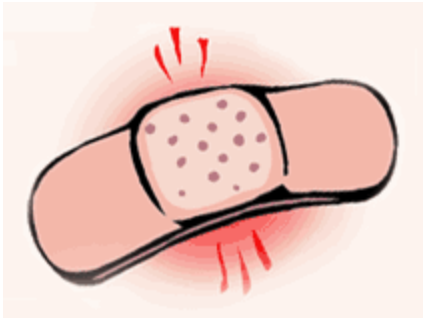
THE FRITSMA FACTOR

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



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Bottom Line at the Start (BLATS)

The participant…

- Gives an overview of hemophilia.
- Recounts the history of hemophilia from the second century, including the story of the Russian royal family.
- Reviews the events leading to the discovery of coag factors concentrates' transmission of viral disease.
- Plans factor VIII concentrate therapy, differentiating between plasma-derived and recombinant preparations.
- Manages prolonged half-life factor VIII and IX.
- Describes current efforts to manage hemophilia through gene transfer therapy and antithrombin suppression.

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Hemophilia A, B, and C Congenital Single-factor Deficiencies

- 85% FVIII deficiency (hemophilia A)
  - Incidence: 1 in 10,000 male births
- 14% FIX deficiency (hemophilia B)
  - Christmas disease, incidence 1 in 30,000 male births
- 1% FXI deficiency (hemophilia C)
  - Rosenthal syndrome: 50% in Ashkenazi Jews
- Autosomal recessive single factor deficiencies
  - Composite incidence ~1 in 1,000,000 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 U/dL FVIII	1–5 U/dL	6–30 U/dL
Spontaneous bleeds	Bleed after minor trauma	Bleed after major trauma

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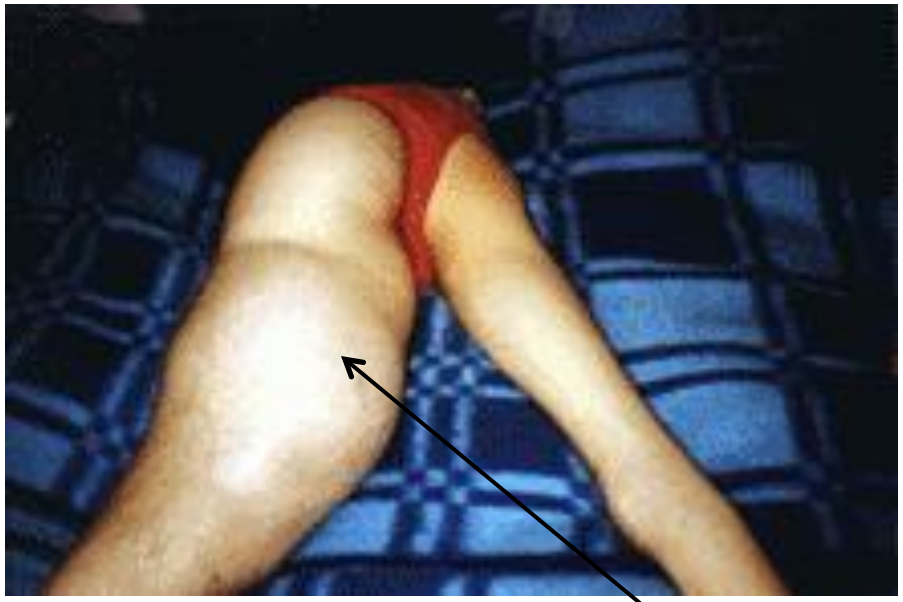

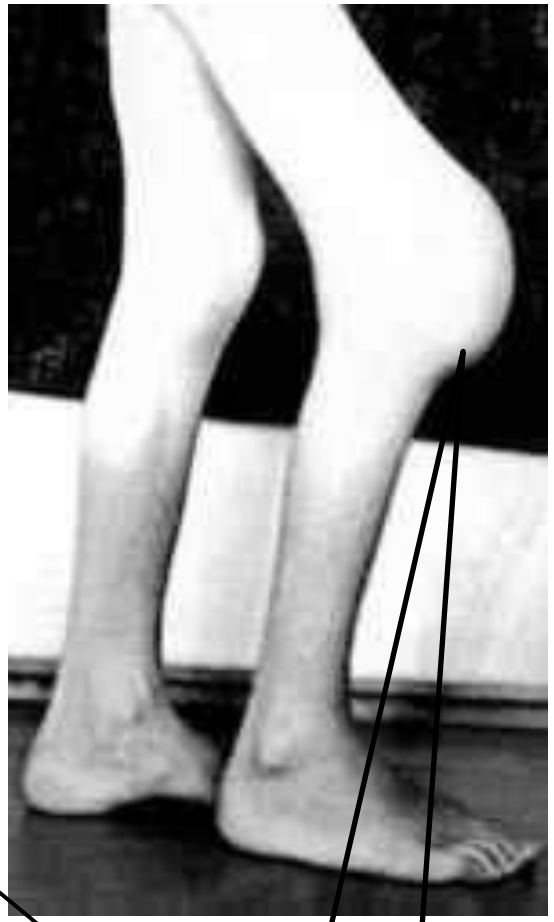

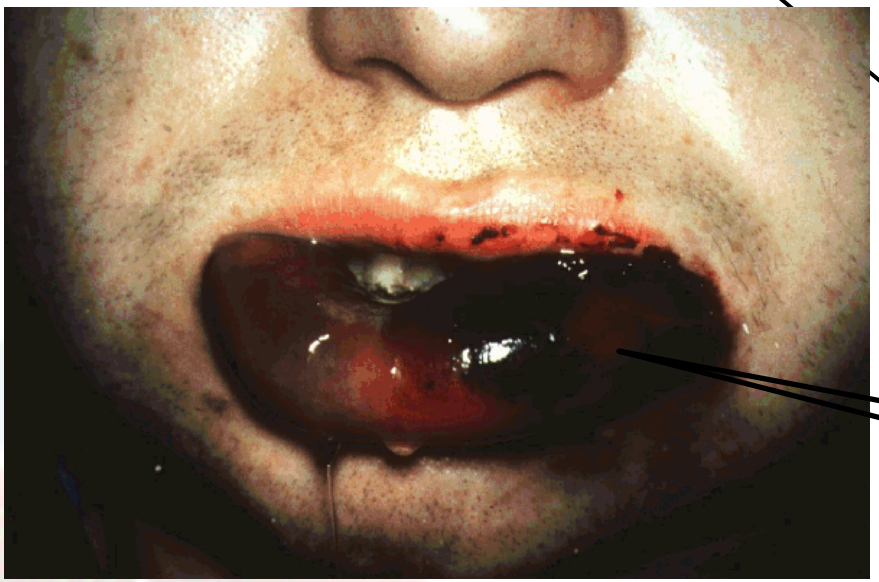

Hemarthroses

Airway Obstruction

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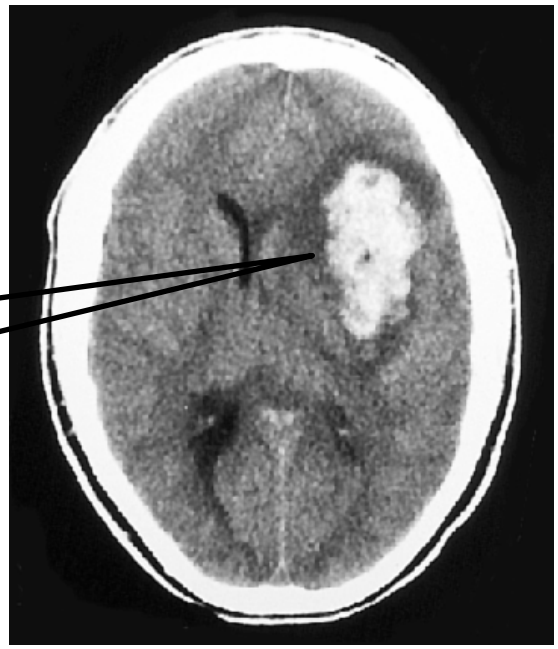
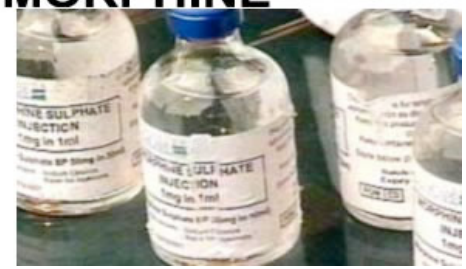
Cerebral Bleeds and Other Complications

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



MORPHINE



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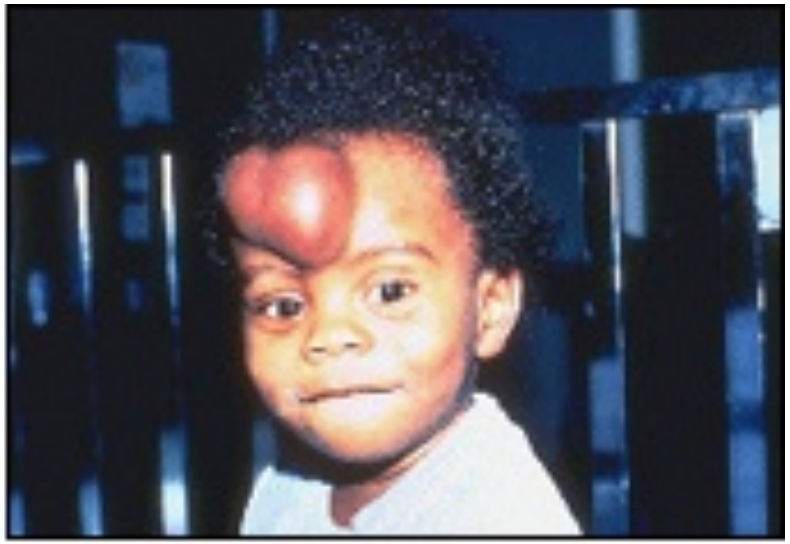
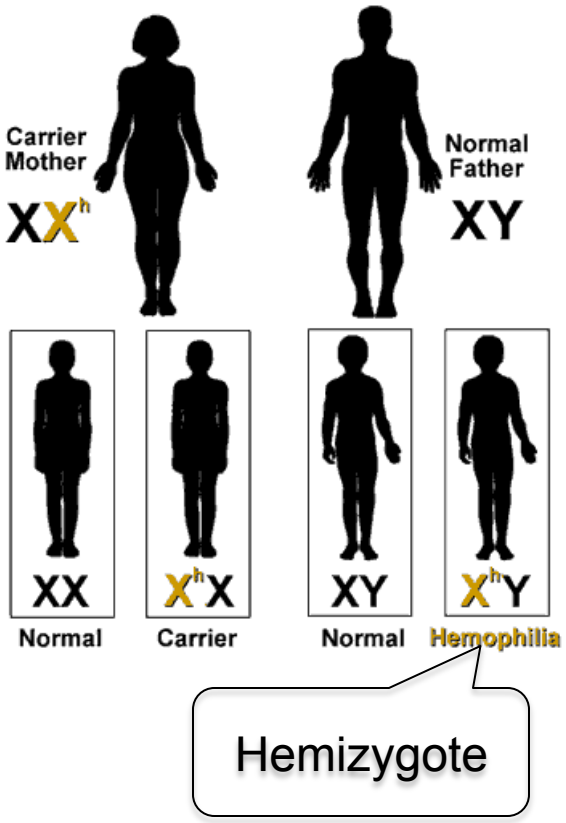
Hemophilia A Inheritance

