THE FRITSMA FACTOR
Your Interactive Hemostasis Resource

Managing Hemophilia Therapy
Rasputin to Recombinants

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

Hemophilia case study
Hemophilia in history
Alexis and Rasputin
AIDs and recombinants
Extended half-life recombinants
Gene transfer

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40 YO Af-Am Hemophilic Male

- Bleeding into ankle at midnight
  - Anatomic soft-tissue and joint bleeds
  - Tried RICE, DDAVP (Stimate®) inhaler
- Ran out of factor VIII (FVIII) concentrate
  - Subsidized by Medicare and Medicaid through ~100 national hemophilia centers
  - National Hemophilia Foundation: www.hemophilia.org
- Night tech and on call clin path resident
  - Determine residual patient factor VIII activity
  - Compute FVIII concentrate dosage
  - Order FVIII concentrate from transfusion service
  - Reconstitute with sterile water, administer as IV push
  - Subsequently determine therapeutic factor VIII activity

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Calculating FVIII Concentrate Dosage

- One unit = activity in 1 mL normal plasma or 100%
- Determine plasma volume based on patient's weight
  - Blood volume (BV, mL) = weight (kg) x 70 mL/kg
    - Use 60 mL/kg for obese, BMI 25–30
    - Use 50 mL/kg for morbidly obese, BMI >30
  - Plasma volume (PV, mL) = BV x (100%–HCT%)
- Decide on units (%) of FVIII concentrate desired

Units of FVIII required =
(desired FVIII in units/mL – initial units/mL) x PV (mL)

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If Factor Assay not Available

- When timing is critical, assume <1% activity or…
- Estimate residual FVIII from PTT

Factor VIII	PTT
40%	35 s
30%	50 s
20%	65 s
10%	90 s
1%	120 s
Example only, do not use	

- Collect baseline plasma
  - Assay or freeze and confirm with a real assay during next day shift
- Maintain patient database

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40 YO Af-Am Hemophilic Compute FVIII Dosage

- Weight is 80 kg, HCT 40%, factor level <1%
- Determine plasma volume
  - Blood volume (mL) = 80 kg x 70 mL/kg = 5600 mL
  - Plasma volume (mL) = blood volume (5600 mL) × (100%−40%) = 60% × 5600 = 3360 mL
- Determine units of FVIII required:
  - Choose to reach 80% FVIII (0.8 units/mL), therefore…
  - Units of FVIII required = (0.8 units/mL−0) x 3360 mL = 2688 (2700) units
  - Typical concentrate vial provides ~1000 units, use 3
- Avoid overdose: potentially thrombotic and wasteful

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Plasma-derived FVIII Concentrates

- Theoretical risk of HBV, HCV, HIV transmission
  - Hemofil-M®, Monarc-M®(Baxter), Monoclate-P®
  - Human and animal plasma matrix
  - Purification: immunoaffinity column, solvent-detergent, Pasteurization, viral filtration, combinations
- Seroconversions per CDC surveillance: 0
  - Data from 2003; predicted risk, 1:60,000
- <25% of FVIII concentrates used in industrialized countries

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Recombinant FVIII Concentrates

- Serum or albumin in culture medium
  - Helixate, Kogenate, Recombinate
  - Abundant, used for prophylaxis
  - No HBV, HCV, HIV seroconversions
- No protein in culture or preparation
  - No viral risk: Advate
- B-domain-deleted FVIII concentrate
  - Human albumin in culture: ReFacto
  - No protein: Xyntha
  - Extended half-life: Eloctate
  - Caution when using clot-based FVIII assay, use chromogenic FVIII

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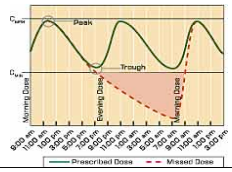
40 YO Af-Am
Advate FVIII Concentrate Therapy

- Peak: collected 15 m after administration: 0.3 units/mL
  - Should have been 0.8 units/mL, what happened?
  - Suspect anti-FVIII inhibitor
  - If peak reaches expected value, go to next administration
- Nadir (trough): 12 h after administration
  - Reflects half-life, should reach 50% of desired activity
  - Administer new AHF, use half the dosage second time



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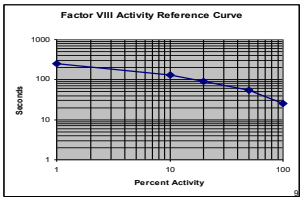
One-stage Factor VIII Assay

- Dilute pt plasma and control 1:10 in imidazole buffer
- Add FVIII-depleted reagent plasma 1:1 (FVIIIDP)
  - Typical: 100 uL pt plasma dilution/100 uL FVIII-depleted plasma
  - Provides all factors except FVIII
- Add PTT reagent, incubate 3 minutes
- Add $CaCl_2$, record interval to clot formation
- Confirm control
- Compare result in seconds to calibration curve to derive plasma activity:



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Factor VIII Assay Plasma Dilutions
Parallelism Indicates No Inhibitor

Automated Plasma Dilution	Seconds	Raw Factor VIII Activity	Computed Factor VIII Activity (× dilution)
1:10 "undiluted"	90 s	20%	20%
1:20	105 s	10%	20% (parallel)*
1:40	107 s	5%	20% (parallel)
1:80	110 s	2.5%	20% (parallel)
* <10% difference from undiluted indicates parallelism, no inhibitor			

