factor IX activity following etranacogene dezaparvovec gene therapy in the treatment of hemophilia B. Curr Med Res Opin. 2023;39:227-237.

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Roctavian® Valoctocogene Roxaparvovec Hemophilia A FVIII Gene Transfer Trials

- BDD *f8* gene with minimal glycosylation
  - Vector optimization: rAAV "8" with BDD *f8* gene capacity
- FDA-approved 2023: clinical trial on 134 HA patients:
  - Median FVIII activity levels at 1 year = 41.9 IU/dL
  - 98.6% ABR decline
  - 83.8% reduction in FVIII concentrate usage
  - Elevated LFTs in 85.8% of participants; treated with prednisolone
  - Rack-rate charge is $2.9 million
  - Estimated alternative prophylactic lifetime therapy cost is $25 million

- Nathwani AC. Gene therapy for hemophilia. Hematology Am Soc Hematol Educ Program Dec 9;2022:569-578. doi: 10.1182/hematology.2022000388.
- Blair HA. Valoctocogene roxaparvovec: first approval. Drugs. 2022;82:1505–10. doi: 10.1007/s40265-022-01788-y.

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Post-Market HA Gene Transfer Rx

- Current recombinant adeno-associated virus (rAAV) vectors are limited…
  - FVIII levels 5–20 U/dL remain stable for ≥3 years but now…
  - Initially high FVIII levels decline after year 1 or 2 in post-market usage.
- No HA gene therapy recipient has developed a persistent inhibitor.
- Gain of function FVIII would allow for durable, low FVIII antigen levels but high hemostatic function.
  - Analogous to FIX Padua in all current rAAV gene therapies for HB.
- Current OSA or CSA methods underestimate computed plasma Rx factors.
  - Factors are synthesized in hepatocytes, not Kupffer cells

Samelson-Jones BJ, Doshi BS, Georg LA. Coagulation factor VIII: biological basis of emerging hemophilia A therapies. Blood 2024.

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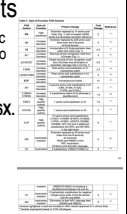
Gain of Function FVIII Attempts

- FVIII-ET3 high secretion **human/porcine** hybrid where 125 synthetic porcine amino acids from the A1 and A3 domains are substituted into rFVIII along with a porcine B-domain replacement linker: **activity/antigen ~5X** normal FVIII
- FVIII-K12 with synthetic **canine FVIII** sequence, **activity/antigen ~6X.**
- **K1813A substitution in BDD-FVIII activity/antigen ~2.4X** with "increased in vivo hemostatic potency."
- **Inactivation-resistant FVIII**, **activity/antigen ~12X.**



Samelson-Jones BJ, Doshi BS, Georg LA. Coagulation factor VIII: biological basis of emerging hemophilia A therapies. Blood 2024.

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Hemophilia Gene Transfer Rx Considerations

- One Rx: can't stop or modify Rx once started
- Post-infusion liver care with corticosteroids
- "Quiescent" joint inflammation markers based on reported pain
- No further need for prophylaxis but treat occasional bleeds?
  - Patient activity is emboldened leading to joint deterioration.
- Long-term monitoring required: what we know about durability…
  - Factor levels must remain >5mg/dL.
  - Hemophilia B: 8 years, steady
  - Hemophilia A: 6 years study per clinical trial but deteriorates in post-market patient Rx.
- Patient resistance: expense, imagined *mutagenesis, malignancy risk, vector shedding*
- Pfizer D/Ced Beqves HB gene cure 2-20-25, lack of demand

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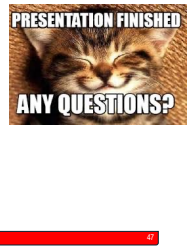
Have We Cured Hemophilia?

The participant…

- Reviewed symptoms, prevalence, pathophysiology.
- Described factor concentrate Rx from 1980–2014.
- Assays extended half-life (EHL) concentrate Rx.
- Assays factor levels in *bypassing* Rx.
- Assays control levels in *rebalancing* Rx.
- Assays factor levels following gene transfer Rx.



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