- 186 kb gene on X chromosome
  - Deletions, stop codons; missense and nonsense point mutations
  - Predominantly quantitative deficiency, 10% are qualitative
  - *25−30% spontaneous mutation rate*
- Sex-linked recessive
  - Carrier mom, hemizygous son:





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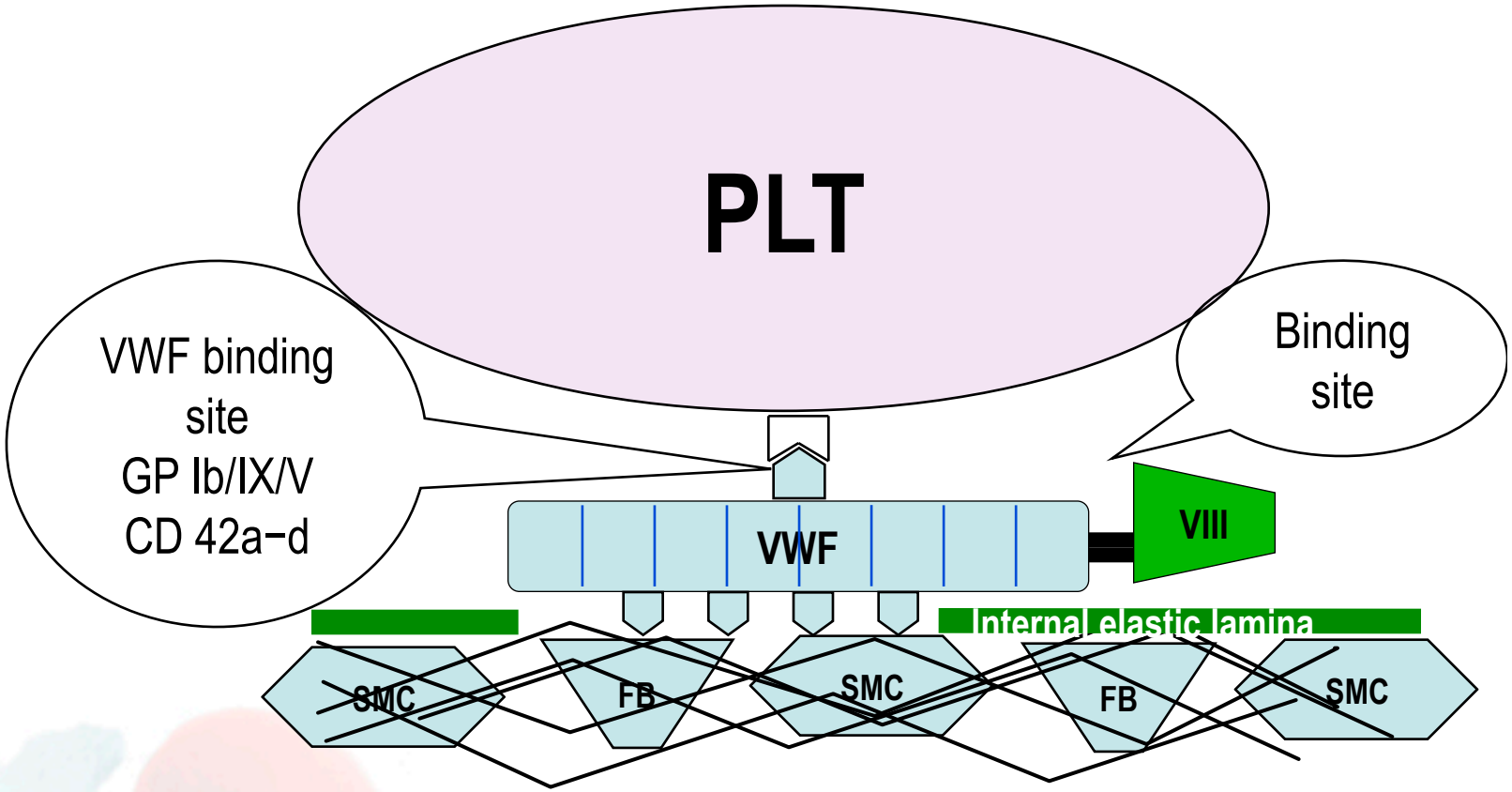
FVIII Requires VWF as Cofactor



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

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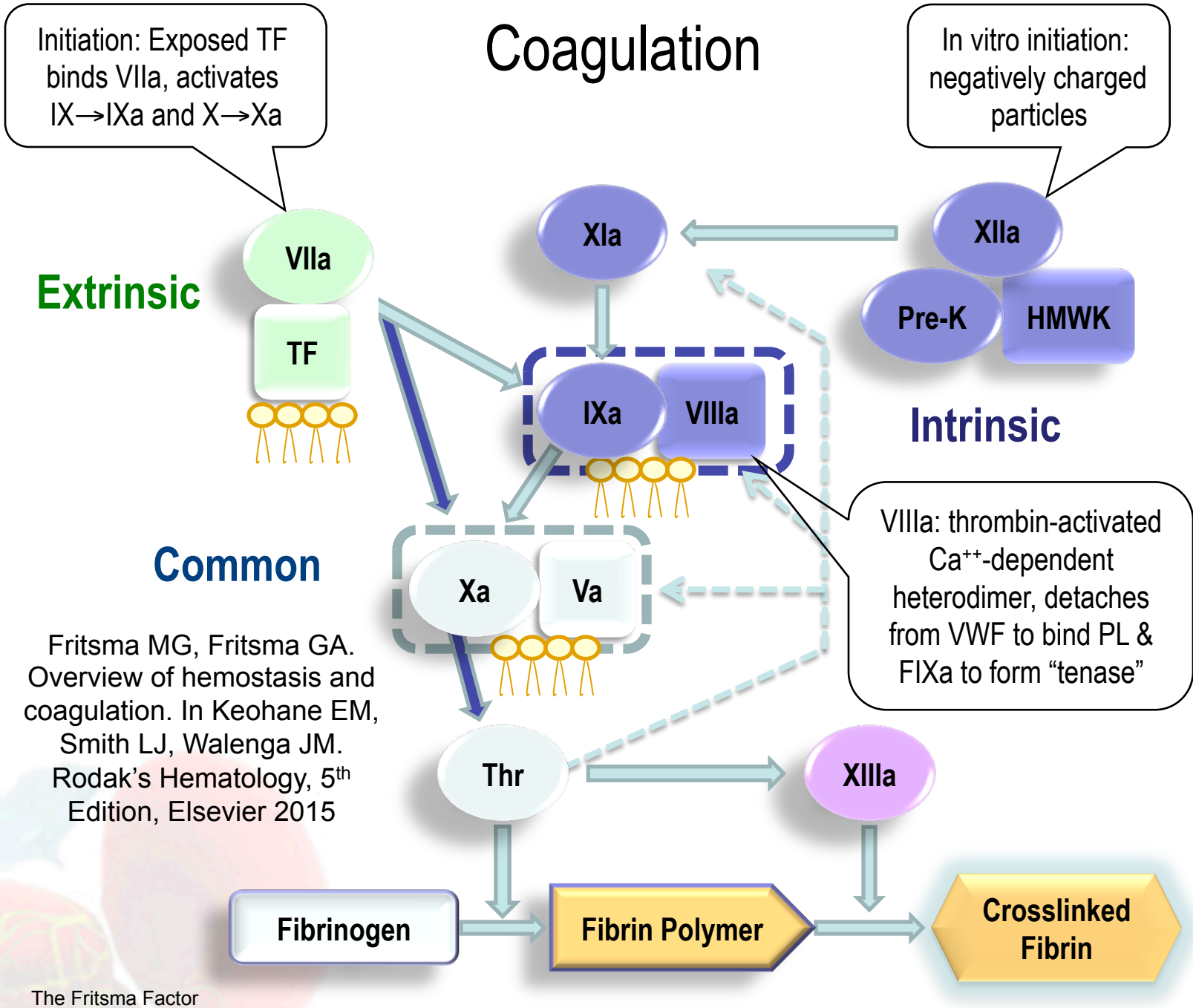
Coagulation



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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40 YO Hemophilic

- 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
  1. Assay residual factor VIII activity
  2. Compute FVIII concentrate dosage: package insert
  3. Order FVIII concentrate from transfusion service
  4. Reconstitute with sterile water, administer as IV push
  5. Avoid overdose: wastes resources and potentially thrombophilic
  6. Subsequently assay therapeutic factor VIII activity

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If Factor Assay not Available

When timing is critical, assume <1U/dL activity or…

Estimate residual FVIII from PTT:

Factor VIII	PTT
40 U/dL	35 s
30 U/dL	50 s
20 U/dL	65 s
10 U/dL	90 s
1 U/dL	120 s
Example only, do not use *Varies by reagent sensitivity*	