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FVIII Assay Plasma Dilutions
non-Parallelism Indicates Inhibitor

Plasma Dilution	Seconds	Raw Factor VIII Activity	Computed Factor VIII Activity (× dilution)*
1:10 (undiluted)	80 s	10%	10%
1:20	93 s	8%	16%
1:40	107 s	5%	20%
1:80	108 s	4%	32%
* >10% difference from undiluted = non-parallel & rising, implies inhibitor			

- Inhibitor: IgG alloantibody to FVIII concentrate
  - 30% incidence, almost all in severe hemophilia

Kasper CK. Laboratory diagnosis of factor VIII inhibitors. In Kessler C, Garvey MB, Green D, Kasper C, Lusher J. Acquired Hemophilia 2nd Edition. Excerpta Medica 1995

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CDC Modified Nijmegen Bethesda Assay

Heat patient and control plasmas 56C/30m & cfg to remove residual FVIII

Make serial dilutions of patient plasma in FVIIIDP

Patient mix
Mix 1 part patient dilution with 1 part NPP

Control mix
Mix 1 part FVIIIDP with 1 part NPP

Incubate 37C/2h, measure FVIII activity

Patient mix FVIII/control mix FVIII X 100 = % residual activity (RA)

Nijmegan-Bethesda Units (NBU) = (2-log %RA)/0.301

Miller CJ, Platt SJ, Rice AS, et al. Validation of Nijmegan-Bethesda assay modifications to allow inhibitor measurement during replacement therapy and facilitate inhibitor surveillance. J Thrombos Haemostas 2012;10:1055–61.

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FVIII Inhibitor Therapy: Factor IX Complex

- If ≤5 NBU, give high-dose FVIII concentrate
- Prothrombin complex concentrate (PCC, 1980)
  - >5 NBU
  - $BaSO_4$ extracted human plasma; II, VII, IX, X: Proplex
  - *Activated* PCC: FEIBA, Autoplex
- FEIBA or Autoplex dosage
  - 50 units/kg/12 h standard
  - 70 units/kg/8 h in hemorrhage
  - Limit 200 units/kg/24 h to avoid DIC
  - Cannot monitor: only generalized coag evaluation with PTT

Ludlam DA, Morrison AE, Kessler C. Treatment of acquired hemophilia. Semin Hematol 1994;31 (Suppl 4) 16–19

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Recombinant FVIIa Concentrate; NovoSeven®

- Dose: 90 μg/kg for inhibitor
  - Repeat every 3–6 h; 6 h FVII half-life
- $0.83/μg
  - For our 80-kg patient, one dose = $6000
- Cannot monitor
  - Generalized coag evaluation using PTT
  - No risk of DIC

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Hemophilia A, B, and C

- *Anatomic* bleeding caused by congenital single-factor deficiencies
- 85% FVIII deficiency (hemophilia A)
  - 1 in 10,000 male births
- 14% FIX deficiency (hemophilia B)
  - Christmas disease, 1 in 30,000 male births
- 1% FXI deficiency (hemophilia C)
  - Rosenthal syndrome: 50% in Ashkenazi Jews
- Rare autosomal recessive single factor deficiencies
  - Prothrombin, V, VII, X, XIII

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Hemophilia A Symptoms

Spontaneous anatomic (soft-tissue) bleeds

- Bleeding at umbilical stump and circumcision
- Delayed bleeding triggered by injury
  - Joints, large muscles, body cavities, GI, soft tissue, tongue, kidney, testicles, brain, CNS
- Spontaneous bleeds, especially into joints
- Inflammation, hematomas, hemarthroses

Severe	Moderate	Mild
70%	15%	15%
<1% FVIII	1–5% FVIII	6–30% FVIII
Spontaneous bleeds	Bleed after minor trauma	Bleed after major trauma

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Hemarthroses

Airway Obstruction

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Cerebral Bleeds and Other Complications

- Lifestyle
- Economic
- Vocational
- Neurologic
- Psychological
- Lack of insurance
- Narcotics addiction

Cerebral hemorrhage

MORPHINE

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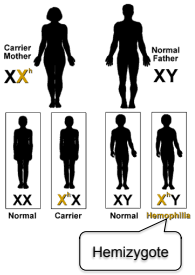
Hemophilia A Inheritance

- Sex-linked recessive, 1/10,000 males
- Carrier mother, hemizygous son



Hemizygote

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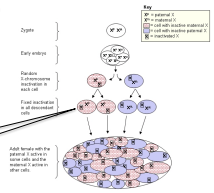
Hemophilia A Genetics

- 186 kb gene on X chromosome
  - Deletions, stop codons; missense and nonsense point mutations
  - Predominantly quantitative deficiency, a few are qualitative
  - *25–30% spontaneous mutation rate*
- Hemophilia A in females is rare
  - Acquired anti-FVIII inhibitor (IgG_4)
  - Autosomal hemophilia or VWD type N (Normandy)
  - Hemophilic father with carrier mother
  - Random "excess Lyonization"

