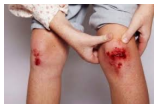
Have We Cured Hemophilia?



George A. Fritsma, MS MLS
The Fritsma Factor; Your Interactive Hemostasis Resource
Click FritsmaFactor for your 6PP handout.

1

Bottom Line at the Start (BLAST)

The participant…
- Recognizes hemophilia symptoms.
- Recounts pre-1980 hemophilia treatments (Rx).
- Describes factor concentrate Rx from 1980–2014.
- Measures extended half-life (EHL) concentrate Rx.
- Monitors factor bypassing (HemLibra, 2018) Rx.
- Explains rebalancing (fitusiran in clinical trials) Rx.
- Describes gene transfer Rx.

2

Hemophilias: Congenital Single-factor Deficiencies

- Hemophilia A, B, and C prevalence: 1 in 8,000 male births
  - ~300,000 worldwide
  - 85% hemophilia A: FVIII
  - 14% hemophilia B: FIX
  - 1% hemophilia C: FXI
    - Rosenthal syndrome: 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.

3

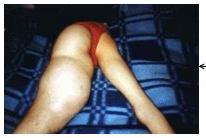
Severe HA and HB Symptoms

- 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
  - Annualized bleed rates (ABR) 0–50
- Chronic joint damage: inflammation, hematomas, hemarthroses

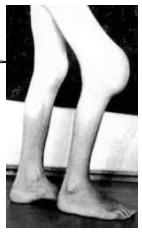
Severe	Moderate	Mild
Activity of FVIII or FIX		
<1 U/dL	1–5 U/dL	6–40 U/dL
70%	15%	15%
Spontaneous bleeds	Bleed after minor trauma	Bleed after major trauma

4

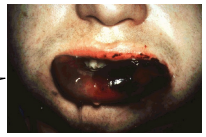
Hemophilias: Congenital Single-factor Deficiencies



Hemarthroses



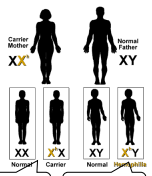
Airway Obstruction



5

HA & HB Sex-linked Recessive Inheritance

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



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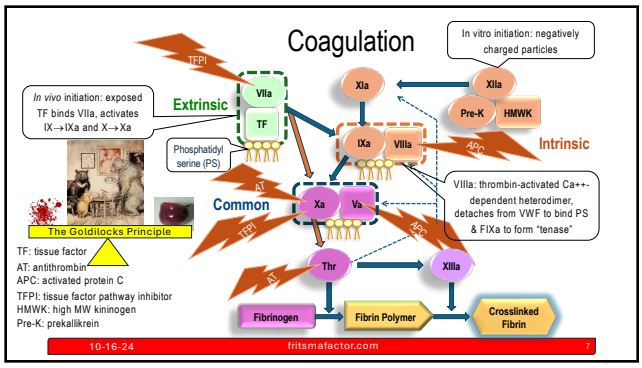
Coagulation
In vitro initiation: negatively charged particles
In vivo initiation: exposed TF binds VIIa, activates IX→IXa and X→Xa
Extrinsic
Intrinsic
Common
Phosphatidyl serine (PS)
VIIIa: thrombin-activated Ca++-dependent heterodimer, detaches from VWF to bind PS & FIXa to form "tenase"
The Goldilocks Principle
TF: tissue factor
AT: antithrombin
APC: activated protein C
TFPI: tissue factor pathway inhibitor
HMWK: high MW kininogen
Pre-K: prekallikrein
Fibrinogen
Fibrin Polymer
Crosslinked Fibrin
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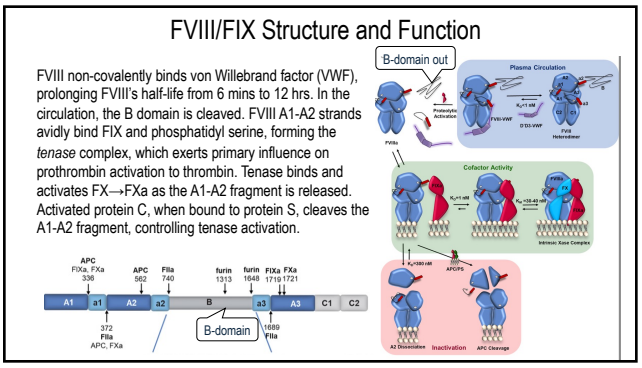
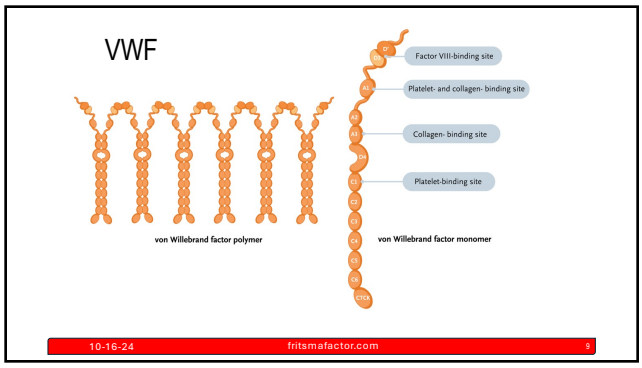
7

7


FVIII/FIX Structure and Function
FVIII non-covalently binds von Willebrand factor (VWF), prolonging FVIII's half-life from 6 mins to 12 hrs. In the circulation, the B domain is cleaved. FVIII A1-A2 strands avidly bind FIX and phosphatidyl serine, forming the tenase complex, which exerts primary influence on prothrombin activation to thrombin. Tenase binds and activates FX→FXa as the A1-A2 fragment is released. Activated protein C, when bound to protein S, cleaves the A1-A2 fragment, controlling tenase activation.
B-domain out
B-domain
Plasma Circulation
Cofactor Activity
Inactivation
APC Cleavage