Collect baseline plasma

Assay or freeze and confirm with a real assay during next day shift

Maintain patient database

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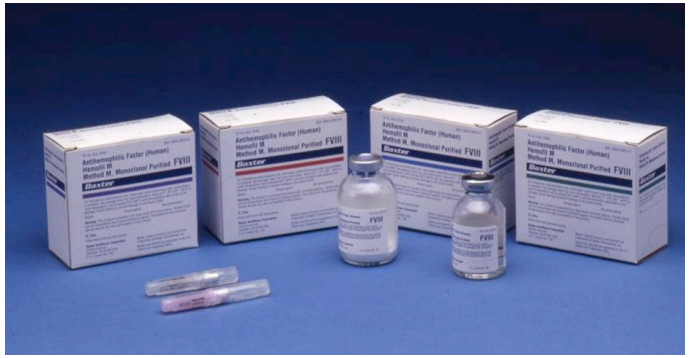
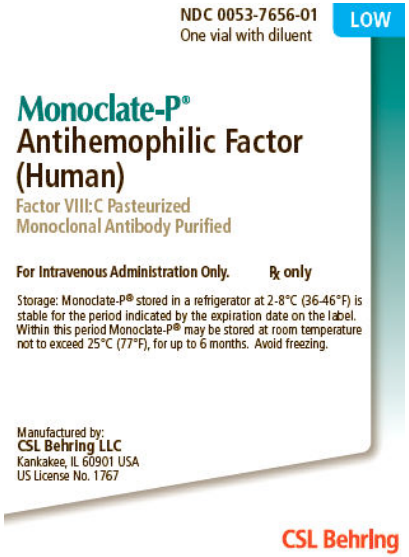
Plasma-derived FVIII Concentrates

- Theoretical risk of HBV, HCV, HIV transmission
  - Hemofil-M, Monarc-M, Monoclate-P
  - Human and animal plasma matrix
  - Purification: immunoaffinity column, solvent-detergent, Pasteurization, viral filtration, combinations



- Seroconversions per CDC surveillance: 0
  - MMWR 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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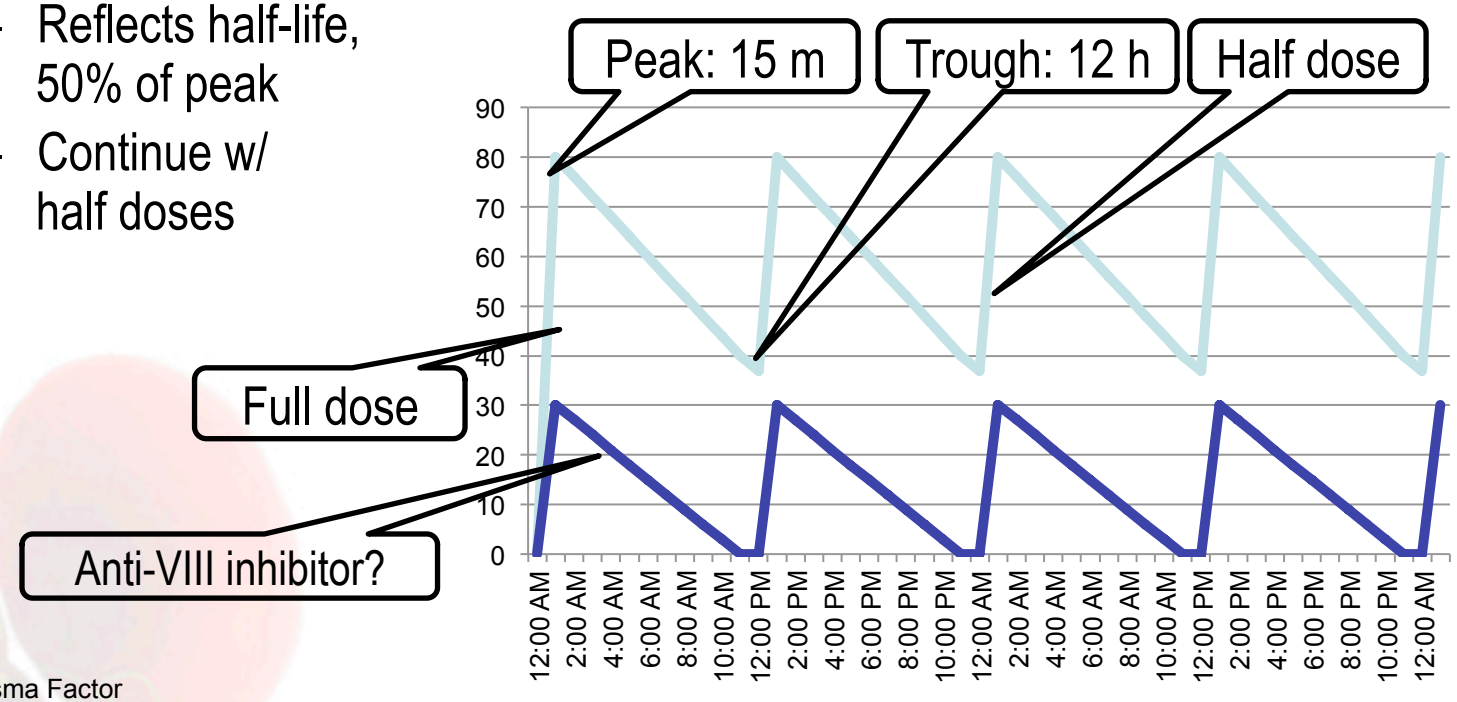
40 YO Hemophilic Receives Advate

- Peak: collect 15 m after administration
  - If peak reaches expected value, plan for next administration at 12 h
  - Factor assay result: 30 U/dL
  - Should have reached 80 U/dL, what happened?
  - Suspect anti-FVIII inhibitor
- Trough: collect 12 h after administration
  - Reflects half-life, 50% of peak
  - Continue w/ half doses



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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 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)
* <10% difference from undiluted indicates parallelism, no inhibitor			

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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FVIII Assay Plasma Dilutions non-Parallelism Indicates Inhibitor

Plasma Dilution	Seconds	Raw Factor VIII Activity	Computed Factor VIII Activity (× 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
• >10% difference from undiluted = non-parallel & rising, implies inhibitor			

- Inhibitor: IgG alloantibody to FVIII concentrate
  - 30% incidence, most arise in severe hemophilia
  - Reflex to inhibitor assay

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Nijmegen Bethesda Assay

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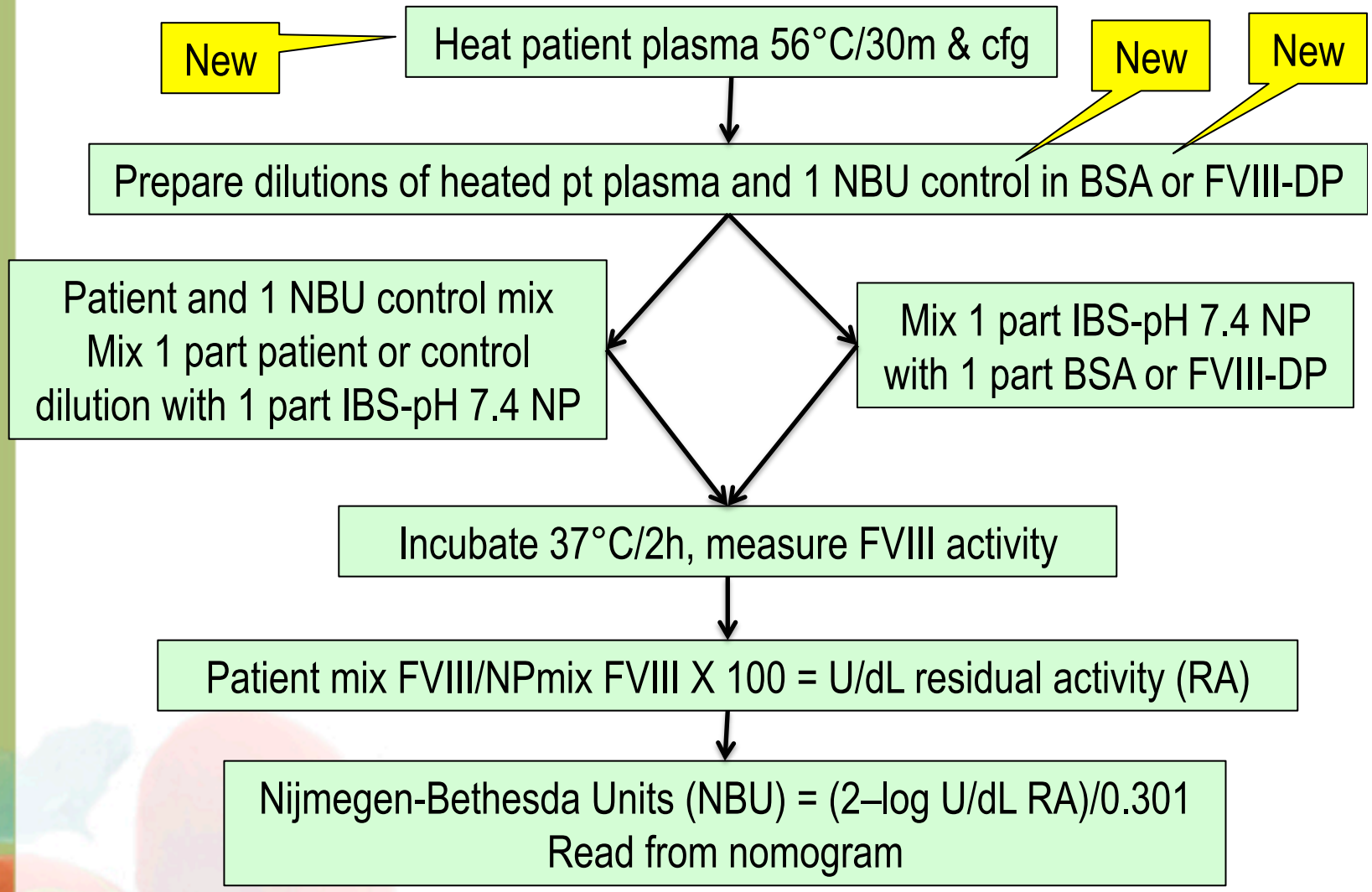
CDC Nijmegen Bethesda Assay (NBA)



Courtesy of Connie Miller, PhD, CDC

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FVIII Inhibitor Therapy: PCC

- If ≤5 NBU, give high-dose FVIII concentrate
- If >5 NBU, prothrombin complex concentrate
  - Proplex PCC, 1980
  - $BaSO_4$ extracted human plasma; II, VII, IX, X
  - *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 factor VIII inhibitor
  - Repeat every 3–6 h; 6 h FVII half-life
- $1.80/μg
  - For our 80-kg patient, one dose = $14,4000
- Cannot monitor
  - Generalized coag evaluation using PTT
  - No risk of DIC