Unbalanced excess of maternal X



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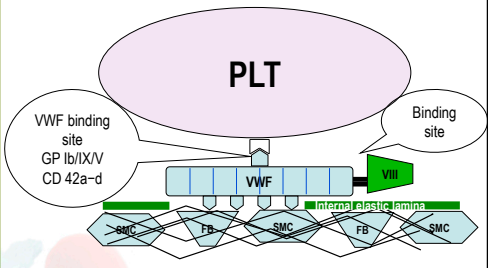
FVIII Requires VWF Carrier



VWF: von Willebrand factor
SMC: smooth muscle cell
FB: fibroblast
Lnes: collagen

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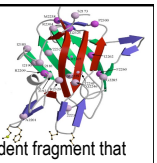
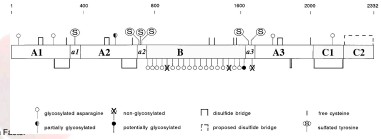
FVIII is a Glycoprotein Cofactor



- 285,000-D heterodimer
- Cleaved by thrombin, leaving a Ca^{++}-dependent fragment that detaches from VWF and binds factor IXa and platelet phospholipid: *phosphatidyl serine*
- Stabilizes IXa to form the "tenase" complex
- Deficiency slows thrombin production
- In vitro, deteriorates 5%/hour at 18–24°C



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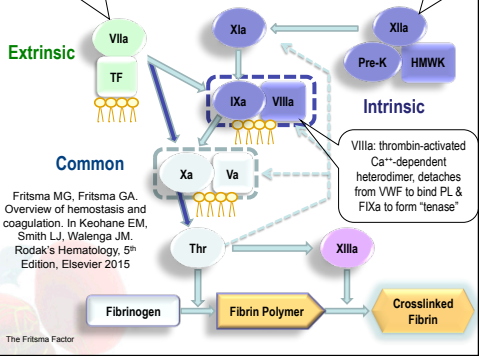
Coagulation

Initiation: Exposed TF binds VIIa, activates IX→IXa and X→Xa

In vitro initiation: negatively charged particles



VIIIa: thrombin-activated Ca^{++}-dependent heterodimer, detaches from VWF to bind PL & FIXa to form "tenase"

Fritsma MG, Fritsma GA. Overview of hemostasis and coagulation. In Keohane EM, Smith LJ, Walenga JM. Rodak's Hematology, 5th Edition, Elsevier 2015

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1st Documented Bleeder's Disease

2nd century: Talmudic ruling of *Rabbi Judah the Patriarch* exempts a 3rd son from circumcision if two elder brothers had died of bleeding after circumcision

2nd century: *Rabbi Simon ben Gamaliel* forbade a boy to be circumcised after sons of his mother's three elder sisters had bled to death after circumcision

11th century: Arabic surgeon *Albucasis* describes village males who bled to death from "trivial" wounds

Ingram GIC. The history of haemophilia. J Clin Pathol 1976; 29: 469-79.

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1791–1803: British & American Families

- 1000–1800: Several written references to "bleeders"
- 1791 (Britain), Zoll: 6 brothers
  - Each bled to death after minor injuries
  - Half-siblings by a different mother were unaffected
- 1803 (Philadelphia), Otto: "A hemorrhagic disposition existing in certain families"
  - Recorded males in his own family with symptoms and recognized transmission through asymptomatic women
  - Traced pedigree to a woman named Smith in Plymouth, 1720–30
- 1820 (Germany) Nasse accurately defines the inheritance pattern, named "Nasse's law."

Otto quoted in Bulloch W, Fildes P. Treasury of human inheritance, parts V & VI, section XIVa, Haemophilia, 1911.

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1800–28: Documented Names

- Haemorrhoea
- Bleeding disease
- Idiosyncrasia haemorrhagica
- Hereditary haemorrhagic diathesis
- 1828: first use of "haemophilia" (blood-loving) appears in the title of a treatise by Hopff at University of Zurich

Hopff F. Cited by United States Surgeon General's catalogue, 1st series: Hemophilia, 1828.

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Bulloch and Fildes

Bulloch W, Fildes P. *Treasury of human inheritance, parts V and VI, section XIVa, haemophilia*.

Published as Eugenics Laboratory memoirs XII, Francis Galton Laboratory for National Eugenics, University of London; 1911, Dulau and Co, 37 Soho Square, London.

- 949 references & case reports from 235 pedigrees
- Identified hemophilia as hereditary and sex-linked
- Didn't understand hemophilia carrier status
- Meticulously traced the current spread of the mutation throughout Queen Victoria's family

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Alexandrina Victoria; May 24, 1819–Jan 22 1901, was *Queen of the United Kingdom of Great Britain and Ireland* from her coronation at 18, June 20, 1837 until her death, altogether 63 years and 7 months. The Victorian era was a time of UK industrial, political, imperial, and military progress.

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Queen Victoria: Hemophilia Carrier

- Presumed spermatogenesis mutation in her father; Edward, Duke of Kent, in his 50s when Victoria was conceived
  - FVIII mutation in men double prevalence in women
- Victoria's seventh child, Leopold, was hemophilic
  - Stigmatized as a "weak" invalid by mother, hidden from public, married at 29 contrary to medical advice
  - Died of cerebral hemorrhage following a fall at 31
- Two daughters of Victoria, Alice (2nd) and Beatrice (8th) turned out to be carriers, as learned later

Massie RK. Nicholas and Alexandra. (1968). Gollancz, London.

