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INVESTICE DO ROZVOJE VZDĚLÁVÁNÍ

Přehled rekombinantních FVIII

MUDr. Ivan Vonke, OKH, Nemocnice České Budějovice

Differentiation parameters

- Pathogen Safety
- Molecule structure
- Clinical efficacy
- Clinical safety
- Convenience
- Company reputation/R&D programs
- Leadership claims

PATHOGEN SAFETY

Consideration son the risk of transmission of blood-borne pathogens by FVIII therapies

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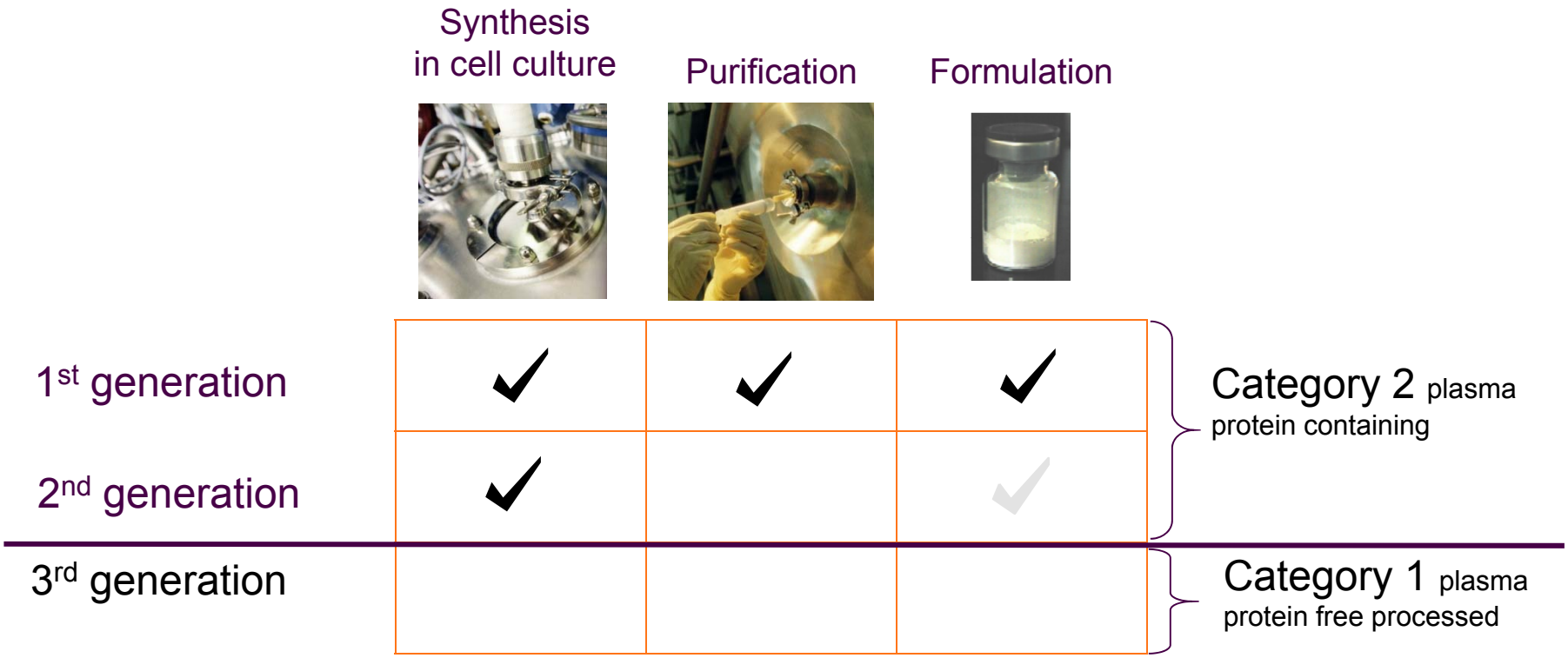
3 manufacturing steps
for rFVIII concentrates

	FVIII Synthesis in cell culture	FVIII Purification	FVIII final formulation	
				
1st generation	✓	✓	✓	Recombinate
2nd generation	✓		✓	Kogenate FS, Helixate NexGen, ReFacto
3rd generation therapies produced without the use of human or animal derived additives				ADVATE, (XYNTHA*)