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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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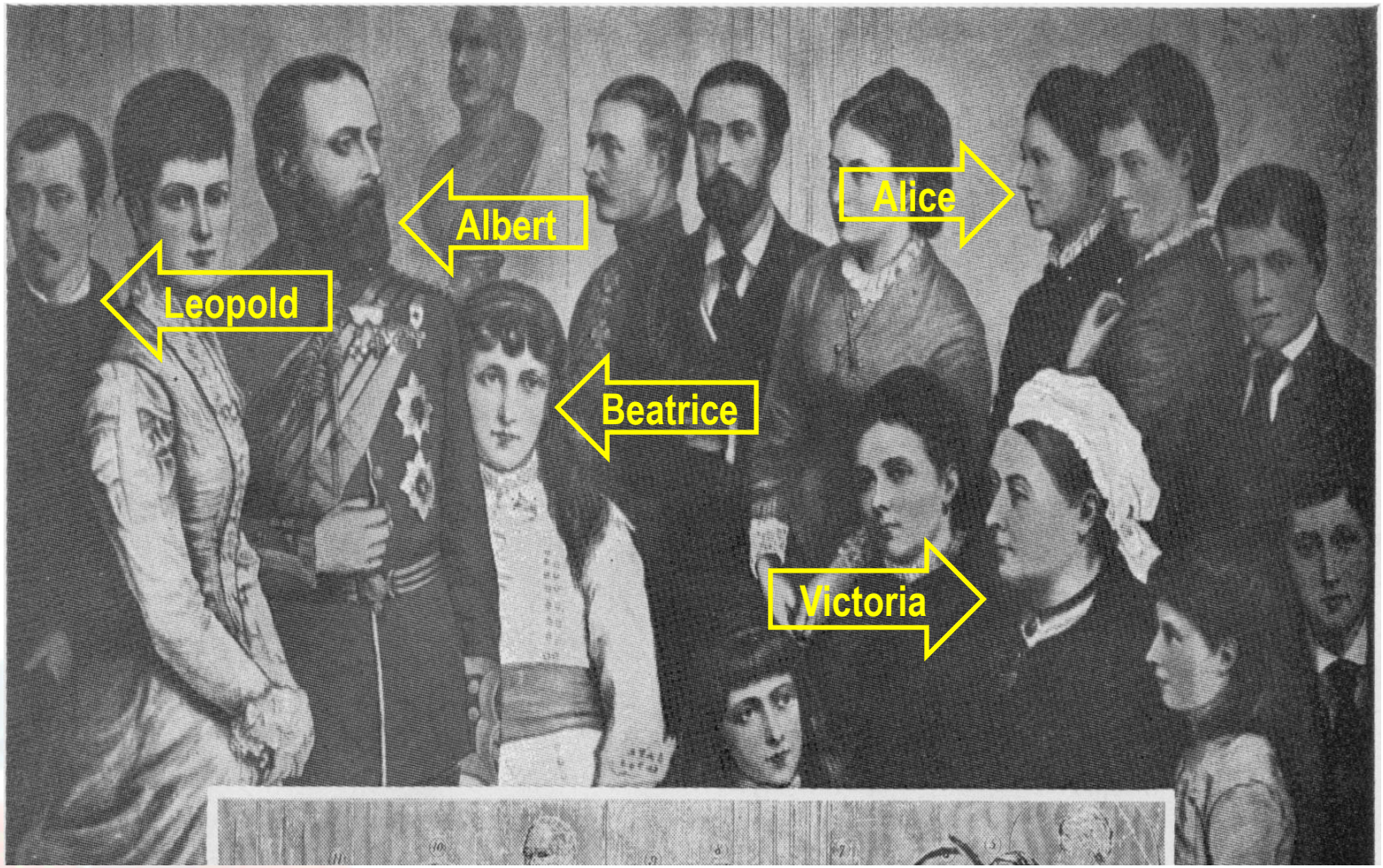
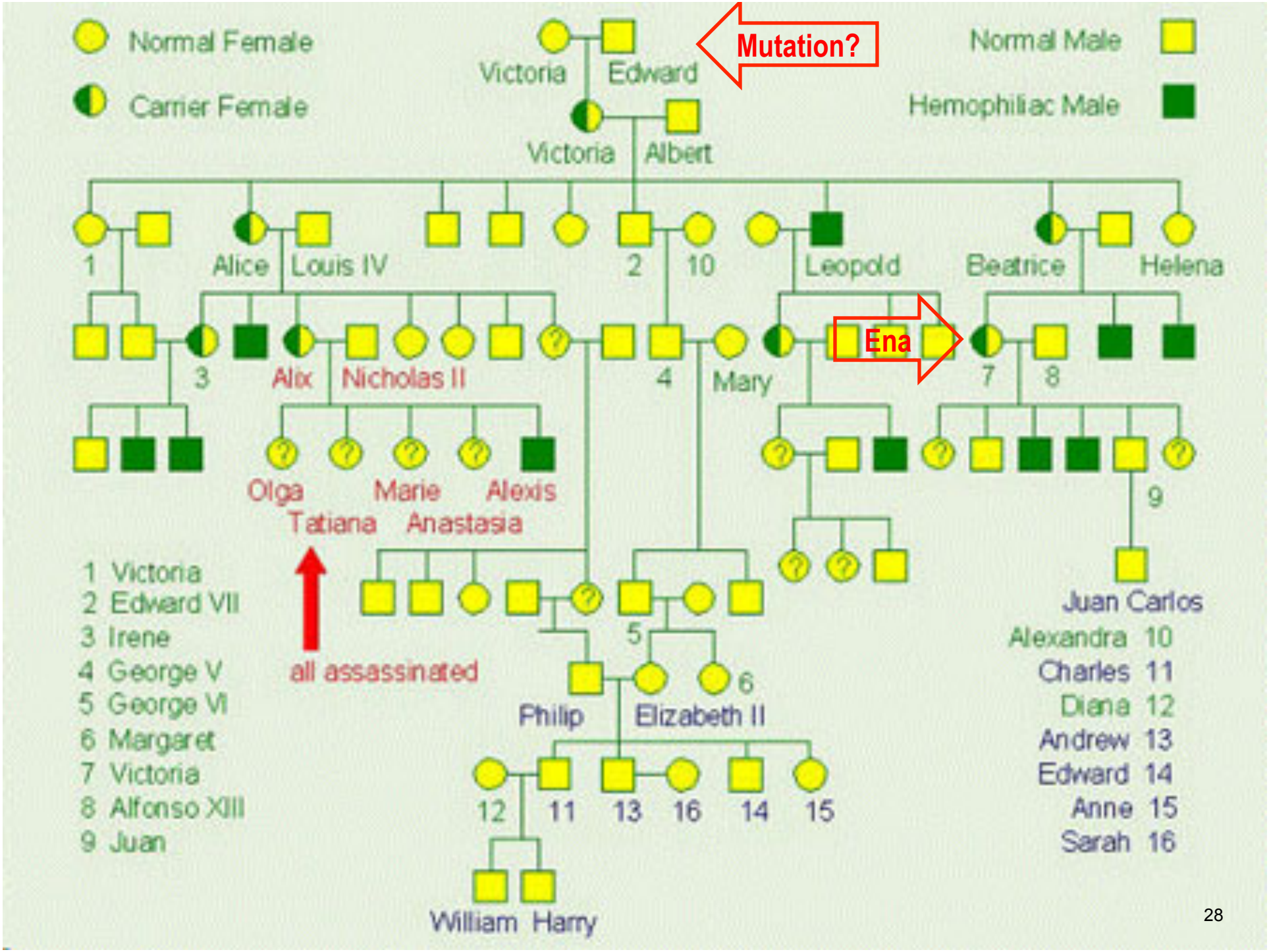
Queen Victoria and Nine Children




Normal Female
Carrier Female
Victoria
Edward
Mutation?
Normal Male
Hemophiliac Male
Victoria
Albert
1
Alice
Louis IV
2
10
Leopold
Beatrice
Helena
3
Alix
Nicholas II
4
Mary
Ena
7
8
Olga
Tatiana
Marie
Anastasia
Alexis
9
1 Victoria
2 Edward VII
3 Irene
4 George V
5 George VI
6 Margaret
7 Victoria
8 Alfonso XIII
9 Juan
all assassinated
5
6
Philip
Elizabeth II
12
11
13
16
14
15
William
Harry
Juan Carlos
Alexandra 10
Charles 11
Diana 12
Andrew 13
Edward 14
Anne 15
Sarah 16

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Nicholas II (Romanov) and Alexandra

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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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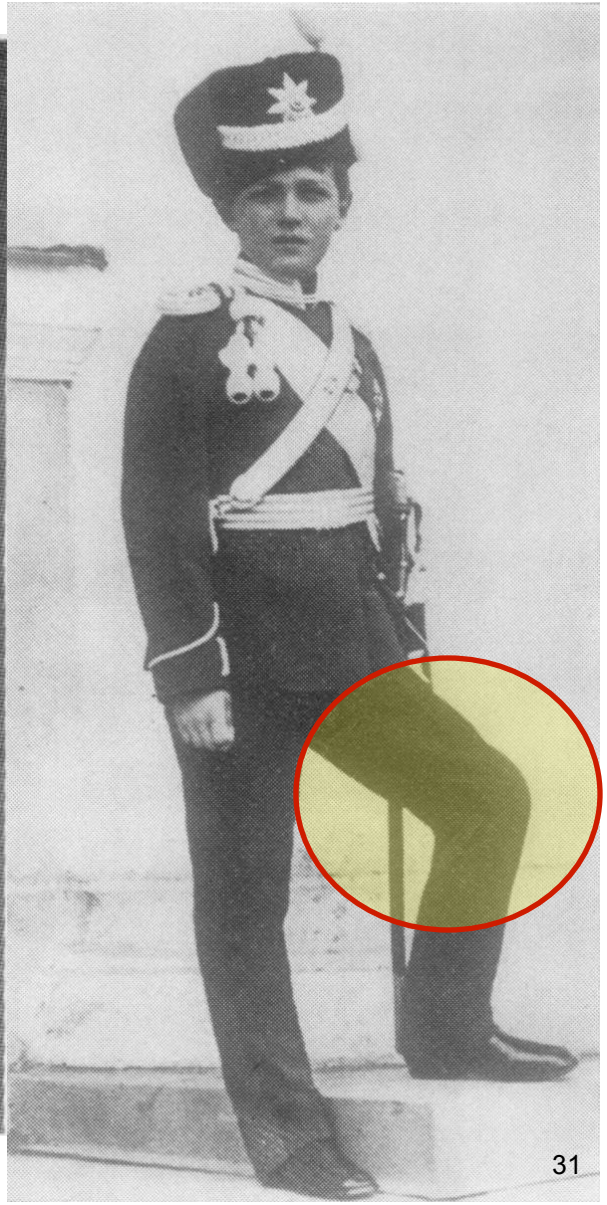
Prince Alexis, 1912