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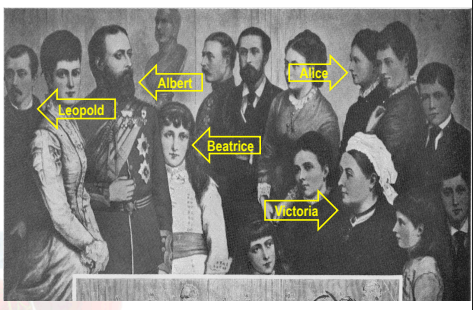
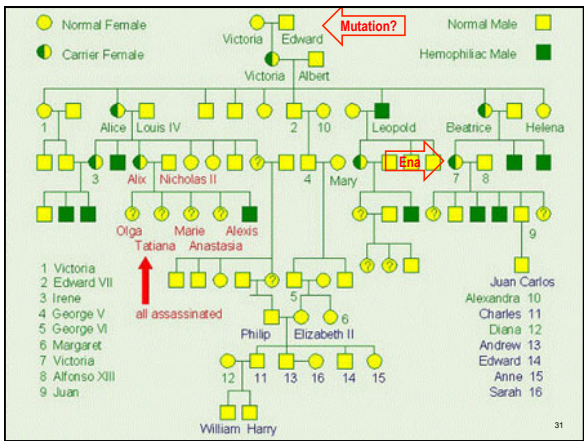
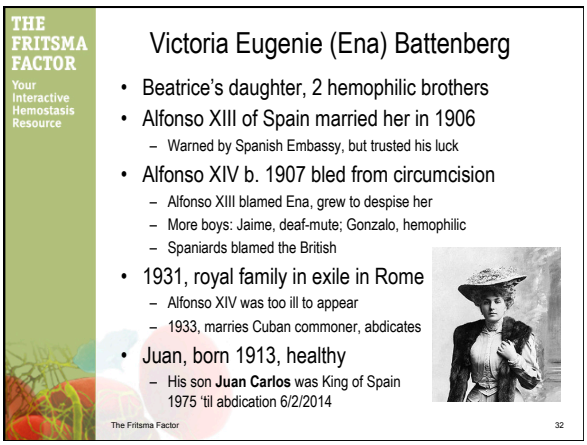
Queen Victoria and Family




Normal Female
Carrier Female
Mutation?
Normal Male
Hemophiliac Male
Victoria
Edward
Victoria
Albert
Alice
Louis IV
Leopold
Beatrice
Helena
Alix
Nicholas II
Mary
Ena
Olga
Tatiana
Marie
Anastasia
Alexis
all assassinated
Philip
Elizabeth II
William
Harry
1 Victoria
2 Edward VII
3 Irene
4 George V
5 George VI
6 Margaret
7 Victoria
8 Alfonso XIII
9 Juan
Juan Carlos
Alexandra 10
Charles 11
Diana 12
Andrew 13
Edward 14
Anne 15
Sarah 16
31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Victoria Eugenie (Ena) Battenberg
• Beatrice's daughter, 2 hemophilic brothers
• Alfonso XIII of Spain married her in 1906
– Warned by Spanish Embassy, but trusted his luck
• Alfonso XIV b. 1907 bled from circumcision
– Alfonso XIII blamed Ena, grew to despise her
– More boys: Jaime, deaf-mute; Gonzalo, hemophilic
– Spaniards blamed the British
• 1931, royal family in exile in Rome
– Alfonso XIV was too ill to appear
– 1933, marries Cuban commoner, abdicates
• Juan, born 1913, healthy
– His son **Juan Carlos** was King of Spain 1975 'til abdication 6/2/2014
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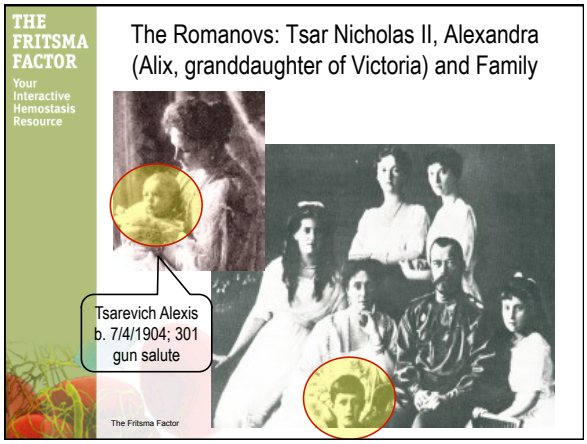
32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The Romanovs: Tsar Nicholas II, Alexandra (Alix, granddaughter of Victoria) and Family
Tsarevich Alexis b. 7/4/1904; 301 gun salute
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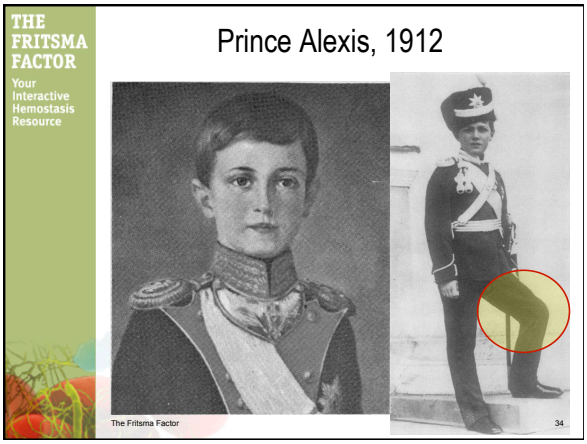
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Prince Alexis, 1912
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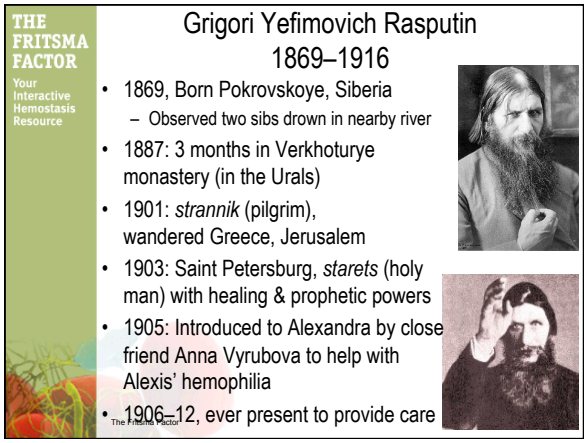
34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Grigori Yefimovich Rasputin
1869–1916
• 1869, Born Pokrovskoye, Siberia
– Observed two sibs drown in nearby river
• 1887: 3 months in Verkhoturye monastery (in the Urals)
• 1901: *strannik* (pilgrim), wandered Greece, Jerusalem
• 1903: Saint Petersburg, *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 care
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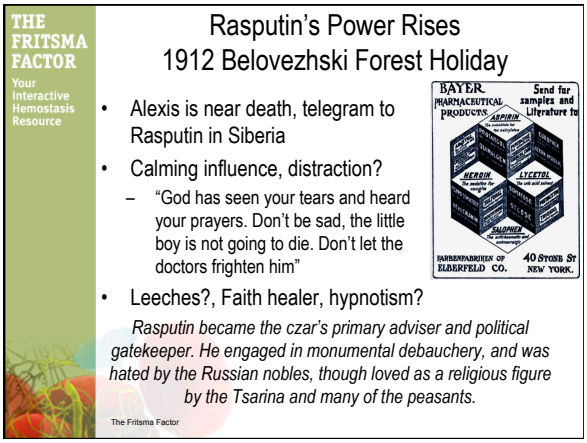
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Rasputin's Power Rises
1912 Belovezhski Forest Holiday
• Alexis is near death, telegram to Rasputin in Siberia
• Calming influence, distraction?
– "God has seen your tears and heard your prayers. Don't be sad, the little boy is not going to die. Don't let the doctors frighten him"
• Leeches?, Faith healer, 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.
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FARBENFABRIKEN OF ELBERFELD CO.
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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.

