Inibitori in emofilia

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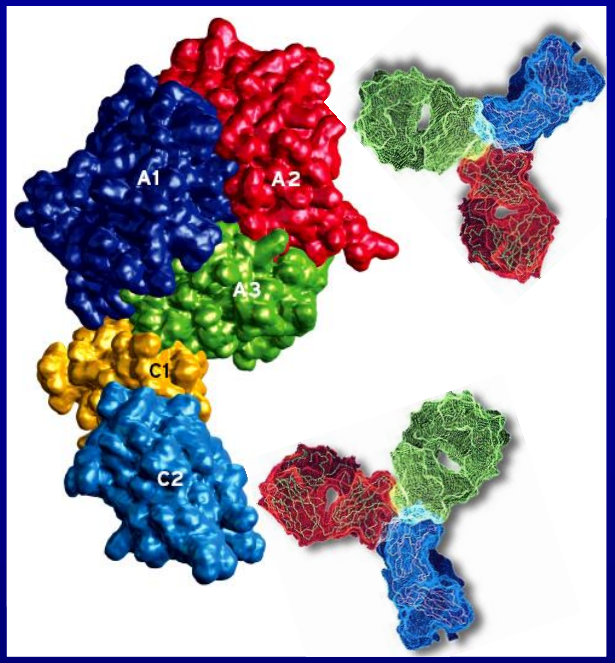
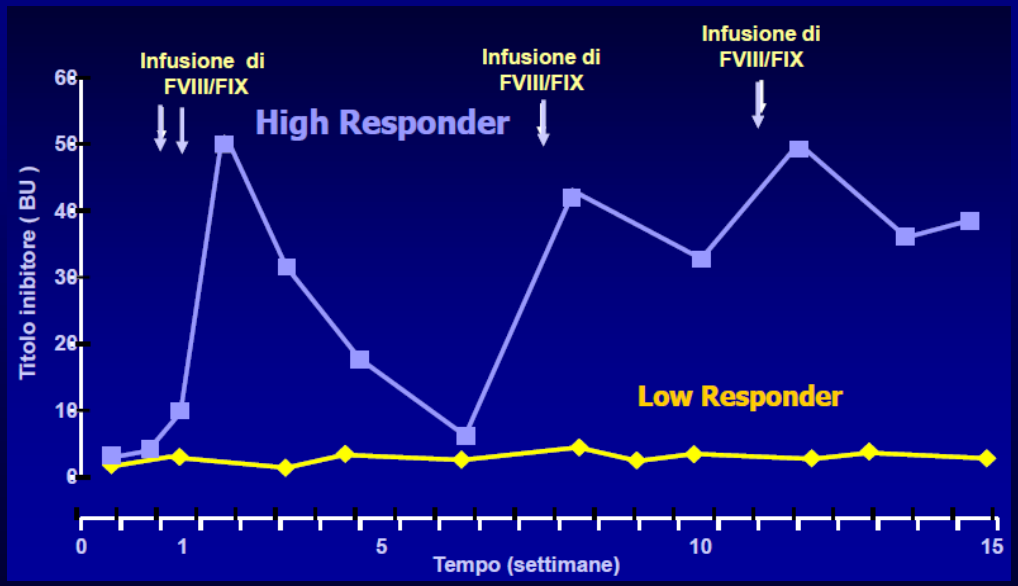
Inhibitor development in hemophilia...

Alloantibodies (IgG1-IgG4) against the therapeutically administered clotting factor, usually developing over the first 20 exposure days



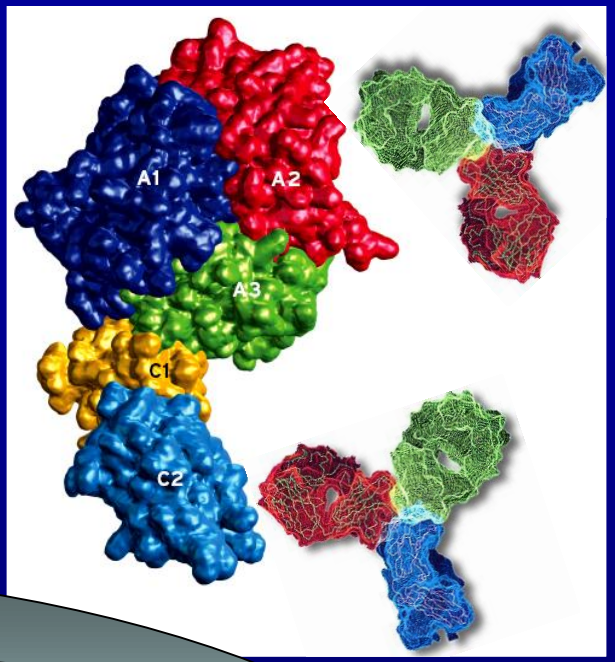


HIGH RESPONSE (HR)
>5 BU/ml

LOW RESPONSE (LR)
< 5 BU/ml

TRANSIENT: disappearance on factor concentrate exposure, usually **within 6 months** (LR in the majority of cases)
PERSISTENT

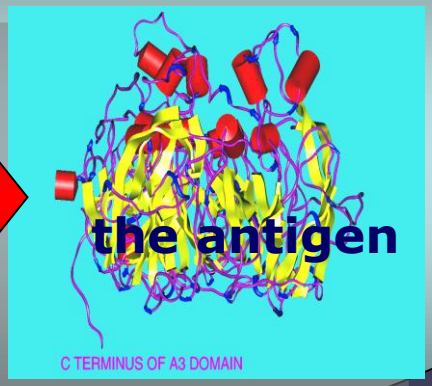
Inhibitor development in hemophilia...

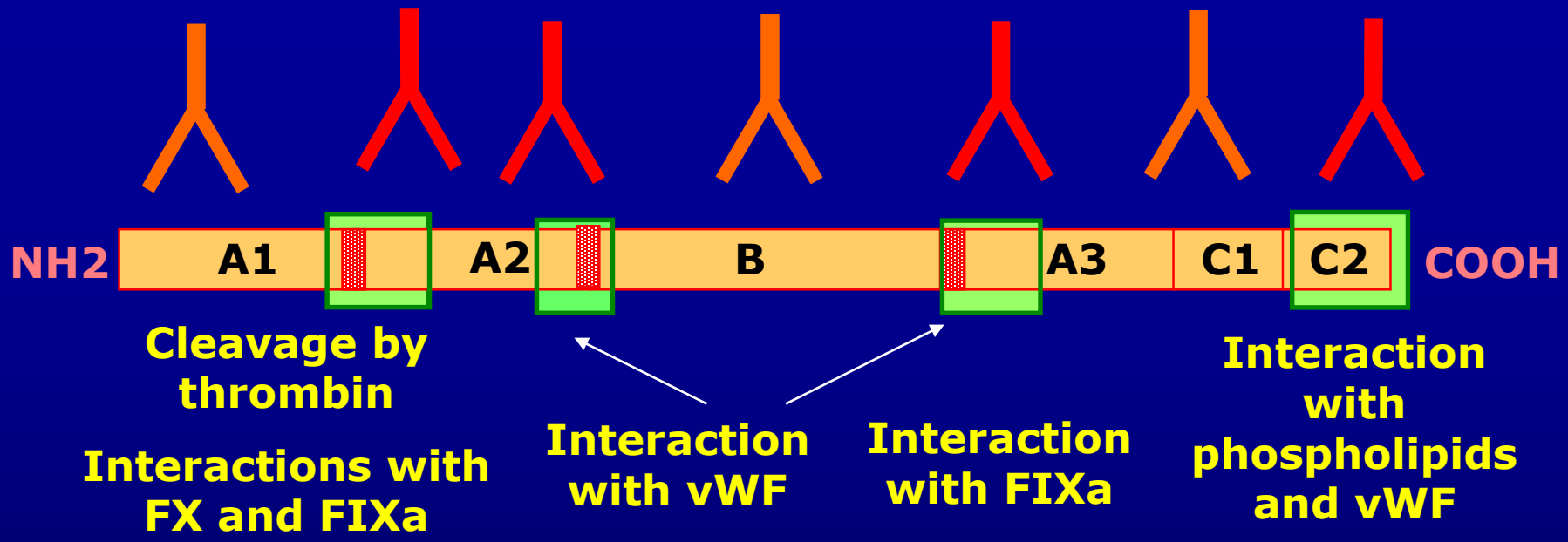


25-35% severe HA
5-10% non-severe HA
<5% HB

the environment of their interaction

the modality of their interaction





A2, A3, C2

Functional epitopes involved in the formation of Xase complex

↓

neutralizing activity

↓

detectable by Bethesda assay

A1, B, C1

non functional epitopes

↓

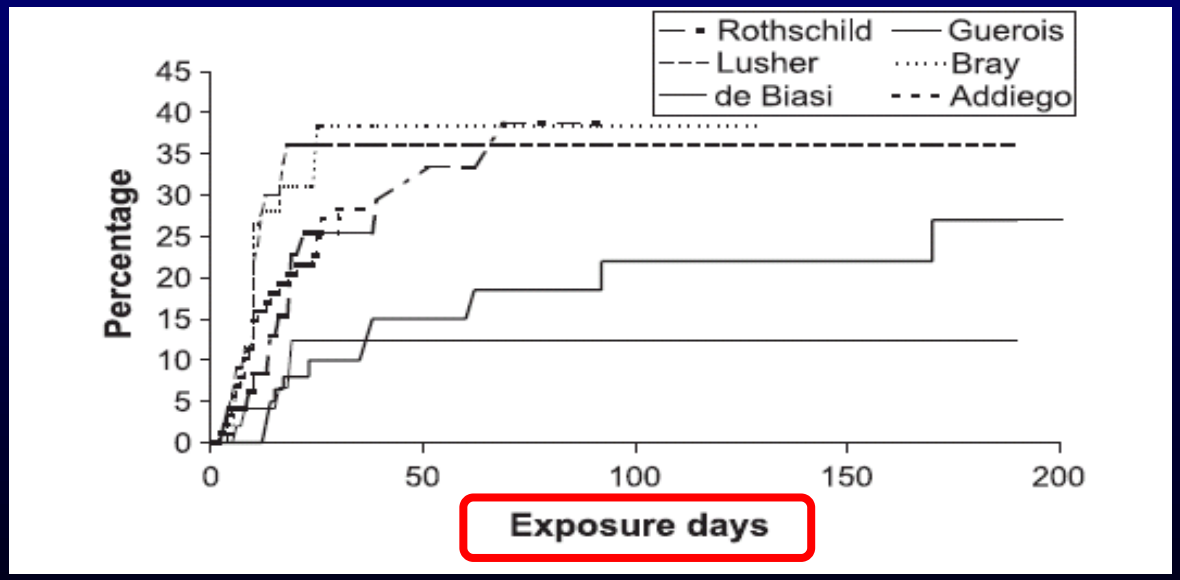
enhanced in vivo clearance of FVIII

↓

non detectable by Bethesda assay

reduced FVIII half-life

Incidence of inhibitors



Cumulative risk

At age of 5	16%
At age of 15	20%
At age of 50	30%
At age of 75	36%

Incidence

Age (yrs)	***all inh***	***HR inh***
0-4	64.3	36.1
5-9	9.4	1.1
10-49	5.3	1.0
50-59	5.25	1.6
60+	10.9	2.6

per 1000 pt-yrs at risk

Wight & Paisley, Haemophilia, 2003

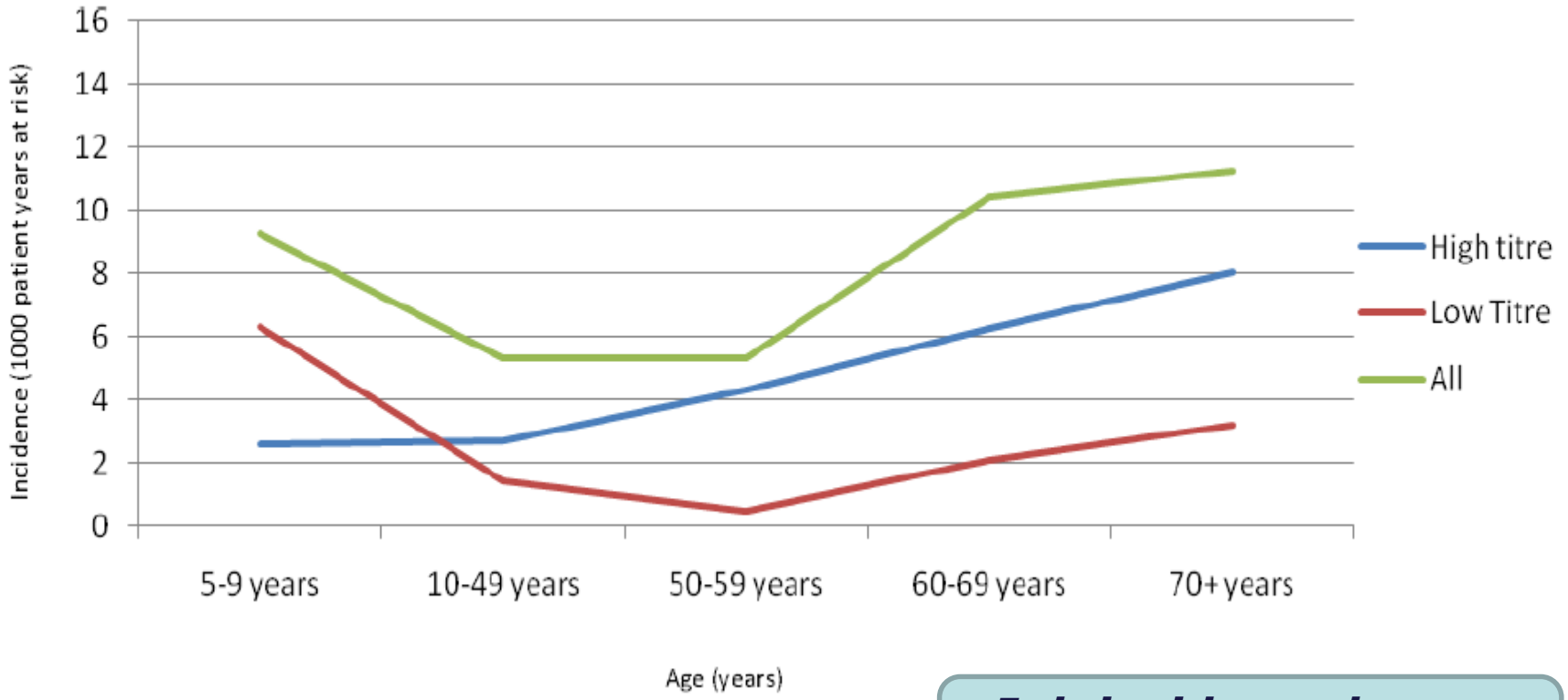
Hay et al, Blood 2011
UKHCDO, J Thromb Haemost, 2004

blood

Prepublished online April 6, 2011;
doi:10.1182/blood-2010-09-308668

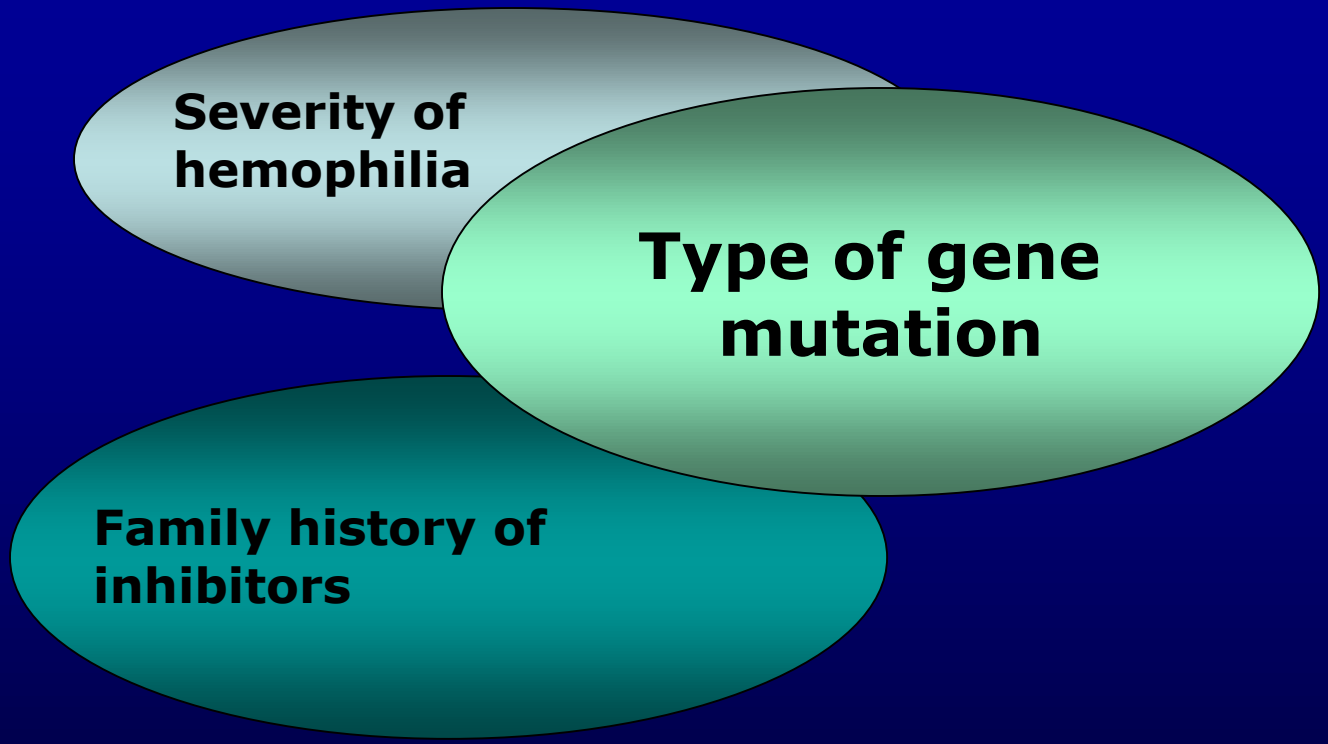
The incidence of factor VIII inhibitors throughout life in severe hemophilia A in the United Kingdom

Charles RM Hay, Ben Palmer, Elizabeth Chalmers, Ri Liesner, Rhona Maclean, Savita Rangarajan, Michael Williams and Peter W Collins



Inh incidence by age in the UK, 1990-2009

Why do inhibitors develop:
Genetic factors



Oldenburg, 2005, mod

HIGH RISK

100

deletions spanning the intron 22

Multi-domain

75

50

LARGE DELETIONS

Light chain

NONSENSE

Single domain

25

Heavy chain

INTRON 22/1 INVERSIONS

Non A-run

SMALL DEL/INS

A-run

large duplications

conserved positions

SPLICE SITE

C1-C2

MISSENSE

non C1-C2

non conserved positions

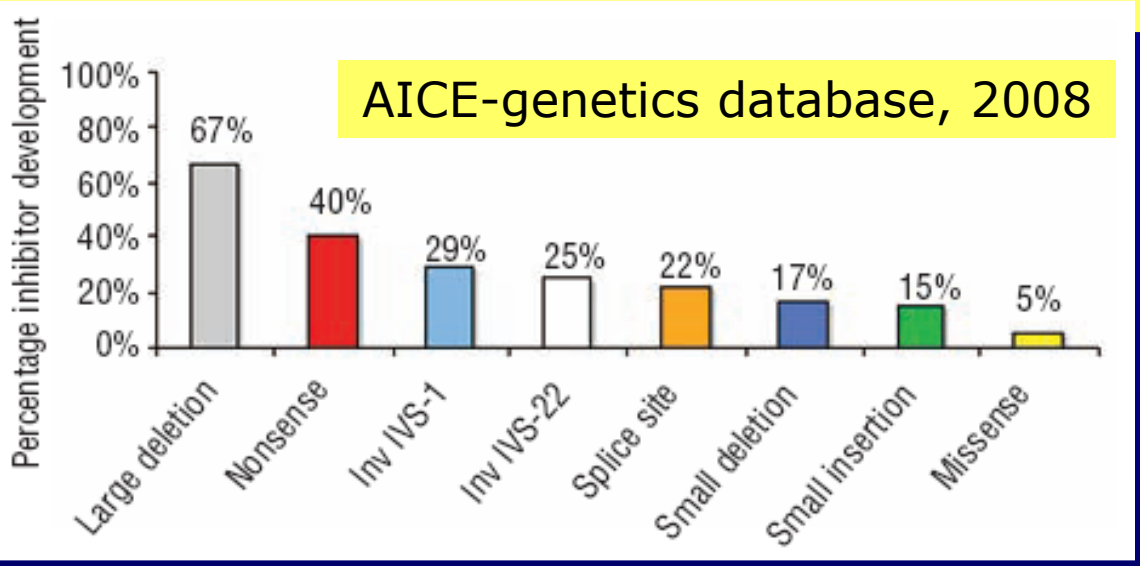
0

LOW RISK

Oldenburg et al, 2006

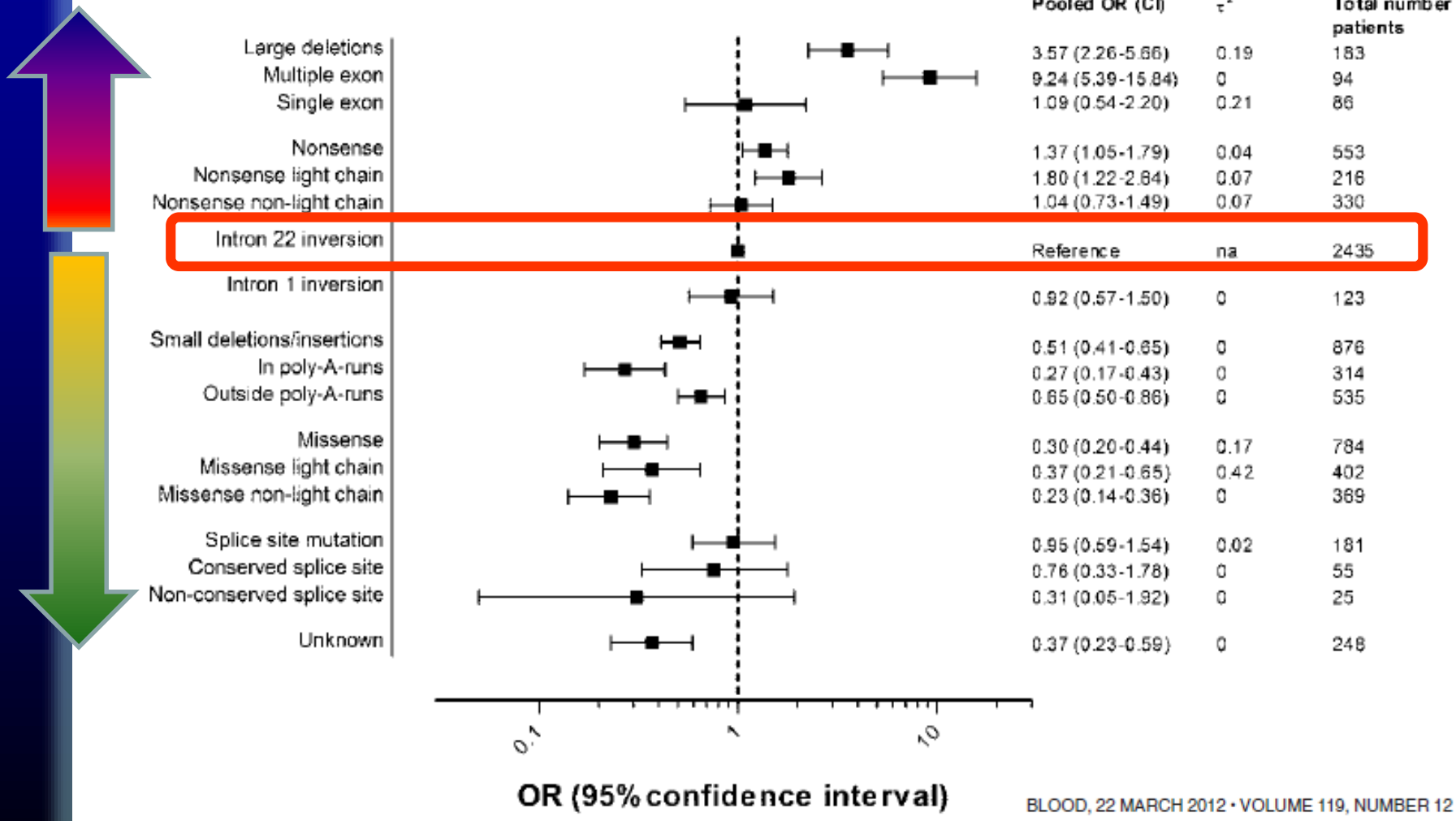
Oldenburg et al, 2008

Type of mutation and inhibitor prevalence in patients with haemophilia A



F8 gene mutation type and inhibitor development in patients with severe hemophilia A: systematic review and meta-analysis