8


VWF
Factor VIII-binding site
Platelet- and collagen- binding site
Collagen- binding site
Platelet-binding site
von Willebrand factor polymer
von Willebrand factor monomer
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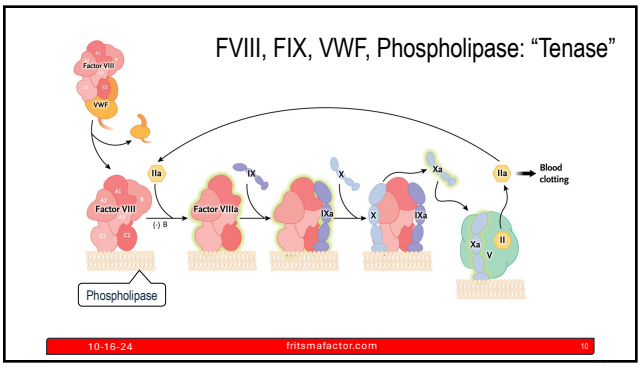
9

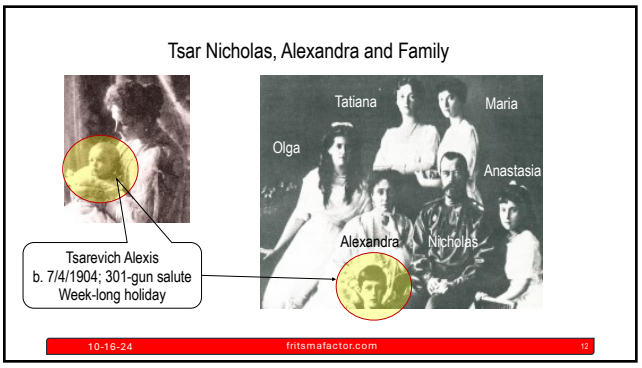
9


FVIII, FIX, VWF, Phospholipase: "Tenase"
Factor VIII
VWF
Factor VIIIa
Blood clotting
Phospholipase
10-16-24
fritsmafactor.com
10

10

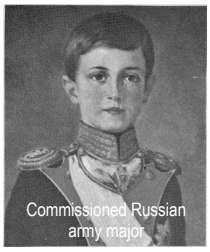

The Romanovs: Tsar Nicholas II and Alexandra (Alix, Prussian granddaughter of Queen Victoria)
Victoria, hemophilia carrier
Alix bore four girls
10-16-24
fritsmafactor.com
11

11


Tsar Nicholas, Alexandra and Family
Tsarevich Alexis
b. 7/4/1904; 301-gun salute
Week-long holiday
Tatiana
Maria
Olga
Anastasia
Alexandra
Nicholas
10-16-24
fritsmafactor.com
12

12

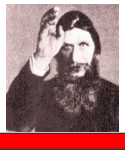
Prince Alexis, 8 YOA, 1912



13

Grigori Yefimovich Rasputin, 1869–1916

- 1869, Born Pokrovskoye, Siberia
  - Observed two sibs drown in nearby river
- 1887: 3 months in Verkhoturye monastery (in the Urals)
- 1901: *Strannik* (pilgrim), wandered Greece, Jerusalem
- 1903: Saint Petersburg, *starets* (holy man) with healing & prophetic powers
- 1905: Introduced to Alexandra by close friend Anna Vyrubova to help with Alexis' hemophilia
- 1906–12, ever present to provide Alexis' care

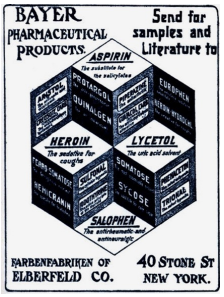


14

Rasputin's Power Rises 1912 Belovezhski Forest Holiday

- Alexis is near death, telegram to Rasputin in Siberia
  - "God has seen your tears and heard your prayers. Don't be sad, the little boy is not going to die. Don't let the doctors frighten him"
- Calming influence? distraction? leeches? faith healer? man of God? hypnotism?

Rasputin became the czar's primary adviser and political gatekeeper. He engaged in monumental debauchery, and was hated by the Russian nobles, though loved as a religious figure by the Tsarina and many of the peasants. Russians hated Alexandra because she was Prussian and hid her shyness with arrogance.

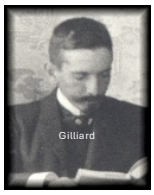


15

The Romanovs 1912–16

Pierre Gilliard, Alexis' tutor, wrote in 1921: "*The illness of the Tsarevich cast its shadow over the whole of the concluding period of Tsar Nicholas II's reign. Without appearing to be, it was one of the main causes of his fall, for it made possible the phenomenon of Rasputin and resulted in the fatal isolation of the sovereigns who lived in a world apart, wholly absorbed in a tragic anxiety that had to be concealed from all eyes.*"

Historians have since disputed the contribution of Alexis' hemophilia to Russian politics, but the strain on the royal household is clear enough.