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Rasputin and Admirers, 1914

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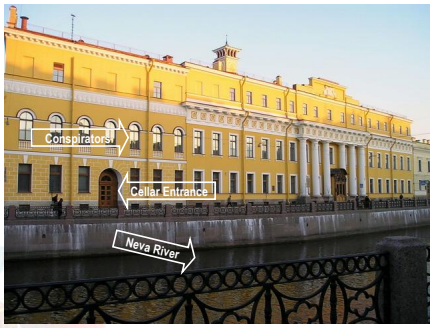
Yusupov Palace, St. Petersburg



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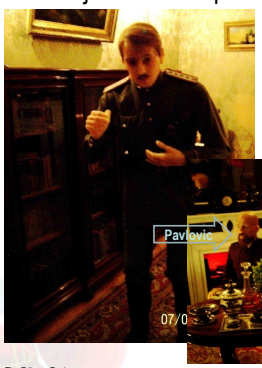
Maj. Felix Yusupov: Dec 16, 1916



Wax figures at the Yusupov palace in St. Petersburg, photographed in 2008

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Poisoning Rasputin

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Treatment Attempts 1901–1942

- Lime
- Gelatin
- Oxygen
- Splenectomy
- Bone marrow
- Sodium citrate
- Calcium lactate
- Witte's peptone
- Hydrogen peroxide
- Induced anaphylaxis
- Antidiphtheric serum
- The 'galvanic needle'
- Animal and human sera
- Adrenaline
- Bird's muscle
- IV oxalic acid
- Vitamin therapy
- 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

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Effective Hemophilia Treatments

- 1926, Surgeon General: 12 published attempts at whole blood transfusion beginning after WW I
  - Blood groups defined by Landsteiner in 1900
- 1934, McFarlane: topical Russell viper venom
- 1938, Patek and Taylor, Brinkhouse: first characterization of anti-hemophilic globulin
- 1938, McFarlane: fresh whole plasma
- 1944: EJ Cohn fractionation of plasma
  - Animasl: Biggs and Macfarlane, 1954; Bidwell, 1955
  - Kekwick and Wolf, 1957; Soulier, Gobbi, Larrieu, 1957; Blomback, Blomback, Nilsson, 1958

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1952: Stephen Christmas, Canadian Hemophilia Soc: Delineated F*IX def (Christmas disease) from FVIII def (Alexis was factor IX deficient)*

Nine-year-old Donald Burns smiles on father's knee. His is believed to be first successful appendectomy operation to a haemophiliac in North America.