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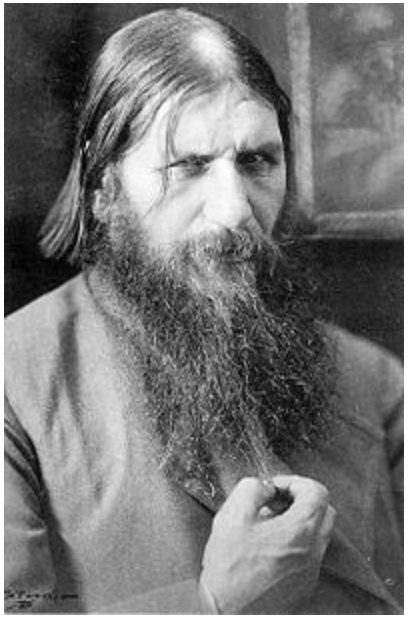
Grigori Yefimovich Rasputin 1869–1916



- 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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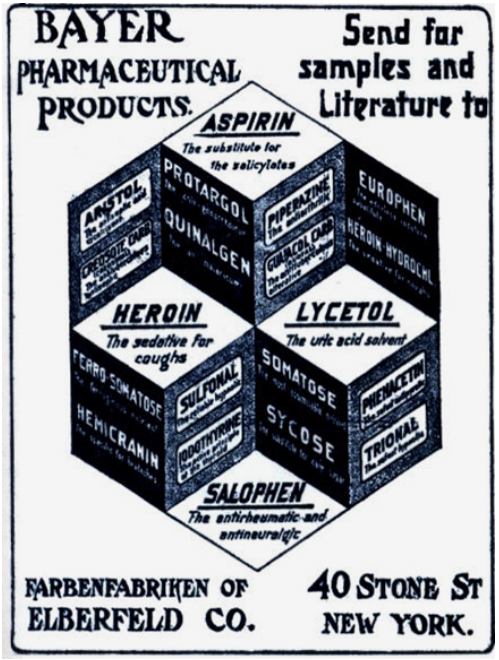
Rasputin's Power Rises
1912 Belovezhski Forest Holiday

- 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.

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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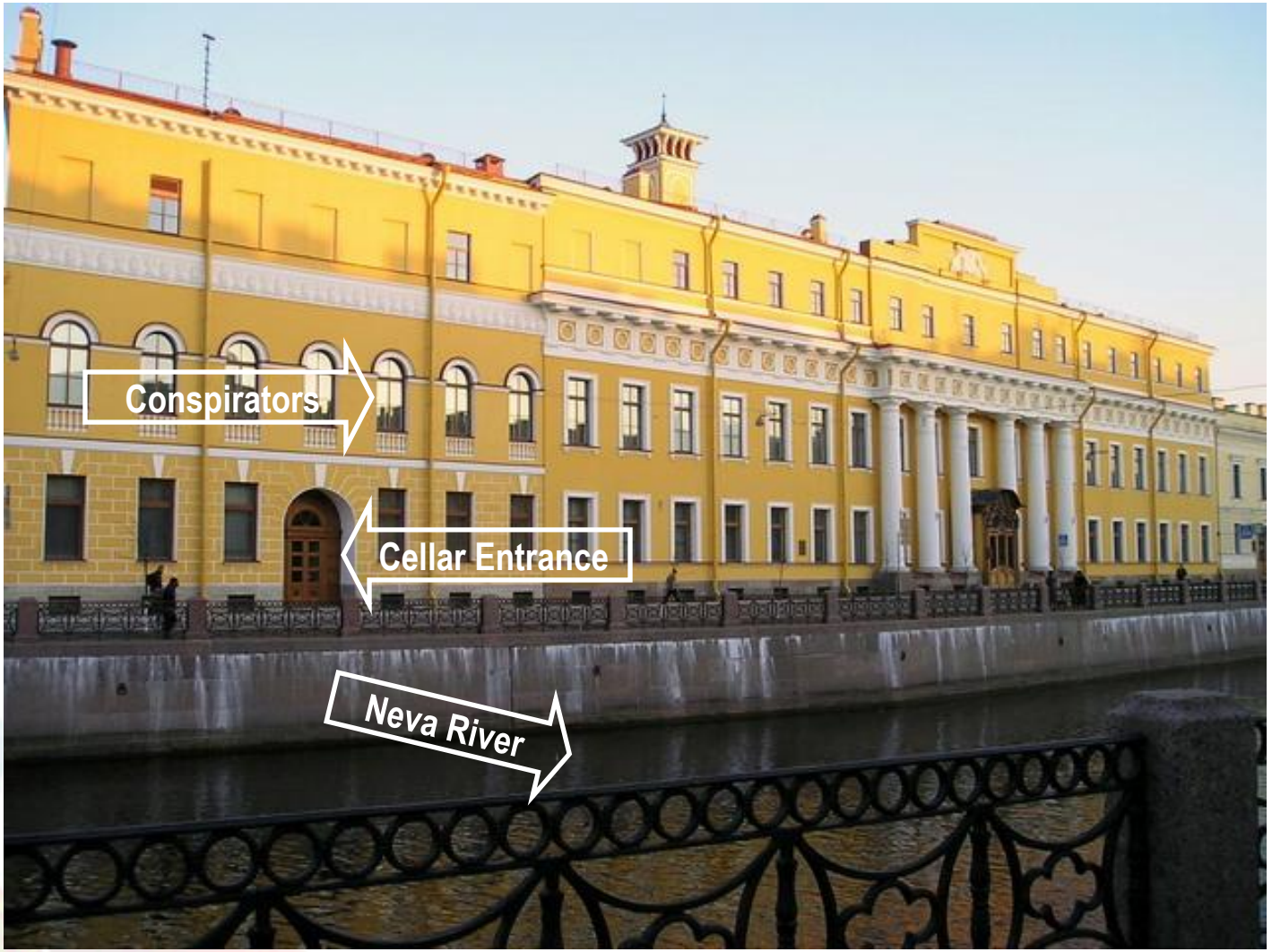
Rasputin and Admirers, 1914

Yusupov Palace, St. Petersburg



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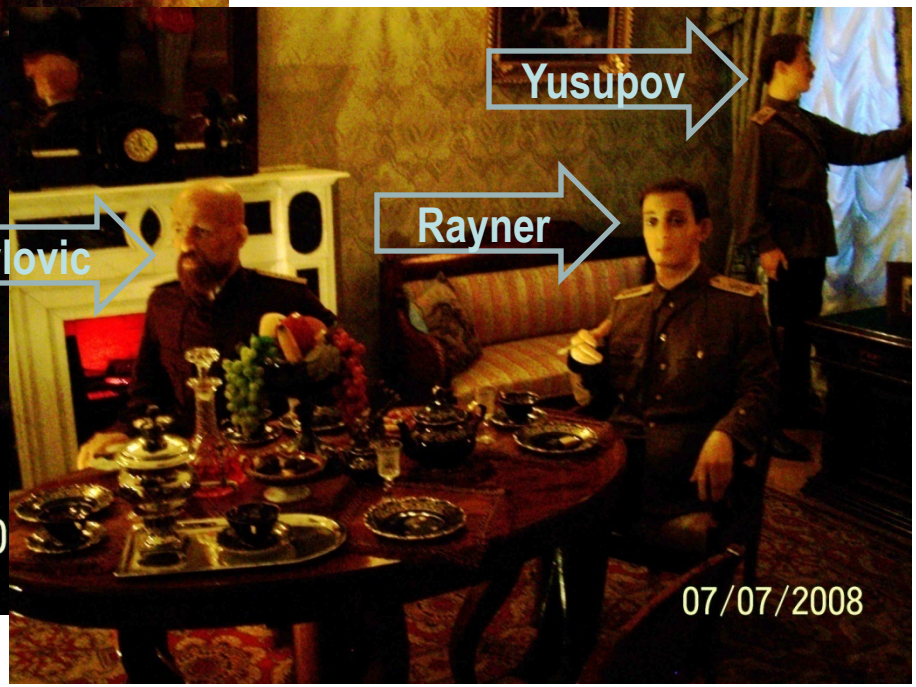
Maj. Felix Yusupov: Dec 16, 1916



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

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Poisoning Rasputin

Cyanide-laced cakes

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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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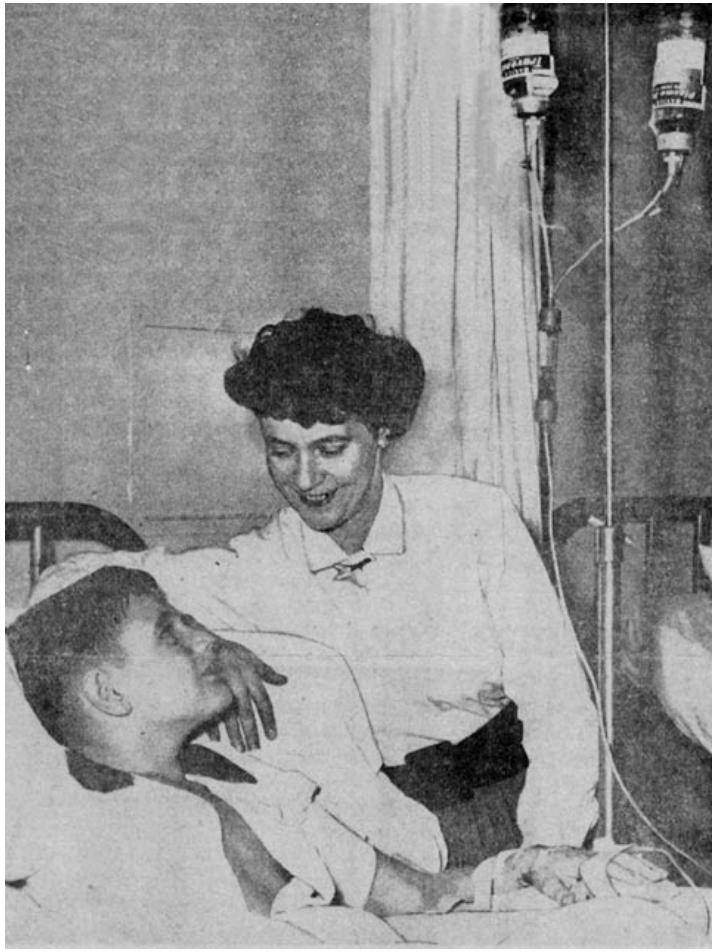

1952: Stephen Christmas, Canadian Hemophilia Soc: Delineated F*IX def (Christmas disease) from FVIII def (BTW, Alexis was factor IX deficient)*

Stephen Christmas
2/12/47–12/20/93

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: Kohn fraction to prevent bleeds during extractions and minor procedures
- 1960: AHF correction of hemarthroses
- 1970: home therapy
- 1973: hemophilia treatment centers
- 1980: prothrombin complex concentrate
- 1980: activated PCC 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
  - I. e. amyl nitrite, anti-sperm antibodies, 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.