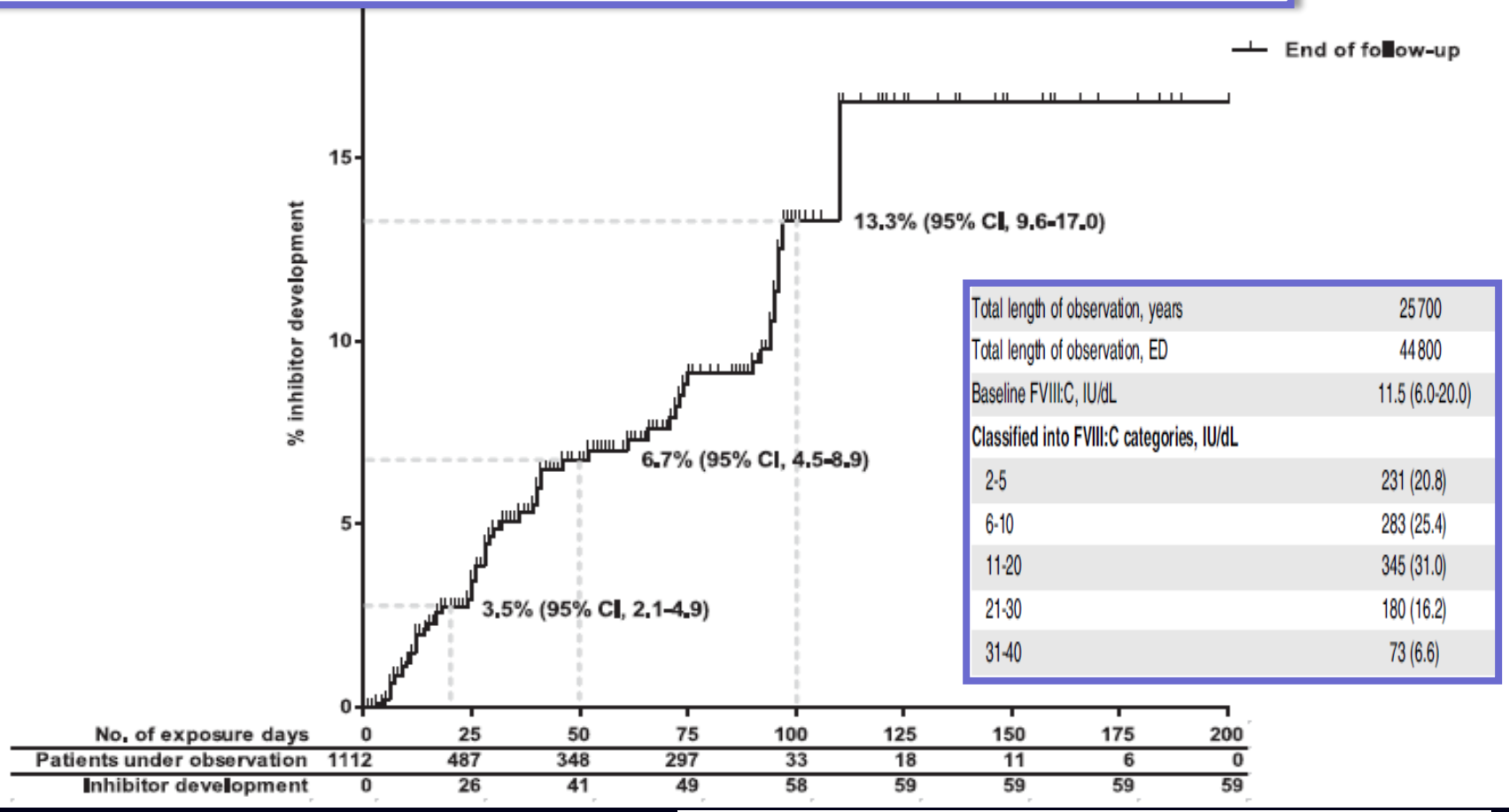
Samantha C. Gouw,[1,2] H. Marijke van den Berg,[2] Johannes Oldenburg,[3] Jan Astermark,[4] Philip G. de Groot,[2] Maurizio Margaglione,[5] Arthur R. Thompson,[6] Waander van Heerde,[7] Jorien Boekhorst,[7] Connie H. Miller,[8] Saskia le Cessie,[9,10] and Johanna G. van der Bom[10,11]



BLOOD, 22 MARCH 2012 • VOLUME 119, NUMBER 12

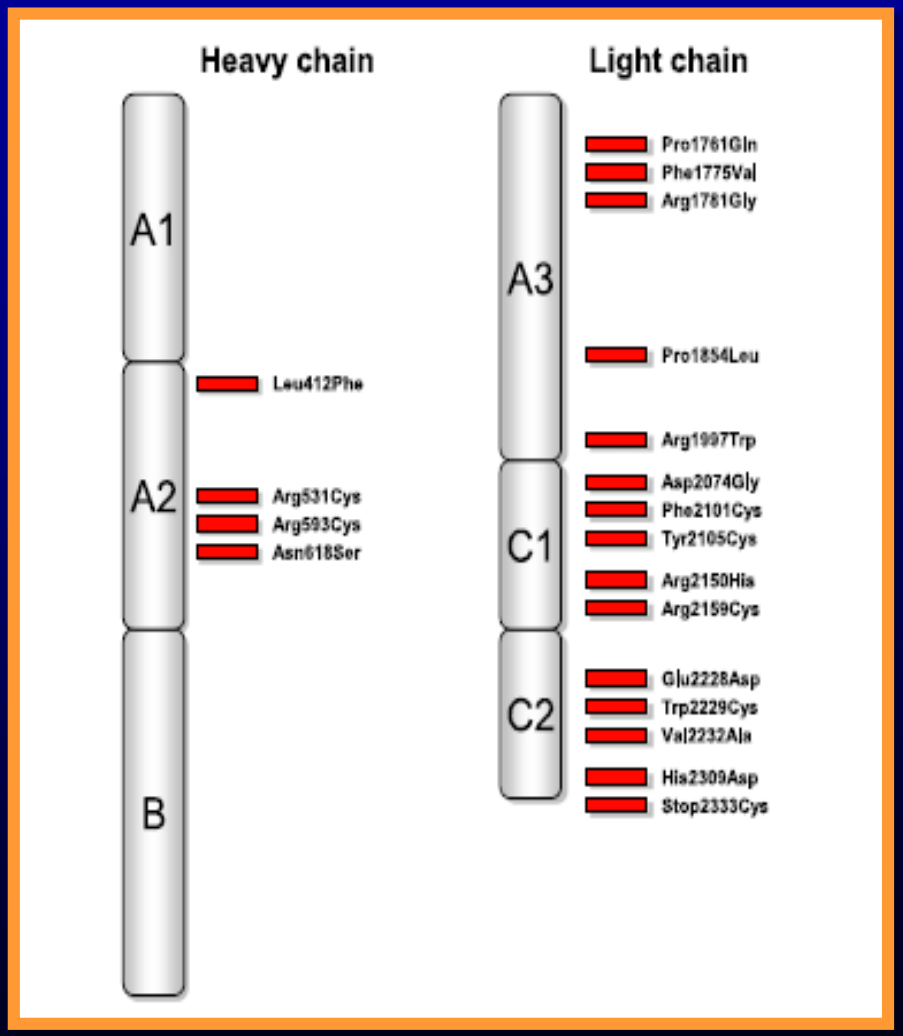
Factor VIII gene (*F8*) mutation and risk of inhibitor development in nonsevere hemophilia A

Corien L. Eckhardt,[1] Alice S. van Velzen,[1] Marjolein Peters,[1] Jan Astermark,[2] Paul P. Brons,[3] Giancarlo Castaman,[4] Marjon H. Cnossen,[5] Natasja Dors,[6] Carmen Escuriola-Ettingshausen,[7] Karly Hamulyak,[8] Daniel P. Hart,[9] Charles R. M. Hay,[10] Saturnino Haya,[11] Waander L. van Heerde,[3] Cedric Hermans,[12] Margareta Holmström,[13] Victor Jimenez-Yuste,[14] Russell D. Keenan,[15] Robert Klamroth,[16] Britta A. P. Laros-van Gorkom,[3] Frank W. G. Leebeek,[5] Ri Liesner,[17] Anne Mäkipernaa,[18] Christoph Male,[19] Evelien Mauser-Bunschoten,[20] Maria G. Mazzucconi,[21] Simon McRae,[22] Karina Meijer,[23] Michael Mitchell,[24] Massimo Morfini,[25] Marten Nijziel,[26] Johannes Oldenburg,[27] Kathelijne Peerlinck,[28] Pia Petrini,[13] Helena Platokouki,[29] Sylvia E. Reitter-Pfoertner,[19] Elena Santagostino,[30] Piercarla Schinco,[31] Frans J. Smiers,[32] Berthold Siegmund,[33] Annarita Tagliaferri,[34] Thynn T. Yee,[35] Pieter Willem Kamphuisen,[1] Johanna G. van der Bom,[32,36] and Karin Fijnvandraat,[1] for the INSIGHT Study Group



Total length of observation, years	25 700
Total length of observation, ED	44 800
Baseline FVIII:C, IU/dL	11.5 (6.0-20.0)
Classified into FVIII:C categories, IU/dL	
2-5	231 (20.8)
6-10	283 (25.4)
11-20	345 (31.0)
21-30	180 (16.2)
31-40	73 (6.6)

Inhibitors in nonsevere hemophilia A

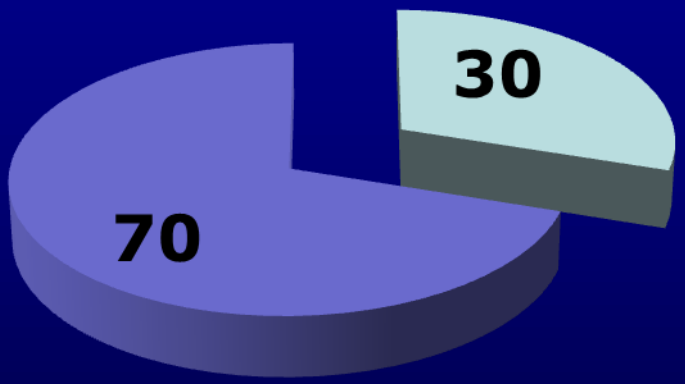


- 19 mutations associated with inh development from 214 missense mut identified
- Importance of F8 genotyping also in nonsevere patients

BLOOD, 12 SEPTEMBER 2013 • VOLUME 122, NUMBER 11

Inhibitors in hemophilia A and B

Severe Hemophilia A



Hemophilia B

Study, yr (country)	All HB patients	Severe HB
Sultan, 1992 (F)	11/565 **2%**	**4%**
Katz, 1996 (US)	29/1967 **1.5%**	28/728 **3.8%**
Miller, 2012 (US)	2/153 **1.3%**	
Castaman, 2013 (I)	8/282 **2.8%**	
Ljung, 2001 (S)		**11/48 22.9%**

Inhibitor rate: HB<HA

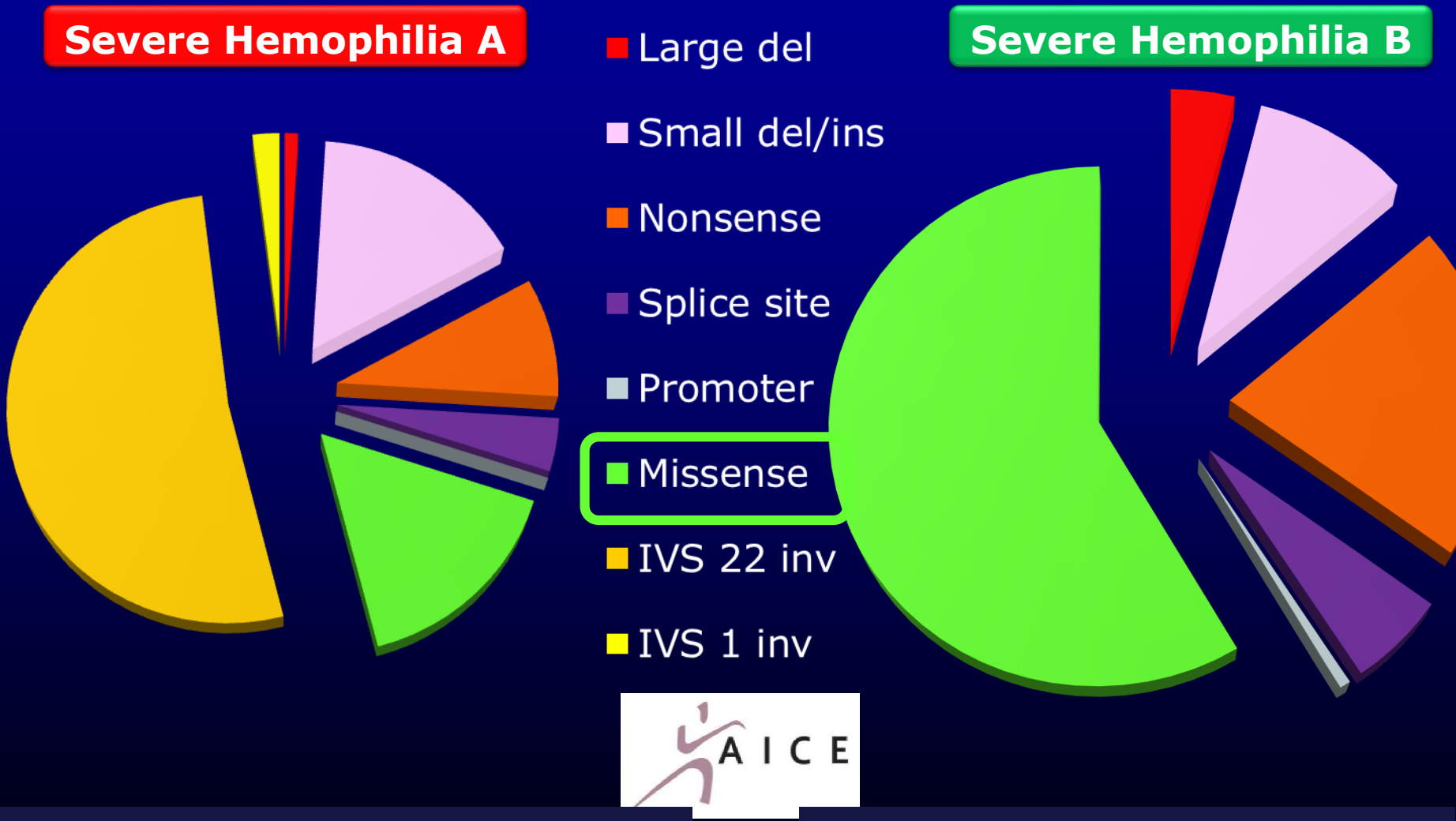
Untested hypothesis

- Lower proportion of severe patients in HB (30-40%) vs. HA (~60%)[1]
- More CRM+ patients (detectable FIX polypeptides inducing tolerance)[2,3]
- FIX less immunogenic than FVIII (acquired deficiency much less common): conservation of amino-acid sequence among vit. K-dependent proteins[4,5]
- Different genetic background[6]

1. High, Adv Exp Med Biol 1995; 2. Ljung et al, Br J Haematol 2001; 3. Lollar, J Thromb Haemost 2005;

4. Warrier, Textbook of Haemophilia, 2005; 5. Camassi et al, Haemophilia 2007; 6. F9 mut database, 2004

Type of causative gene defect in hemophilia A and B



Margaglione et al, Haematologica 2007

Tagariello et al, Haematologica 2005

Type of causative gene defect in hemophilia B and inhibitors

Tagariello et al,
Haematologica 2005

Ljung et al,
Br J Haematol 2001

Table 4. Characteristics of patients with an inhibitor.

Patient	*Mutation*	*Type of treatment*	*Exposure days*	*Peak titer (BU)*
CF 26	Trp 194 Stop	pd/od	15	25
FI 1071	Trp 194 Stop	r/od	45	36
VI 880	Arg 248 Stop	pd/od	30	2.6
VR 1357*	Arg 248 Stop	r/od	9	0.8
NA 4	Arg 252 Stop	pd/od	14	117
BA 1111°	Del. from ex A to H	pd/od	nk	25
FI 718°	Complete gene del.	pd/od	48	4.8
TO 1386	Complete gene del.	r/od	31	6.4

Table IV. Mutations in patients who developed inhibitors ($n = 11$).

Mö-ID	Mutation	Protein change
2	del total	–
68	del total	–
82	del exons A–D, F–H	–
44	20398 del 1 bp (fs)	fs Thr 140, stop 156
44	20398 del 1 bp (fs)	fs Thr 140, stop 156
1	30950 del 8 bp (fs)	fs Asp 276, stop 288
4	C 6460 T	Arg 29, stop
69	C 6460 T	Arg 29 - stop
5	G 20561 A	Trp 194, stop
3	C 30863 T	Arg 248, stop

Higher rate of large deletions in Sweden (8%)

Lower inhibitor rate, but…

- With the exception of F9 mutations, few data on other host- and treatment-related risk factors
- Safety and efficacy of by-passing agents less studied and characterized than for patients with FVIII inhibitors
- **Additional morbidity issues of allergic phenotype**

Inhibitors in hemophilia B

- Allergic up to frank, even life-threatening anaphylactic reactions, almost exclusively prior to or concomitant with inhibitor development
- In approximately 60% of inhibitor patients
  - 10/16 (63%) in the NAITR
  - 59/94 (60%) in the Int'l Reg.
- Etiology remains unclear: the unpredictable and often serious reactions thwarted any attempts at immunologic studies

Warrier et al, 1997; Thorland et al, 1999; Warrier et al, 2005; DiMichele, 2007

Allergic/anaphylactic inhibitor co-manifestations

Unproven, poorly studied hypothesis

- **Extravascular distribution** of the small FIX molecule, mast-cell activation and IgE-mediated hypersensitivity response[1]
- Complement activation by transient IgG1 antibody formation (polyclonal, predominant IgG4)[2]
- Excessive immune complex formation resulting from the infused high FIX concentrations (double than for FVIII to allow for FIX increased volume of distribution)[3]

…again a role for the genetic background…?

1.Ketterling et al, Am J Hum Gen 1994; 2. Sawamoto et al, Thromb Res 1996; 3. Warrier, Textbook of Hemophilia 2005

Allergic phenotype and F9 genotype

- Higher risk in patients with large deletions and major F9 rearrangements[1-2]
- F9 deletions are often extremely large (up to a Mb).
- Co-deletion of immune response modifier genes could trigger these phenomena?[3]

1.Thorland et al, Haemophilia 1999; 2. DiMichele et al Thromb Haemost 2002; 3. Ketterling et al, Am J Hum Gen 1994.

Addressing the risk of allergic reactions in hemophilia B

- Routine **early molecular diagnosis** to identify genetically 'higher-risk' patients
- Prolonged period (at least 10-20 ED) of **hospital-based FIX administrations** prior to transition to home treatment…

Di Michele, Br J Haematol 2007
AICE recommendations, 2013

- …preferably with access to pediatric resuscitation facilities
- Inhibitor screen at every third ED. ***Any reaction should prompt inhibitor testing before FIX re-exposure***, as even low-titer inhibitor may cause anaphylaxis

Collins et al UKHCDO guidelines, Br J Haematol 2013

So much lower ?

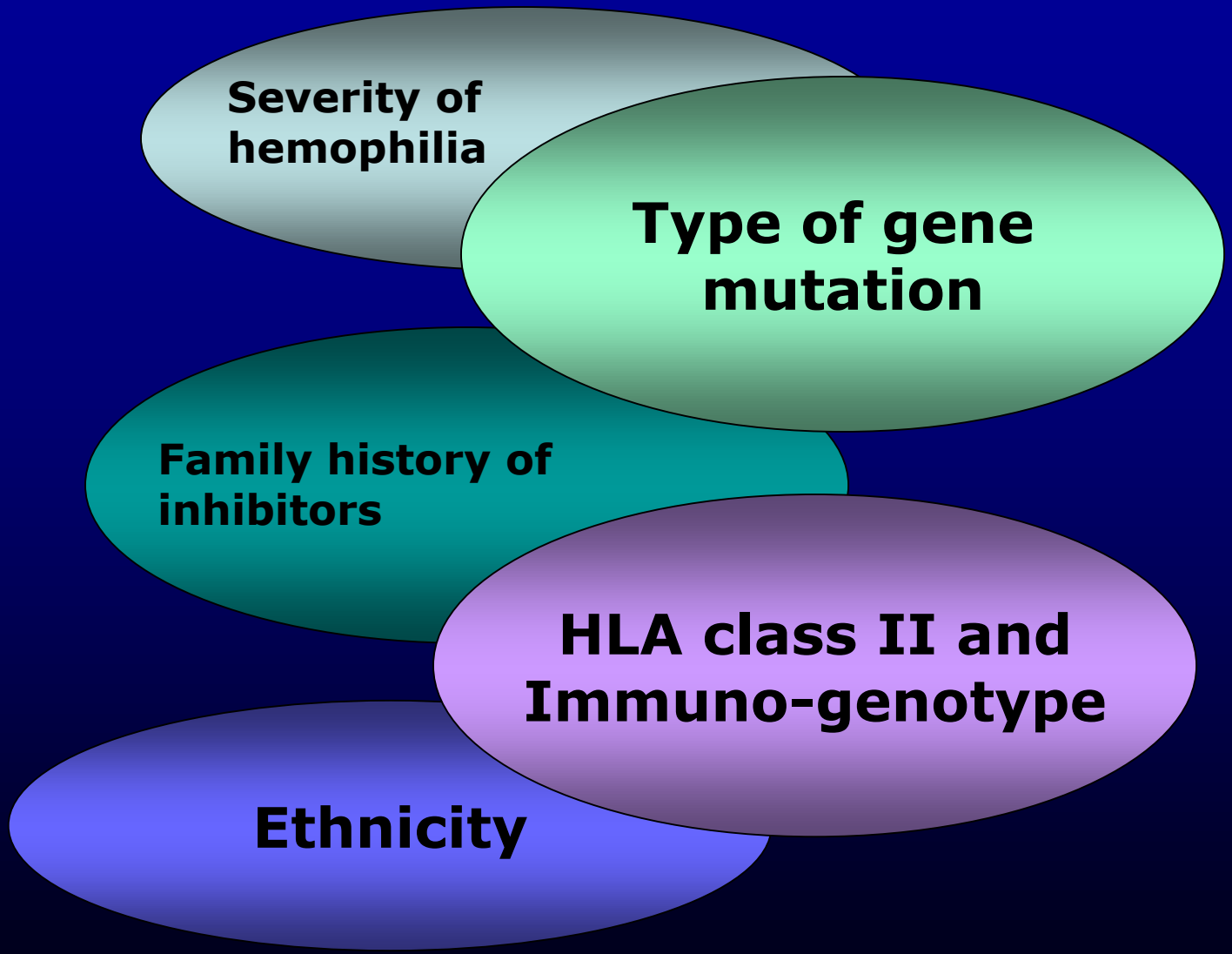
Inhibitor incidence in previously untreated patients with severe haemophilia B: a systematic literature review

Thrombosis and Haemostasis 116.1/2016

Massimo Franchini[1]; Cristina Santoro[2]; Antonio Coppola[3]

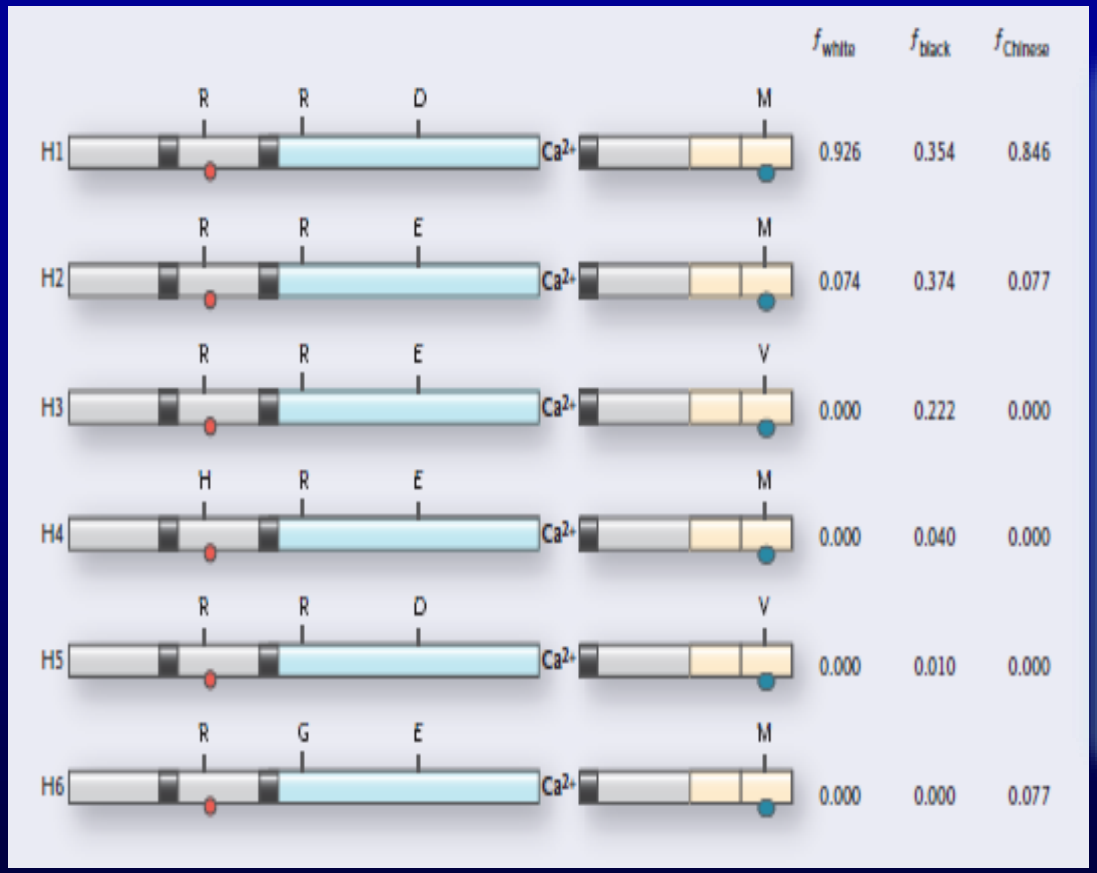
First author, year [Ref]	Study design	SHB PUPs, n	FIX product	Inhibitors n (%)				
				Total, n (%)	HR[1], n (% inh)	LR[1], n (% inh)	Allergy/anaphylaxis, n (% inh)	High-risk F9 mutations[2], n (% inh)
Shapiro, 1996 [11]	Prospective	11	pdFIX (Mononine)	1/11 (9)	1/1 (100)	0/1	1/1 (100)	1/1 (100)
Parquet, 1999 [12]	Retrospective/ prospective	15	pdFIX (LFB)	1/15 (7)	1/1 (100)	0/1	0/1	1/1 (100)
Knobe, 2002 [13]	Prospective	16	Intermediate/high-purity pdFIX	6/16 (37)	2/6 (25)	4/6 (75)	NR	4/6 (75)
Shapiro, 2005 [14]	Prospective	40	rFIX (BeneFIX)	2/40 (5)	2/2 (100)	0/2	2/2 (100)	1/2 (50)
Kreuz, 2005 [15] GTH	Prospective	15	13 pdFIX, 2 rFIX (BeneFIX)	2/15 (13)	1/2 (50)	1/2 (50)	NR	NR
Monahan, 2010 [16]	Prospective	7[3]	rFIX (BeneFIX)	1/7 (14)	0/1	1/1	1/1 (100)[4]	1/1 (100)
Fischer, 2015 [17] EUHASS	Prospective	72	30 pdFIX 42 rFIX (BeneFIX)	5/72 (7) 3 (10) pdFIX 2 (5) rFIX	NR	NR	NR	NR
Total		176	85 pdFIX, 91 rFIX	18/176 (10.2) pdFIX: 11/72 (15.3) rFIX: 5/89 (5.6)[5]	7/13 (54)	6/13 (46)	4/5 (80)	8/11 (73)