16

Rasputin and Admirers, 1914

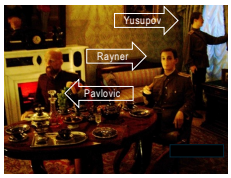
17

Yusupov Palace, St. Petersburg, 2008

18

Maj. Felix Yusupov: Dec 16, 1916

Wax figures at the Yusupov Palace



19

Shooting Rasputin

20

Hemophilia Rx Attempts 1901−1942

- Lime
- Oxygen
- Splenectomy
- Bone marrow
- Sodium citrate
- Calcium lactate
- Hydrogen peroxide
- Induced anaphylaxis
- Antidiphtheric serum
- The 'galvanic needle'
- Animal and human sera
- Adrenalin
- IV oxalic acid
- X-ray irradiation
- Serum from the mother
- Tissue fibrinogen by mouth
- Bromide extract of egg white; sedative
- Blood—both withdrawn and injected therapeutically
  - "Auto-hemotherapy"
- Female hormone therapy
  - in the belief that femininity prevents expression of the hemophilic gene

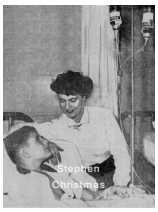
21

Effective Hemophilia Treatments

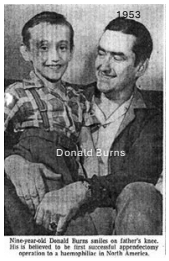
- 1900: Landsteiner defines blood groups
- 1926: Surgeon General: 12 published attempts at WB transfusion after WW I
- 1934: McFarlane: topical Russell viper venom
- 1938: Patek and Taylor, Brinkhous: first characterization of anti-hemophilic globulin
- 1938: McFarlane: fresh whole plasma
- 1944: EJ Kohn plasma chemical fractionation
  - Animals: Biggs and Macfarlane, 1954; Bidwell, 1955
  - Kekwick and Wolf, 1957; Soulier, Gobbi, Larrieu, 1957; Blomback, Blomback, Nilsson, 1958

22

Stephen Christmas 2/12/47–12/20/93



1952: Canadian Haemophilia Soc: Delineated F*IX (Christmas disease) from FVIII deficiency*
BTW, Alexis was factor IX deficient



23

Breakthroughs: CRYO, Anti-hemophilic Factor

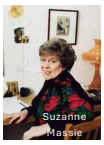
- 1964, Judith Graham Pool: 1919−75, University of Chicago
  - Developed cryoprecipitate; first home care opportunity
  - Still used for fibrinogen and VWF Rx
  - Died at 56 of brain tumor
  - In 1980 NHF named the Judith Graham Pool research fellowship
- 1968, Kenneth M. Brinkhous: 1908–2000, U North Carolina Chapel Hill
  - First to chemically characterize factor VIII in 1938
  - Developed AHF using hemophilic dog experiments
  - AHF released through Hyland in 1968
  - In 1972 NHF awarded Brinkhous the Murray Thelin Award

National Hemophilia Foundation (NHF)

24

Advances in the 1960–70s

- 1960: Kohn fraction to prevent bleeds during extractions and minor procedures
- 1968: AHF correction of hemarthroses
- 1970: CRYO/AHF home therapy (expensive)
- 1973: Hemophilia Foundation, treatment centers
- 1980: prothrombin complex concentrate for HB
- 1980: activated PCC if inhibitors present
- 1980: life expectancy was 60
  - No viral inactivation
  - High hepatitis rate, 20,000 donors/pool

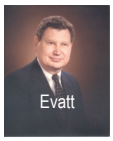


Read *Journey, 1973*, by Susan and Robert K. Massie

25

HIV; AIDS and the CDC

- Fall 1980: *Pneumocystis carinii* pneumonia (PCP) and Kaposi sarcoma (KS) in homosexual males, immunodeficiency-related conditions
  - Searched for non-infectious causes: amyl nitrite "poppers," and anal intercourse
- Spring 1982: CDC recorded three fatal PCP cases in hemophilic men receiving FVIII concentrate, reported by CDC president Dr. Bruce Evatt.
  - Paralleled reports of similar symptoms in Haitian hemophilics and in US drug abusers.
  - No homosexual behavior, led to concept of blood-borne viral infection



26

CDC Report Delivered 7/27/82

- Evatt coined the term AIDS and reported to blood and plasma industries, gay organizers, hemophilia organizations, FDA and NIH
- Consensus to defer action
  - Evidence of three patients too weak to conclude hemophilics were a risk group.
  - Concern it would stigmatize homosexual community
  - Loss of homosexual donors to blood and plasma services
  - It would cost millions to change manufacturing practices.
- Dec 1982: 6 more fatal cases: 2 non-hemophilic RBC recipients, one an infant
  - Donor services refused to reveal donor identities to avoid embarrassing and subsequently losing donors.
  - One definite identification slipped through, presumed condition.

CDC
CENTERS FOR DISEASE CONTROL AND PREVENTION

27

CDC Report #2, Delivered 1/4/1983

- CDC reported to the same groups including ARC, AABB, National Hemophilia Foundation, National Gay Task Force, Pharmaceutical Mfrs Association, Council of Community Blood Centers, State and Territorial Epidemiologists.
- CDC reported the statistical prevalence of hepatitis B (surrogate association) was the same in hemophilics and AIDS risk groups.
- Again, consensus to not act, debate was irrational, acrimonious and public, harshly critical of Dr. Evatt and CDC

28

Public Responses 1/4/1983

- CCBC and AABB: "*Transfusions are life-saving procedures; some adverse reactions are acceptable to save lives. The rare disorder affecting nine cases is not enough to force a policy change.*"
- ARC president stated: "*It has long been noted that CDC increasingly needs a major epidemic to justify its existence… In short, we can not depend on the CDC to provide scientific, objective, unbiased leadership.*"

American Red Cross

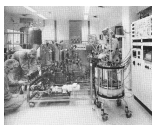
- Meanwhile, the National Hemophilia Foundation, alarmed, had contacted plasma processors in December 1982.

NATIONAL HEMOPHILIA FOUNDATION
for all bleeding and clotting disorders

29

National Hemophilia Foundation Initiative: 1983

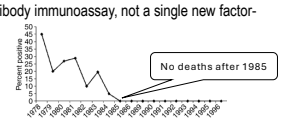
- 20% of commercial plasma came from services who refused to screen donors for sexual orientation
- Dec 1982: *Alpha Therapeutics* began to screen donors with a questionnaire
- US Public Health Service guidelines, March 4, 1983
  - CDC (Evatt) bypassed FDA, sent guidelines direct to the Public Health Service
  - Donor questionnaire and surrogate testing using hepatitis markers
- March 1983, Baxter Hyland began heat treating plasma
- August 1983, 26 confirmed cases of transfusion transmitted AIDS, including one FIX deficiency



30

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.
- Since 1/1/1985, following adoption of AIDS antibody immunoassay, 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

Frequency of HIV infection in US hemophilia birth cohorts.