CDC Report Delivered 7/27/82

- Evatt 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
- Dec 1982: 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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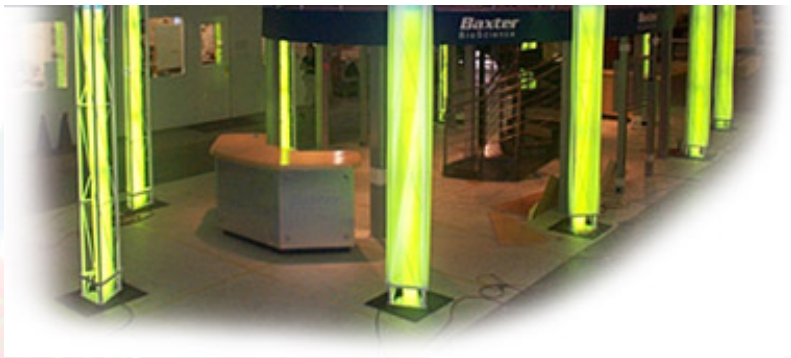
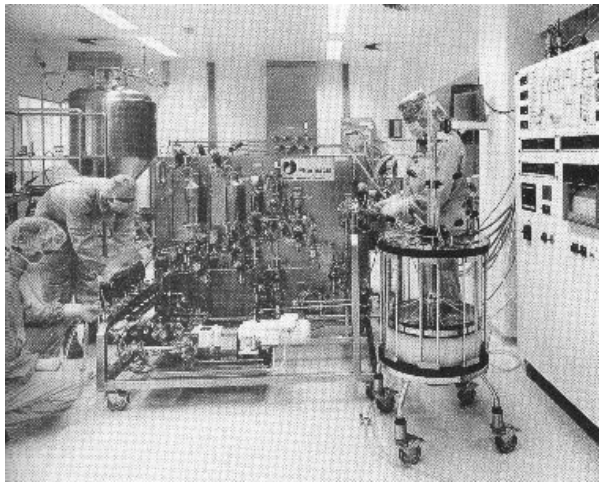
National Hemophilia Foundation Initiative: 1983

- 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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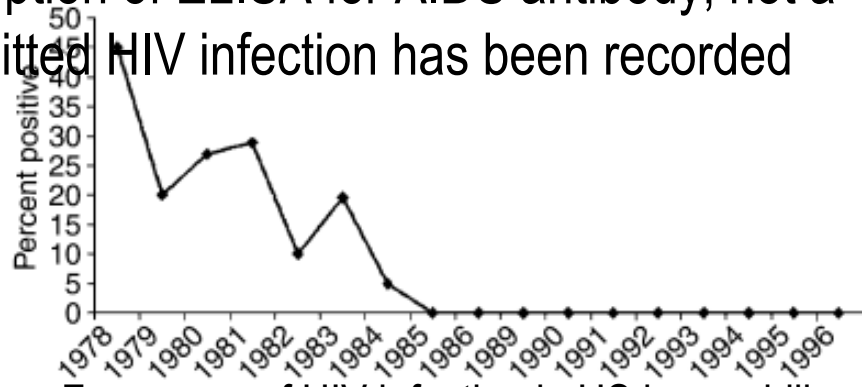
Virus Isolation: 1983

- 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

The Fritsma Factor



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

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Robert Gallo, NIH

Established link between HIV and AIDS, but only Montagnier was awarded Nobel prize.

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Recombinant Clotting Factors

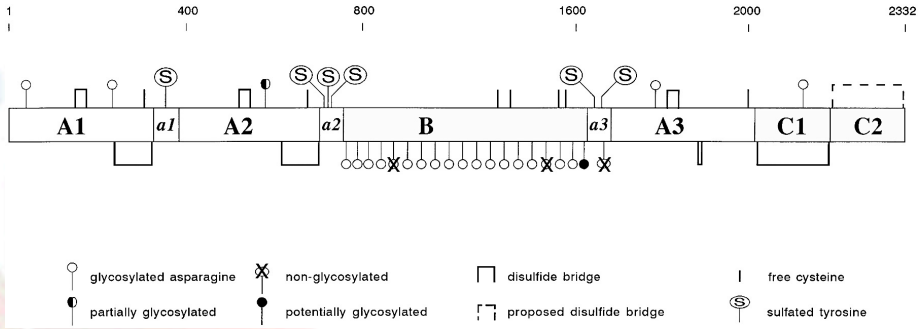
- 4/7/1976: *Genentech* incorporated
- 1981: *Genetics Institute* incorporated
- 1982: rFIX cloned by both (small molecule)
- Aug, 1984: Both cloned rFVIII gene and produced the protein, but yields are small
- 1985: rVWF coexpressed, improves rFVIII yields

genesis
genetics institute

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

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.

ReFacto Antihemophilic Factor (Recombinant)

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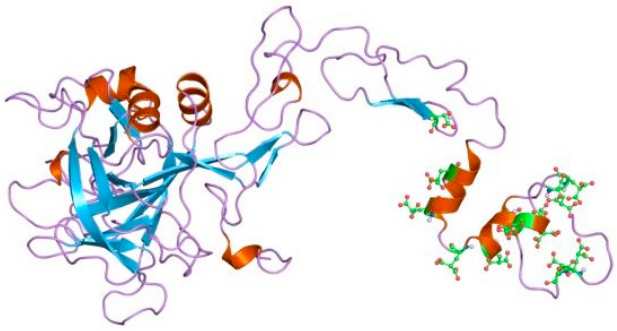
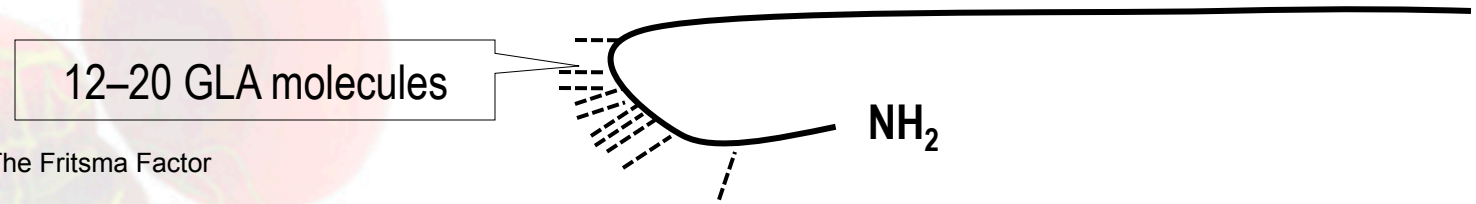
rFIX



17 YEARS YOUR EXPERIENCE MATTERS

BeneFix®
Coagulation Factor IX (Recombinant)
Room Temperature Storage

- 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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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 Factor VIII: Eloctate

- Recombinant B-domain deleted Fc fusion factor VIII
- Extended by Fc receptor and IgG recycling pathway
  - 96 HA adult males with >12 annual bleeds, 3–4 doses/week
  - rFVIIIFc half-life 19 h vs rFVIII 12h; 1.6–3.6 annual bleeds
- Prophylactic Rx interval 5 days Vs. 3–4 doses/week
- Monitor using FVIII assay with non-kaolin-based PTT
- Improved monitoring using chromogenic FVIII assay for all B-domain-deleted FVIII preparations

8/15/16 stock price $313.94

- Shapiro AD, Ragni MV, Kulkarni R, et al. Recombinant factor VIII Fc fusion protein: extended-interval dosing maintains low bleeding rates…. J Thromb Haemost. 2014;12:1788–800.
- Mancuso ME, Mannucci PM. Fc-fusion technology and recombinant FVIII and FIX…. Drug Des Devel Ther. 2014 28;365–71.

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Additional rFVIII Preparations

Name	MFR	Comment	Progress
Bax 111	Baxter	rVWF (not rFVIII)	At FDA
Kovaltry	Bayer	Full-length rFVIII with no human or animal proteins, Reduced inhibitors, normal to slightly extended half-life	Approved
NovoEight	Novo Nordisk		
NuWiq	Octapharma		
Bay 94-9027	Bayer	Pegylated, plasma/albumin free, full-length rFVIII, up to 7.5 d frequency	At FDA
Bax 855	Baxter "Baxalta"	Pegylated plasma/albumin free, full-length rFVIII, 1.5 X Advate half-life	At FDA
rFVIII single-chain	CSL Behring	rFVIII covalently bonds VWF reduces clearance, extends half-life; no inhibitors	Phase II
ACE 910	Chugai & Genentech	Bispecific protein mimics FVIII cofactor, activates IX & X, bypasses inhibitors, SC 1/wk, generates no immune response	FDA breakthrough status

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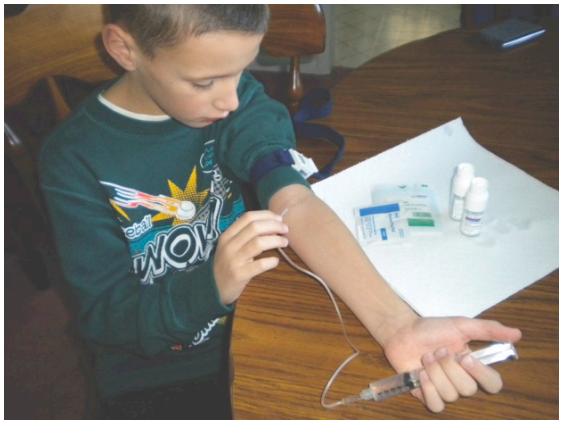
Extended Half-life Factor IX

- Recombinant Fc fusion factor IX Alprolix, 2014
  - rFIXFc half-life 70–80 h versus 24 h, 7–10 day intervals
  - Monitor using FIX assay with non-kaolin-based PTT
  - Chromogenic FIX valid but not available in the US
- Recombinant albumin fusion FIX Idelveon
  - FDA 3/4/2016
  - Patients <12 years old: 40–55 U/kg 7 day interval
  - Patients ≥12 years old: 25–40 U/kg 7 day interval
  - For ≥12 YO if controlled, go to 14-d interval at 50–75 U/kg

CSL Behring

Biotherapies for Life®



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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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BioMarin BMN270 FVIII Transfer Trial

- B-domain-deleted FVIII gene with minimal glycosylation
- Vector optimization: adenovirus-associated vector "8" with adequate capacity for the FVIII gene
  - University College London
  - St. Jude's Research Hospital
- Phase 1 & 2 trial completed
- Preparing phase III trial

- 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.