Genetic factors

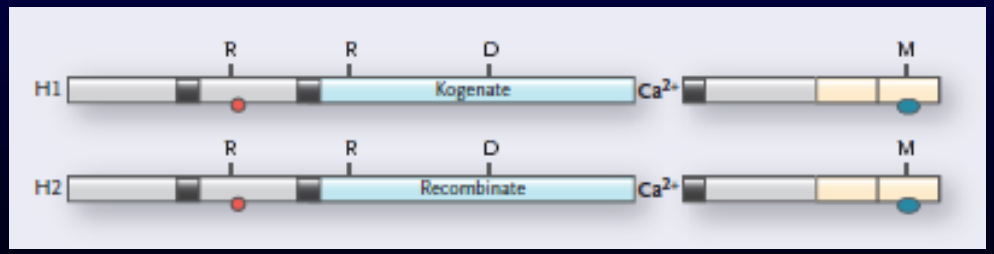


Oldenburg, 2005, mod

F8 polymorphisms



Variable	Development of Factor VIII Inhibitor		Odds Ratio (95% CI)
	Yes	No	
	no. of patients		
Factor VIII haplotype			
H4	2	0	Undefined*
H3	7	10	2.5 (0.6–10.7)†
H2	8	31	0.9 (0.2–3.5)†
H1	4	14	Reference group
Haplotype group			
H3+H4	9	10	3.4 (1.1–10.2)‡
H1+H2	12	45	



Viel et al, NEJM 2009

Immuno-genotype and inhibitor risk

Gene	Polimorphism(s)	Inhibitor (%)	Inhibitor risk
Interleukin 10	allele 134 bp no allele 134 bp	**72.7** **37.5**	↑ **5.4**
TNF-α	**-308 AA** -308 GA -308 GG	**72.7** 46.9 39.7	↑ **4.0**
CTLA-4	-318 CC -318 CT	57.6 **31.2**	↓ **3.3**

Astermark et al, Blood 2006; J Thromb Haemost 2007

Immuno-genotype and inhibitor risk

Interleukin 10

Haemophilia 2013 Sep;19(5):706-10.
Thromb Haemost 2012;107:30–6
Haemophilia 2011;17:641–9
Int J Immunogenet 2010;37:79–82
J Thromb Haemost 2009;7:2006–15
Blood 2006;107:3167–72

Interleukin 1

Haemophilia 2011;17:641–9

Interleukin 2

Haemophilia 2011;17:641–9

TGFβ

J Pharm Sci 2012;101:48–55

Cytotoxic T-lymphocyte Antigen 4

J Thromb Haemost 2009;7:2006–15
Haemophilia 2008;14:355–60
J Thromb Haemost 2007;5:263–5

Interleukin 12

Haemophilia 2011;17:641–9

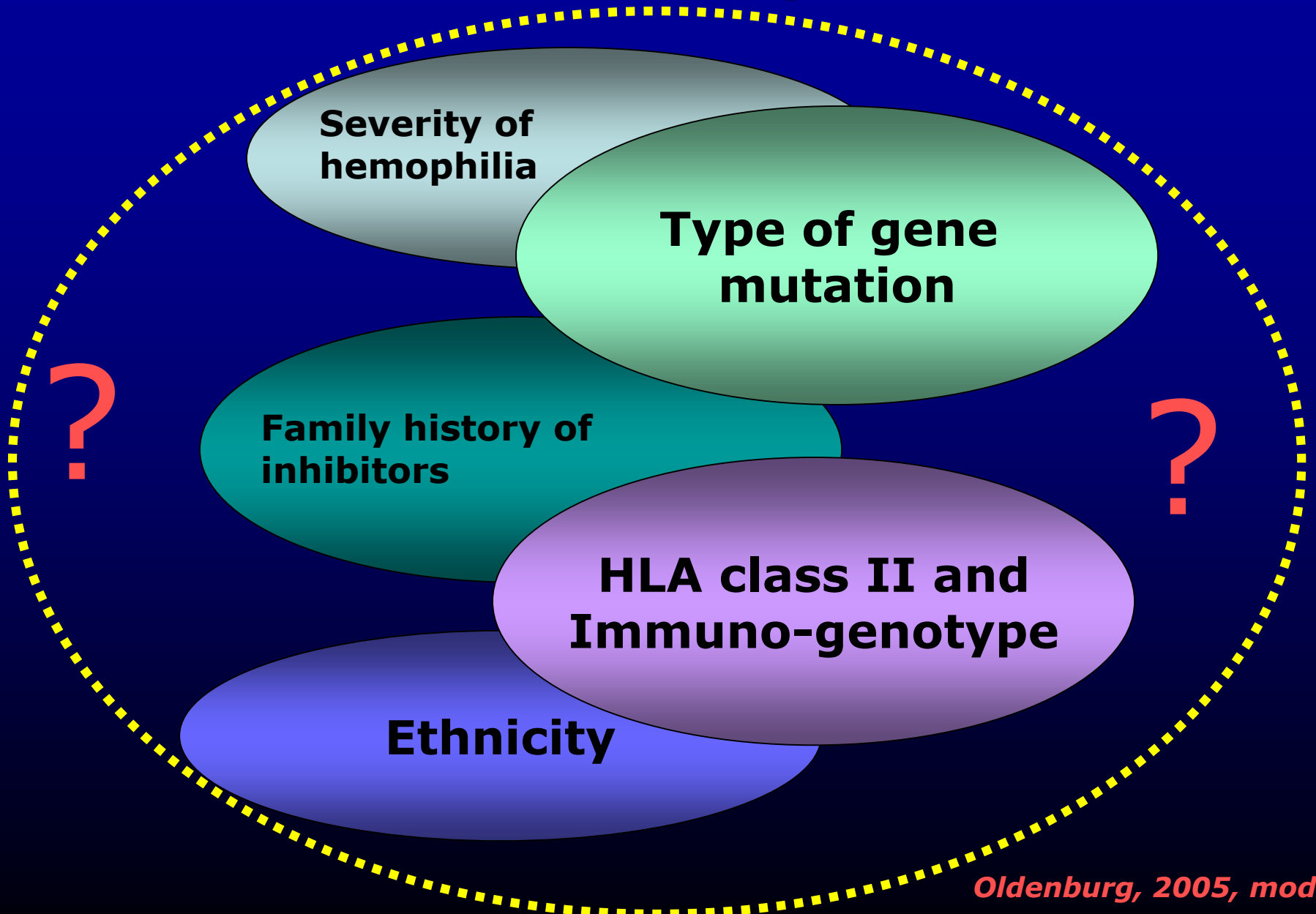
CD40/CD40L

J Pharm Sci. 2012;101:48–55

Tumor Necrosis Factor α

J Thromb Haemost 2009;7:2006–15
Blood 2006;108:3739–45

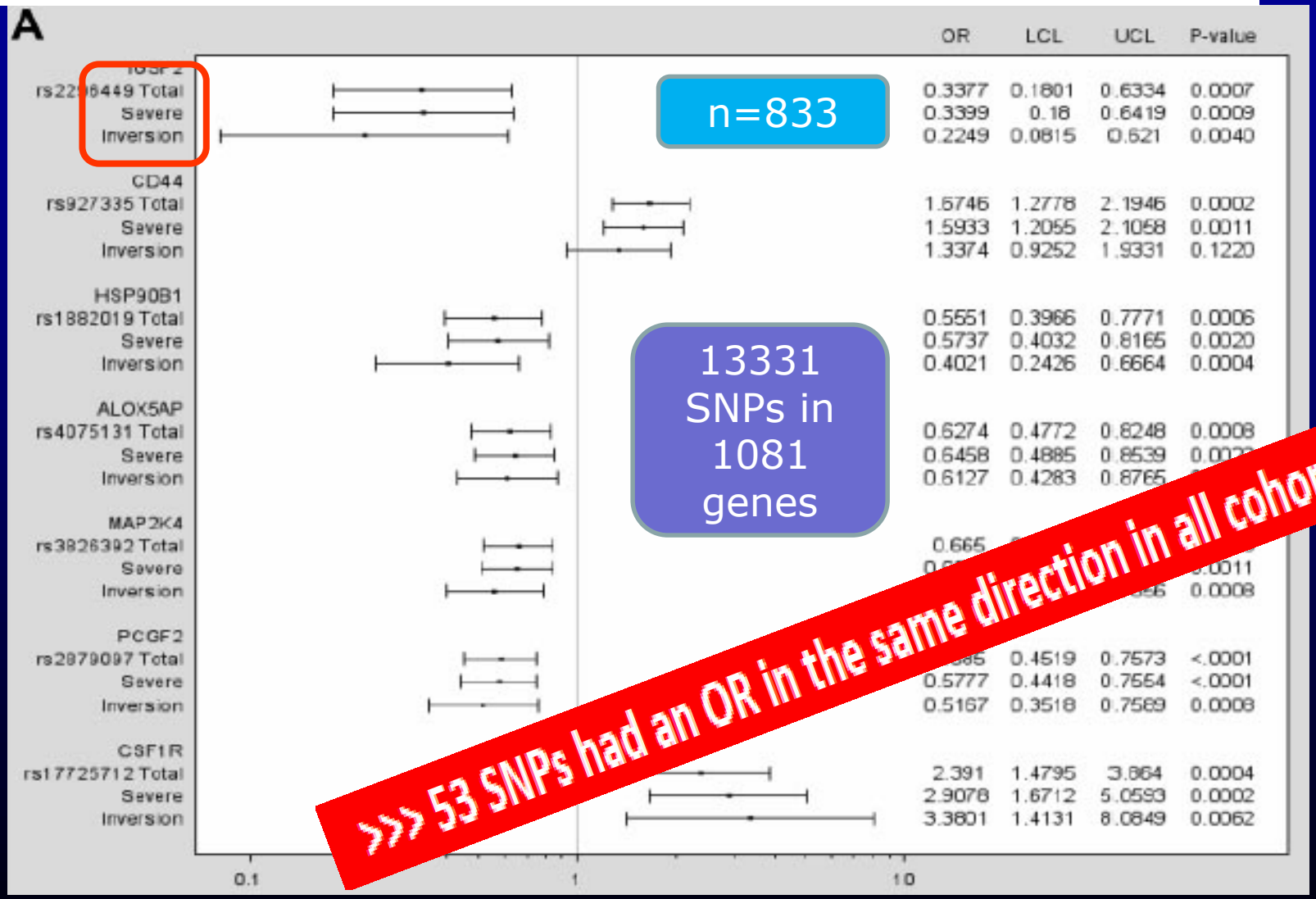
Indicators of genetic predisposition to inhibitor development



Oldenburg, 2005, mod

The polygenic nature of inhibitors in hemophilia A: results from the Hemophilia Inhibitor Genetics Study (HIGS) Combined Cohort

Jan Astermark,[1] Sharyne M. Donfield,[2] Edward D. Gomperts,[3] John Schwarz,[2] Erika D. Menius,[2] Anna Pavlova,[4] Johannes Oldenburg,[4] Bailey Kessing,[5] Donna M. DiMichele,[6] Amy D. Shapiro,[7] Cheryl A. Winkler,[5] and Erik Berntorp,[1] on behalf of the Hemophilia Inhibitor Genetics Study (HIGS) Combined Cohort



Genetic factors and inhibitor development

- Genetic contribution to inh development in hemophilia A more complex than previously thought

- Multiple immune response genes and intracellular signalling potentially of significant importance, requiring further and specific evaluation

Astermark et al, Blood 2013

Delving deeper into immunology of inhibitors

The Journal of Clinical Investigation RESEARCH ARTICLE

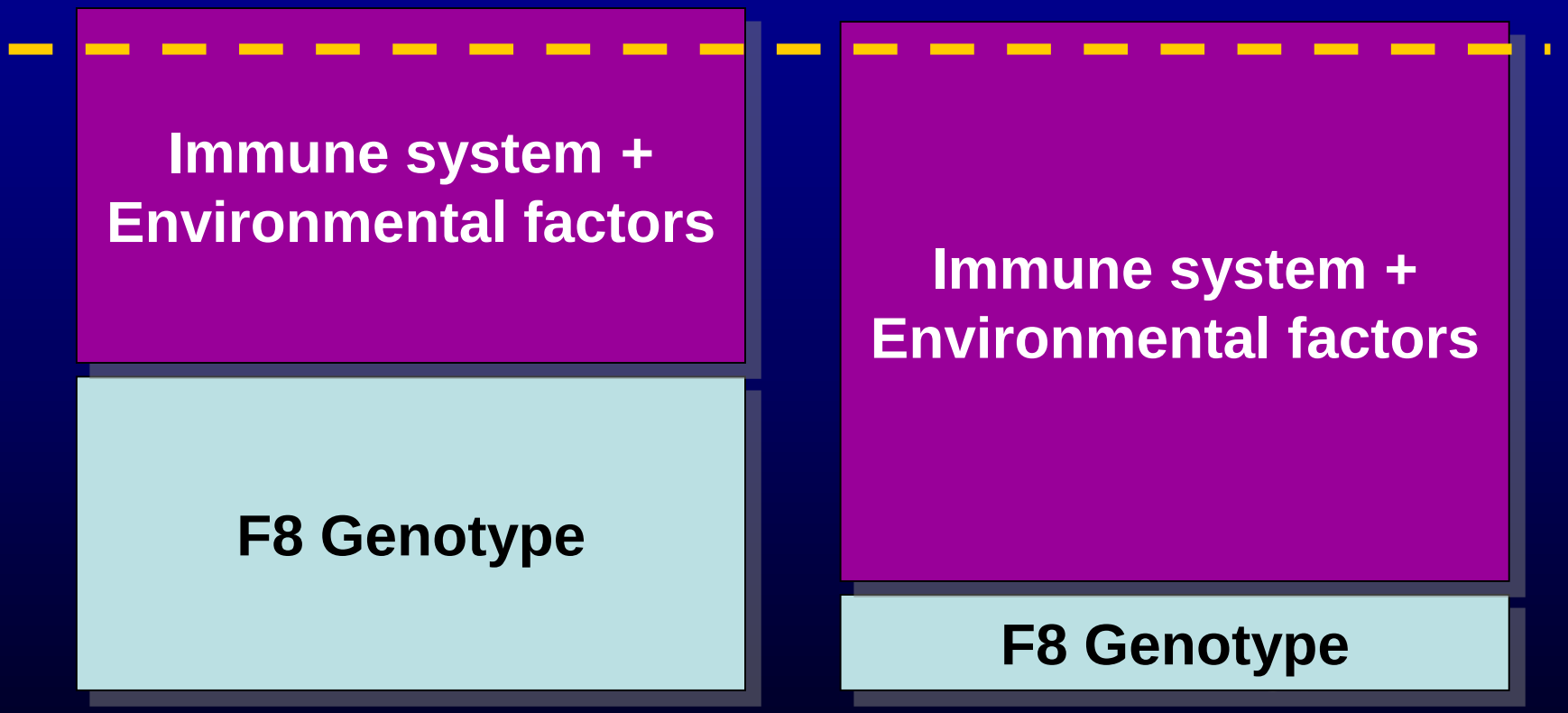
IDO1 suppresses inhibitor development in hemophilia A treated with factor VIII

2015

Davide Matino,[1] Marco Gargaro,[1] Elena Santagostino,[2] Matteo N.D. Di Minno,[3] Giancarlo Castaman,[4,5] Massimo Morfini,[5] Angiola Rocino,[6] Maria E. Mancuso,[2] Giovanni Di Minno,[3] Antonio Coppola,[3] Vincenzo N. Talesa,[1] Claudia Volpi,[1] Carmine Vacca,[1] Ciriana Orabona,[1] Rossana Iannitti,[1] Maria G. Mazzucconi,[7] Cristina Santoro,[7] Antonella Tosti,[8] Sara Chiappalupi,[1] Guglielmo Sorci,[1] Giuseppe Tagariello,[9] Donata Belvini,[9] Paolo Radossi,[9] Raffaele Landolfi,[10] Dietmar Fuchs,[11] Louis Boon,[12] Matteo Pirro,[13] Emanuela Marchesini,[13] Ursula Grohmann,[1] Paolo Puccetti,[1] Alfonso Iorio,[14] and Francesca Fallarino[1]

- Indoleamine 2,3 dioxygenase 1 (IDO1) plays a key role in the development of peripheral tolerance, by inducing T-reg lymphocites after TLR9-mediated activation on antigen presentation by dendritic cells.
- Defective IDO1 induction has been associated with inhibitor development in severe hemophiliacs with null F8 mutations (50 inhibitor positive vs. 50 inhibitor negative)
- In hemophilic mice tryptophan metabolites derived from IDO1 activity prevent inhibitor development and treatment with TLR9-agonist may suppress FVIII specific B-cells via IDO-1 induced T-regs.

Inhibitor risk



...in a pathophysiological background of increasing complexity...

The danger model: a renewed sense of self

Self-nonself model:

the immune system, functions by discriminating between self (defined early in life) and nonself (anything that comes later) tolerating self and attacking nonself.

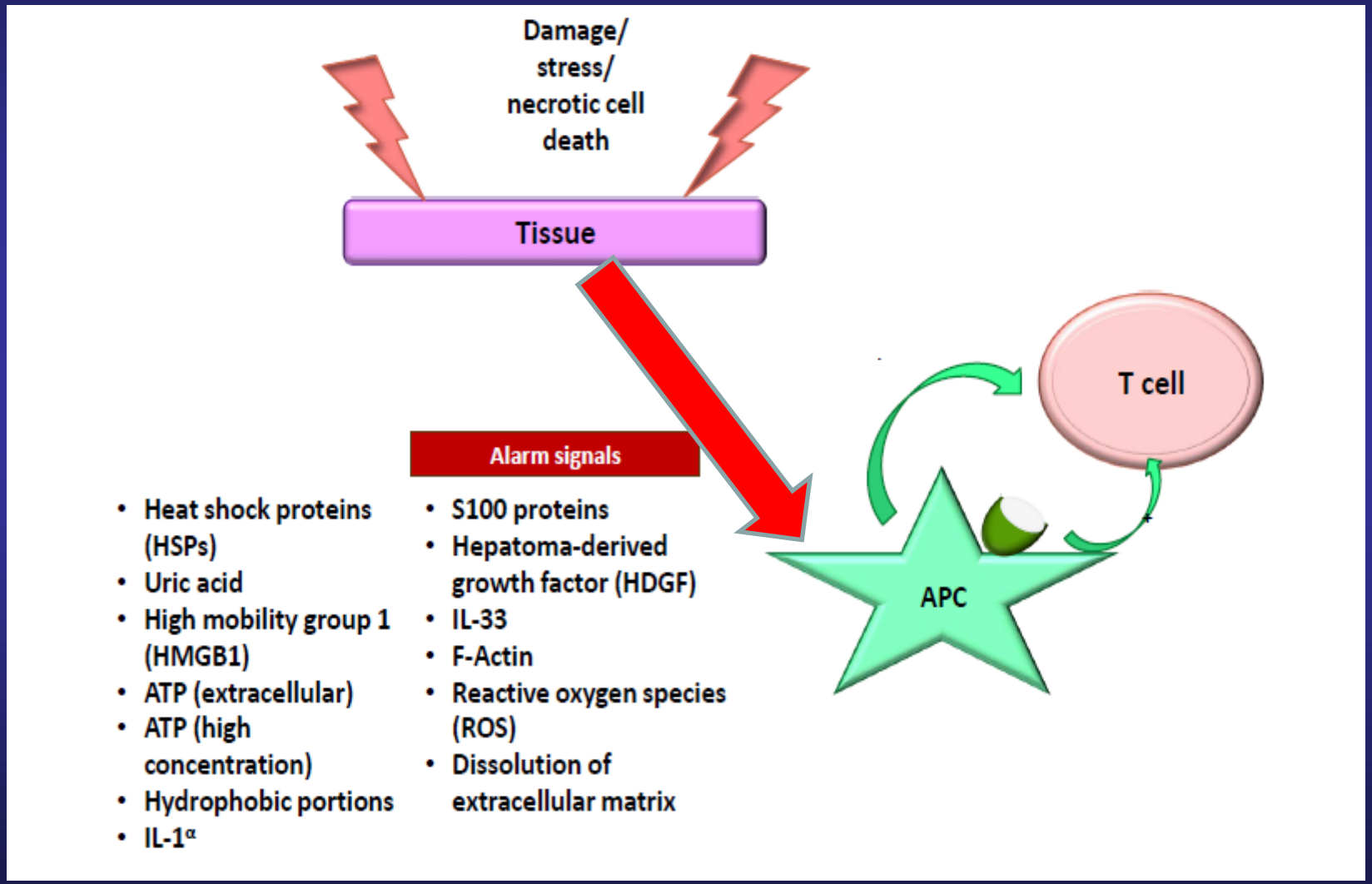
The Danger Model:

the immune system is more concerned with damage than with foreignness, and is called into action by **alarm signals from injured tissues, rather than by the recognition of nonself**.

Matzinger, Science 2002

The danger theory

danger signals from injured tissues required to elicit the immune response



Pradeau and Cooper, Front Immunol 2012

Environmental factors

- Intensity of treatment at first FVIII exposures

- Prophylaxis

- Type of FVIII product

Age and intensity at first FVIII exposure

Study (pts)	Main results
Lorenzo, BJH 2003 (62)	Cumulative incidence at 3 years from first exposure: 41% (<6 mo), 29% (6-12 mo), 12% (>12 mo). *P* = .03. Multivariate analysis: YES
Van der Bom, Th 2003 (81)	Cumulative incidence at 100 exposure days: 34% (<6 mo) 20% (6-12 mo), 13% (1-1.5 y), 0 (>1.5 y). *P* = .03
Santagostino, BJH 2005 (108)	⬆ risk (OR 2.8) for early age (≤ 11 mo vs. > 16 mo) at univariate analysis. Multivariate analysis after adjusting for genetic factors: NO
Goudemand, Blood 2006 (148)	⬇ risk (RR 0.3) for late age (≥ 12 vs. <6 mo.), in all inhibitors. Multivariate analysis: YES
Chalmers, Haemophilia 2007 (348)	Inverse relationship with inhibitor development (20-26% at ages 0-18 mo vs. 9% at ages >18 mo, P = .018), but not at different time during first year, at univariate analysis. Multivariate analysis: NO
Gouw, Blood 2007 (366) CANAL	⬆ risk (RR 2.4-2.7) of all and high inhibitors for early age (<1 mo. vs >18 mo.) at univariate analysis. Multivariate analysis: NO. Surgical procedures and peak treatment moments (≥ 5 days) at start of treatment increased inhibitor risk (RR 3.7-3.3).
Gouw, JTH 2007 (236)	Age at first exposure (< 6 mo, 6-12 mo, >12 mo) was not associated with inhibitor development. Surgical procedures and peak treatment moments at start of treatment increased inhibitor risk (RR 2.7-1.6).
Maclean, Haemophilia 2011 (78)	No association was found between inhibitor development and age at first FVIII exposure. High intensity treatment increased inhibitor risk around 2.5 folds at multivariate analysis.