* Brand name of ReFacto AF recently licensed in the US by Wyeth

	Synthesis in cell culture	Purification	Formulation	
				
1st generation	✓	✓	✓	Category 2 plasma protein containing
2nd generation	✓		✓	Category 1 plasma protein free processed
3rd generation				

Opinion: *„There is no difference between 2nd and 3rd gen rFVIII regarding pathogen safety. 2nd gen products are safe“.*



Opinion: *„All rFVIII are safe with regard to known pathogens. However regarding new emerging hitherto unknown blood-borne pathogens only 3rd gen rFVIII products have eliminated the risk of transmission“.*

3rd generation products are the „ADVATE generation“

The CHO cell line used to produce ReFacto is grown in culture medium that contains human serum albumin; no albumin is added during purification or in final formulation. Following purification, the albumin concentration is below the detection limit of the assay.

Ref.: ReFacto brochure distributed at ASH 2003

Serum albumin is eliminated in the final formulation. The cell culture medium is completely defined and contains only 2 protein components of biological origin: HSA, which is produced by licensed manufacturers for the cell culture process and recombinant insulin.

Ref.: Eriksson RK et al (2001) The manufacturing process for B-domain deleted rFVIII. Seminars in Hematology 38 (2) Suppl 4:24-31

In 1999 and 2000 three virtually albumin-free formulations (ReFacto, Kogenate Bayer and Helixate NexGen) were introduced. They contain 1000-times less pd albumin than the former formulations

Ref.: Zwart-van Rijkom JEF et al. (2002) The uptake of rFVIII in the Netherlands. BJ Haematol 119:332-341

ReFacto, one of several rFVIII products, does contain human albumin. ReFacto is a 3rd generation product, which also uses human albumin in its manufactutre and is thus a potential source of exposure (potential source of PVB19 transmission)

Ref.: Schlesinger KW and Ragni MV (2002) Safety of the new generation rFVIII concentrates. Expert opinion drug Safety 1 (3):213-223

In addition, human plasma protein and traces of host cell proteins and murine IgG, which derive from the fermentation and purification process, are found in the product.

Ref.: Kogenate FS European Public Assessment Report
http://www.emea.europa.eu/humandocs/Humans/EPAR/kogenatebayer/kogenatebayer.htm

For your information: HPPS (Human Plasma Protein Fraction) used in the cell culture medium is not a highly purified albumin concentrate (such as Buminate used for Recombinate) but is an early albumin fraction with relatively undefined plasma contaminants.

Kogenate FS, as an advanced rFVIII, contains less albumin than Recombinate

Ref.: Bayer brochure ASH 2003

Product	Baxter ADVATE	Baxter RECOMBINATE	Bayer & CSL Behring Kogenate FS Helixate NexGen	Wyeth ReFacto	VWF containing Pd-FVIII
Pathogen Safety	3rd generation rFVIII The first and only plasma and albumin free	1st generation rFVIII Human albumin is used as stabilizer and bovine proteins as cell culture medium components	2nd generation Albumin containing plasma fraction in cell culture and albumin detectable in final product	2nd generation Albumin in cell culture and in final product	Pd double virus inactivated

Main advantage of ADVATE compared to all other FVIII products !

All products are safe with regard to known pathogens but only ADVATE is safe with regard to new emerging hitherto unknown blood-borne pathogens.

Disadvantage for pdFVIII concentrates as having the highest risk of transmission of blood borne pathogens.