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Breakthroughs: CRYO, Anti-hemophilic Factor

- 1964, Judith G. Pool (1919−75, U of Chi)
  - Developed cryoprecipitate
  - First opportunity for hemophilic home care

- 1968, Kenneth M. Brinkhous
  - 1908−2000, UNC Chapel Hill
  - First to chemically characterize factor VIII in 1938
  - Developed AHF with hemophilic dog experiments
  - AHF released through Hyland in 1968

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Advances in the 1960–70s

- 1960: prevent bleeds during dental extractions and minor procedures using Kohn fraction
- 1960: surgical orthopedic correction of hemarthroses using AHF
- 1970: home therapy
- 1973: hemophilia treatment centers
- 1980: prothrombin complex concentrate
  - Proplex®, II, VII, IX, X for IX deficiency
- 1980: activated PCC, FEIBA® for inhibitors
- 1980: life expectancy was 60
  - But high hepatitis rate, 20,000 donors/pool
  - No viral inactivation

Rev. Robert K.Massie III

Robert K. Massie

Susan Massie

Massie R, Massie K. *Journey*. Knopf, USA 1973

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HIV; AIDS and the CDC

- Fall 1980: *Pneumocystis carinii* pneumonia (PCP) and Kaposi sarcoma (KS) in homosexual males
  - Searched for non-infectious immunodeficiency causes such as amyl nitrite "poppers," anti-sperm antibodies or anal intercourse
- Spring 1982: CDC recorded three cases of PCP in hemophilic men receiving FVIII concentrate, all died
  - Paralleled reports of similar symptoms in Haitian hemophilics and in US drug abusers
  - No homosexual behavior or illegal drug use
  - Led to concept of blood-borne viral infection

Evatt BL. The tragic history of AIDS in the hemophilia population, 1982–1984. J Thrombos Haemost 2006; 4: 2295–301.

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CDC Report Delivered 7/27/82

CDC
CENTERS FOR DISEASE CONTROL AND PREVENTION

- CDC reported to blood and plasma industries, gay organizers, hemophilia organizations, FDA and NIH; coined the term AIDS
- 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
- Dec1982: 6 more CDC cases: two non-hemophilic blood recipients, one an infant
  - Donor services refused to reveal donor identities to avoid embarrassing and subsequently losing donors
  - One definite identification slipped through, presumed infection

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CDC Report #2, Delivered 1/4/83

- CDC reported the statistical prevalence of hepatitis B was the same in hemophilics and AIDS risk groups (surrogate association)
- 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, and individuals.
- Again, consensus to not act, debate was irrational, acrimonious and public, harshly critical of Dr. Evatt and CDC

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January 13, 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 head Dr Cumming wrote: "*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.*"
- The National Hemophilia Foundation, however, alarmed, had already contacted plasma processors in December, 1982

American Red Cross

National Hemophilia Foundation
for all bleeding and clotting disorders

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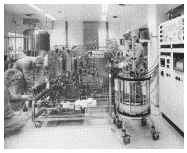
National Hemophilia Foundation Initiative: 1983

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



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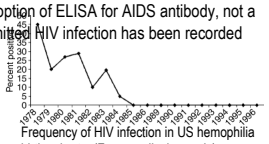
Virus Isolation: 1983

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



Frequency of HIV infection in US hemophilia birth cohorts. (From medical records)

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Recombinant Clotting Factors

- 4/7/1976: *Genentech* incorporated
- 1981: *Genetics Institute* incorporated
- 1982: rFIX cloned by both (small molecule)
- Aug, 1984: Both cloned rFVIII gene and produced the protein, but yields are small
- 1985: rVWF coexpressed, improves rFVIII yields

genesis
genetics institute

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

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rFVIII Barriers

- Extensive post-translational modification
  - Requires mammalian cloning system; Chinese hamster ovary cells
- rFVIII affinity for CHO membrane phospholipid
  - CHO membrane binds and degrades FVIII
  - CHO cells transfected with FVIII and VWF genes
  - Resolved by rVWF coexpression—higher affinity than membranes
- Need for serum-free cloning system
  - Eliminate animal viruses, ease purification
- High-volume fermentation process
  - Immunoaffinity, ion-exchange, Pasteurization, solvent-detergent, nanofiltration reduces 7000 L to 1 L.

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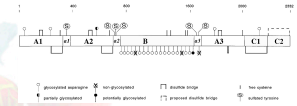
rFVIII to Market

- Regulatory uncertainty
  - Sterility, consistency questions generate 600 tests/lot
- Baxter Hyland and Genetics Institute produced the first rFVIII, Recombinate
- Bayer Miles Lab/Cutter Biological and Genentech produced Kogenate
- 3/27/1987: UNC Chapel Hill, first phase II infusion into a 39 year-old hemophilic
- Recombinate approved1992, Kogenate 1993



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rFVIII Market Efforts and Inhibitor Formation

- B-domain deleted: Genetics Institute ReFacto, 2000
- No human or animal albumin in formulation: Advate, 2003
- Stabilizing matrix of sucrose, glycine, histidine: Kogenate FS
- Non-protein B-domain deleted: Wyeth Xyntha, 2008
- Hemostatic efficacy: 90% cessation of bleeding
- Risk of inhibitor formation in PUPs is approximately double the risk from plasma-derived FVIII concentrate
- Risk of additional inhibitor formation in response to rFVIII in previously treated persons (PTPs) is <1%

Pipe SW. The promise and challenges of bioengineered recombinant clotting factors. J Thromb Haemost 2005; 3: 1692–1701.
Barnes C, Lillicrap D, Pazmino-Canizares J, et al: Pharmacokinetics of recombinant factor VIII (Kogenate-FS®) in children and causes of inter-patient pharmacokinetic variability. Haemophilia 2006:2 (Suppl 4): 40–9.