Intensity of factor VIII treatment and inhibitor development in children with severe hemophilia A: the RODIN study

Samantha C. Gouw,[1] H. Marijke van den Berg,[2] Kathelijn Fischer,[2,3] Günter Auerswald,[4] Manuel Carcao,[5] Elizabeth Chalmers,[6] Hervé Chambost,[7] Karin Kurnik,[8] Ri Liesner,[9] Pia Petrini,[10] Helen Platokouki,[11] Carmen Altisent,[12] Johannes Oldenburg,[13] Beatrice Nolan,[14] Rosario Pérez Garrido,[15] M. Elisa Mancuso,[16] Anne Rafowicz,[17] Mike Williams,[18] Niels Clausen,[19] Rutger A. Middelburg,[20] Rolf Ljung,[21] and Johanna G. van der Bom[20,22] for the PedNet and Research of Determinants of INhibitor development (RODIN) Study Group

Condition		All inh HR' (95%CI)	HR inh HR' (95%CI)
Peak moment at 1.st exposure°	5-10 d	2.0 (1.3-3.0)	2.4 (1.4-4.1)
°vs none	≥ 10 d	1.7 (1.0-2.9)	2.7 (1.5-4.9)
Peak tratement moment at subsequent exposures	≥ 3 d	1.5 (1.0-2.2)	1.6 (1.0-2.6)
Dose of FVIII product*	35-50	2.4 (1.2-5.2)	2.4 (0.95-5.8)
**vs. <35 IU/Kg*	>50	2.3 (1.0-4.8)	1.8 (0.7-4.7)

'adjusted for possible confounders

BLOOD, 16 MAY 2013 • VOLUME 121, NUMBER 20

Journal of Thrombosis and Haemostasis, 6: 2048–2054 DOI: 10.1111/j.1538-7836.2008.03187.x

IN FOCUS

Risk stratification for inhibitor development at first treatment for severe hemophilia A: a tool for clinical practice

P. C. TER AVEST,* K. FISCHER,*† M. E. MANCUSO,‡ E. SANTAGOSTINO,‡ V. J. YUSTE,§ H. M. VAN DEN BERG* and J. G. VAN DER BOM*¶ ON BEHALF OF THE CANAL STUDY GROUP

PREDICTIVE SCORE from the CANAL Cohort

- Positive family history of inhibitor = 2
- High-risk F8 gene mutation = 2
- Intensive treatment (>5 ED) at initial exposure = 3

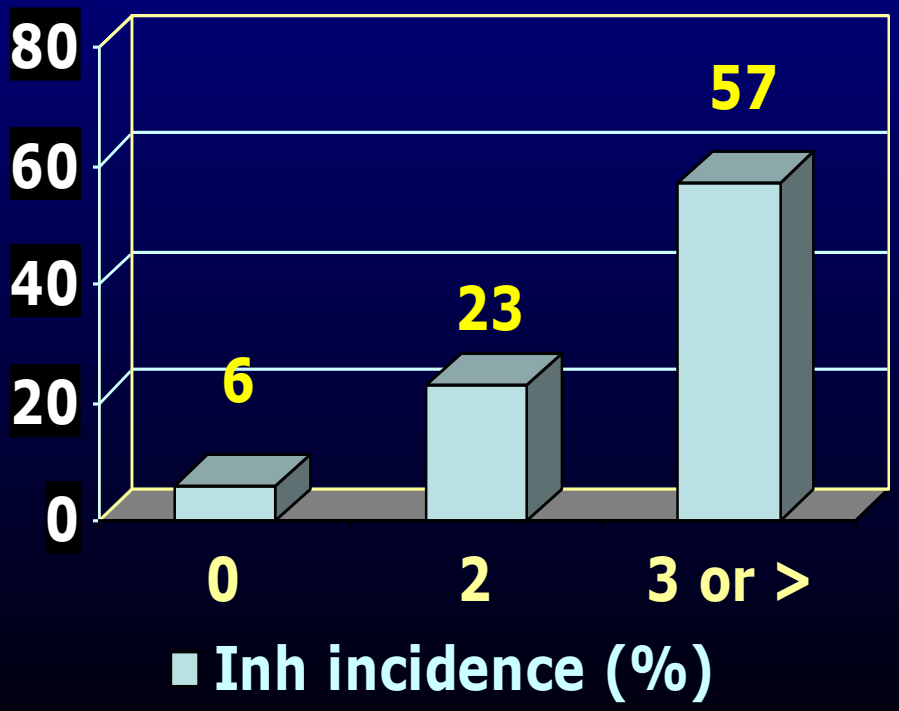


Table 4 Positive and negative predictive values and calibration of the risk score in the CANAL cohort and validation cohort

	Total number of patients	Predicted inhibitors	Observed inhibitors	Positive predictive value	Negative predictive value
Risk categories					
CANAL cohort					
Low (0 points)	95	8	6	0.06	0.68
Medium (2 points)	170	38	39	0.23	0.73
High (3 points or higher)	67	36	38	0.57	0.83
Validation cohort					
Low (0 points)	20	2	1	0.05	0.64
Medium (2 points)	28	6	8	0.29	0.75
High (3 points or higher)	16	8	8	0.50	0.81

Journal of Thrombosis and Haemostasis, **9**: 1948–1958 DOI: 10.1111/j.1538-7836.2011.04467.x

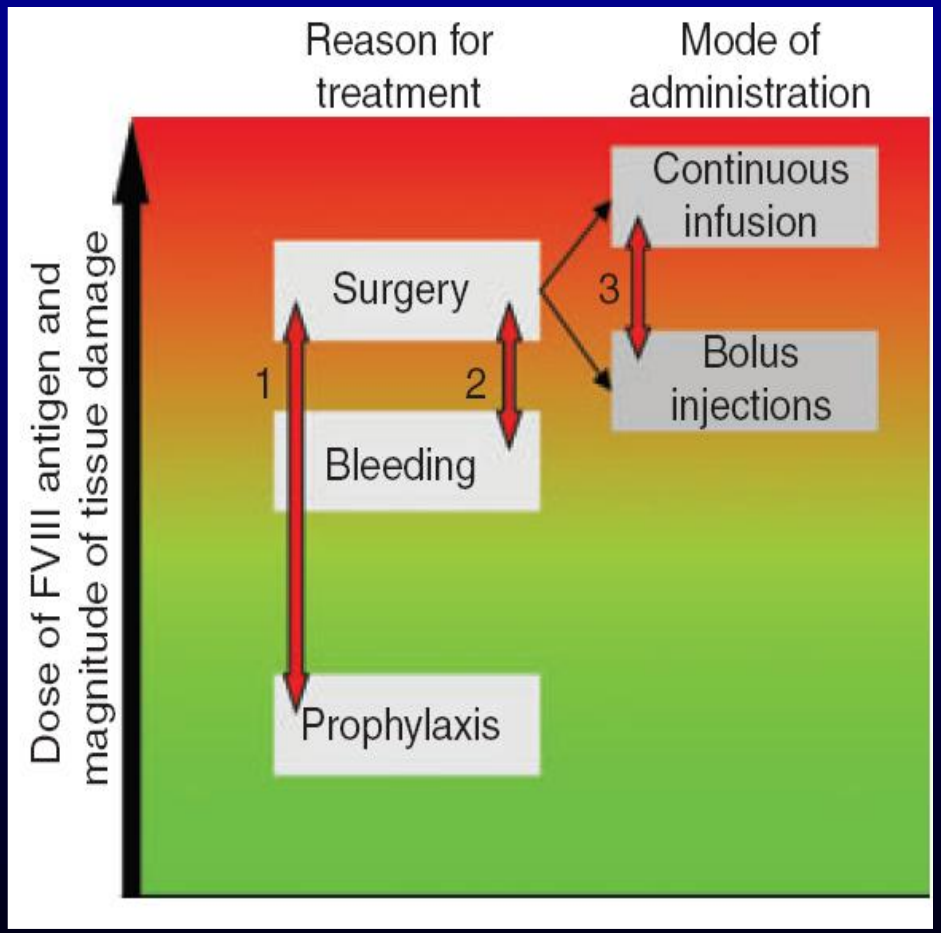
ORIGINAL ARTICLE

Surgery and inhibitor development in hemophilia A: a systematic review

C. L. ECKHARDT,*† J. G. VAN DER BOM,‡ M. VAN DER NAALD,† M. PETERS,* P. W. KAMPHUISEN† and K. FIJNVANDRAAT*

Results: **Intensive treatment increased the inhibitor risk, most pronounced with intensive treatment of ≥ 5 exposure days (EDs) compared with < 3 EDs (OR, 4.1; 95% CI, 2.6−6.5). Pooled odds ratio for inhibitor development in severe hemophilia patients that received intensive treatment for surgery at first exposure was 4.1 (95% CI, 2.0−8.4) compared with treatment for bleeding or prophylaxis.**

Conclusions: **Intensive FVIII treatment for surgery at first exposure leads to a higher inhibitor risk in hemophilia A patients compared with intensive treatment for bleeding.**



Treatment-related risk factors

Early regular Prophylaxis

?

Early intensive treatment

the Environmental Risk Factor Italian study

Table I. Characteristics of 60 cases and 48 controls with severe or moderately severe haemo

Santagostino et al, Br J Haematol, 2005

	Cases (n = 60)	Controls (n = 48)	P-value
Age at study entry, months* (range)	77 (2–151)	106 (38–200)	0·007
Italian origin (%)	57 (95)	46 (96)	NS
Plasma FVIII <1% (%)	49 (82)	39 (81)	NS
Family history of haemophilia (%)	23 (38)	15 (31)	NS
Family history of inhibitors (%)	12 (20)	1 (2)	0·001
Null mutations† (%)	43/52 (83)	27/42 (64)	0·04
Amniocentesis or villocentesis (%)	7 (12)	9 (19)	NS
Premature birth (%)	7 (12)	3 (6)	NS
Caesarean birth (%)	25 (42)	19 (40)	NS
Breast-fed (%)	47 (78)	34 (71)	NS
Duration, months* (range)	4‡ (1–24)	6 (1–36)	NS
Age at first FVIII infusion, months* (range)	11 (2 days-64)	13 (1 day-57)	NS
FVIII infusions during infections or vaccinations (%)	12‡ (20)	11§ (23)	NS
Surgery (%)	15‡ (25)	11§ (23)	NS
At first FVIII infusion (%)	7 (12)	2 (4)	NS
CNS bleeding (%)	5‡ (8)	2§ (4)	NS
Prophylaxis (%)	7‡ (12)	34§ (71)	<0·0001

multivariate analysis

te analysis of prophylaxis and other putative risk factors for inhibitor development in a subgroup of 25 cases and

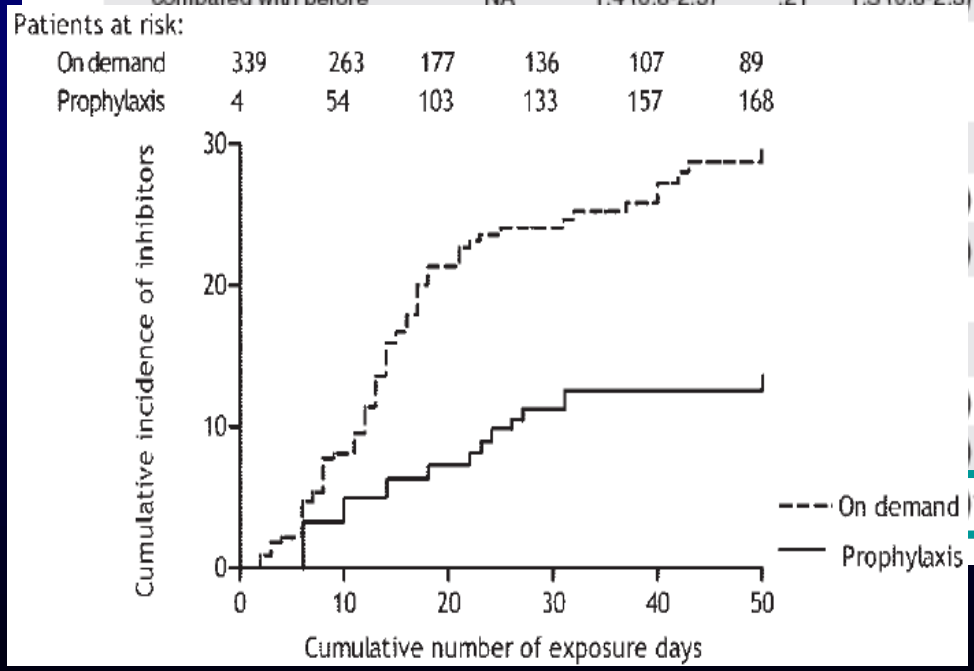
	Cases (n = 25§)	Controls (n = 48)	Crude OR (95% CI)	Adjusted OR* (95% CI)
Family history of inhibitors (%)	6 (24)	1 (2)	14·8 (1·7–131·7)	4·5 (0·3–62·8)
Null mutations (%)	15/18 (83)	27/42 (64)	2·8 (0·7–11·2)	2·5 (0·5–12·5)
Breast-fed				
No (%)	5 (20)	14 (29)	1 (ref.)	1 (ref.)
≤6 months (%)	12† (48)	20 (42)	1·7 (0·5–5·8)	2·2 (0·4–12·8)
>6 months (%)	8† (32)	14 (29)	1·6 (0·4–6·1)	4·0 (0·7–23·1)

After excluding controls who started late (>35 mo.) prophylaxis (n=16):
7/25 cases, 28% *vs* 18/32 controls, 56%
OR 0.2, 95% CI: 0.06-0.9

The CANAL cohort study

risk factor multivariate analysis

	All inhibitors					HR inhibitors				
	Crude RR (CI)	p	Adjusted RR (CI)	p		Crude RR (CI)	p	Adjusted RR (CI)	p	
During first 50 exposure days										
After peak treatment moment compared with before	NA	1.6 (1.0-2.7)	.06	1.5 (0.9-2.5)¶	.14	NA	1.7 (1.0-2.9)	.07	1.5 (0.8-2.6)¶	.18
After major peak treatment moment compared with before	NA	2.0 (1.3-3.1)	.002	1.6 (1.0-2.6)¶	.03	NA	2.3 (1.4-3.7)	.001	1.9 (1.1-3.1)¶	.02
After major surgical procedure compared with before	NA	1.4 (0.8-2.5)	.21	1.3 (0.8-2.3)¶	.32	NA	1.3 (0.7-2.5)	.43	1.2 (0.6-2.3)¶	.61
					.26	NA	1.0	.06	1.0#	.62
						NA	0.6 (0.3-1.4)		0.6 (0.3-1.4)	
						NA	1.9 (1.1-3.4)		1.3 (0.7-2.5)	
					.01	NA	1.0	< .001	1.0**	.01
						NA	1.7 (0.7-4.0)		1.5 (0.6-3.6)	
						NA	4.2 (1.9-9.3)		3.0 (1.3-6.9)	
					.02	NA	0.5 (0.2-0.9)	.03	0.5 (0.2-1.0)††	.05



Gouw et al, Blood, 2007

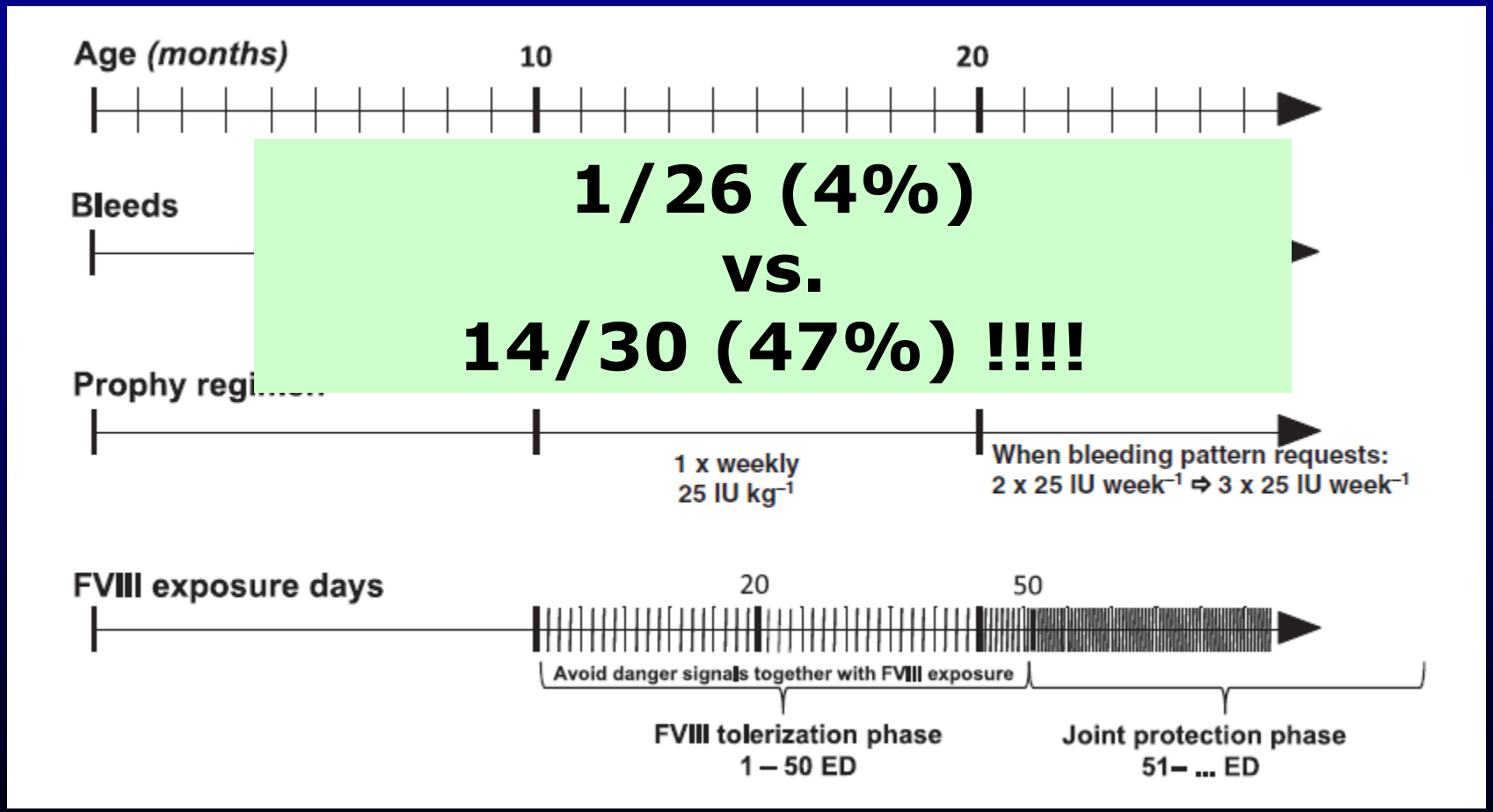
Haemophilia

Haemophilia (2010), **16**, 256–262 DOI: 10.1111/j.1365-2516.2009.02122.x

ORIGINAL ARTICLE *Clinical haemophilia*

New early prophylaxis regimen that avoids immunological danger signals can reduce FVIII inhibitor development

K. KURNIK,* C. BIDLINGMAIER,* W. ENGL,† H. CHEHADEH,† B. REIPERT† and G. AUERSWALD‡



1/26 (4%)
vs.
14/30 (47%) !!!!

The **EPIC Study**: a lesson to learn

- **Aims and Methods**

designed to test the hypothesis that inhibitor incidence in PUPs with severe or moderately severe haemophilia A could be reduced when a once-weekly FVIII prophylaxis starts with 25 IU kg^{-1} rAHF-PFM before 1 year of age and immunological danger signals are minimized

- **Results**

Eight of the 19 treated subjects (42.1%) developed confirmed inhibitors. Eleven of the 19 treated subjects were PUPs without any prior exposure to FVIII. Three of them (27.3%) developed a confirmed inhibitor together with FVIII-binding antibodies. The study was stopped because the likelihood to reach the primary objective was minimal (decision by DSMB).

- **Conclusion**

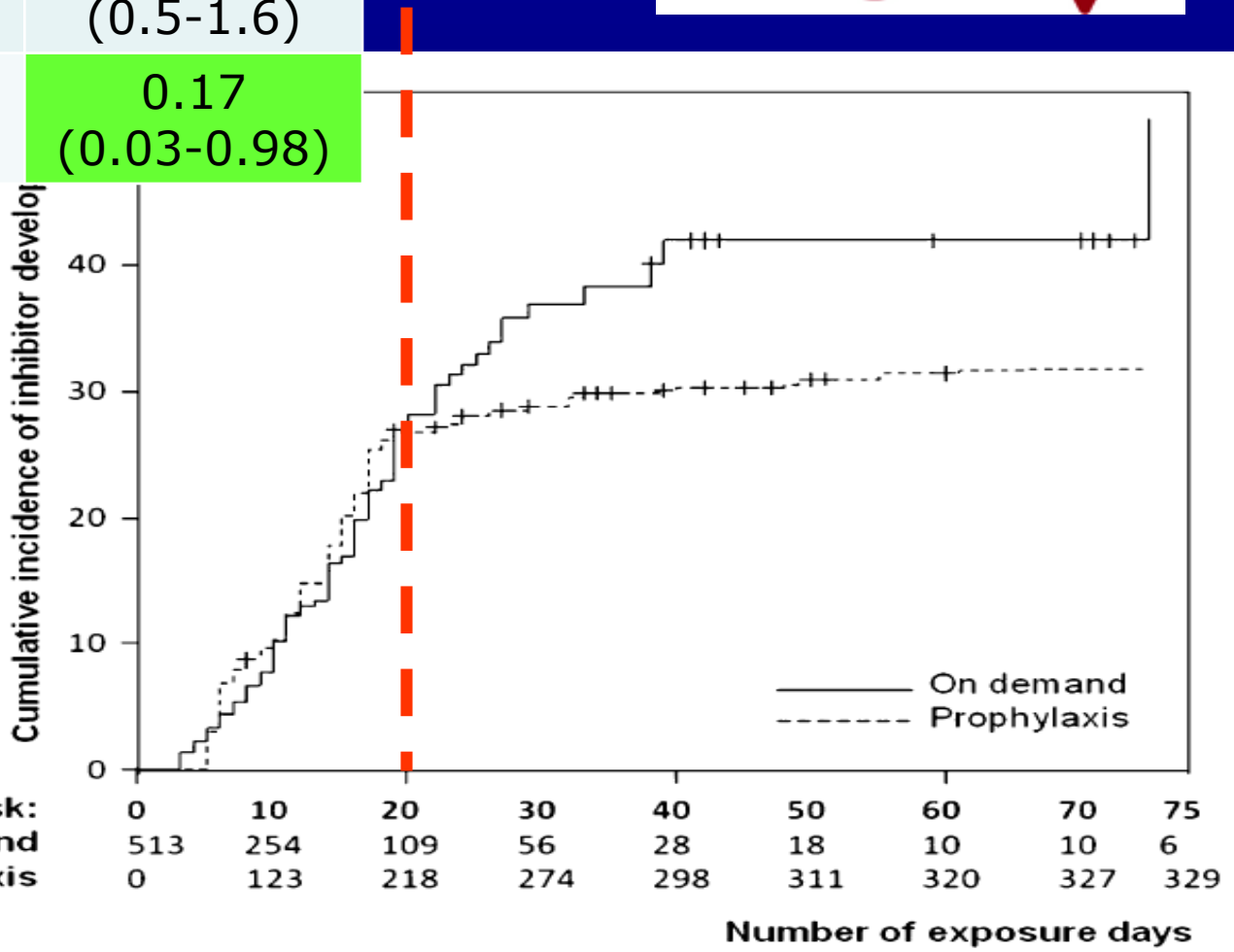
Because of early termination, the EPIC study hypothesis could not be corroborated. Nonetheless, our data analyses indicate that the current definition of an inhibitor only based on plasma inhibitor activity ≥0.6 BU mL^{-1} may not always reflect the presence of FVIII-neutralizing antibodies. The findings of this study teach us that low-level inhibitor activity results need in addition a confirmatory test and/or the assessment of the therapeutic response.