Newly licensed in the US XYNTHA (=ReFacto AF). 3rd generation B-domain depleted rFVIII

Q&A available (please contact Paul Woo Global Marketing)

Hot Topic Training planned

FVIII Molecule Structure

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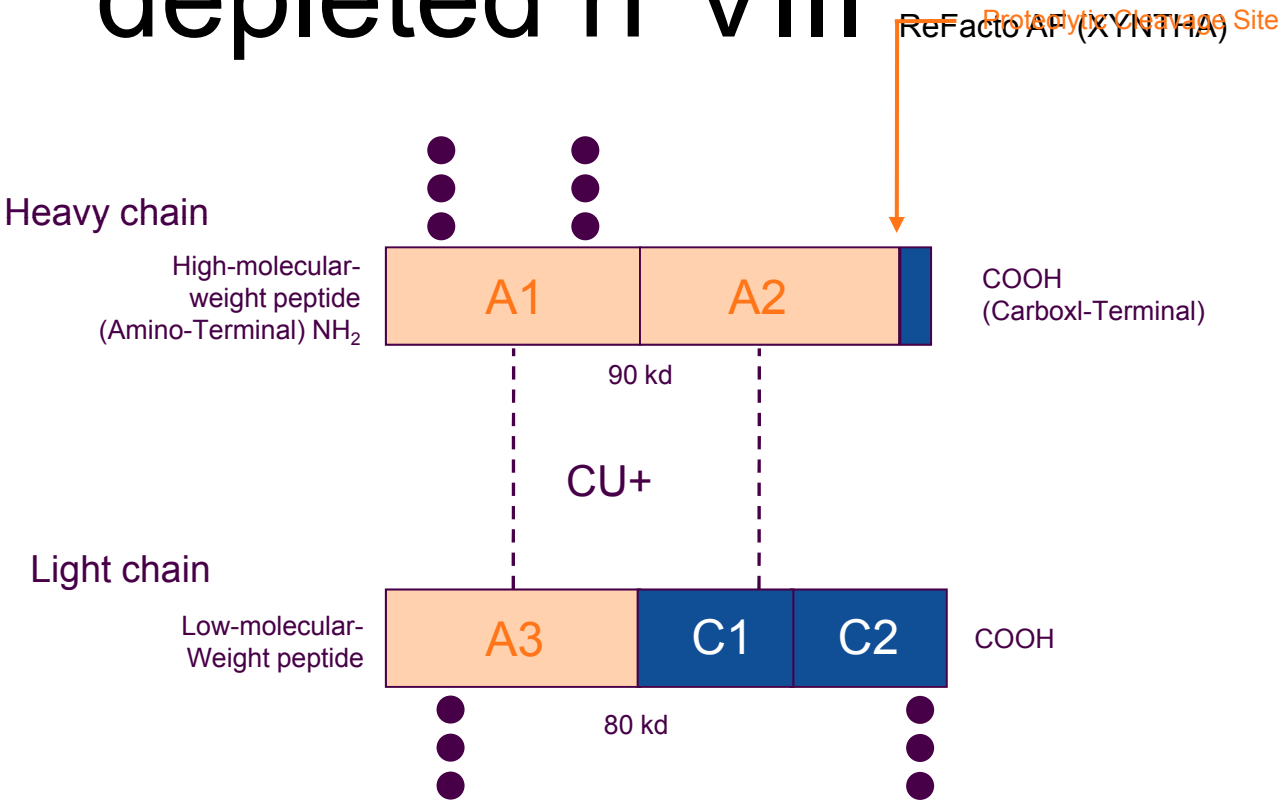
The structure of the B-domain depleted rFVIII



Sandberg H et al (2001) Structural and functional characterization of B-domain deleted recombinant factor VIII. Sem Hematol 38 (2) suppl 4:4-12

and Thromb Haemost (2001) 856:93-100

The B-domain ....

Synthesis	mediates recycling of misfolded molecules	Kaufman 1988[6], Pipe 1998[7]
Secretion	enhances ER - to - Golgi transport	Zhang et al. 2005[10]; Pipe et al. 2005[11]
Platelet binding	increases binding to activated platelets	Li & Gabriel 1997[12], Kaufman et al., 1997[3]
Activation	prevents accelerated proteolysis attacks by thrombin	Eaton, 1986[1]
Co-factor activity	has no apparent function	Kaufman et al. 1988[6]
Inactivation	slows down proteolysis by APC and FXa	Khrenov 2006[13]
Catabolism	interacts with endothelial endocytic receptor during removal of FVIII from circulation	Bovenschen 2005[18]

Immediately after synthesis of the FVIII protein within the CHO cell, cell own chaperons bind to the FVIII b-domain and check whether the FVIII protein is correctly folded.