BioMarin Phase 1 and 2 Results 4/20/16: 8 Severe Hemophilics

Dose	Week	U/dL	Outcome
$6X10^{12}$ vg/kg	20	<1	Severe
$2X10^{13}$ vg/kg	16	2	Moderate
$6X10^{13}$ vector genomes/kg	16	57	Normal
	8	60	Normal
	7	8	Mild
	7	4	Moderate
	6	21	Mild
	5	10	Mild
Prednisolone controls liver toxicity as measured by ALT			

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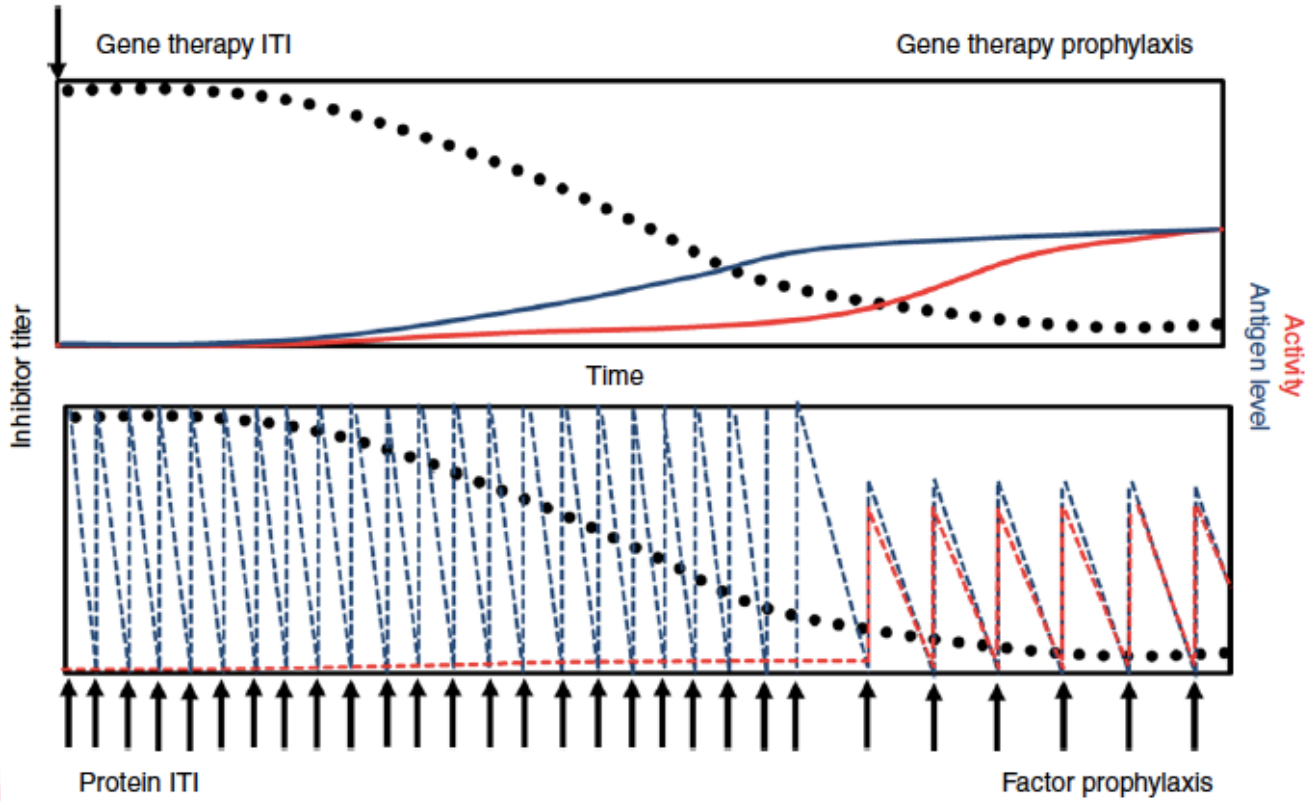
Gene Transfer Therapy May Reduce Inhibitor Formation: Animal Models



Nichols TC, Hough C, Agerso H, Ezban M, Lillicrap D. Canine models of inherited bleeding disorders in the development of coagulation assays, novel protein replacement and gene therapies. J Thromb Haemost 2016; 14: 894–905.

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Target Antithrombin to Decrease Inhibition

- Silencing RNA (siRNA): synthetic RNA complementary to mRNA sequence, blocks mRNA translation
- siRNA-AT3 binds antithrombin mRNA and silences hepatic antithrombin production
- siRNA can be produced against any gene product: first accomplished in petunias

- 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

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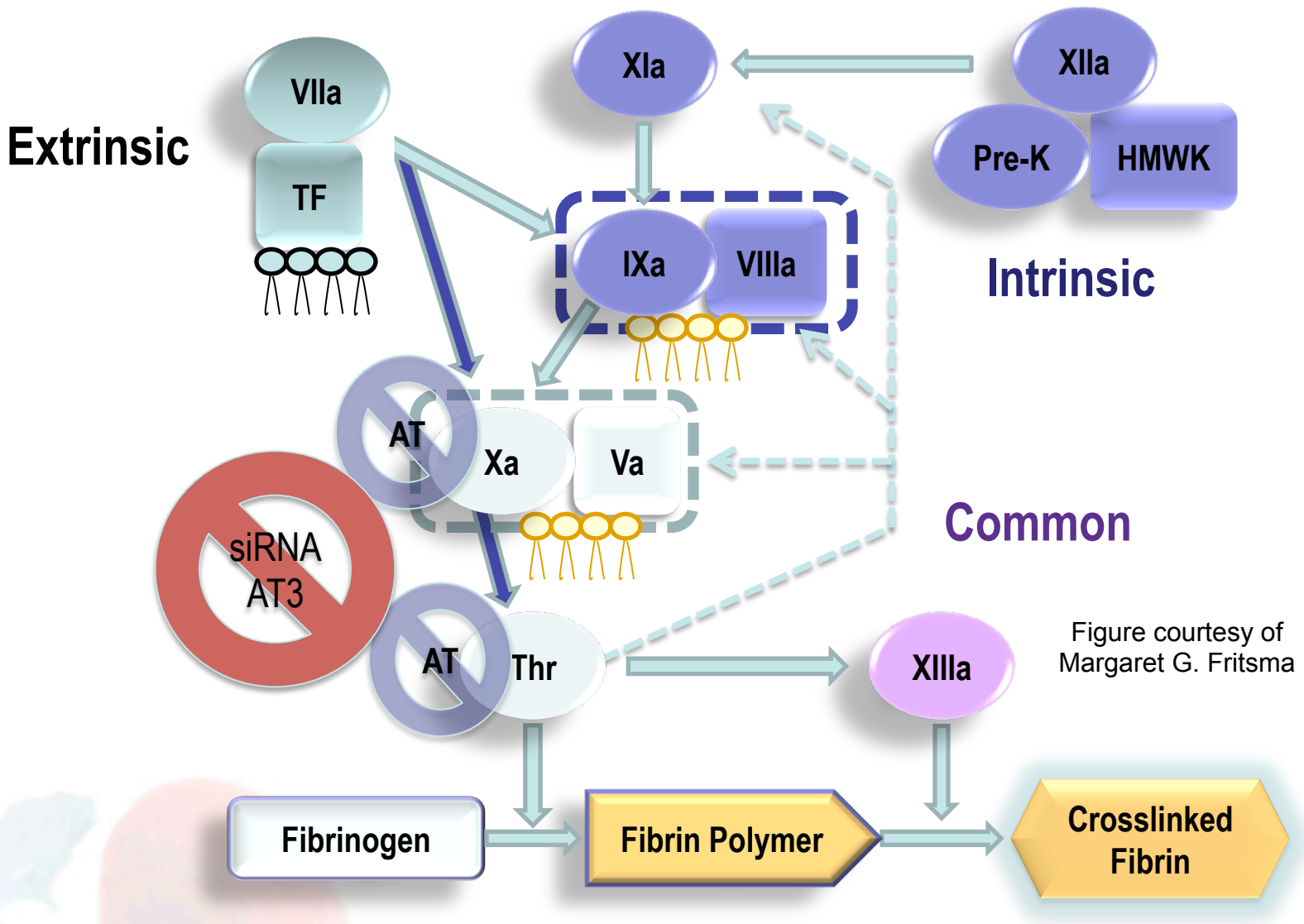