Auerswald et al, Haemophilia 2015

A protective role for prophylaxis?

aHR	All inh	HR inh
High-risk genotype	0.85 (0.5-1.4)	0.83 (0.5-1.6)
Low-risk genotype	0.61 (0.2-2.0)	0.17 (0.03-0.98)

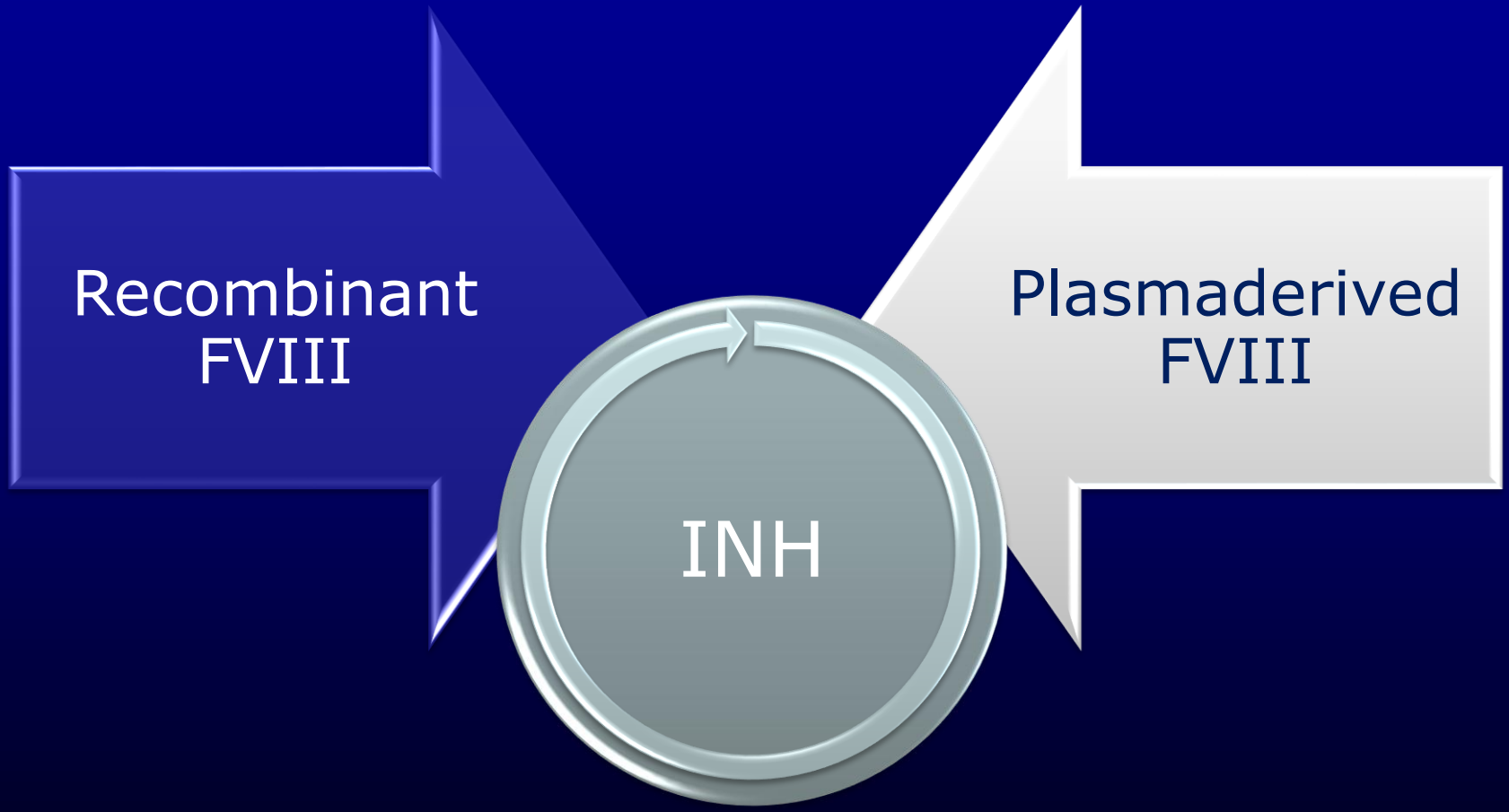
Gouw et al, Blood 2013



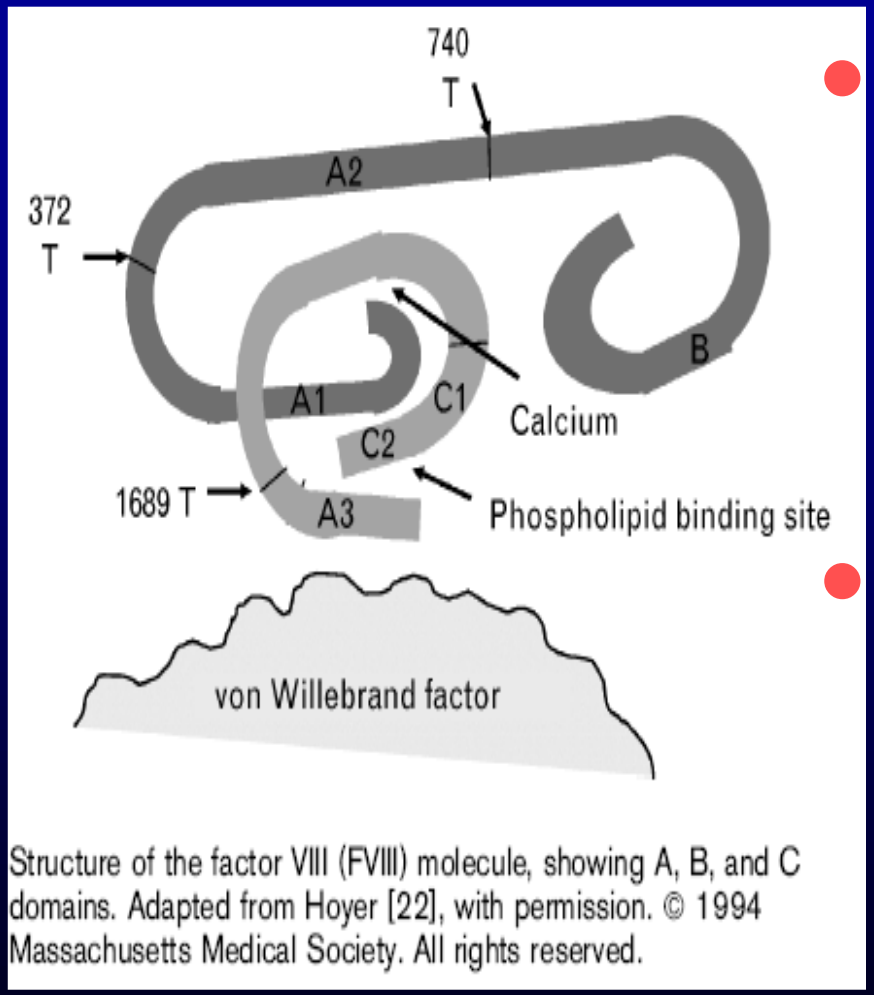
Apparently, there are 3 types of patients with severe hemophilia A: (1) patients in whom inhibitors will never develop; (2) patients in whom inhibitor development depends on the treatment regimen; and (3) patients in whom inhibitors will develop in all situations. The results of this study suggest that the potentially protective effect of prophylaxis may be more pronounced in patients with low-risk *F8* genotypes than in patients with high-risk *F8* genotypes, suggesting that the patients with high-risk *F8* genotypes are more likely to be type 3 patients and, thus, are not susceptible to the protective effect of prophylaxis.

Gouw et al, Blood 2013

The type of FVIII product



FVIII – VWF interaction



Structure of the factor VIII (FVIII) molecule, showing A, B, and C domains. Adapted from Hoyer [22], with permission. © 1994 Massachusetts Medical Society. All rights reserved.

Role of vWF

- FVIII : vWF = 1:70
- Transportation
- Protection against proteolysis

Mechanisms of protection

- Epitope masking
- Protection from FVIII endocytosis by dendritic cells

Impact of different inhibitor reactivities with commercial factor VIII concentrates on thrombin generation

G. L. SALVAGNO,* J. ASTERMARK,† M. EKMAN,† M. FRANCHINI,‡ G. C. GUIDI,* G. LIPPI,* G. POLI* and E. BERNTORP†

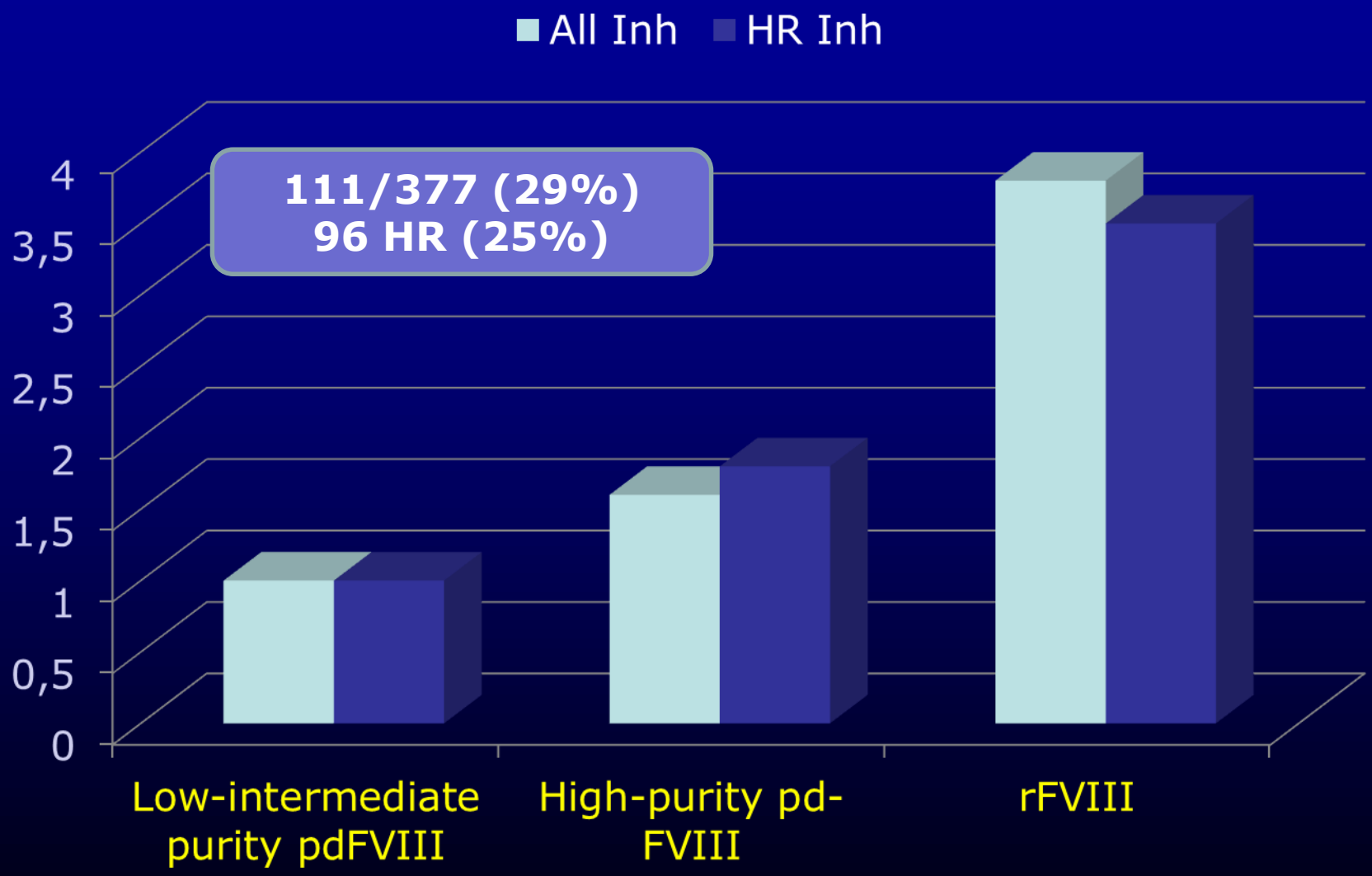
**Department of Clinical Chemistry, Verona University Hospital, Verona, Italy; †Malmö Centre for Thrombosis and Haemostasis, Malmö University Hospital, Malmö, Sweden; and ‡Haemophilia Center, City Hospital, Verona, Italy*

Summary. In order to describe the haemostatic role of a variation in inhibitor reactivity with different factor VIII (FVIII) concentrates, we have compared inhibitor titres against a panel of FVIII concentrates and correlated titre with the capacity to inhibit thrombin generation. Three plasma-derived concentrates were tested *in vitro* in mixing experiments with inhibitor plasmas from 11 patients with severe haemophilia A: Fanhdi, which contains von Willebrand factor (VWF) with a final ratio of approximately 1:1 (VWF IU per IU FVIII:C); Haemate-P with a ratio of 2.5:1 and Hemofil-M containing only trace amounts of VWF. In addition, the recombinant FVIII concentrate Kogenate Bayer containing no VWF was included. Inhibitor titres and the capacity to generate thrombin were measured. A statistically significant difference in measured titres was found with the highest titres recorded against Hemofil-M. The inhibitor titres needed to inhibit 50% maximum thrombin generation were the lowest for Kogenate Bayer and the highest and similar for Fanhdi and Haemate-P with intermediate titres needed for inhibition of Hemofil-M. In this study, the thrombin generation assay provides additional indications for the role of VWF in the treatment of patients with inhibitors. The VWF-containing concentrates Fanhdi and Haemate-P, added to FVIII-deficient plasma with the presence of inhibitor, generate more thrombin than do the purified concentrates Hemofil-M and Kogenate Bayer.

Keywords: factor VIII, haemophilia A, inhibitor, thrombin generation

Haemophilia (2007), **13**, 51–56

Product purity and inh development



Mancuso et al, JTH 2012

Comparative studies

Study (pts)	Main results
Mauser Bunschoten Haemophilia 2001 (81)	No SS difference was observed in inhibitor incidence in the two groups (24% with CP/pdFVIII vs. 23% mpFVIII/rFVIII)
Kreuz, STH 2002 (72)	No SS difference was observed in the development of high-titer inhibitor (37% with pdFVIII vs. 36% with rFVIII)
GTH-PUP, Haematologica 2003 (112)	A trend to statistical difference (0.08) observed in inhibitor incidence in the two groups (21% with pdFVIII vs. 36% rFVIII)
Goudemand, Blood 2006 (148)	A more than doubled RR (2.4; 31% vs. 11%; P 0.049) of inhibitors was found in rFVIII compared with pdFVIII group. HR inh. no SS
Chalmers, Haemophilia 2007 (348)	No SS difference was observed in the development of high-titer inhibitor (10% with pdFVIII vs. 15% with rFVIII) or at MA

Limitations of studies reporting inhibitors in PUPs

NON-HOMOGENEOUS

STUDY POPULATIONS

- Severity
- Mutation type
- Ethnicity
- Pre-treatment (minimally treated)
- Regimens and modality of treatment (intensive exposure, early/late prophylaxis, on demand)

STUDY DESIGNS

- Prospective/retrospective
- Duration of follow-up and number of exposure days
- Inhbitor testing
- Frequency of testing

«comparing apples with oranges»
Scharrer & Ehrlich, Haemophilia, 2004

Gouw et al, Blood, 2007

Type of concentrate: the CANAL study. *pdFVIII vs rFVIIIa*

- **Multicentre (13 Europe, 1 Canada), retrospective cohort of 376 severe (≤2 IU/ml) PUPs, born between 1990 and 2000**
- **Data on treatment recorded up to 50 ED or inh development**
- **23 pdFVIII (135 pts, 43%) and 4 rFVIII products (181, 57%)**
- **No. inhibitors 82 (26%); high-titer 66 (21%); median 14 ED**

Table 2. Risk of inhibitor development according to type of factor VIII product

		All clinically relevant inhibitor development				High-titer inhibitor development*			
		Crude		Adjusted		Crude		Adjusted	
	NED	RR (CI)	*P*	RR (CI)	*P*	RR (CI)	*P*	RR (CI)	*P*
Recombinant	8493	1.0		1.0		1.0		1.0	
Plasma-derived	4425	0.8 (0.5-1.3)	.34	0.7 (0.4-1.1)	.14	0.9 (0.5-1.5)	.72	0.8 (0.4-1.3)	.33
Recombinant	8493	1.0		1.0		1.0		1.0	
Plasma-derived									
Low VWF content†	1272	0.3 (0.1-1.1)	.07	0.4 (0.1-1.1)	.08	0.3 (0.1-1.2)	.09	0.3 (0.1-1.3)	.11
High VWF content†	3153	1.0 (0.6-1.6)	.91	0.8 (0.5-1.4)	.45	1.1 (0.7-2.0)	.61	0.9 (0.5-1.6)	.79
Kogenate	4267	1.0		1.0		1.0		1.0	
Kogenate Bayer	378	1.1 (0.2-4.5)	.94	1.2 (0.3-5.4)	.79	1.5 (0.3-6.5)	.60	1.6 (0.3-7.3)	.55
Recombinate	1639	1.1 (0.5-2.3)	.75	1.0 (0.5-2.1)	.99	1.4 (0.6-3.1)	.39	1.2 (0.5-2.7)	.70
Refacto	2209	1.4 (0.8-2.6)	.24	1.6 (0.9-3.2)	.14	1.5 (0.7-3.0)	.30	1.4 (0.6-3.1)	.38

Adjusted for baseline factor VIII activity level, ethnicity, factor VIII gene mutation type, age at first exposure, duration between exposure days, dose of factor VIII, and regular prophylaxis.

NED indicates number of exposure days on the concerning product type; CI, 95% confidence interval; and RR, relative risk.

*High-titer inhibitor was defined as a clinically relevant inhibitor with inhibitor titers of at least 5 Bethesda units/mL at any time.

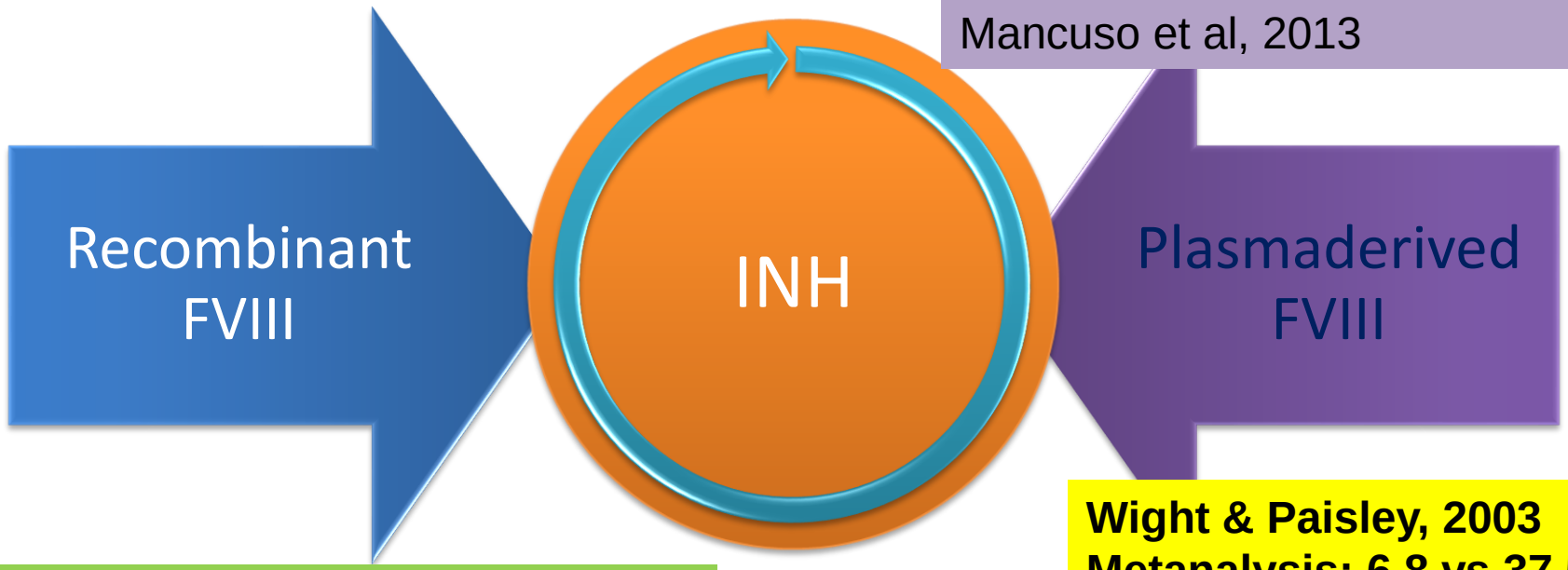
†Low VWF content was defined as less than 0.01 IU VWF antigen per IU factor VIII antigen; high VWF content was defined as more than 0.01 IU VWF antigen per IU factor VIII antigen.

The source of FVIII product

Cohort studies:
Chalmers et al, 2007
CANAL Study, 2007

In vitro findings: role of VWF (epitope masking, protection from endocytosis; lower inhibitor reactivity)

Cohort studies: GTH PUPS 2003
Goudemand et al, 2006
Mancuso et al, 2013



Franchini et al, 2012; Metanalysis, prospective studies:
ALL 21% vs 27% HR 14% vs 16%

Wight & Paisley, 2003
Metanalysis: 6.8 vs 37.5%

Iorio et al, 2010; Metanalysis:
ALL 14.3% vs 27.4%; HR 9.1% vs 23.7%

ORIGINAL ARTICLE

Rate of inhibitor development in previously untreated hemophilia A patients treated with plasma-derived or recombinant factor VIII concentrates: a systematic review

A. IORIO,* S. HALIMEH,† S. HOLZHAUER,‡ N. GOLDENBERG,§ E. MARCHESINI,* M. MARCUCCI,* G. YOUNG,¶ C. BIDLINGMAIER,‡‡ L. R. BRANDAO,§§ C. E. ETTINGSHAUSEN,¶¶ A. GRINGERI,** G. KENET,*** R. KNÖFLER,††† W. KREUZ,¶¶ K. KURNIK,‡‡ D. MANNER,†† E. SANTAGOSTINO,** P. M. MANNUCCI** and U. NOWAK-GÖTTL††

24 studi – 2094 patients

14.3% pdFVIII vs. 27.4% rFVIII (p<0.001)

HR 9.3% pdFVIII vs. 17.4% rFVIII (p=0.004)

At multivariate analysis the source of concentrate lost statistical significance

19 prospective studies

9.1% PDFVIII vs. 23.7% RFVIII (p<0.001)

HR 6.0% PDFVIII vs. 19.4% RFVIII (p=0.195)

" Conclusions: these findings underscore the need for randomized controlled trials to address whether or not the risk of inhibtor in PUPs with hemophilia A differ between rFVIII and pdFVIII."

ELSEVIER

Critical Reviews in Oncology/Hematology 81 (2012) 82–93

CRITICAL REVIEWS IN
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Cumulative inhibitor incidence in previously untreated patients with severe hemophilia A treated with plasma-derived versus recombinant factor VIII concentrates: A critical systematic review

Massimo Franchini [a,*], Annarita Tagliaferri [b], Carlo Mengoli [c], Mario Cruciani [d]

25 prospective studies (1990-2007) – 800 patients

Quality assessments: NOS and STROBE

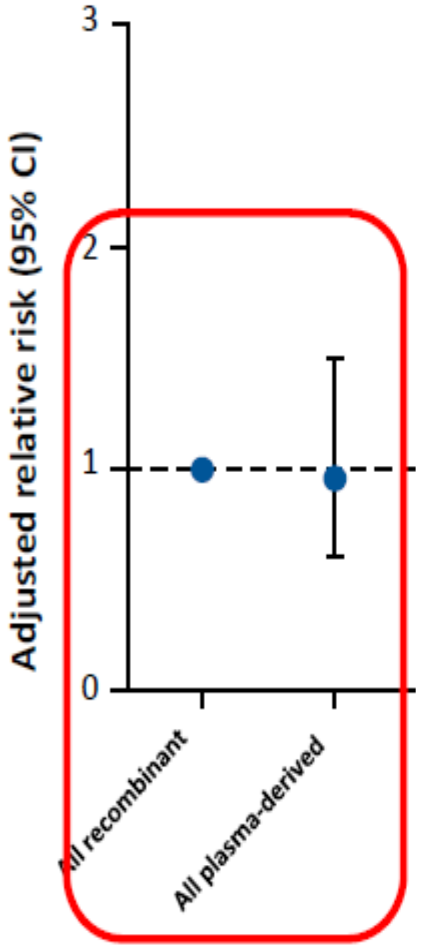
Inclusion criteria: only prospective, > 10 pts., severe hemophilia (<1%), PUPS (no MTP).