- If it is correctly folded this specific FVIII molecule is released into the culture medium.
- If it is not correctly folded this FVIII molecule is never released but destroyed within the cell.

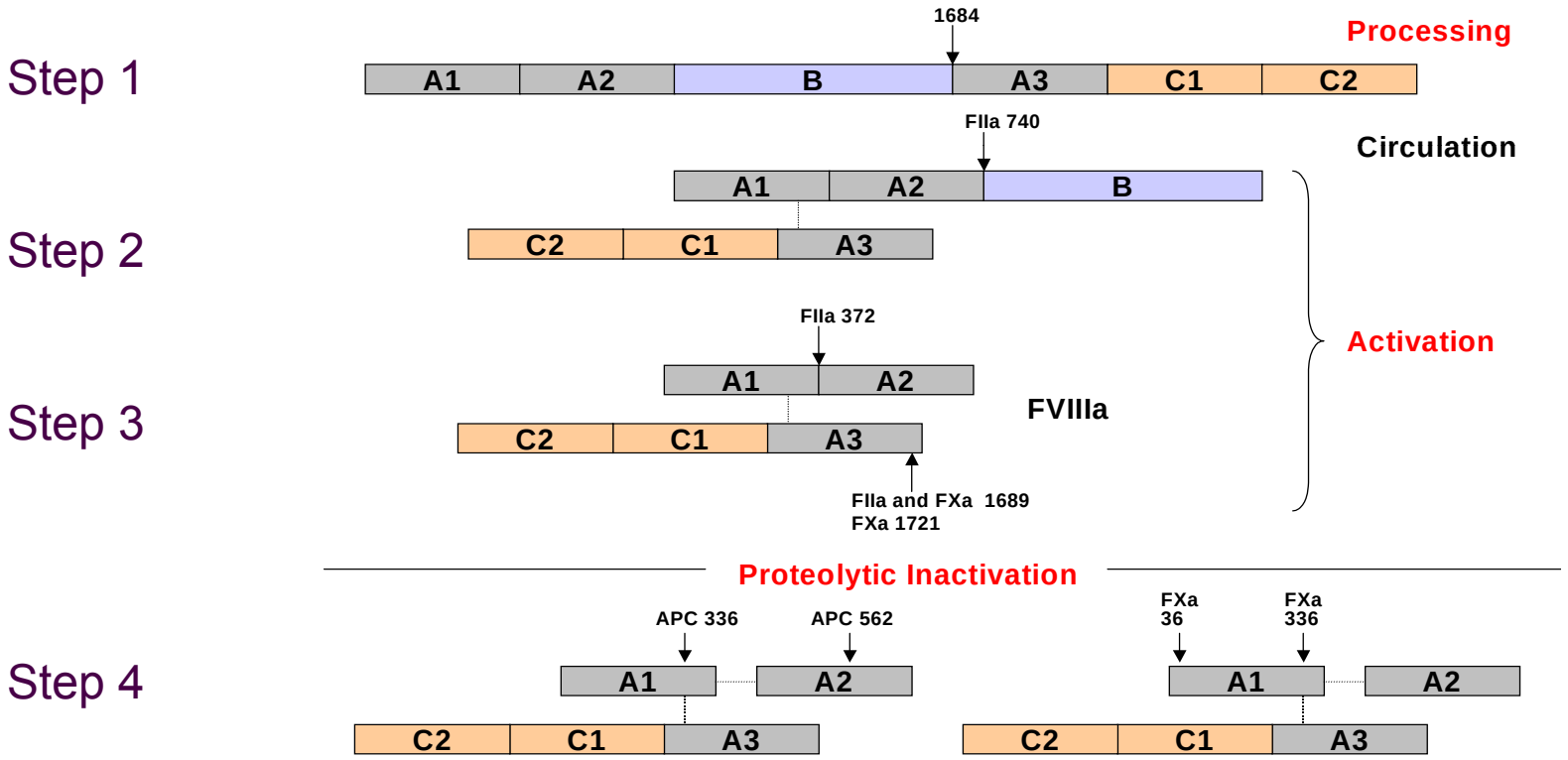
Thus the chaperon – b-domain interaction would be needed to guarantee proper folding what is a prerequisite for correct FVIII function.