31

Recombinant Clotting Factors

- 1976: *Genentech* incorporated
- 1981: *Genetics Institute* incorporated
- 1982: both cloned *f9* gene and produced rFIX, a small molecule
  - r = recombinant; compared to plasma-derived = pdFIX
- 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.

32

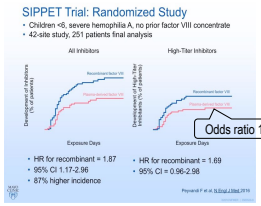
Standard Half-life (SHL) Recombinant FVIII (rFVIII) to Market

- Regulatory uncertainty: sterility, consistency questions generate 600 tests/lot
- Genetics Institute with Baxter Hyland produced the first clinically viable rFVIII
  - Recombinate®—now distributed by Takeda
- Bayer and Genentech produced Kogenate® now distributed by Bayer
- 3/27/1987: UNC Chapel Hill, successful phase II infusion into a 39 YOA hemophilic
- Recombinate® FDA approved 1992, Kogenate® 1993
- Cultured in human serum albumin (HSA) matrix, viral transmission risk

33

Hemophilia A Inhibitor: pdFVIII Versus rFVIII

- Inhibitor: IgG4 alloantibody to FVIII concentrate Rx
- 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
- High titer: 8.8% in pdFVIII, 12.3% in rFVIII

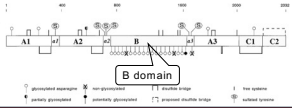


- Iorio A, Marcucci M, Marchesini E, Mannucci P. Plasma derived, and recombinant factor VIII concentrates include a different rate of inhibitor development in hemophilia A patients. A systematic review of the literature. J Thromb Haemost 2009;7
- Prezotti ANL, Frade-Guanaes JO, Yamaguti-Hayakawa GG,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

34

2003: Standard Half Life (SHL) B-domain Deleted (BDD) rFVIII

- Hemostatic efficacy: 90% cessation of bleeding
- Available for prophylaxis in severe hemophilia
- Elevated risk of inhibitor formation
- Advate® (Takeda) 2003: no HSA
- ReFacto® (SOBI-Pfizer) 2000, cultured in HSA D/Ced 2024
  - Xyntha® (Wyeth, SOBI-Pfizer) 2008: no HSA



35

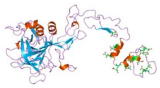
FVIII Bypassing Therapeutics

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 • Use chromogenic substrate FVIII assay when needed
Emicizumab (HemLibra®): prophylaxis w/ or w/o inhibitors	• Approved 2017 for on-demand or prophylaxis • 70% of hemophilics in industrialized countries • Use bovine-based chromogenic substrate FVIII assay

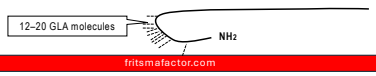
36

SHL Factor IX (pdFIX, rFIX)

- Immunine® (Baxter) only remaining pdFIX; Mononine® (Behring) D/Ced 2020
- 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



BeneFix® Coagulation Factor IX (Recombinant) Room Temperature Storage



37

2024 HA and HB Rx Regimens

- On-demand with porcine (pFVIII), pdFVIII, rFVIII; pdFIX, rFIX
  - Moderate or mild HA, HB w/ occasional bleeds, before procedures
  - Breakthrough bleeds in non-factor Rx
  - Underserved communities
- Prophylaxis with concentrates
  - Reduce annual bleed rate (ABR)
  - HA 2–3 infusions per week
  - HB 2 infusions per week
- Extended half-life (EHL) concentrates
- Factor bypassing Rx
  - Emicizumab HemLibra®
  - Mim8 in clinical trials
- Rebalancing Rx in trials
  - Fitusiran AT mRNA suppression
  - TFPI and APC mRNA suppression
- Genetic transfer Rx
  - HB: 2 meds
  - HA: 1

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.

38

Extended Half-Life (EHL) rFVIII BDD Concentrates

On-demand or prophylaxis

Name	MFR	Comment	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

Use CSA FVIII assay except Altuviiio

No inhibitors

39

EHL rFIX Concentrates

On-demand or prophylaxis

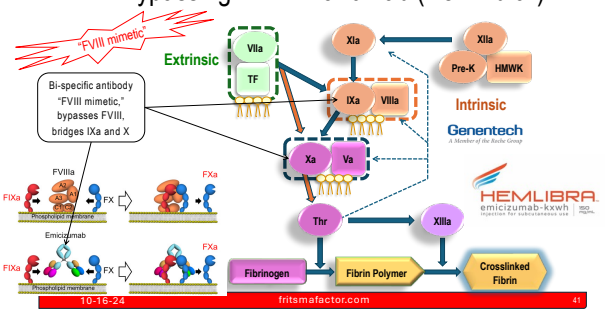
Name	MFR	Comment	Half-life (H)
Alprolix	Bioverative/SOBI	IgG1 Fc fragment fusion, 2014	86.5 ± 32.2
Idelveon	CSL Behring	Albumin fusion, 2016	104 ± 18.7
REBININE/ Refixia	Novo Nordisk	40-kDa glycoPEGylated, 2017	70–89

Chromogenic FIX assay

No inhibitors

40

FVIII Bypassing Rx: Emicizumab (HemLibra®)