All inh: 21% PDFVIII vs. 27% RFVIII (p NS)

HR inh: 14% PDFVIII vs. 16% RFVIII (p=0.195)

"Thus, the main conclusion of this systematic review performed using selective criteria is that the type of FVIII product does not seem to influence the inhibitor rate in PUPs"

Adjusted HR
0.96 (95% CI 0.62-1.49)



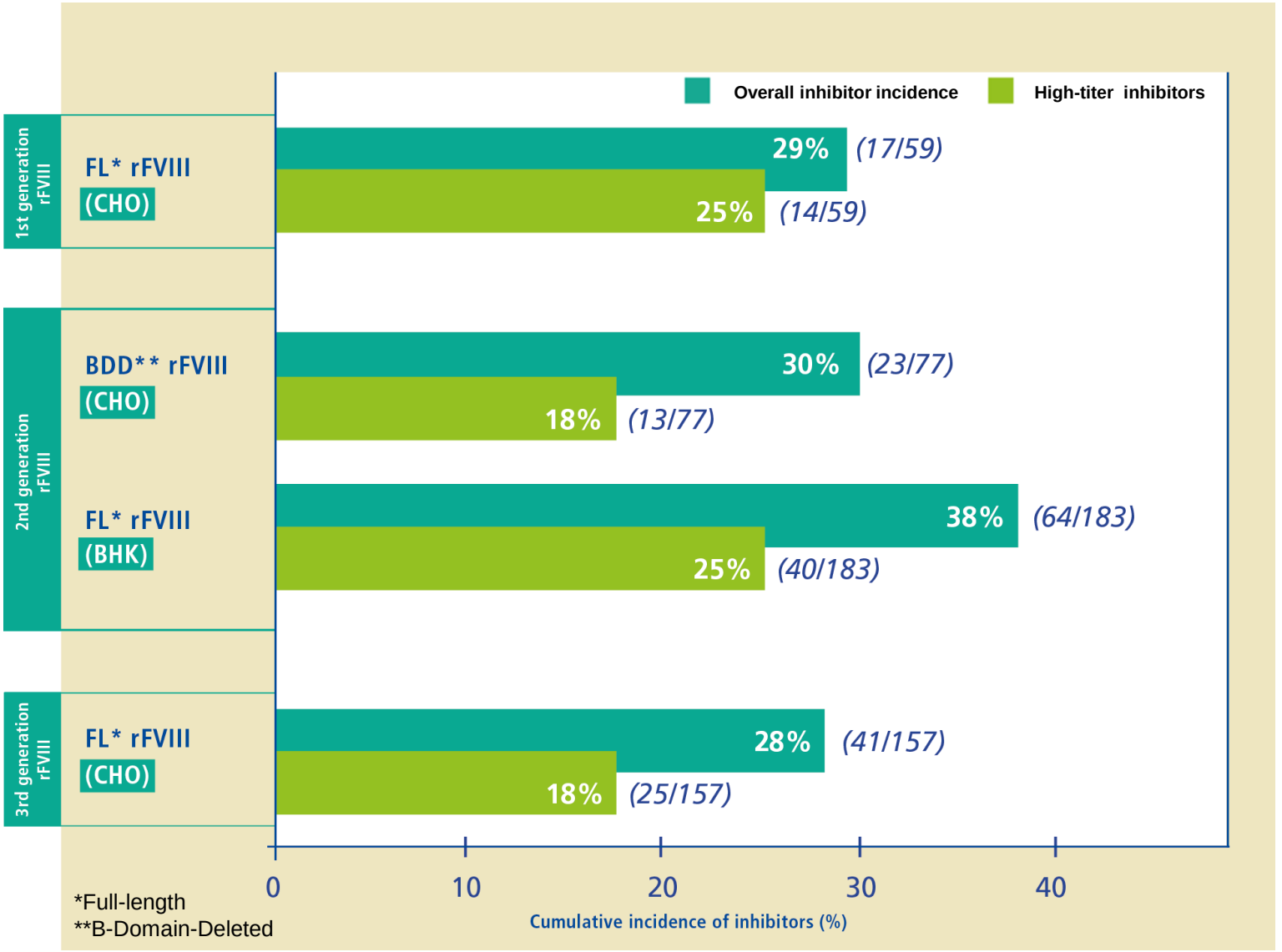
574 children with severe HA
born Jan 01 2000 – Jan 01 2010
consecutively enrolled at 29 Centres
(Europe, Israel and Canada)
followed up to 75 ED

Inhibitors	Plasmaderived n=88, n (%)	Recombinant n=486, n (%)
Clinically relevant*	29 (33.1)	145 (29.8)
High-titre	21 (25.7)	92 (18.9)

Gouw et al, NEJM 2013

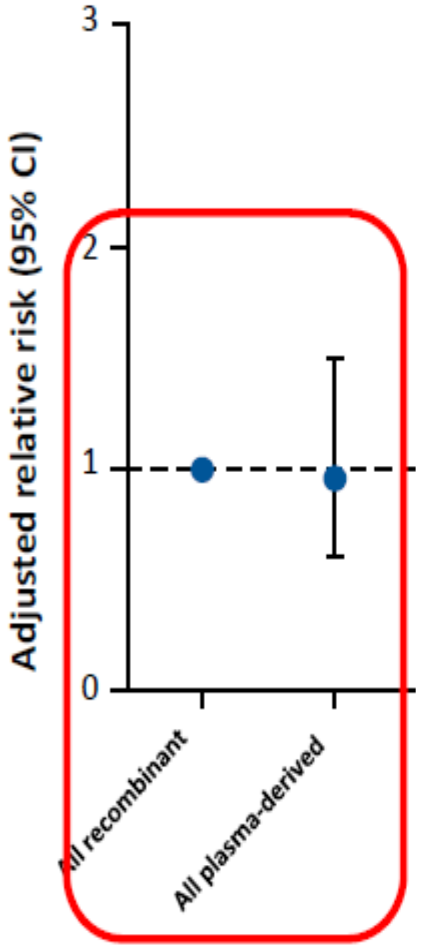
*≥2 positive titres with decreased recovery

Inhibitor incidence with different rFVIII products the RODIN Study



Gouw S et al. New Engl J Med 2013; 368 (3): 231-39

Adjusted HR
0.96 (95% CI 0.62-1.49)



Gouw et al, NEJM 2013

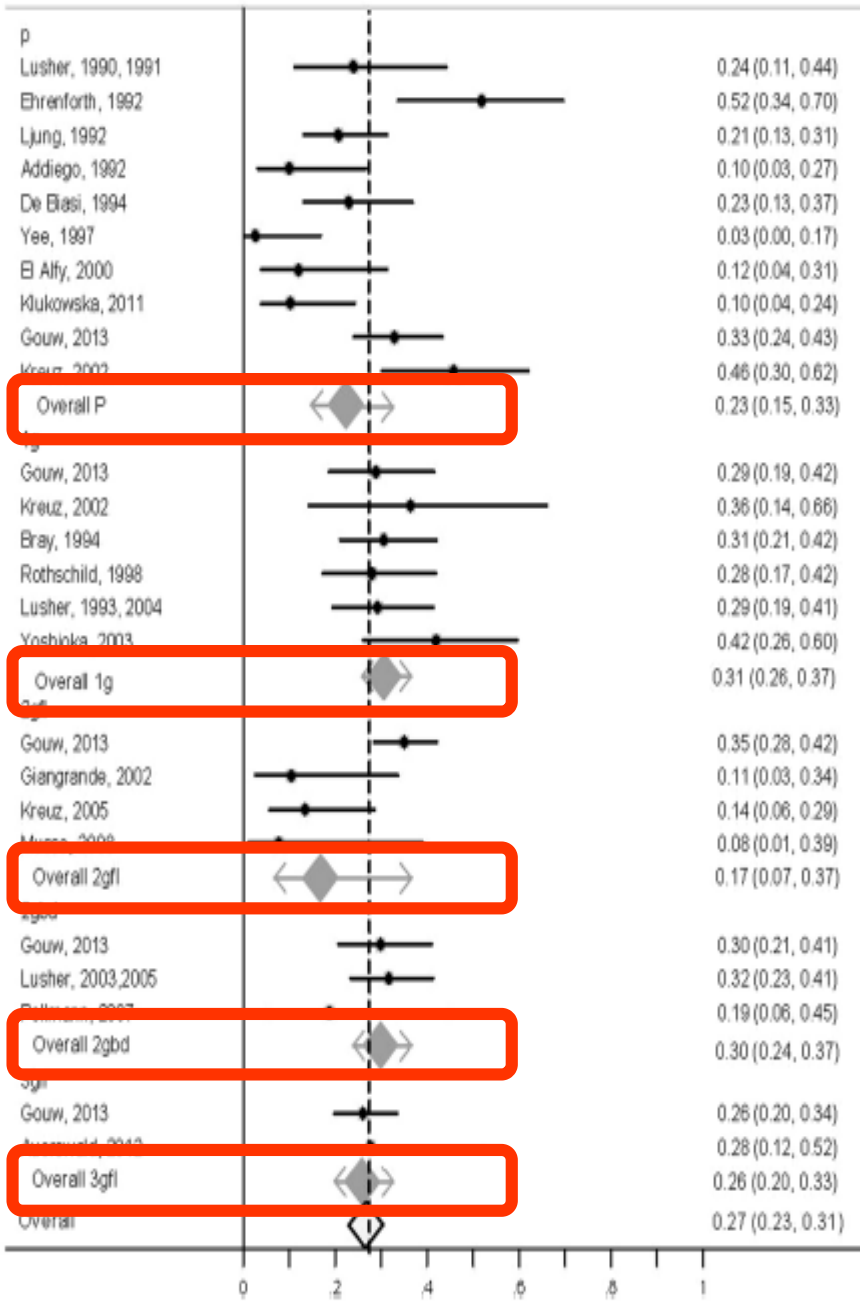
Adjusted HR
1.60 (95% CI 1.08-2.37)

Systematic Review of the Role of FVIII Concentrates in Inhibitor Development Previously Untreated Patients with Sev Hemophilia A: A 2013 Update

Massimo Franchini, MD[1] Antonio Coppola, MD[2] Angiola Rocino, MD[3] El Annarita Tagliaferri, MD[5] Ezio Zanon, MD[6] Massimo Morfini, MD[7]; Italian Hemophilia Centers (AICE) Working Group

28 prospective studies
1421 patients
only severe (FVIII <1%)
only PUPs (MTPs excluded)

	ALL INHIBITORS	HR INHIBITORS
Plasma-derived	**23%**	**16%**
Recomb-inant	**29%**	**18%**



Inhibitor development in haemophilia according to concentrate

Four-year results from the European HAemophilia Safety Surveillance (EUHASS) project

Kathelijn Fischer[1,2]; Riita Lassila[3]; Flora Peyvandi[4,5]; Gabriele Calizzani[6]; Alex Gatt[7]; Thierry Lambert[8]; Jerzy Windyga[9]; Alfonso Iorio[10,11]; EstelleGilman[12]; Michael Makris[13]; on behalf of the EUHASS participants*

- Inyernational multicentre pharmacosurveillance (68 Centres, 26 EU Countries) covering an open population
- Started Oct 1, 2008 or later, reports every 3 mo by participating centres
- Number of PUPs at risk for inhibitor development established by those reaching 50 Eds without developing an inhibitor

	Inh/total (cumul. Inc.)	Lower/ Upper CI
pd-FVIII	**11/51 (21.6%)**	11.3-35.3
r-FVIII	**97/366 (26.5%)**	22.1-31.3
Excluding RODIN patients		
pd-FVIII	**8/38 (21.1%)**	9.6-37.3
r-FVIII	**62/259 (23.9%)**	19.1-29.5

	Inhibitors (N)	Treatment years (N)	Inhibitors (N/100 years) (95 % CI)
FVIII rec	19	12959	0.15 (0.09–0.23)
FVIII pd	7*	4708	0.15 (0.06–0.31)

Thromb Haemost 2015;113(5):968-75

The source of FVIII product

Cohort studies:
Chalmers et al, 2007
CANAL Study, 2007

In vitro findings: role of VWF (epitope masking, protection from endocytosis; lower inhibitor reactivity)

RODIN Study, 2013

Cohort studies: GTH PUPS 2003
Goudemand et al, 2006
Mancuso et al, 2013

Awaiting for the randomized SIPPET Study

Recombinant FVIII

INH

Plasmaderived FVIII

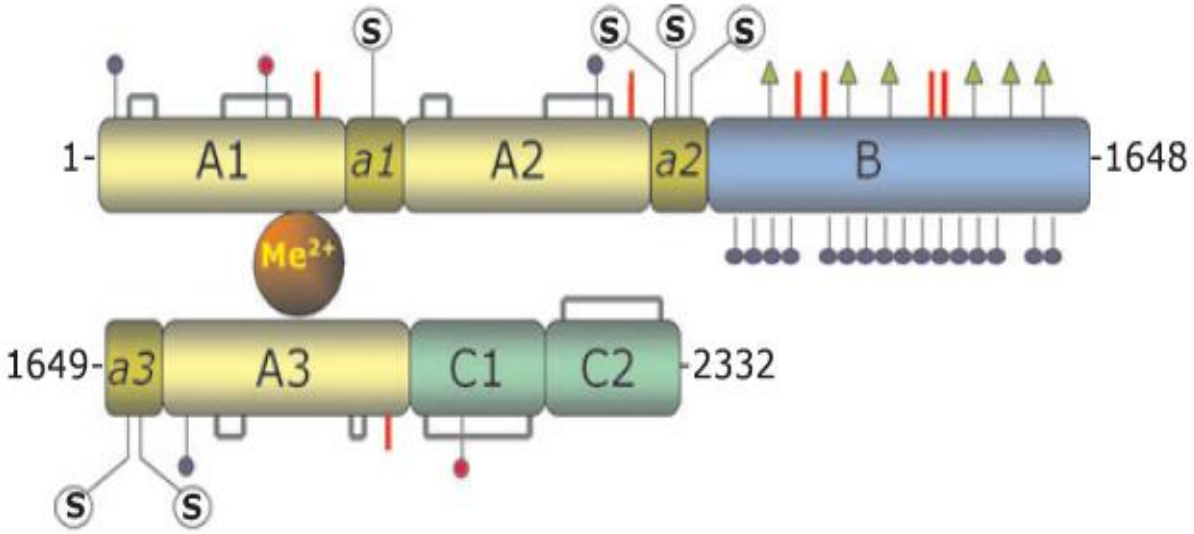
Franchini et al, 2012; Metanalysis, prospective studies:
ALL 21% vs 27% HR 14% vs 16%

Franchini et al, 2013; Metanalysis, prospective studies:
ALL 23% vs 29%; HR 16% vs 18%

Wight & Paisley, 2003
Metanalysis: 6.8 vs 37.5%

Iorio et al, 2010; Metanalysis:
ALL 14.3% vs 27.4%; HR 9.1% vs 23.7%

The unexpected results of RODIN study: differences in inhibitor risk of rFVIII concentrates ? Searching for biological plausibility…



Brand of rFVIII

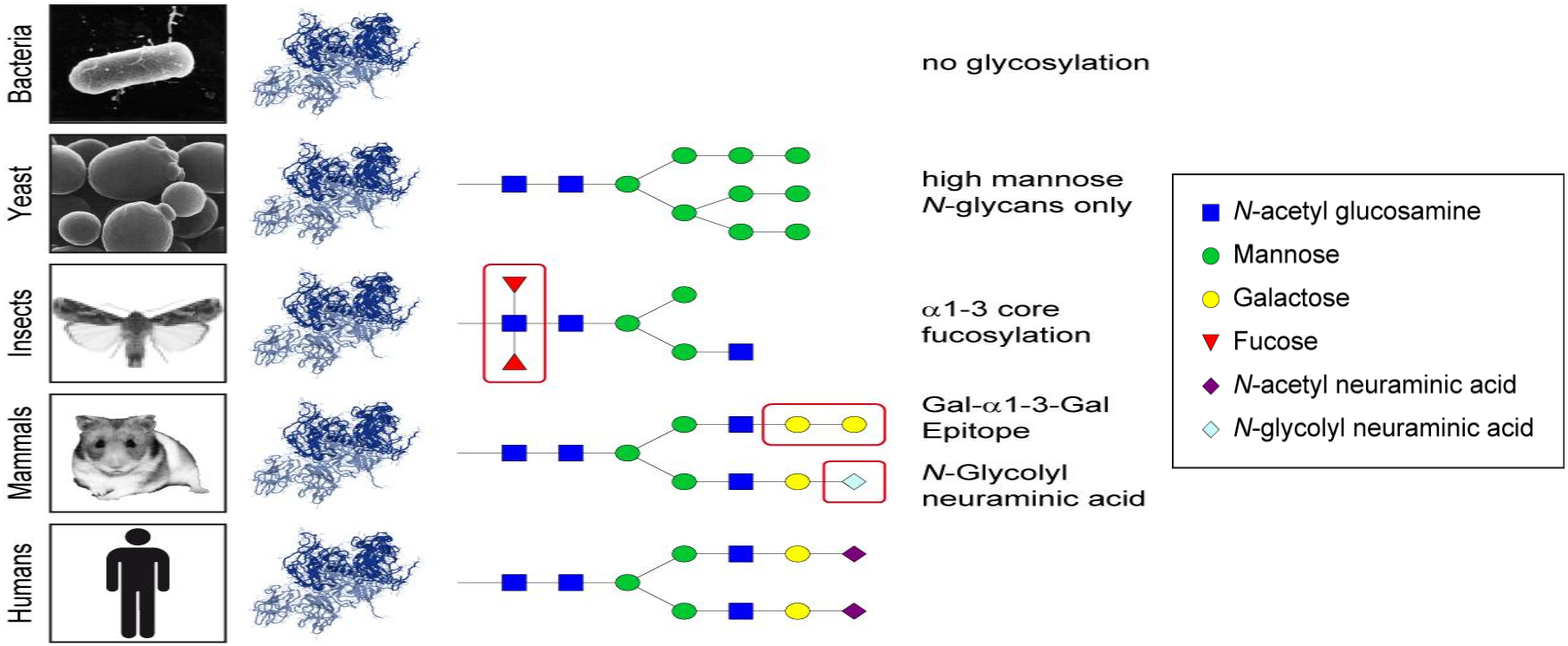
The gene

The host cell

Post-translational modifications

Gouw et al, 2013
Calvez et al, 2014
Collins et al, 2014
Fischer et al, 2015
Marcucci et al, 2015

Host cell and post-translational FVIII modifications: N-glycosilation



Product	Cell line	Galα1→3Gal	Neu5Gc
Human-cl rhFVIII	HEK 293F	Not found	Not found
Advate®	CHO DUKX-B11	Not found	Present (0.5%)[a]
Kogenate FS®	BHK 21	Present (3%)[b]	Not found

Hironaka et al, J Biol Chem 1992
Macher et al, Biochem Biophys Acta 2008
Kannicht et al, Thromb Res 2013

Host cell and post-traslational FVIII modifications: Tyr sulphation

- Sulphation at Tyr-1680 is essential for binding of FVIII to vWF: in the absence of sulphation at Tyr 1680 the affinity for vWF is reduced fivefold

Proportion of non-sulfated Y1680 in five FVIII products.

Origin	FVIII product	Lot	Proportion non-sulfated Y1680 (%)
	Plasma-derived FVIII, Octanate®	I	Below detection limit
		II	Below detection limit
HEK	Human-cl rhFVIII	I	Below detection limit
		II	Below detection limit
CHO	Advate®	I	5.3
		II	8.0
BHK	Kogenate FS®	I	1.6
		II	1.5
CHO	ReFacto®	I	5.3
		II	3.7

Kannicht et al, Thromb Res 2013

CHO Turoctocog — Below detection limit

Nielsen et al, Haemophilia 2012

Further support from the French and UK cohorts

CLINICAL TRIALS AND OBSERVATIONS

Factor VIII brand and the incidence of factor VIII inhibitors in previously untreated UK children with severe hemophilia A, 2000-2011

Peter W. Collins,[1] Benedict P. Palmer,[2] Elizabeth A. Chalmers,[3] Daniel P. Hart,[4] Ri Liesner,[5] Savita Rangarajan,[6] Katherine Talks,[7] Michael Williams,[8] and Charles R. M. Hay,[9] on behalf of the UK Haemophilia Centre Doctors' Organization

(*Blood.* 2014;124(23):3389-3397)

CLINICAL TRIALS AND OBSERVATIONS

Réseau FranceCoag

Recombinant factor VIII products and inhibitor development in previously untreated boys with severe hemophilia A

Thierry Calvez,[1,2] Hervé Chambost,[3,4] Ségolène Claeyssens-Donadel,[5] Roseline d'Oiron,[6] Véronique Goulet,[7] Benoît Guillet,[8] Virginie Héritier,[7] Vanessa Milien,[3] Chantal Rothschild,[9] Valérie Roussel-Robert,[10] Christine Vinciguerra,[11] and Jenny Goudemand,[12] for the FranceCoag Network

(*Blood.* 2014;124(23):3398-3408)

Inhibitor incidence in PUPs cohorts and rFVIII brand

	Inhibitor Development Rate (%)				
	Kogenate B./ Helixate N. (BHK)	**Advate (CHO)**	**Recombinate (CHO)**	**ReFacto (CHO)**	**ReFacto AF (CHO)**
Collins et al. *Blood*, 2014	**35.2**	24.4	36.4	23.1[+]	34.1[+]
Calvez et al. *Blood*, 2014	**49.5**	34.0	27.1	25.9	NA
Gouw et al. *NEJM*, 2013	**37.7**	28.2	29.0	30.3	NA

[+] Small number of patients studied in these groups

Adjusted inhibitor risks

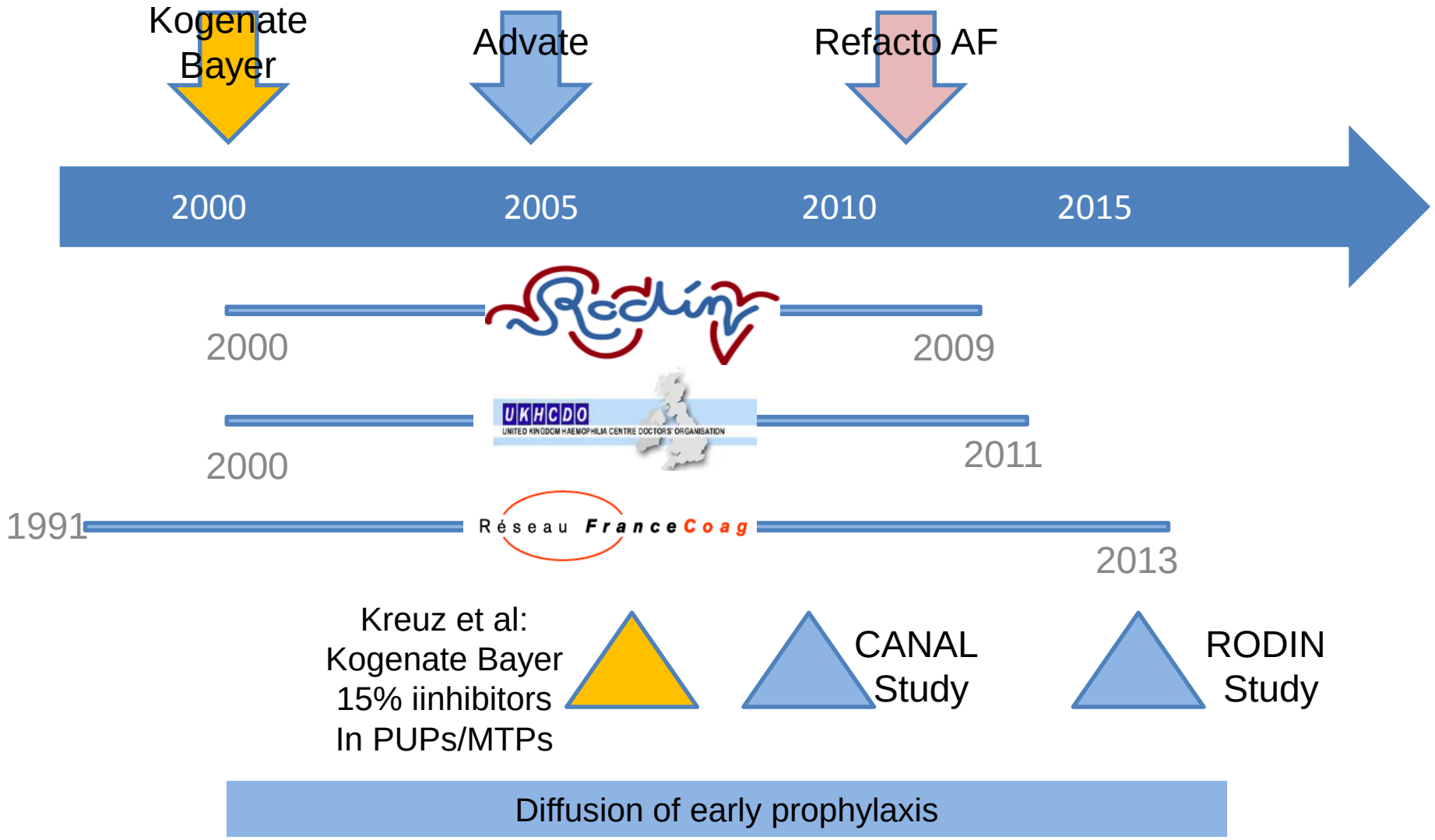
	RODIN*	FranceCoag	UKHCDO
Advate	1.00		
Kogenate Bayer/ Helixate Nexgen	**1.60 (1.08-2.37)**	1.55 (0.97-2.49)	**1.75 (1.11-2.76)**
Recombinate	0.99 (0.53-1.83)	0.97 (0.40-2.37)	1.95 (0.62-6.2)
Refacto	1.01 (0.60-1.70)	1.2 (0.47-3.08)	0.79 (0.36-1.73)
Refacto AF	NA	NA	**2.63 (1.26-5.47)**

Adjusted inhibitor risks – high titer

	RODIN*	FranceCoag*	UKHCDO
Advate	1.00		
Kogenate Bayer/ Helixate Nexgen	**1.79 (1.09-2.94)**	1.56 (0.82-2.98)	**2.14 (1.12-4.1)**
Recombinate	1.26 (0.61-2.61)	1.87 (0.59-5.89)	3.68 (0.88-15.4)
Refacto	0.97 (0.49-1.91)	1.94 (0.54-6.91)	1.52 (0.57-4.04)
Refacto AF	NA	NA	1.28 (0.33-5.00)

*Treated inhibitors: Kogenate Bayer/Helixate vs. Advate
HR 1.58 (0.94-2.64)

Three to zero… but time (and knowledge) does matter !



Incidence of inhibitors, rFVIII brands and period of study (UKHCDO cohort)

	All patients			Non-RODIN			RODIN		
	No. of inhibitors	No. of patients	%	No. of inhibitors	No. of patients	%	No. of inhibitors	No. of patients	%
2000-2004									
Advate	3	12	25	1	7	14.3	2	5	40
Kogenate Bayer/Helixate NexGen	24	65	37	20	55	36.4	4	10	40
2005-2008									
Advate	26	117	22	20	87	23	6	30	20
Kogenate Bayer/Helixate NexGen	16	31	52	11	25	44	5	6	83.3
2009-2013									
Advate	13	43	30	8	30	26.7	5	13	38.5
Kogenate Bayer/Helixate NexGen	5	32	16	4	27	14.8	1	5	20

22% of the cohort participated in RODIN study

*higher n of intensive exposure and family history of inhibitors

	UK-RODIN*	UK-NON RODIN	P
Inhibitors	33/88 (37.5%)	85/319 (26.7%)	0.05
High-titer	17/88 (19.3%)	43/319 (13.5%)	0.17
HR Kogen vs. Advate	2.90 (0.49-17.13)	2.00 (0.93-4.34)	

Collins et al, 2014

Time and centre effects in the French cohort

	Multivariate analysis		
	Adjusted HR	(95%CI)	P value
All inhibitors			*0.338**
1st period: Dec. 27, 1998 -> Mar. 22, 2005	1.00		
2nd period: Mar. 23, 2005 -> Jul. 06, 2007	0.92	(0.39-2.19)	0.851
3rd period: Jul. 11, 2007 -> Feb. 27, 2011	1.64	(0.81-3.31)	0.171
4th period: Feb. 28, 2011 -> May 19, 2014	1.69	(0.76-3.77)	0.198
High-titer inhibitors			*0.487**
1st period: Dec. 27, 1998 -> Mar. 22, 2005	1.00		
2nd period: Mar. 23, 2005 -> Jul. 06, 2007	1.58	(0.47-5.26)	0.458
3rd period: Jul. 11, 2007 -> Feb. 27, 2011	2.18	(0.76-6.21)	0.145
4th period: Feb. 28, 2011 -> May 19, 2014	2.08	(0.60-7.24)	0.250
Inhibitors subsequently treated with a bypassing agent and/or ITI			*0.348**
1st period: Dec. 27, 1998 -> Mar. 22, 2005	1.00		
2nd period: Mar. 23, 2005 -> Jul. 06, 2007	0.78	(0.29-2.05)	0.608
3rd period: Jul. 11, 2007 -> Feb. 27, 2011	1.60	(0.76-3.37)	0.219
4th period: Feb. 28, 2011 -> May 19, 2014	1.65	(0.70-3.87)	0.253

* P value for global test.