Without a b-domain chaperons cannot interact with FVIII protein, which may cause the release of misfolded FVIII protein by the CHO cells.

Pipe et al (1998) Differential interaction of coagulation factor VIII and factor V with protein chaperones Calnexin and Calreticulin. J Biol Chem 273: 8537-8544

Walter S & Buchner J (2002) Molecular chaperones – cellular machines for protein folding. Angew Chem Int Ed Engl 41 (7):1098-1113

Zhang X et al (2002) Machinery of protein folding and unfolding. Curr Opin Struct Biol 2:231-238



APC = activated protein C

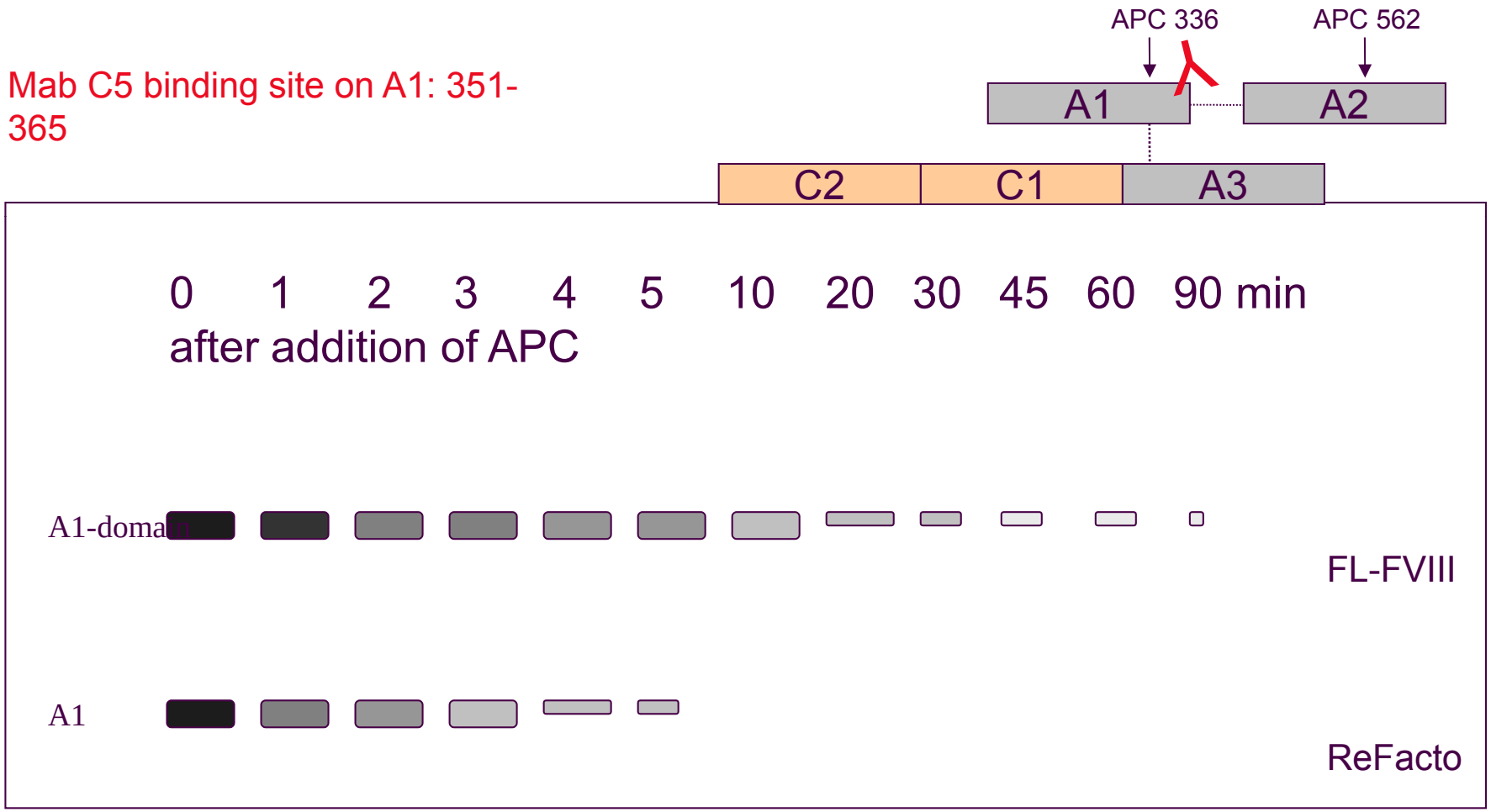
FIIa = activated factor II = thrombin

FXa = activated factor X

Cleavage sites are given based on amino acid numbers

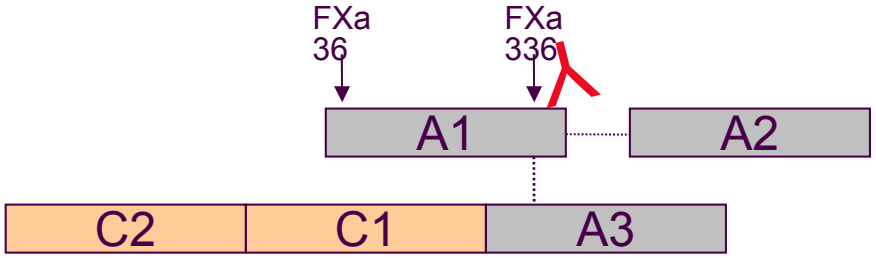
FVIIIa = activated factor VIII = coenzyme function in Xase complex (FIX/FVIIIa/PL/Ca++)