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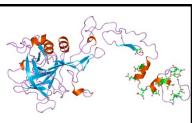
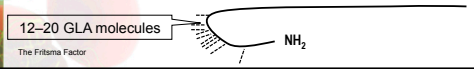
rFIX



- Genetics Institute BeneFix 1998
- Barrier: γ-carboxylation of 12–20 glutamic acids at amino terminus requires…
  - Vitamin K
  - Coexpressed γ-glutamyl carboxylase
  - Coexpression of furin activation enzyme
- 90% bleeding cessation: 1–2 infusions on demand
- PUPs—3% incidence of high titer inhibitors



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Inhibitor Formation in Severe Hemophilia

- Meta-analysis of 20 trials; pd versus 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

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Primary Prophylaxis in Children

- Abundant rFVIII/IX; concern for VAD clotting
- 2005: Joint damage outcome study
  - 25 IU/kg every other day generates 6X decrease in joint deterioration by MRI vs on-demand (OD) Rx up to 6 YO
- 2009 Italian study on prophylaxis
  - 10 Y f/u on 25 IU/kg 3X a week vs OD: 0.52 vs 1.08 total bleeds and 0.2 vs 0.52 joint bleeds/patients/month
- 2009 Danish/Russian study
  - Orthopedic issues 15.6 in OD vs 2.2 in prophylaxis

Franchini M, Coppola A, Molinari AC, et al. Forum on the role of recombinant factor VIII in children with severe haemophilia A. Haemophilia 2009; 1–9.
Gringeri A, Lundin V, von Mackensen S, et al. Primary and secondary prophylaxis in children with haemophilia A reduces bleeding frequency and arthropathy development compared to on demand treatment; a 10-year, randomized clinical trial. J Thromb Haemost 2009;7
Ingerslev J, Lethagen S, Poulsen L, et al. A case-controlled Danish-Russian comparative study of clinical outcomes in younger severe haemophilia patients treated with prophylaxis compared to those managed with on-demand treatment. J Thromb Haemost 2009;7

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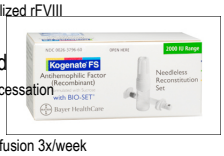
On-demand Versus Primary in Adults

- 19 hemophilics 30–45 YO, all <1 unit/dL
  - Mean 2 bleeds/month, no additional bleeding disorder
  - ≥3 bleeds in elbow, ankle or knee, prior 6 months
  - Bayer Kogenate FS: sucrose-stabilized rFVIII
  - Prospective, open-label
- Six months OD Rx per bleed
  - 20–100 units/kg every 12–24 h to cessation
- Six months' prophylaxis
  - Home: 20–40 units/kg 2 mL/min infusion 3x/week
  - 1st month of prophylaxis was run-in, six months data collection
- Outcomes
  - Primary: patient self-assessed joint bleeds
  - Secondary: all bleeds, joint function, QOL, health economics, safety



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Results

Median of 19 subjects	6 m on-demand	6 m prophylaxis
Infusions	29	88
Total consumption, units	70,421	211,933
Joint bleeds	15	0
All bleeds	20.5	0
Spontaneous bleeds	13.5	0
Trauma bleeds	2.5	0

- Baseline: median joint bleeds: 14.0; 24% mild, 63% moderate, 13% severe
- Fourteen used rFVIII, two used plasma-derived FVIII, three used both
- Prophylaxis median trough: 48 h: 6 units, 72 h: 4 units
- Mean total Gilbert joint function score (pain, swelling, atrophy, deformity, range of motion, instability): on-demand, 25.3%, prophylaxis 19.8%
- Safety: no Rx-related adverse events

Collins P, Faradji A, Morfini M, Enriquezs MM, Schwartz L. J Thromb Haemost 2010;8:83–9.

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Extended Half-life Factors VIII and IX

- Recombinant B-domain deleted Fc fusion factor VIII
  - Biogen Idec Eloctate, FDA-approved 2014
  - 96 adult hemophilic males with > 12 annual bleeds, 3–4 doses/week
  - Extended by Fc receptor and IgG recycling pathway
  - rFVIIIFc half-life 19 h versus rFVIII 12; 1.6–3.6 annual bleeds
  - Prophylactic Rx frequency up to 5 days versus 3–4 doses/week
  - Monitor using clot-based FVIII assay with non-kaolin-based PTT
- Recombinant Fc fusion factor IX
  - Biogen Idec Alprolix, FDA-approved 2014
  - Monitor using FIX assay with non-kaolin-based PTT or chromogenic
  - rFIXFc half-life 70–80 h versus 24 h, 7–10 day intervals

biogen idec

Shapiro AD[1], Ragni MV, Kulkarni R, et al. Recombinant factor VIII Fc fusion protein: extended-interval dosing maintains low bleeding rates and correlates with von Willebrand factor levels. J Thromb Haemost. 2014;12:1788–800.
Mancuso ME, Mannucci PM. Fc-fusion technology and recombinant FVIII and FIX in the management of the hemophilias. Drug Des Devel Ther. 2014 28;365–71.