- 3 Centres providing ~31% of the cohort with higher difference in inhibitor development between Kogenate and Advate:

  58% vs. 17%;
  36% vs. 33%

  in the remaining Centres

Réseau FranceCoag

Calvez et al, 2014

EUHASS surveillance and brand of rFVIII

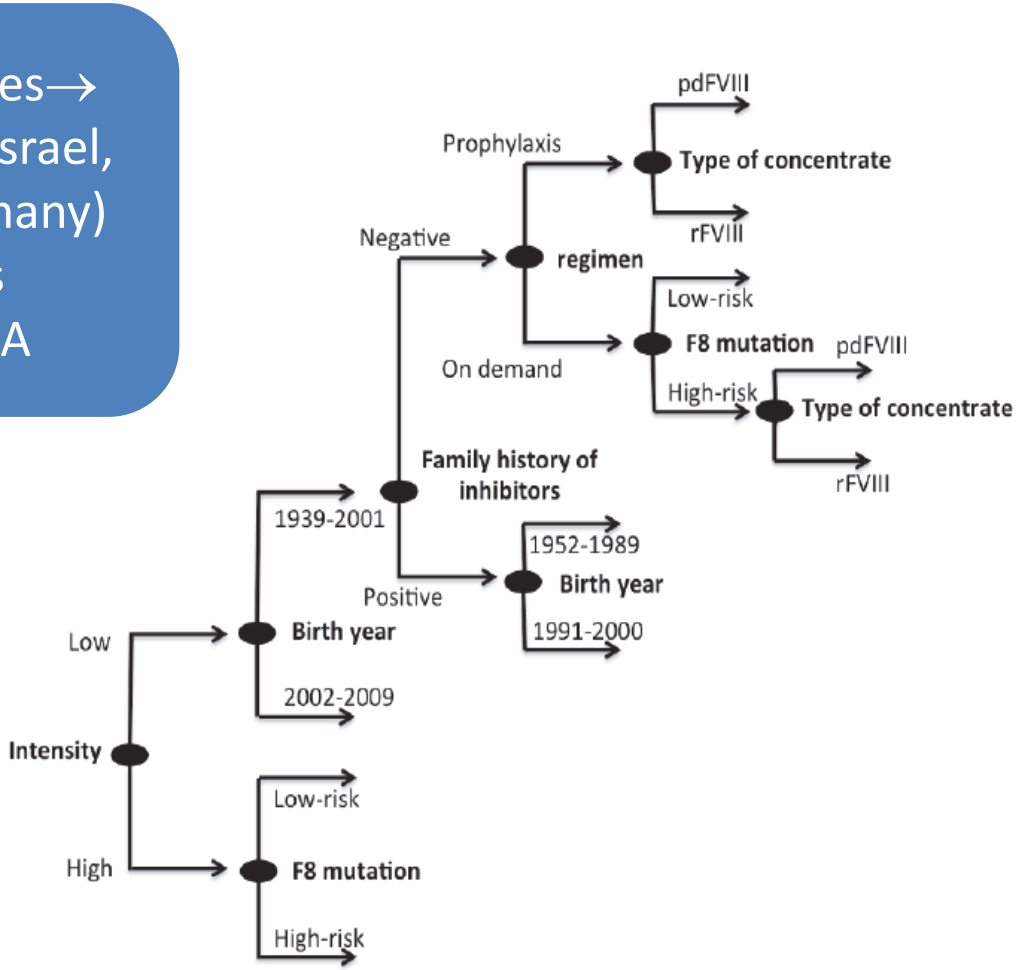
	All PUPs reported to EUHASS					Excluding overlap with RODIN study				
	Inhibitors	Total	Cumulative incidence	Lower CI	Upper CI	Inhibitors	Total	Cumulative incidence	Lower CI	Upper CI
recFVIII BHK	44	143	30.8%	23.3%	39.0%	27	105	25.7%	17.7%	35.2%
recFVIII CHO	53	223	23.8%	18.3%	29.9%	35	154	22.7%	16.4%	30.2%
Advate	37	141	26.2%	19.2%	34.3%	22	85	25.9%	17.0%	36.5%
Helixate NexGen	12	37	32.4%	18.0%	49.8%	11	33	33.3%	18.0%	51.8%
Kogenate Bayer	32	106	30.2%	21.7%	39.9%	16	72	22.2%	13.3%	33.6%
Recombinate	1	24	4.2%	0.1%	21.1%	1	24	4.2%	0.1%	21.1%
Refacto	0	6	0.0%	0.0%	45.9%	0	1	0.0%	0.0%	97.5%
Refacto AF	15	52	28.8%	17.1%	43.1%	12	44	27.3%	15.0%	42.8%

Fischer et al, Thromb Haemost 2015

The need for individual patient analysis: the EAHAD patient-level metanaysis

29 elegible studies→
4 PUPs cohorts (Israel, Italy, Egypt, Germany)
761 patients
86% severe HA

Three different statistical approaches:
Cox regression, propensity scores, CART* analysis



	N	F	RHR
	125	8	.19
	40	7	.55
	101	12	.37
	87	25	.86
	40	18	1.77
	23	11	1.34
	12	11	4.81
	43	26	2.58
	16	2	.40
	75	49	2.99

*classification and regression tree

Marcucci et al, Thromb Haemost 2015

Type and intensity of FVIII exposure on inhibitor development in PUPs with haemophilia A

A patient-level meta-analysis

Maura Marcucci[1,2]; Maria Elisa Mancuso[3]; Elena Santagostino[3]; Gili Kenet[4]; Mohssen Elalfy[5]; Susanne Holzhauer[6]; Christoph Bidlingmaier[7]; Carmen Escuriola Ettingshausen[8]; Alfonso Iorio*[1,9]; Ulrike Nowak-Göttl*[10]

- Higher risks of inhibitors for all rFVIII at univariate analysis compared to pdFVIII, disappearing after adjusting for confounders.
- No significant difference at univariate analysis among different types of rFVIII; however at multivariate analysis:
  - lower inhibitor risk for third generation full-lenght rFVIII than first generation full-lenght and second-generation BDD rFVIII.
  - no difference between third- and second-generation full-lenght rFVIII or between CHO- and BHK-derived products.
- Consistent and pivotal role for intensity of treatment as a risk factor for inhibitor development
  - FVIII type-by-intensity interaction (rFVIII/low intensity - pdFVIII/high intensity)
- Minimal overlap with RODIN Study (49/761 patients, 6%)

http://dx.doi.org/10.1160/TH14-07-0621
Thromb Haemost 2015; 113: ■■■

Three to one (or two): a neat victory* ?

- Different cohorts, different analysis, different results
- No randomized study or pre-specified analysis of different inhibitor risk between rFVIII brands
- Time and centre effects likely play relevant role
- Confounder assessment in the multicausality of inhibitor development fully addressed ?
- Biological plausibility still unravelled
- Findings generate hypotheses to be further tested in experimental or clinical context

**Mannucci and Garagiola, Thromb Haemost 2015*

EUROPEAN MEDICINES AGENCY
SCIENCE MEDICINES HEALTH

13 May 2016
EMA/PRAC/332348/2016
Pharmacovigilance Risk Assessment Committee (PRAC)

Inhibitor development in previously untreated patients with severe haemophilia A treated with recombinant factor VIII products

The PRAC agreed that overall, the currently available evidence does not confirm that Kogenate Bayer/Helixate NexGen is associated with an increased risk of factor VIII inhibitors, compared with other recombinant factor VIII products in previously untreated patients. These conclusions are consistent with the previous conclusions drawn by the PRAC within the review carried out on Kogenate Bayer/Helixate NexGen in 2013.

The PRAC recommended that the marketing authorisation holders of recombinant coagulation factor VIII products should monitor published studies on drug inhibitor development with the aim of keeping the product information up to date.

Conclusions and perspectives

- Caution always applies in the interpretation of differences from clinical studies.
- Long-term assessment of inhibitor formation is needed for all FVIII products
- Randomized studies unlikely. Searching for better methods for prospective assessment
- Consider the evolving scenario of haemophilia treatment

The NEW ENGLAND JOURNAL of MEDICINE

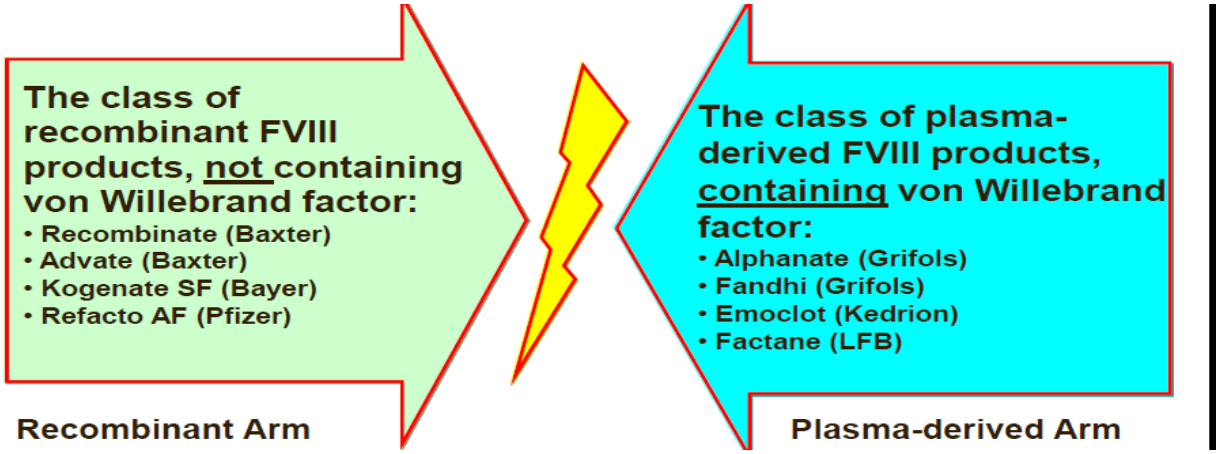
ORIGINAL ARTICLE

A Randomized Trial of Factor VIII and Neutralizing Antibodies in Hemophilia A

F. Peyvandi, P.M. Mannucci, I. Garagiola, A. El-Beshlawy, M. Elalfy, V. Ramanan, P. Eshghi, S. Hanagavadi, R. Varadarajan, M. Karimi, M.V. Manglani, C. Ross, G. Young, T. Seth, S. Apte, D.M. Nayak, E. Santagostino, M.E. Mancuso, A.C. Sandoval Gonzalez, J.N. Mahlangu, S. Bonanad Boix, M. Cerqueira, N.P. Ewing, C. Male, T. Owaidah, V. Soto Arellano, N.L. Kobrinsky, S. Majumdar, R. Perez Garrido, A. Sachdeva, M. Simpson, M. Thomas, E. Zanon, B. Antmen, K. Kavakli, M.J. Manco-Johnson, M. Martinez, E. Marzouka, M.G. Mazzucconi, D. Neme, A. Palomo Bravo, R. Paredes Aguilera, A. Prezotti, K. Schmitt, B.M. Wicklund, B. Zulfikar, and F.R. Rosendaal

SIPPET Study

- First prospective randomized controlled study
- Hypothesis: **2-fold lower inhibitor incidence with plasmaderived** than with recombinant products
- Sample size: 270 patients (α=0.05; statistical power 80%)



- 303 PUPs enrolled in 24 countries in 4 continents
- 251 analyzed (125 pdFVIII e 126 rFVIII)

Peyvandy et al, NEJM 2016

Table 1. Baseline Characteristics of the Randomly Assigned Patients.*

Characteristic	Plasma-Derived Factor VIII (N=125)	Recombinant Factor VIII (N=126)
Country — no. (%)		
India	40 (32.0)	43 (34.1)
Egypt	38 (30.4)	37 (29.4)
Iran	14 (11.2)	18 (14.3)
United States	9 (7.2)	9 (7.1)
Italy	5 (4.0)	4 (3.2)
Other†	19 (15.2)	15 (11.9)
Age at first treatment — mo		
Median (range)	15.0 (0–67)	16.0 (0–75)
Mean	19.1±14.3	21.3±16.3
Type of mutation — no./total no. (%)‡		
Intron 22 inversion	57/117 (48.7)	53/118 (44.9)
Intron 1 inversion	5/117 (4.3)	1/118 (0.8)
Nonsense	14/117 (12.0)	20/118 (16.9)
Large deletion	8/117 (6.8)	8/118 (6.8)
Frameshift	17/117 (14.5)	14/118 (11.9)
Missense	10/117 (8.5)	12/118 (10.2)
Splice site	3/117 (2.6)	9/118 (7.6)
Only polymorphisms	3/117 (2.6)	0/118
No mutation	0/117	1/118 (0.8)
Mutation status — no./total no. (%)‡		
Non-null mutation	16/117 (13.7)	21/117 (17.9)
Null mutation	101/117 (86.3)	96/117 (82.1)
Family history of hemophilia — no./total no. (%)		
Yes	59/124 (47.6)	52/122 (42.6)
No	65/124 (52.4)	70/122 (57.4)
Family history of inhibitor development — no./total no. (%)		
Yes	13/113 (11.5)	12/119 (10.1)
No	100/113 (88.5)	107/119 (89.9)
Race or ethnic group — no. (%)§		
White	39 (31.2)	45 (35.7)
Black	5 (4.0)	2 (1.6)
Asian	41 (32.8)	43 (34.1)
Other	40 (32.0)	36 (28.6)
Previous treatment — no. (%)		
Yes¶	56 (44.8)	53 (42.1)
No	69 (55.2)	73 (57.9)
Treatment regimen — no. (%)‖		
On-demand	61 (48.8)	56 (44.4)
Standard prophylaxis	21 (16.8)	19 (15.1)
Modified prophylaxis	43 (34.4)	51 (40.5)
Brand of concentrate — no. (%)		
Alphanate	9 (7.2)	
Emoclot	61 (48.8)	
Factane	43 (34.4)	
Fanhdi	12 (9.6)	
Advate		13 (10.3)
Kogenate FS		61 (48.4)
Recombinate		45 (35.7)
ReFacto AF		7 (5.6)

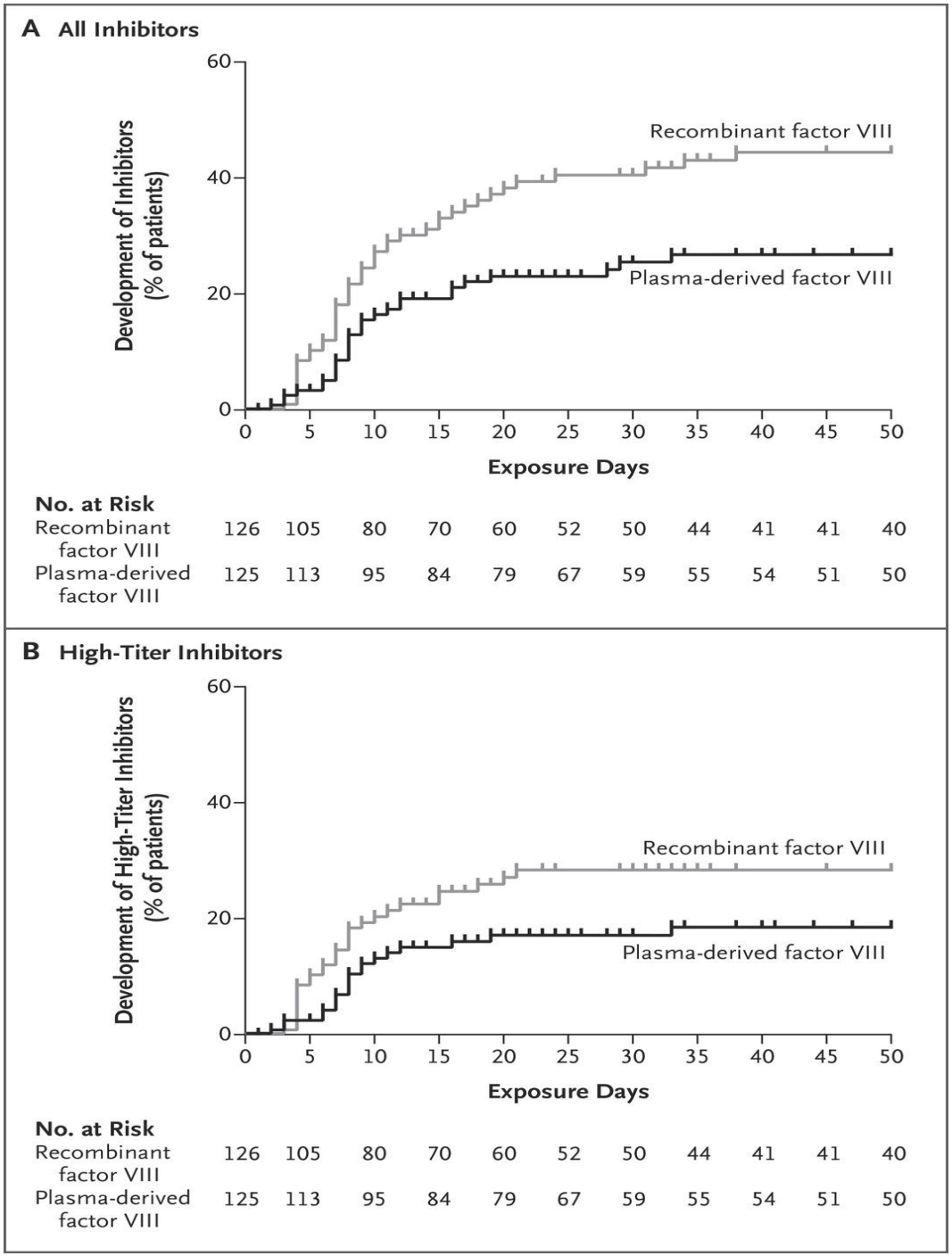
* Plus–minus values are means ±SD.

Table 2. Characteristics of the Patients in Whom Inhibitors Developed.*

Characteristic	Plasma-Derived Factor VIII	Recombinant Factor VIII
Type of inhibitor — no./total no. (%)		
All	29/125 (23)	47/126 (37)
Transient†	7/27 (26)	12/44 (27)
Persistent†	20/27 (74)	32/44 (73)
High titer	20/125 (16)	30/126 (24)
Transient†	3/18 (17)	2/27 (7)
Persistent†	15/18 (83)	25/27 (93)
Low titer	9/125 (7)	17/126 (13)
Transient	4/9 (44)	10/17 (59)
Persistent	5/9 (56)	7/17 (41)
Time of development — exposure days		
All		
Mean	11.2	10.9
Median (range)	8 (3–33)	8 (2–38)
High titer		
Mean	9.8	8.1
Median (range)	8 (3–33)	7 (2–21)
Low titer		
Mean	14.4	15.9
Median (range)	12 (4–29)	11 (7–38)
Peak titer — Bethesda units		
All		
Mean	62.2	124.5
Median (range)	12 (0.8–1100)	16.3 (0.7–1850)
High titer		
Mean	88.9	193.7
Median (range)	17.5 (6–1100)	113.5 (10–1850)
Low titer		
Mean	3.1	2.4
Median (range)	4 (0.8–5)	2 (0.7–5)

* High-titer inhibitors were defined by peak levels of at least 5 Bethesda units. Low-titer inhibitors were defined by levels of 0.4 to less than 5 Bethesda units. Transient inhibitors were those that disappeared spontaneously within 6 months without immunotolerance treatment. Inhibitors developed in 76 patients; during the 6 months of observation after inhibitor development, 19 patients (25%) stopped treatment, 49 (65%) continued with the trial product on demand or as prophylaxis, and 8 (10%) received factor VIII–bypassing agents for bleeding.

† Data at 6-month follow-up were missing for two patients assigned to plasma-derived factor VIII and three patients assigned to recombinant factor VIII.



Cumulative incidence of inhibitors according to the treatment group

HR 1.87 (1.17-2.96)

HR 1.69 (0.96-2.98)

Irrespective of type of rFVIII

	All patients	2.nd generation excluded
All inhibitors	1.87 (1.17-2.96)	1.98 (0.99-3.97)
High-titer inhibitors	1.69 (0.96-2.98)	2.59 (1.11-6.00)

Sensitivity analysis - country

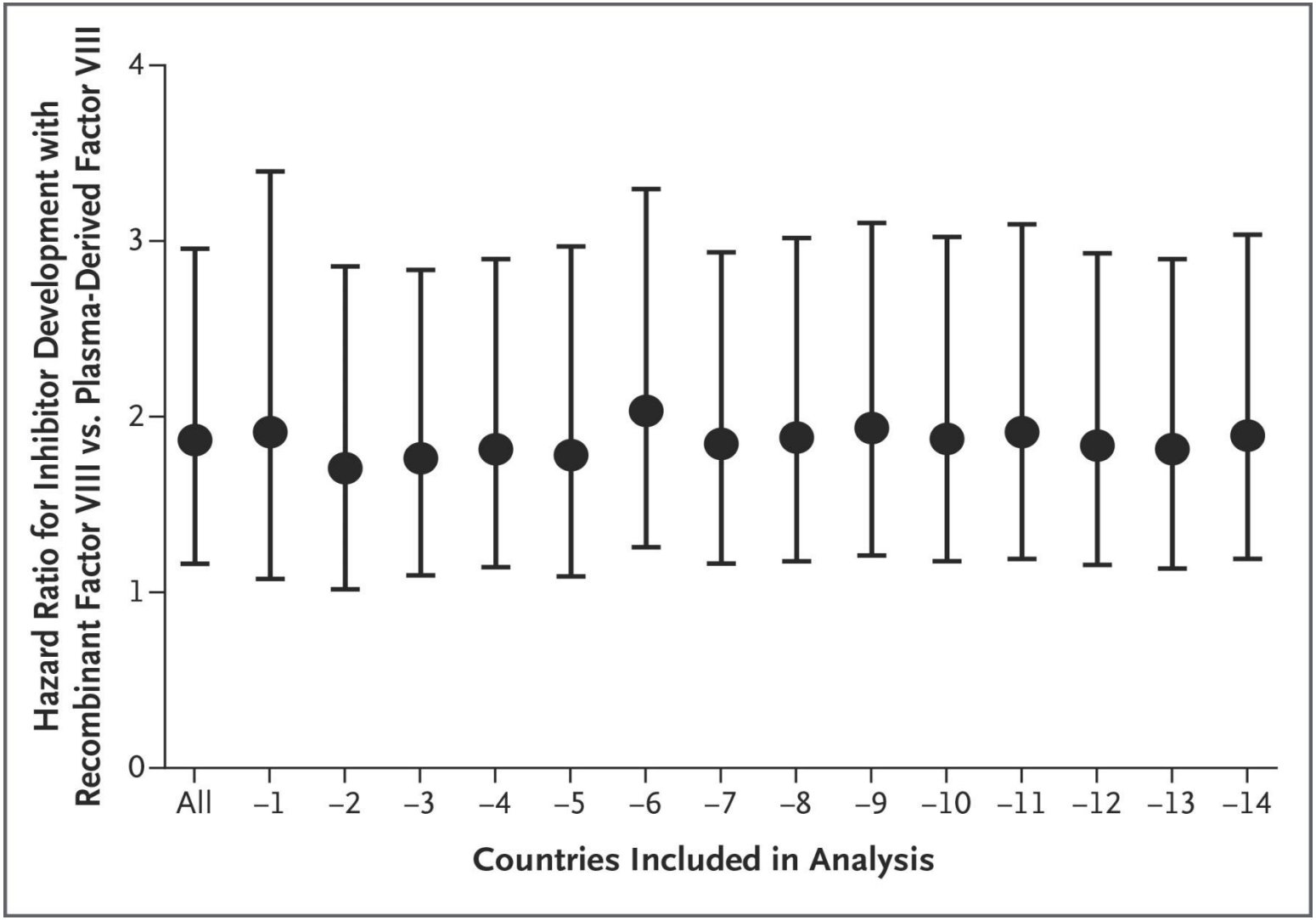


TABLE S1. FOLLOW UP IN PATIENTS WHO DID NOT DEVELOP INHIBITOR.

	Randomized to plasma-derived FVIII *(96)*	Randomized to recombinant FVIII (79)
Early termination, n (%)	4 (4,2%) (median ED = 25; range 20-29)	6 (7,6%) median ED = 25,5; range 8-45
3 years from randomization, n (%)	29 (30,2%) (median ED = 22; range 4-44)	23 (29,1%) median ED = 15; range 1-35
≥50 ED, n (%)	50 (52,1%)	40 (50,6%)
21-49 ED, n (%)	1 (1,0%)	1 (1,3%)
1-20 ED, n (%)	12 (12,5%)	9 (11,4%)

Implications in product choice for PUPs ?