41

Emicizumab Management

- Approved 2017, expedited reviews based on 4 HAVEN trials
- 109 males ≥12 YOA with inhibitors: Emi Vs. on-demand rFVIII
  - Mean 2.9 treated bleeds per year compared to controls' 23.3
  - Less joint pain and swelling, better mobility
  - Thrombotic microangiopathy risk if given with APCC (e. g. FEIBA)
    - Halt APCC 24h before using Emi
- Dosing: all ages, SC injection
  - Loading: 3 mg/kg weekly for 4 weeks
  - Continuous: 1.5 mg/kg once weekly
  - Use FVIII concentrate to reverse breakthrough bleeds
  - Use rFVIIa with caution if FVIII inhibitor is present

NEJM 2016;374:2044–53; NEJM 2017; 377; Scott LJ, Kim ES. Emicizumab-kxwh: first global approval. Drugs. 2018;78:269–74. Rodriguez-Merchan EC, Valentino LA. Emicizumab: Review of the literature and critical appraisal. Haemophilia 2019;25:11–20.

42

FVIII "Mimetic" Mim8

Bagsværd, Denmark, 13 May 2024—Novo Nordisk today announced the results from FRONTIER 2, a pivotal phase 3a, 26-week open-label, randomized, controlled, multi-arm trial in 254 people. The trial investigated the efficacy and safety of once-weekly and once-monthly subcutaneous Mim8 versus no prophylaxis and versus prior coagulation factor prophylaxis treatment in people aged 12 years or older with hemophilia A with or without inhibitors.

The enhanced potency of the FVIII-mimetic Mim8 compared to emicizumab is likely due to Mim8 avidity for the FIX molecule. Anticipated approval: 2026.

novo nordisk®

43

Hemophilia Agencies

- World Federation of Hemophilia (WFH)
- National Organization for Rare Disorders
- American Society of Gene and Cell Therapy
- National Bleeding Disorders Foundation (NBDF)
  - Was National Hemophilia Foundation (NHF)
- European Hemophilia Consortium

NATIONAL BLEEDING DISORDERS FOUNDATION

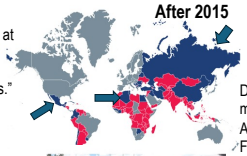
EHC

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

44

WFH Distributes Therapeutics

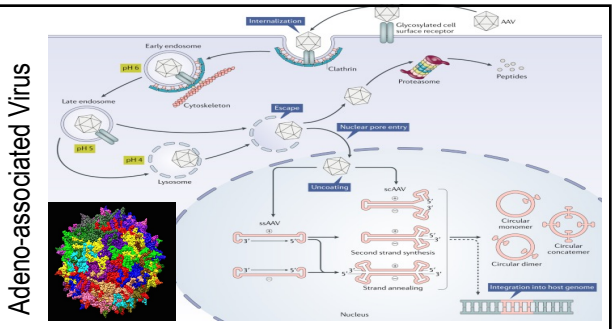
"The WFH Humanitarian Aid Program…provides much-needed factor and non-factor Rx to providers at national member organizations (NMOs), and hemophilia treatment centers (HTCs) in emerging countries."



During the last WFH annual meeting of the General Assembly, the Rwanda Fraternity Against Hemophilia (RFH) was accredited as an NMO—bringing the total of NMOs in the WFH family to 147.

45

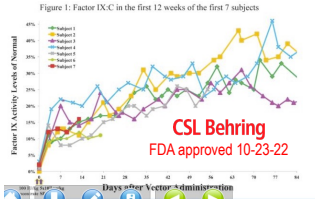
Adeno-associated Virus



46

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 million/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.

47

Roctavian® Valoctocogene Roxaparvovec Hemophilia A FVIII Gene Transfer Trials

BIOMARIN

- B-domain-deleted FVIII gene with minimal glycosylation
- Vector optimization: rAAV "8" with B-domain deleted *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% decline in annual bleed rate
  - 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 current 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.

48

Post-Market HA Gene Transfer Rx

- Current rAAV vectors are limited…
  - initially high FVIII levels decline after year 1 or 2 in post-market usage
  - FVIII levels 5–20 U/dL remain stable for ≥3 years.
- 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.

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

49

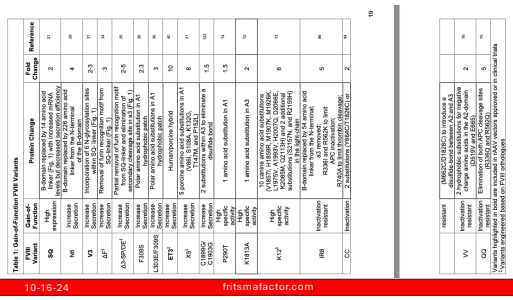
Gain of Function FVIII Examples

- Porcine variant: FVIII-ET3 is a 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 ratio ~5.
- Canine variant: A FVIII variant (FVIII-K12) based on the synthetic canine FVIII sequence, has sixfold higher specific activity than wild-type FVIII.
- K1813A substitution in BDD-FVIII increased specific activity 2.4-fold and increased in vivo hemostatic potency.
- Inactivation-resistant FVIII variant has twelvefold hemostatic function.