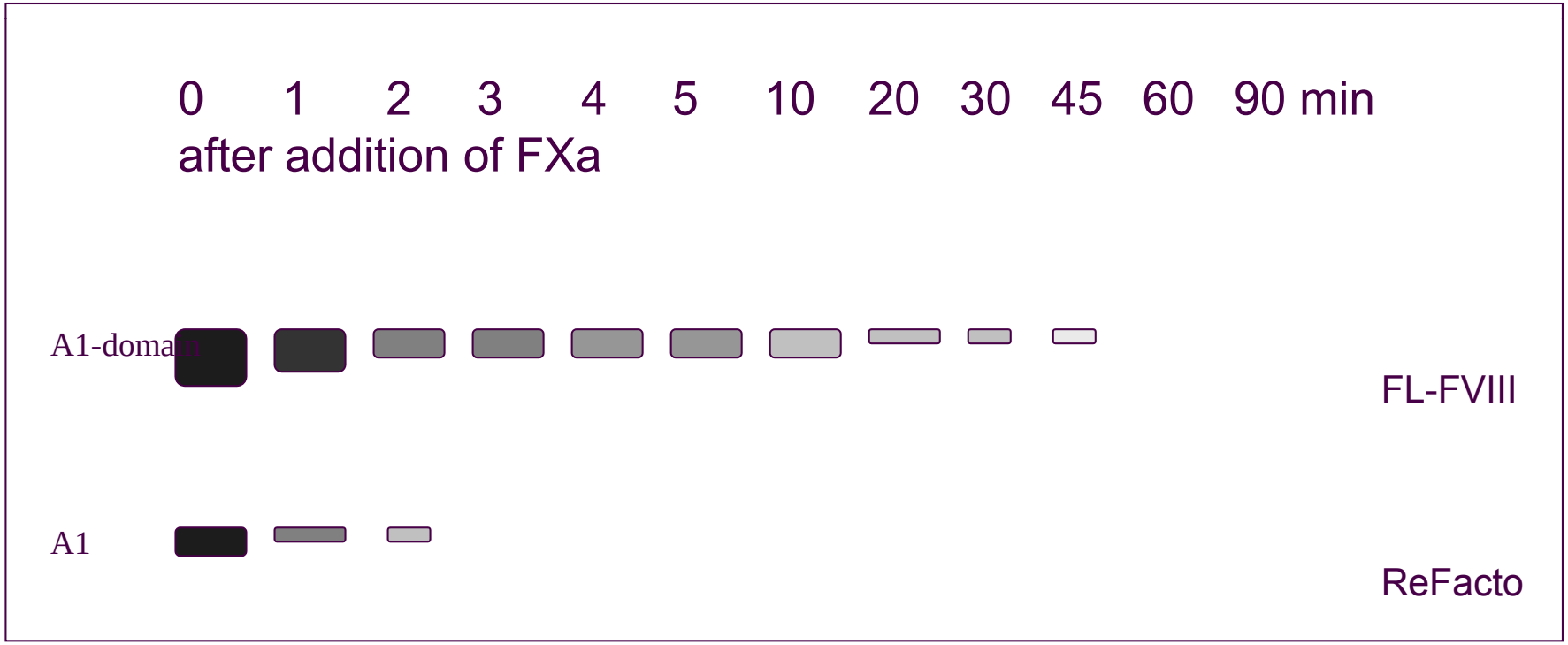
Mab C5 binding site on A1: 351-365



Khrenov AV et al. (2006) Role of the B domain in proteolytic inactivation of activated coagulation factor VIII by activated protein C and activated factor X. Blood Coagulation and Fibrinolysis 17(5):379-388

Mab C5 binding site on A1: 351-365





Khrenov AV et al. (2006) Role of the B domain in proteolytic inactivation of activated coagulation factor VIII by activated protein C and activated factor X. Blood Coagulation and Fibrinolysis 17(5):379-388

Product	Baxter ADVATE	Baxter Recombinate	Bayer & CSL Behring Kogenate FS Helixate NexGen	Wyeth ReFacto	VWF containing Pd-FVIII
FVIII structure	Full-length rFVIII	Full-length rFVIII	Full-length rFVIII	B-domain depleted rFVIII	Full-length natural or WT pdFVIII

Disadvantage for ReFacto: b-domain plays a role in FVIII folding (Pipe) and in FVIII inactivation by APC (Khrenov; FF023/HC; Hot Topic#29). Causing insecurity against b-depleted concept.

Functional role of the FVIII B-domain is summarized in Scientific series #6 brochure by S. Pipe

Downside: Function of B-domain is scientific discussion; direct link to clinical consequences remains difficult.

Advantages for ReFacto: higher rFVIII yield; lower price possible; nano filtration possible;

Advantages for „natural“ pdFVIII; „natural“ glycosylation pattern of the FVIII molecule; better VWF binding capacity?

CLINICAL EFFICACY

Pharmacokinetic

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	Half life	Study size	Data source
Recombinate	14.7	61	White et al 1997
Hemofil M	14.7	61	
Recombinate	11.24	30	Tarantino et al 2004
ADVATE	11.98	30	
(pdFVIII) Hemofil M	(13.1) 13.7	(18) 18	(ReFacto EPAR)
ReFacto	(14.1) 14.8	(18) 18	Kessler et al 2005
Kogenate	15	17	Schwartz et al 1990
Koate HS	13.3	17	
Kogenate (EU) US	(14.3) 13.9	(15) 20	Kogenate FS EPAR
Kogenate FS (EU)US	(14.2) 13.2	(15) 20	