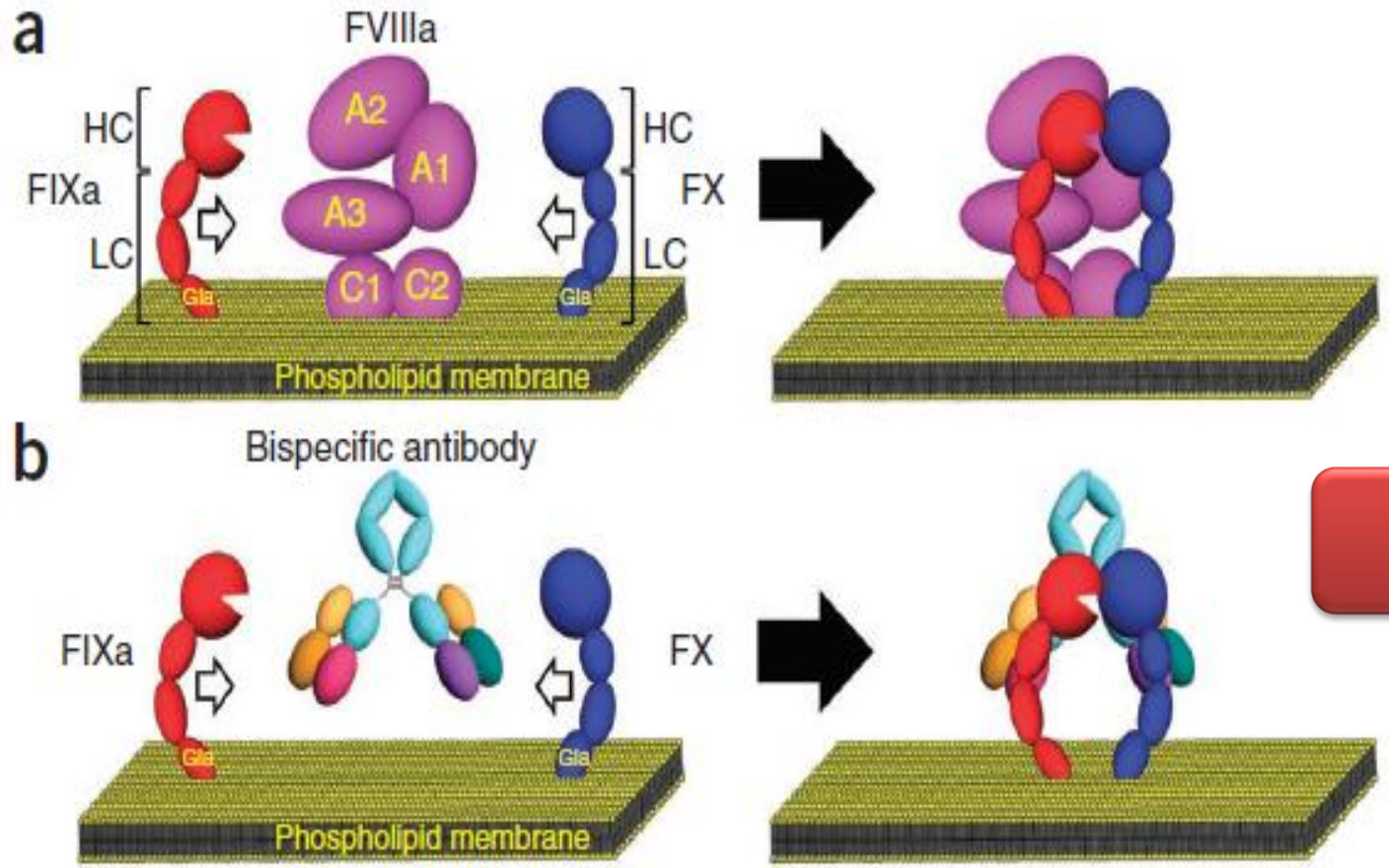
- All PUPs on plasmaderived FVIII ?
- Different choices acccording to the patient inhibitor risk (F8 genotype, family history, intensive treatment) ?
- Plasmaderived during the first 50 Eds, then switch to recombinant FVIII ?
- Newer recombinant products ?

Novel non-FVIII/FIX replacement approaches in hemophilia

Product	Company	Technology	Stage of development	Main characteristics
rFVIIa-FP	CSL Behring	Fusion protein with albumin	Phase II/III study ongoing	Prolonged half-life (8.5 h)
ACE910	Chugai Pharmaceuticals/La Roche Hoffman	Chimeric bispecific humanized antibody	Phase I study ongoing (interim analysis published)	Prolonged half-life (2 wk) SC weekly administration reduced ABR in hemophiliacs
Concizumab	Novo Nordisk	Humanized monoclonal antibody	Phase I studies (Explorer 1–3)	Prolonged half-life (31.1–74.2 h) SC or IV administration improved thrombin generation and reduced TFPI levels for $\geq$ 14 d in hemophiliacs
ALN-AT3	Alnylam Pharmaceuticals	siRNA	Phase I study (interim analysis published)	SC administration improved thrombin generation, whole blood clot formation, and reduced antithrombin levels up to 80% in hemophilia patients
^{super}FVa	–	Bioengineered FVa variant	Preclinical phase	Increased thrombin generation in acquired hemophilia models Synergistic effect with rFVIIa
FXa^{I16L}	–	Bioengineered zymogen-like FXa variant	Preclinical phase	Longer lasting plasma activity than wild-type FXa (60 min Vs. 1 min) Increased thrombin generation in hemophilia models
FXIII	CSL Behring	Plasma-derived product	Preclinical phase	Long half-life (9 d) Improve clot stability alone or in association with rFVIIa

Mannucci et al, Semin Thromb Hemost 2016

The bispecific FVIII-mimetic antibody



s.c. injection

Long half-life

hBS23, Kitazawa et al, Nat Med 2012
Improved variant, **ACE910**, Muto et al, J Thromb Haemost 2014

Thank you

Identifying Nongenetic Risk Factors for Inhibitor Development in Severe Hemophilia A

Samantha C. Gouw, MD, PhD[1] Karin Fijnvandraat, MD, PhD[1]

Seminars in Thrombosis & Hemostasis Vol. 39 No. 7/2013

Non-genetic risk factors	Level of certainty
FVIII exposure	Certainly a risk factor
FVIII product type: specific pasteurized plasma-derived product types	Certainly a risk factor
Periods of intensive treatment	Certainly a risk factor
Prophylaxis	Likely a protective factor
Surgical procedures	Likely a risk factor
Dose of FVIII treatment	Possibly a risk factor
FVIII product type: certain recombinant FVIII products	Possibly a risk factor
Source of FVIII product: recombinant versus plasma	Unlikely a risk factor
Switching between FVIII products	Not a risk factor
Age at first FVIII exposure	Not a risk factor
Breastfeeding	Not a risk factor
Vaccinations, infections, immune modulating medication, allergic constitution	Not enough available data
Extravasation of FVIII product	Not enough available data
Mode of infusion (bolus infusion, continuous infusion)	Not enough available data

Inhibitors in PTPs

- **Incidence: 1.5-5.3 per 1000/yr**

Patients	Source	Findings (reference)
Previously untreated patients	Prospective studies	Cumulative incidence[a]: 25.9 to 37.6%[28–31]
	Registry	Incidence[b]: 64.29 per 1,000 person-years[33]
Previously treated patients	Surveillance systems	EUHASS[c]: 1.47 (95% CI: 1.0–2.2) per 1,000 person-years[31] UDC[d]: 2.14 per 1,000 person-years[34]
	Meta-analysis	Pooled incidence[e]: 3 (95% CI: 1–4) per 1,000 person-years[35]
	Registry	Incidence[f]: 5.31 per 1,000 person-years[33]

Coppola et al, Semin Thromb Hemost 2016

the switch issue: inhibitor risk

- YES: higher for B-domain deleted vs. full lenght rFVIII, OR, 95 CI: 2.61, 1.21-5.53 (Aledort et al, JTH 2012)
- NO effect of any type of FVIII product (Xi et al, JTH 2013)

Coppola et al, Semin Thromb Hemost 2016

Inhibitors in PTPs: the switch issue

- Perceived risk of inhibitor development (outbreaks in the 80's with specific products) by patients but also by physicians results in reluctance to change FVIII products
  - main patient concern (26%; high/very high risk 57%)

    Santagostino et al, Eur J Hematol 2015

  - physicians reassured (>90%; but data about low immunogenicity of new products important)

    Matino et al, Haemophilia 2014;

    Franchini et al, Haemophilia 2013;

    Farrugia et al, Blood Transfus 2015

Safety of Switching Factor VIII Products in the Era of Evolving Concentrates: Myths and Facts

Antonio Coppola, MD[1] Emiliana Marrone, MD[1] Paolo Conca, MD[1] Ernesto Cimino, MD[1]
Rosaria Mormile, BS[1] Erminia Baldacci, MD[2] Cristina Santoro, MD, PhD[2]

Semin Thromb Hemost 2016;42:563–576.

- **First 50-75 EDs**: no increase of risk in 4 available studies (CANAL, RODIN, UKHCDO and French Registries)
- **PTPs** (>150 EDs; > 50 EDs in children) phase II/III clinical trials (2 pd-FVIII and 14 rFVIII studies – 5 in children): 4 *de novo* inhibitors in >1300 patients, all low-titer, transient in 3 cases
- Post-marketing surveillances (6 rFVIII studies, less selected populations): 3 de novo inhibitors, all low-titer

National cohort studies of FVIII switch

Country, y	Product switch	Patients, *n*	Follow-up	Exposure to the new product	Inhibitors	De novo inhibitor rate/incidence	Study (reference)
Canada, 1994	pdFVIII → BHK FL rFVIII	339[a]	24 mo	NR	10 de novo	3%[a]	Giles et al[42]
Canada, 2000	BHK FL rFVIII → BHK FL FS rFVIII	189[b]	24 mo	NR	0	0	Rubinger et al[43]
Ireland, 2003[c]	CHO rFVIII[d] → BHK FL FS rFVIII	94 (89% severe, 94% >100 EDs)	20 mo	54.3% >100 EDs 25.5% 20–100 EDs	One LT de novo[e] Three LT recurrent	1.3%[f]	Singleton et al[44]
Ireland, 2006[c]	BHK FL FS rFVIII → CHO FL PFM rFVIII	113 (89% severe)	30 mo	85% >100 EDs	One LT de novo[g]	1.04%[f]	Bacon et al[45]
UK, 2010	FL rFVIII → CHO BDD AF-CC rFVIII	516 switched versus 682 nonswitched	12 mo	NR	Four versus one	7.5 versus 1.5 per 1,000 patient-years[h]	Hay et al[19]

Abbreviations: AF-CC, albumin-free cell culture; BDD, B-domain deleted; BHK, Baby Hamster Kidney; CHO, Chinese Hamster Ovary; EDs, exposure days; FL, full length; FS, formulated in sucrose; LT, low titer; NR, not reported; pdFVIII, plasma-derived FVIII concentrate; PFM, protein/albumin free method; rFVIII, recombinant FVIII concentrate.

[a]Data available at 1-year follow-up in 478 patients, with 9 de novo inhibitors (1.9%).

[b]Data available at 1-year follow-up in 225 patients, no inhibitors detected.

[c]Retrospective study, here reported for completeness of data about national product switches.

[d]Largely BDD-rFVIII.

[e]After intensive treatment for surgery. In this study, 17 patients (18%) had a previous inhibitor history and 3 of them developed a recurrent low-titer inhibitor. All patients with inhibitors were able to continue rFVIII replacement treatment on demand and tested negative at the last inhibitor assessment on study.

[f]Excluding patients with positive inhibitor history.

[g]This patient was a child with only 3 EDs before switching product. Another patient with an inhibitor at the time of the switch had an anamnestic response after starting immune tolerance induction. None of 16 patients with inhibitor history (including 5 high-titer) had recurrence of inhibitors.

[h]No significant difference in incidence after adjusting for age and HIV status.

Coppola et al, Semin Thromb Hemost 2016

CLINICAL TRIALS AND OBSERVATIONS

A first-in-human phase 1 study of ACE910, a novel factor VIII–mimetic bispecific antibody, in healthy subjects

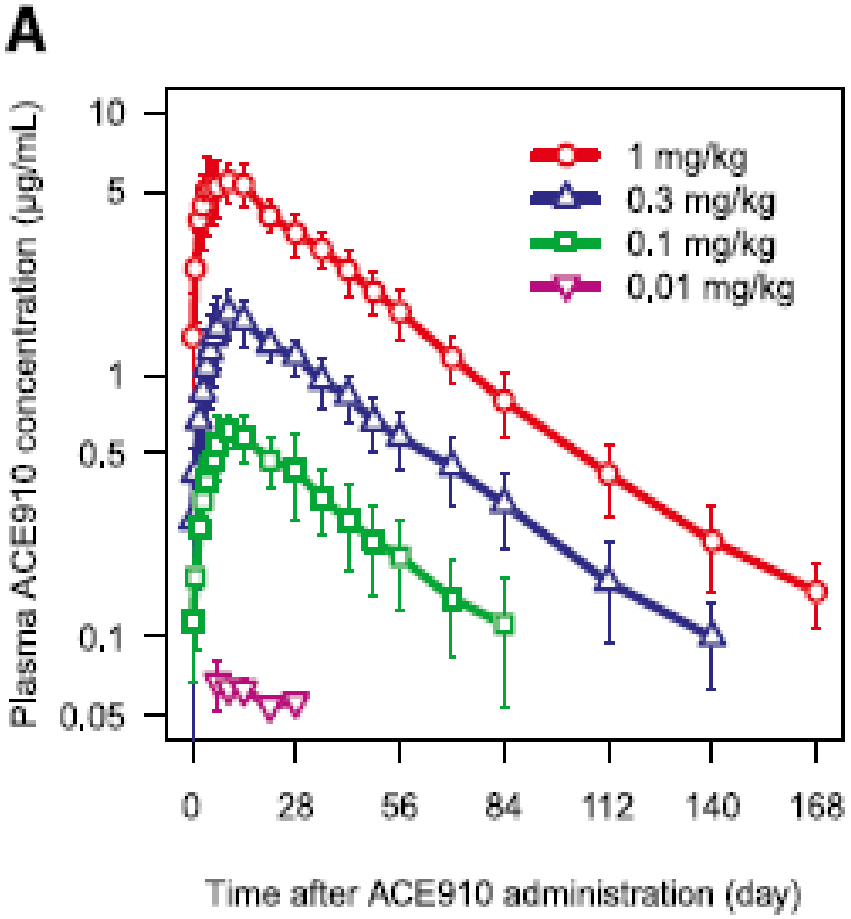
Naoki Uchida,[1,2] Takehiko Sambe,[1,2] Koichiro Yoneyama,[3] Naoki Fukazawa,[3] Takehiko Kawanishi,[3] Shinichi Kobayashi,[1] and Midori Shima[4]

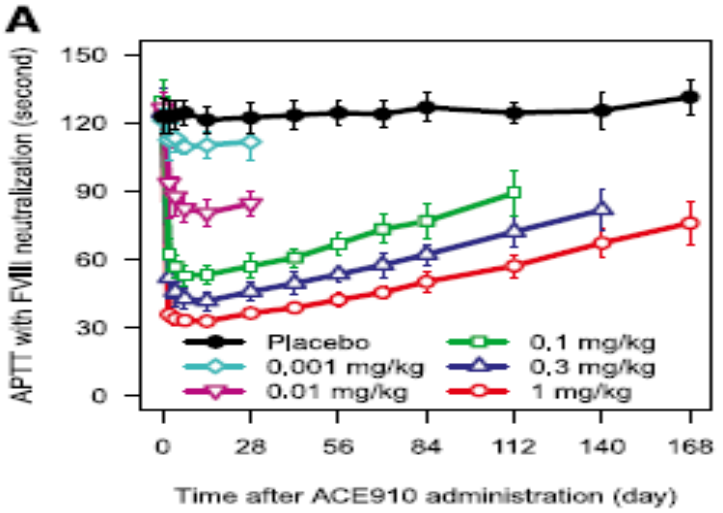
[1]Showa University Clinical Research Institute for Clinical Pharmacology and Therapeutics, Tokyo, Japan; [2]Department of Pharmacology, School of Medicine, Showa University, Tokyo, Japan; [3]Translational Clinical Research Division, Chugai Pharmaceutical Co. Ltd., Tokyo, Japan; and [4]Department of Pediatrics, Nara Medical University, Nara, Japan

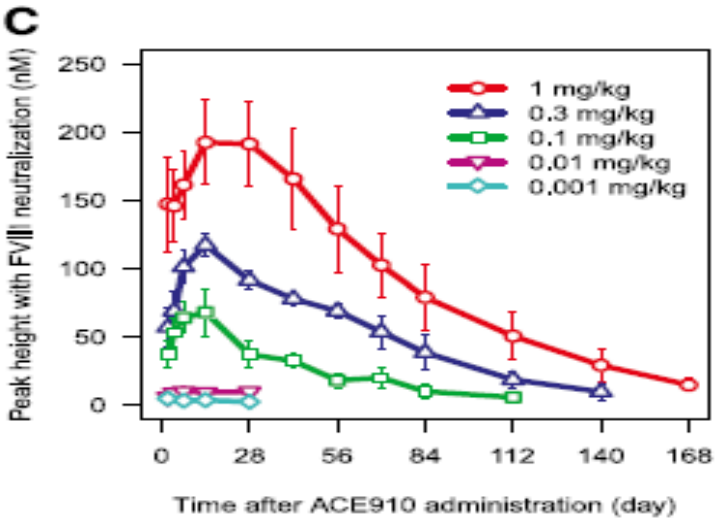
- 40 Japanese and 24 white male subjects, **single s.c. injection** (0.001, 0.01, 0.1, 0.3, or 1 mg/Kg vs. placebo)
- ACE910 exhibited a linear PK profile and a half-life of 4-5 weeks
- Dose-dependent increase of thrombin generation and APTT shortening in FVIII-neutralized plasma
- No serious adverse events or laboratory signs of hypercoagulability
- Anti-drug antibodies in 2/48 subjects receiving ACE910, one both before and after injection, the othe[illegible]

BLOOD, 31 MARCH 2016 · VOLUME 127, NUMBER 13

PK and PD of ACE910







Data from Japanese subjects
(comparable to those from white subjects)

Uchida et al, Blood 2016

AS017

Long-term safety and prophylactic efficacy of once-weekly subcutaneous administration of ACE910, in Japanese hemophilia A patients with and without FVIII inhibitors: interim results of the extension study of a phase 1 study

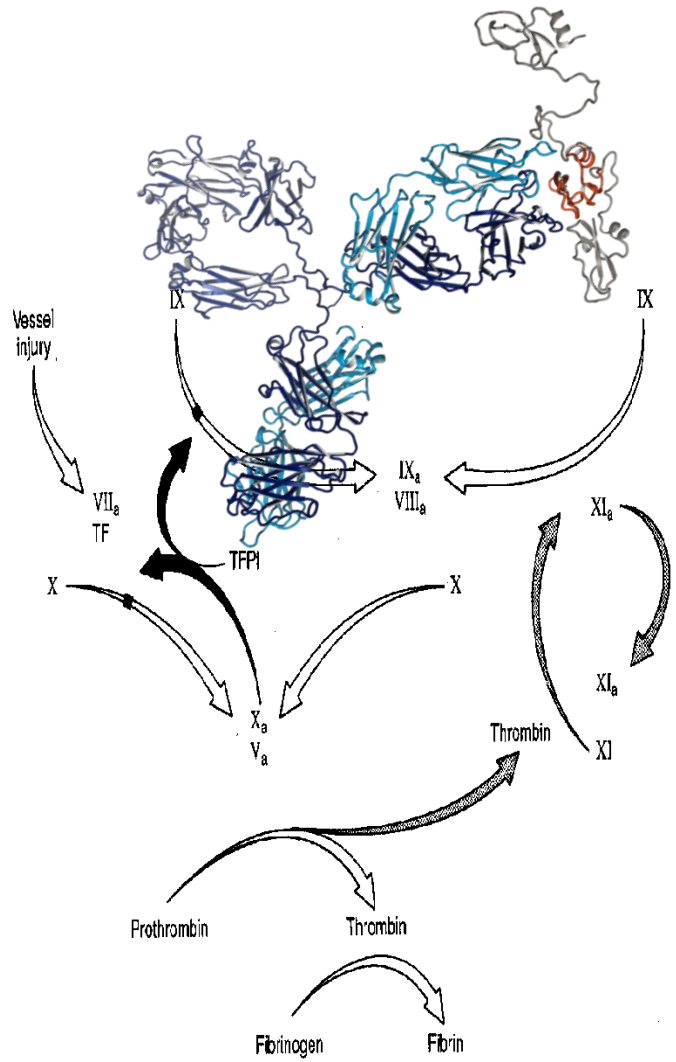
Shima M[1], Hanabusa H[2], Taki M[3], Matsushita T[4], Sato T[5], Fukutake K[6], Fukazawa N[7], Yoneyama K[7], Yoshida H[7], Takahashi H[8] and Nogami K[1]

[1]Department of Pediatrics, Nara Medical University, Kashihara, Nara; [2]Department of Hematology and Pediatrics, Ogikubo Hospital, Suginami-ku, Tokyo; [3]Department of Pediatrics, St. Marianna University School of Medicine, Kawasaki, Kanagawa; [4]Department of Transfusion Medicine, Nagoya University, Nagoya, Aichi; [5]Department of Pediatrics, University of Occupational and Environmental Health, Kitakyushu, Fukuoka; [6]Department of Laboratory Medicine, Tokyo Medical University, Shinjuku-ku, Tokyo; [7]Clinical Research Planning Dept., Chugai Pharmaceutical Co., Ltd., Chuo-ku, Tokyo; [8]Department of Internal Medicine, Niigata Prefectural Kamo Hospital, Kamo, Niigata, Japan

- 18 Japanese HA (11 with inh), 0.3, 1 or 3 mg/Kg for 12 wks
- Offered to continue on the extension (including dose escalation)
- Interim data: **9.5 mo** follow-up
- No serious AEs, no thrombo-embolism
- Anti-ACE910 developed in 2 pts, without any relevant effect on drug PK or PD

ABR	Prior to the study	On ACE910 treatment
0.3 mg/Kg	32.5	2.0
1.0 mg/Kg	18.3	1.2
3.0 mg/Kg	15.2	0.0

TFPI inhibitors: mAb2021, concizumab



High affinity against the KPI-2 domain (FXa binding site), prevents FXa binding to TFPI and inhibition of the TF-FVIIa complex

- **Phase I study**: 24 HA and HB patients and 28 healthy subjects
- Escalating doses (250-9000 mg/Kg i.v. or 1000-3000 mg/Kg s.c.
- Detectable plasma levels up to 43 d
- TFPI activity reduced for ≥14 d
- No serious AEs or anti-

Chowdary et al, J Thromb Haemost 2015

AS019

Thrombin generation is increased in plasma from healthy males who have received concizumab, an antibody against tissue factor pathway inhibitor (ExplorerTM2)

Waters EK, Sigh J, Ezban M and Hilden I

NovoNordisk A/S, Måløv, Denmark

- 4 healthy males
- 250 mg/Kg **s.c**. e.o.d. 8 times
- Thrombin generation (peak, ETP, velocity index) increased compared to baseline
- Correlation (direct) with concizumab and (inverse) with TFPI levels
- Improved thrombin generation in FVIII-neutralized plasma

Preclinical studies for two additional TFPI inhibitors, phase I studies ongoing :
BAY 1093884 (Bayer) - PF-0674186 (Pfizer)

Suppressing antithrombin synthesis: interfering RNA (RNAi) – ALN-AT3

OR213

A subcutaneously administered investigational RNAi therapeutic (ALN-AT3) targeting antithrombin for treatment of hemophilia: interim phase 1 study results in patients with hemophilia A or B

Sorensen B[1], Mant T[2], Georgiev P[3], Rangarajan S[4], John Pasi K[5], Creagh D[6], Bevan DH[7], Austin S[8], Hay C[9], Brand B[10], Simon A[1], Melton L[11], Lynam C[1], Strahs A[1], Sehgal A[1], Hutabarat R[1], Chaturvedi P[1], Barros S[1], Garg P[1], Vaishnaw A[12], Akinc A[1] and on behalf of ALN-AT3 Investigators

[1]Alnylam Pharmaceuticals, Cambridge, MA, USA; [2]Quintiles, London, UK; [3]Division of Hematology, Medical University of Plovdiv, Bulgaria; [4]Southern Hemophilia Network, Basingstoke; [5]Barts and The London School of Medicine and Dentistry, London; [6]Cornwall; [7]Guy's and St Thomas's Hospital; [8]St Georges Hospital, London; [9]Central Manchester University Hospitals NHS Foundation Trust, Manchester, UK; [10]Department of Hematology, Zurich University Hospital, Zurich, Switzerland; [11]Alnylam Pharmaceuticals, Cambridge, MY; [12]Alnylam Pharmaceuticals, Cambridge MA, USA

- Phase 1 study ongoing
- Part A: 4 healthy subjects, 30 μg/Kg s.c. or placebo
- AT knockdown stable and durable over 60 d
- No severe AEs
- Part B/C: 3 weekly doses of 15 and 45 μg/Kg in cohorts of 3 hemophilic patients → AT knockdown ~80% with increased thrombin generation