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

50

Gain of Function FVIII Attempts



51

Hemophilia Gene Transfer Rx Considerations

- Can't stop or modify treatment once started
- Post-infusion care with corticosteroids: reduced pain, "quiescent" joint inflammation markers
- Patients emboldened about activities, finding some joint deterioration
- No further need for prophylaxis but treat occasional bleeds?
- Long-term monitoring required: what we know about durability
  - Hemophilia A: 6 years per clinical trial, but seems to diminish in post-market patient Rx
  - Hemophilia B: 8 years, steady
  - Factor levels must be > 5 mg/dL
- Patient resistance to HS: expense, *imagined mutagenesis, malignancy risk, vector shedding*

52

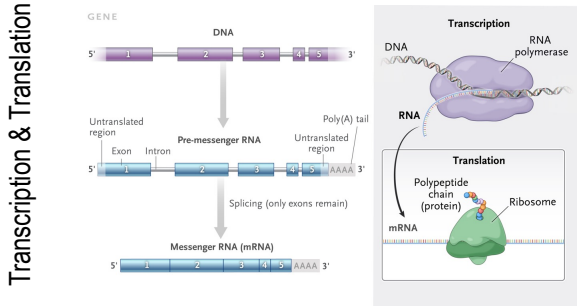
Rebalancing Rx: Fitusiran®: RNAi Targets Antithrombin

- RNAi (interfering RNA): synthetic RNA complementary to an mRNA sequence, reduces mRNA translation
- RNAi can be produced against any gene product: first accomplished in petunias
- RNAi-AT binds antithrombin mRNA and reduces antithrombin production
- Concizumab suppresses TFPI translation
- Same Rx for HA or HB Rx

- 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

53

Transcription & Translation



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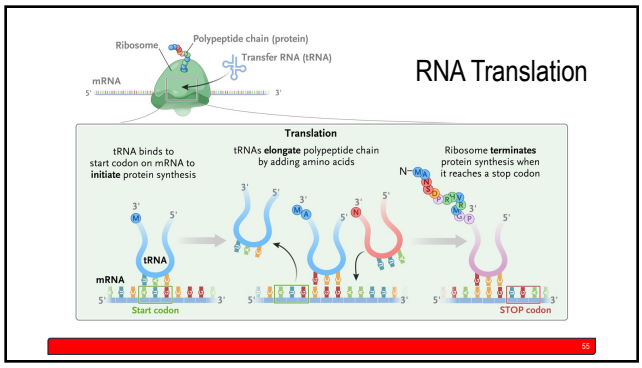

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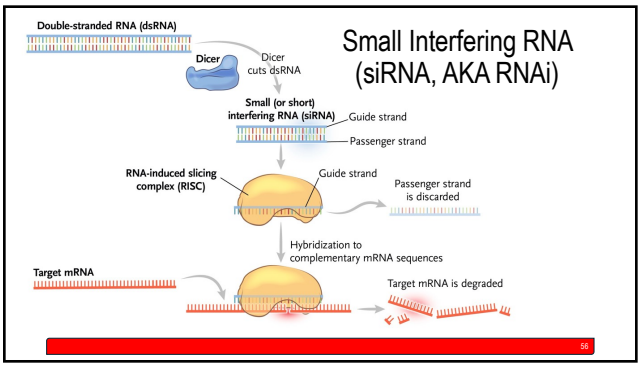

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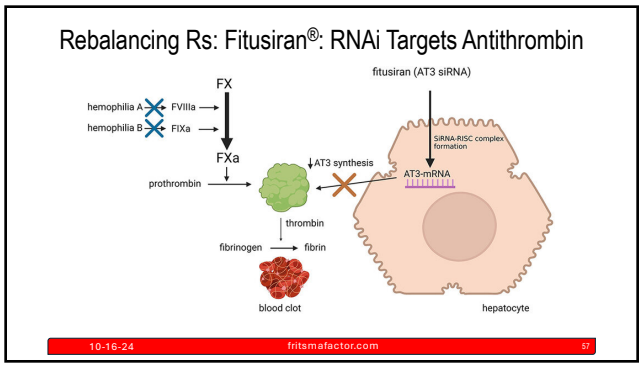

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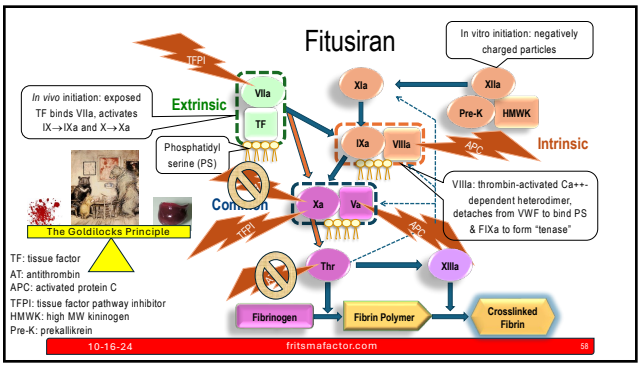

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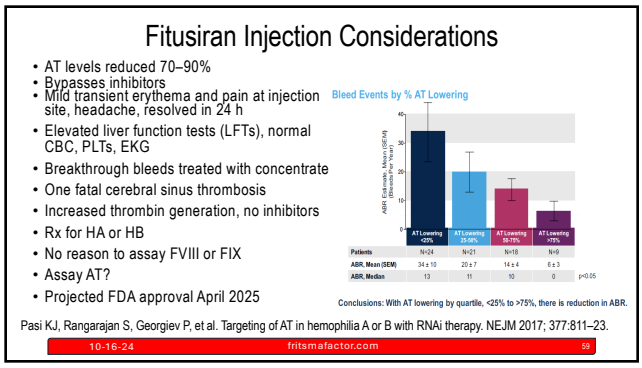

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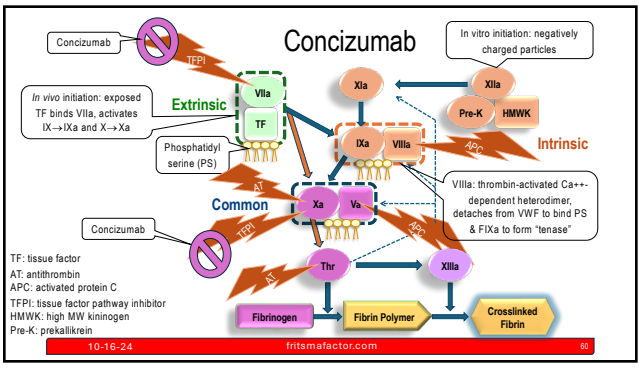
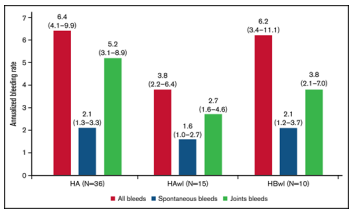