Figure courtesy of Margaret G. Fritsma

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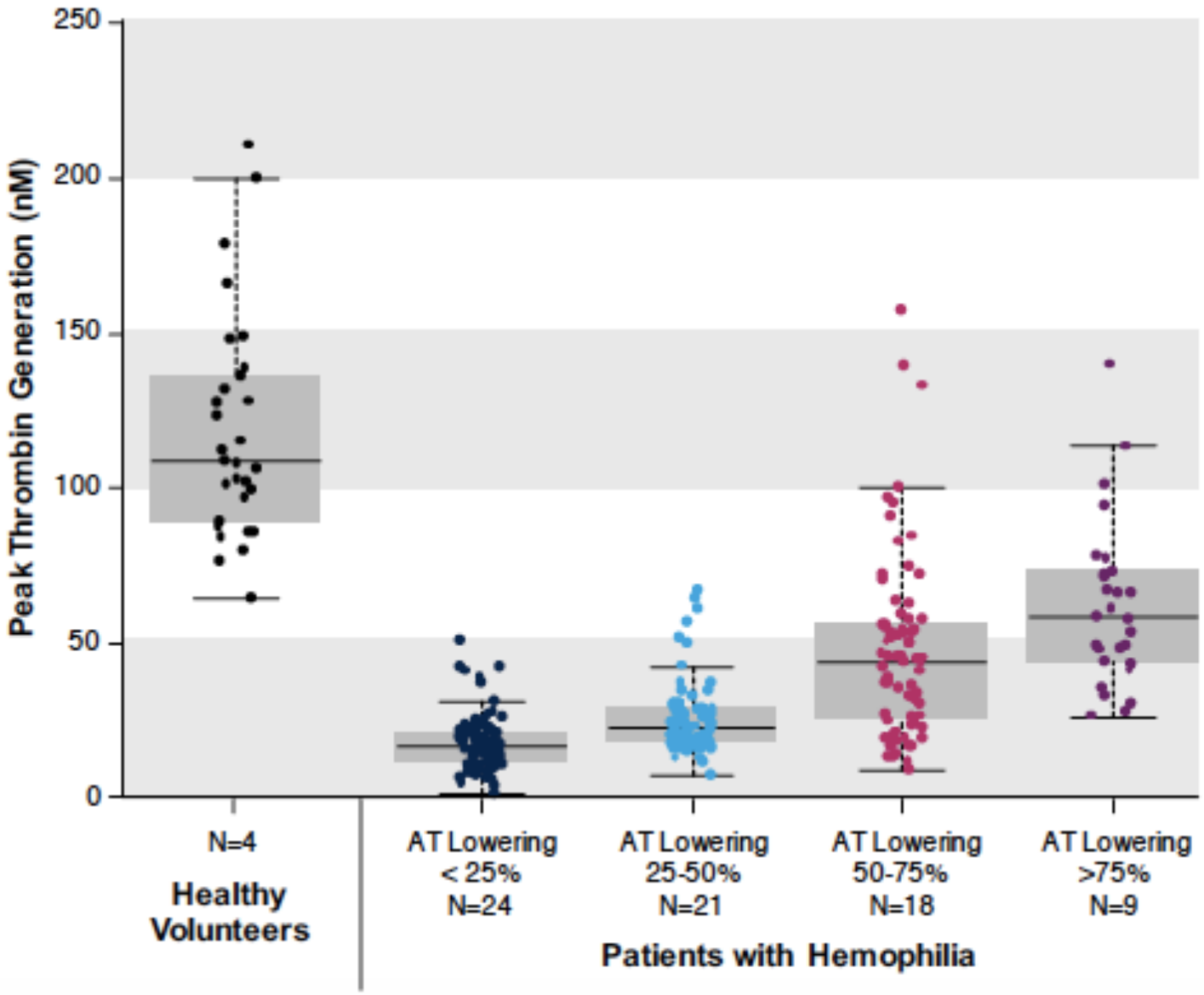
Thrombin Generation by % AT Lowering



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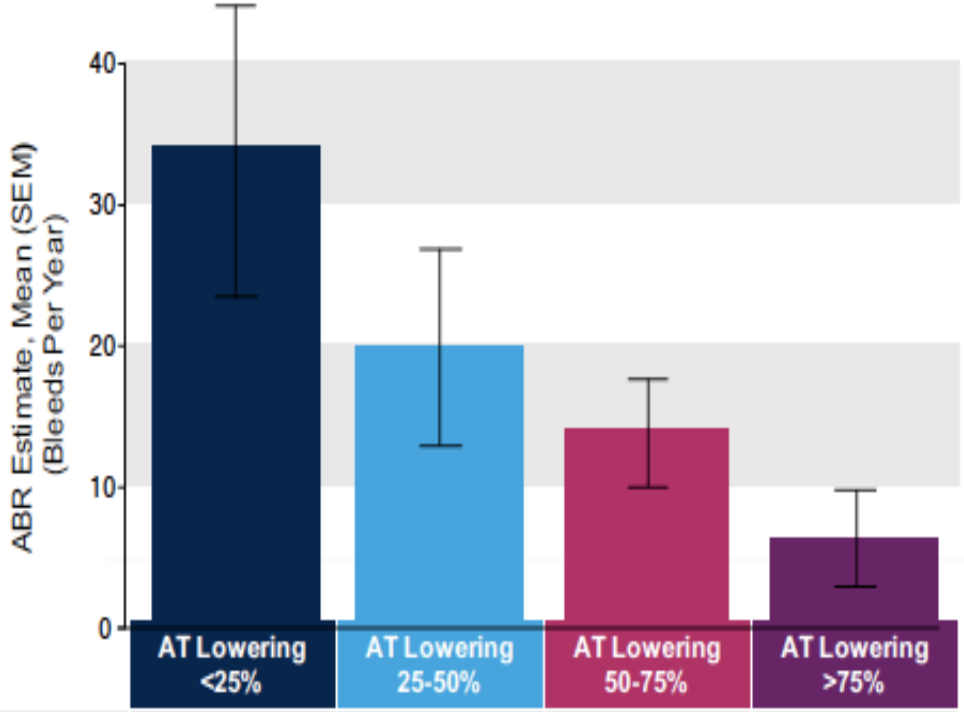
Annual Bleed Rate in Hemophilics Treated with siRNA AT3

Bleed Events by % AT Lowering



	AT Lowering <25%	AT Lowering 25-50%	AT Lowering 50-75%	AT Lowering >75%	
Patients	N=24	N=21	N=18	N=9	
ABR, Mean (SEM)	34 ± 10	20 ± 7	14 ± 4	6 ± 3	
ABR, Median	13	11	10	0	p<0.05

Conclusions: With AT lowering by quartile, <25% to >75%, there is reduction in ABR.

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siRNA AT3 Phase I Safety

- No discontinuation
- Mild adverse events
  - Transient erythema and pain at injection site, resolved at 24 hours
  - Headache
- Bleeds treated with standard therapy
- No antibody formation
- Normal LFTs, CBC, PLTs, FG, EKG

Sehgal A et al, Nat Med 2015

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Bottom Line at the End (BLATE)

The participant…

- Gives an overview of hemophilia.
- Recounts the history of hemophilia from the second century, including the story of the Russian royal family.
- Reviews the events leading to the discovery of coag factors concentrates' transmission of viral disease.
- Plans factor VIII concentrate therapy, differentiating between plasma-derived and recombinant preparations.
- Manages prolonged half-life factor VIII and IX.
- Describes current efforts to manage hemophilia through gene transfer therapy and antithrombin suppression.

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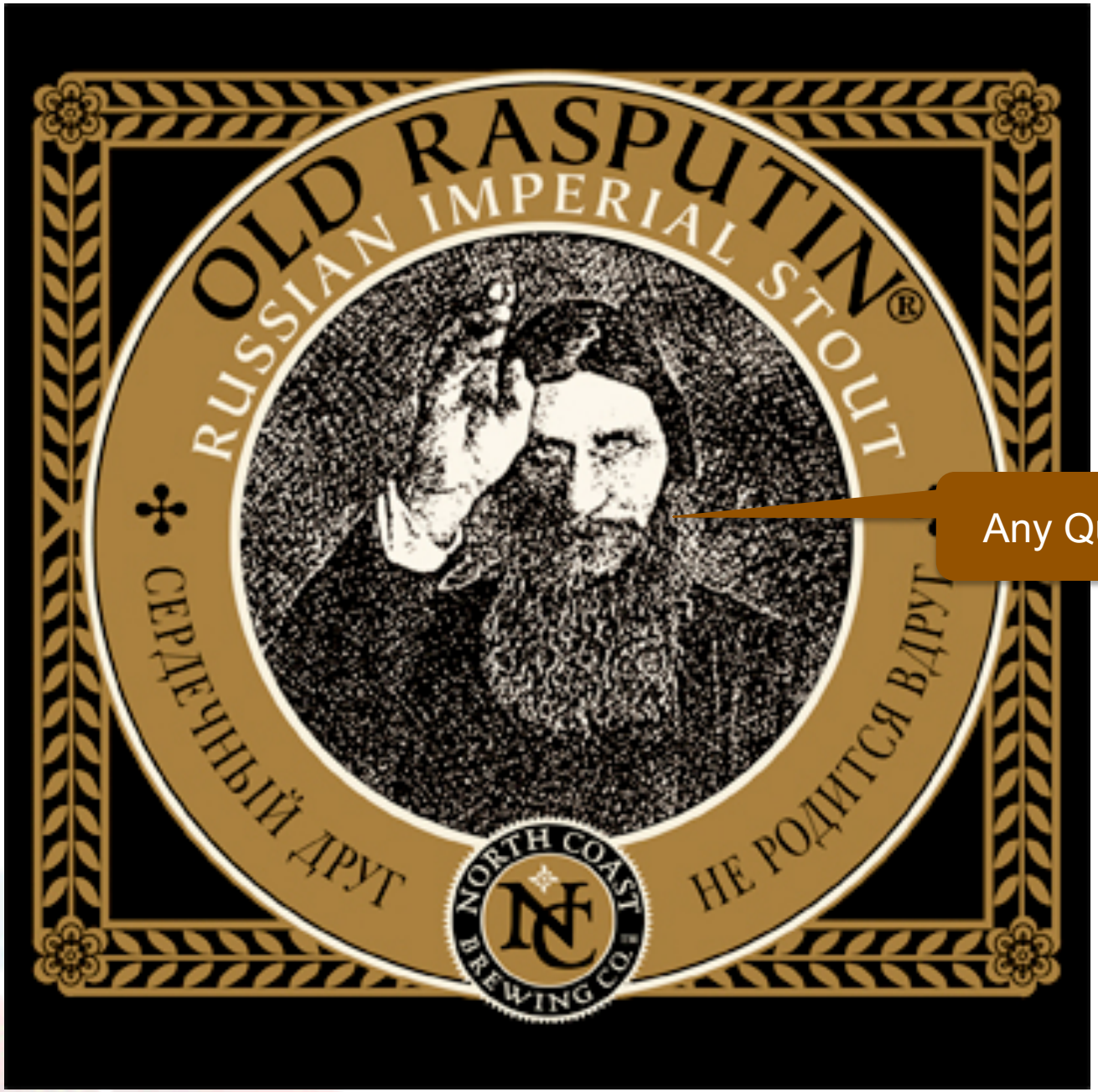

Any Questions?