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Gene-based Hemophilia Therapy

- Genetic delivery targets: liver, skeletal muscle, hematopoietic tissue, endothelial cells
- Vectors: adeno-associated virus (AAV) and liver sinusoidal endothelial cells (LSEC)
- Barriers: theoretical T-cell immune response to vector, FVIII molecule too large for vector, inefficient transduction

- Fomin ME, Togarriti P, Muench MO. Progress and challenges in the development of a cell-based therapy for hemophilia A. J Thromb Haemost 2014; 12: 1954–64.
- Chuah MK, Nair N, Vandendriessche T. Recent progress in gene therapy for hemophilia. Hum Gene Ther 2012; 23:557–65.
- Lozier J. Gene therapy of the hemophilias. Semin Hematol 2004; 41:287-96.

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FIX Gene Transfer: Landmark Study

- Adenovirus-associated virus vector (AAV)
  - Codon-optimized human factor IX transgene
  - scAAV2/8-LP1-hFIXco
  - FVIII gene is 7 kb, AAV capacity 4.6 KB
- Six hemophilia B (<1% FIX) patients 29–64
  - 2 with FIX null, 4 with missense mutations (type II deficiency)
  - All receiving prophylactic FIX 3X/week
  - No inhibitors, no antibodies to AAV
- Vector administration dosages
  - $2x10^{11}$ vector genomes/kg body weight (vg/kg)
  - $6x10^{11}$ vg/kg
  - $2x10^{12}$ vg/kg

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FIX Transfer Study Outcomes

- 4 of 6 required no FIX Rx
- 2 required on-demand FIX Rx
  - One ran the half-marathon
- High-dose subjects had rise in ALT
  - Immune response to hepatocytes?
  - Controlled with prednisolone
- No anti-FIX antibodies
- Did not detect cellular immunity to vector

Nathwani, AC, Tuddenham GD, Rangarajan S, et al, Adenovirus-associated virus vector–mediated gene yransfer in hemophilia B. NEJM 2011;365: 2357–65.

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BioMarin BMN270 FVIII Transfer Trial

- B-domain-reduced FVIII with minimal glycosylation
- Codon optimization: AAV8 variant adequate capacity
  - University College London and St. Jude's Research Hospital
  - Successful in mice and macaques
- Phase 1 & 2 study: enrolled first severe hemophilia A patient 9/28/15, inviting 11 more

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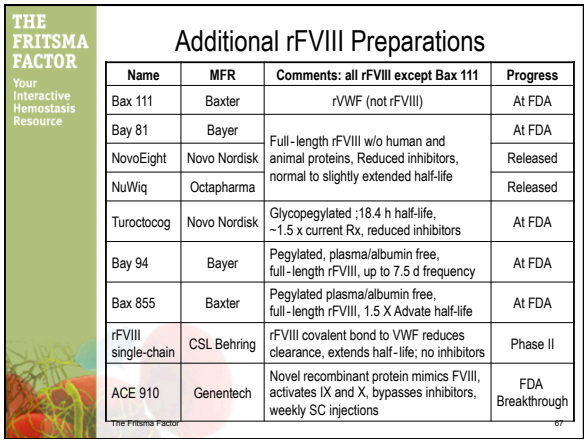
BIOMARIN RARE OPPORTUNITIES. PERSONAL IMPACT.

McIntosh J, Lenting PJ, Rosales C, et al. Therapeutic levels of FVIII following a single peripheral vein administration of rAAV vector encoding a novel human factor VIII variant. Blood. 2013 25;121:3335-44
Ward NJ, Buckley SM, Waddington SN, et al. Codon optimization of human factor VIII cDNAs leads to high-level expression. Blood 2011;117:798–807.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Additional rFVIII Preparations
Name | MFR | Comments: all rFVIII except Bax 111 | Progress
Bax 111 | Baxter | rVWF (not rFVIII) | At FDA
Bay 81 | Bayer | Full-length rFVIII w/o human and animal proteins, Reduced inhibitors, normal to slightly extended half-life | At FDA
NovoEight | Novo Nordisk | | Released
NuWiq | Octapharma | | Released
Turoctocog | Novo Nordisk | Glycopegylated ;18.4 h half-life, ~1.5 x current Rx, reduced inhibitors | At FDA
Bay 94 | Bayer | Pegylated, plasma/albumin free, full-length rFVIII, up to 7.5 d frequency | At FDA
Bax 855 | Baxter | Pegylated plasma/albumin free, full-length rFVIII, 1.5 X Advate half-life | At FDA
rFVIII single-chain | CSL Behring | rFVIII covalent bond to VWF reduces clearance, extends half-life; no inhibitors | Phase II
ACE 910 | Genentech | Novel recombinant protein mimics FVIII, activates IX and X, bypasses inhibitors, weekly SC injections | FDA Breakthrough
The Fritsma Factor
67