60

Concizumab Considerations

Phase 2 trial paused in 2020, 29 patients:

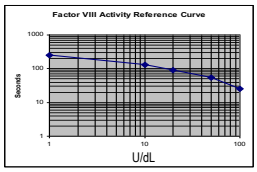
- Acute myocardial infarction in HA patient
- Renal infarction in HB patient with FIX inhibitor
- DVT, pulmonary embolism, and superficial venous thrombosis in HA patient
- Low-titer anti-drug antibodies in 9 subjects



61

One-stage PTT-based FVIII Assay (OSA)

1. Dilute pt plasma 1:10 in buffered saline (BS)
2. Add FVIII-depleted reagent plasma 1:1
   - Provides all factors except FVIII
   - Typical : 100 uL pt plasma dilution + 100 uL FDP
3. Add 100 uL PTT reagent, incubate 3 min
4. Add 0.025M $CaCl_2$, record interval to clot formation
5. Compare result in seconds to calibration curve to derive plasma activity



Our little secret: external survey CVs 20–30%

62

OSA Considerations

- PTT reagent particulate activator: silica, kaolin, ellagic acid, polyphenols, Celite
- PTT phospholipid (PL) source: animal, plant, or synthetic
  - PLs: phosphatidylserine, phosphatidylcholine, phosphatidylinositol, sphingomyelin, phosphatidylethanolamine
- Lower limit of quantification (LLOQ) <1 U/dL
- Coagulometer: optical or electromechanical, sole-source reagents
- Coagulometer: multiple dilutions, may offer chromogenic substrate assay channel
- Factor-depleted or deficient plasma (FDP): <1 U/dL factor, with or without VWF
  - FDP immunodepleted or congenital, frozen or lyophilized
- Calibrator: traceable, 3–7 dilutions, must match EHL concentrate
- Calibration curve: linear, log–log; linear–log, polynomial
- Plasma diluent: Owren's, Owren-Koller, Owren-veronal, HEPES, imidazole buffer

63

FVIII Assay Plasma Dilutions Parallelism Indicates No Inhibitor

Automated Plasma Dilution	Seconds	Raw Factor VIII Activity	Computed Factor VIII Activity (Raw × Dilution)
1:10 "undiluted"	90 s	20 U/dL	20 U/dL
1:20	105 s	10 U/dL	20 U/dL (parallel)*
1:40	107 s	5.5 U/dL	22 U/dL (parallel)
1:80	110 s	2.6 U/dL	20.8 U/dL (parallel)
* <30% difference from undiluted indicates parallelism, no inhibitor			

64

FVIII Assay Plasma Dilutions non-Parallelism Indicates Inhibitor

Plasma Dilution	Seconds	Raw Factor VIII Activity	Computed Factor VIII Activity (Raw × Dilution)*
1:10 (undiluted)	95 s	10 U/dL	10 U/dL
1:20	99 s	8 U/dL	16 U/dL
1:40	107 s	5 U/dL	20 U/dL
1:80	108 s	4 U/dL	32 U/dL
* >20% difference from undiluted = non-parallel & rising, implies inhibitor			

65

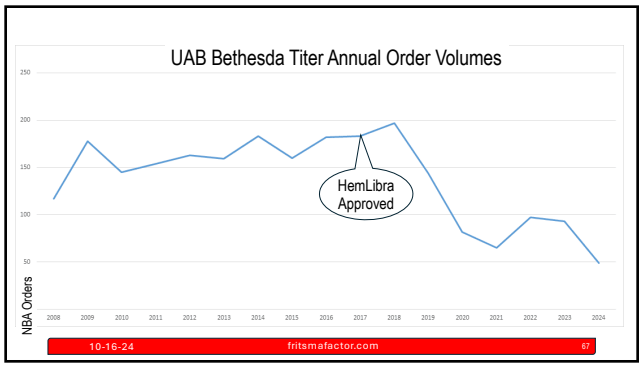
Why Measure Inhibitors?

- Nijmegen-Bethesda assay, high complexity
- Confirm refractory response to factor therapy
- Monitor efficacy of prophylactic FVIII or FIX therapy
- Distinguish low response from high response patients
  - Low responders: Rx with factor concentrate
  - High response when bleeding: Rx with bypass preparations
  - High response when not bleeding initiate immune tolerance therapy
- Monitor immune tolerance therapy efficacy
- Establish prevalence and population trends
  - Hemophilia severity, correlate to mutation, correlate to therapy

Irrelevant? HemLibra? Mim8 Fitusiran Concizumab

Courtesy of Connie Miller, PhD, CDC

66



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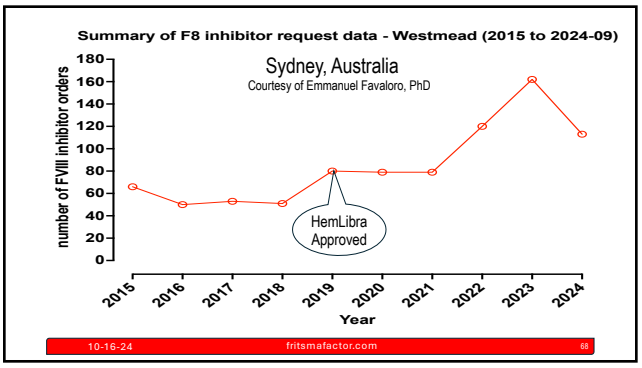

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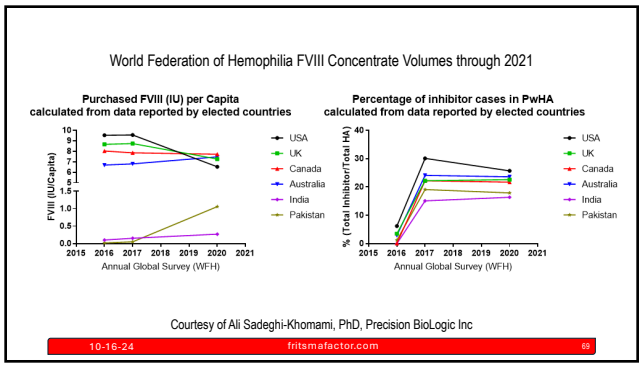

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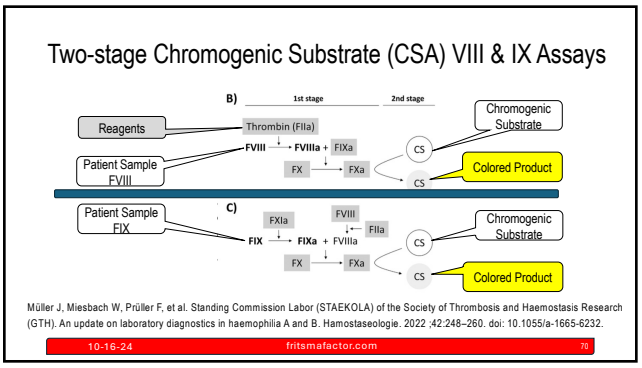

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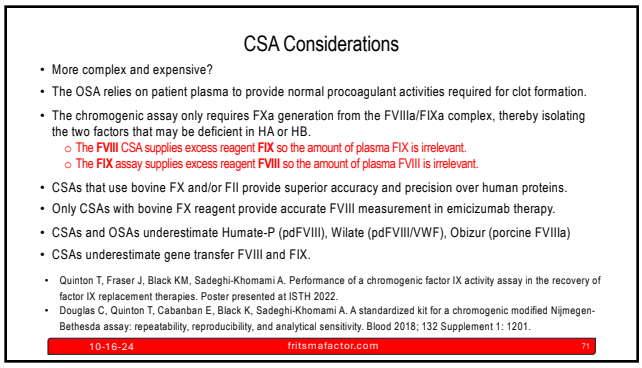

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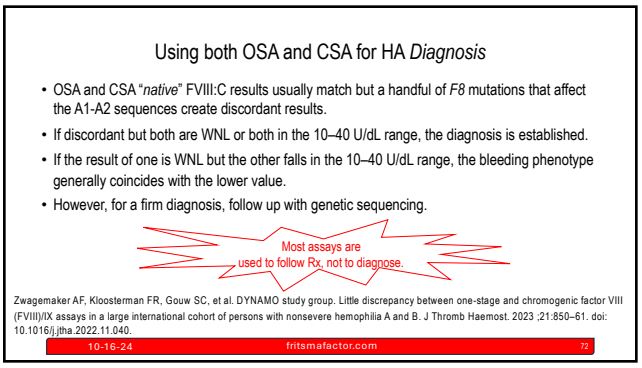
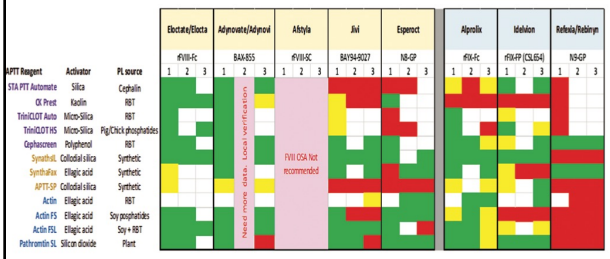


72

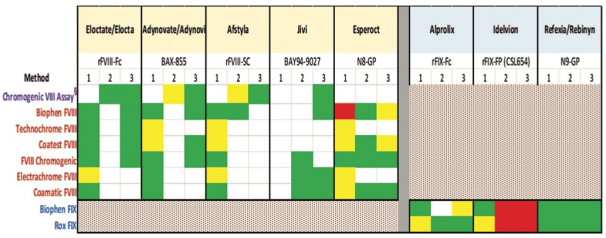
EHL FVIII & FIX Concentrates Assayed by PTT



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..

73

EHL FVIII & FIX Concentrates Assayed by CSA



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..

74

Summary: Monitoring EHL Concentrates

- PTT-based OSA may underestimate or overestimate FVIII and FIX.
- Match and validate with each Rx concentrate & document the activator.
- Check published data, document correction factors.
- Use dedicated calibrators and controls.
- Participate in external quality assessments.
- Communicate limitations with hemophilia center

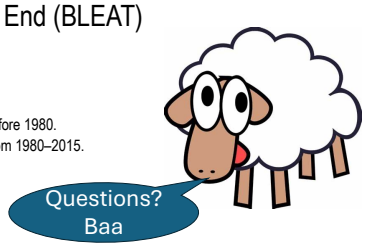
Sommer JM, Sadeghi-Khomami A, Barnowski C, Wikén M, Willemze AJ. Real-world assay variability between laboratories in monitoring of recombinant factor IX Fc fusion protein activity in plasma samples. Int J Lab Hematol. 2020 Jun;42(3):350-358. doi: 10.1111/ijlh.13189.

75

Bottom Line at the End (BLEAT)

The participant…

- Recognizes hemophilia symptoms.
- Recounts hemophilia Rx history before 1980.
- Describes factor concentrate Rx from 1980–2015.
- Measures EHL concentrate Rx.
- Monitors factor bypassing Rx.
- Monitors rebalancing Rx.
- Describes gene transfer Rx.



76