Answer: No

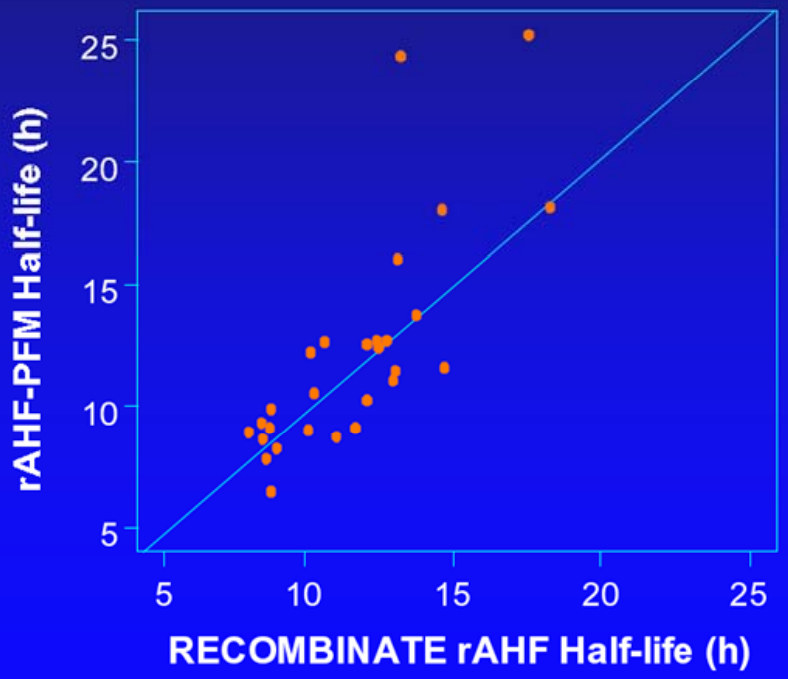
- Only data from cross-over studies can be compared and show bioeqivalency
- Different mean half lives reflect variable PK values of different study populations
- Older studies show tendency to longer half life due to a change in PK calculation methods. (today: removal of extremes)
- PK results from ADVATE and Recombinate in a cross over study were not significantly different.
-

PTP Pivotal Study Interim Report

Pharmacokinetics Part 1: Half-life

Per-protocol analysis (n=30)

7-26h



8-18h

Data on file. Baxter Healthcare Corporation.

Parameter	RECOMBINATE	ADVATE
$AUC_{0\text{-}48h^*}$ (IU•h/dL)	1530 ± 380	1534 ± 436
Adjusted recovery	2.6 ± 0.5 (1.5 – 3.9)	2.4 ± 0.5 (1.5 – 3.9)
Half-life (h)	11.2 ± 2.5	12.0 ± 4.3

Tarantino et al. (2004) Haemophilia 10: 1-10

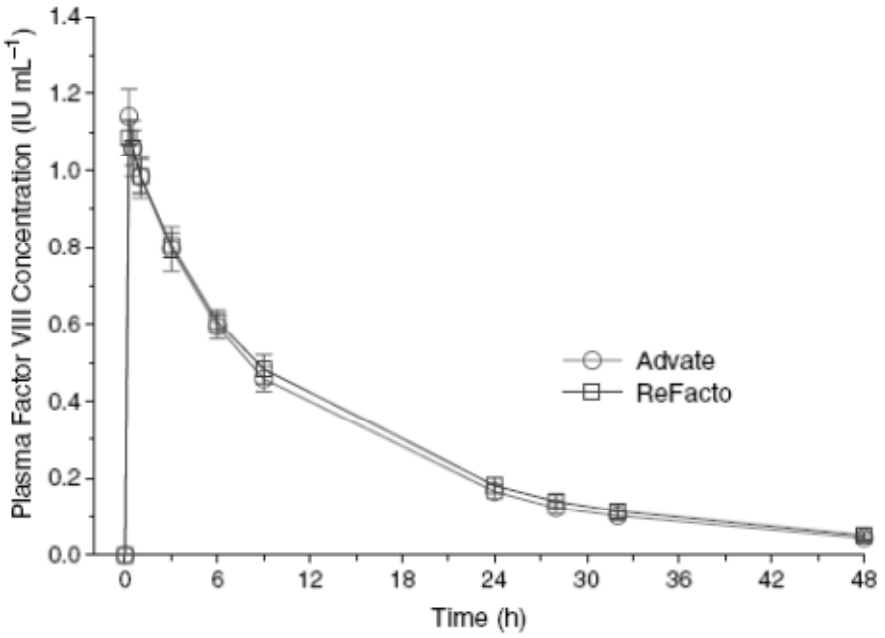


Fig. 1. Mean (±SE) plasma FVIII concentration (IU mL^{-1}) vs. time profiles of ReFacto and Advate in subjects ($n = 17$) with haemophilia A.

Note that a PK study is done in a non bleeding situation.

ReFacto and ADVATE may be bioequivalent regarding PK parameters but the pharmacodynamic of the two FVIII molecules (in a bleeding situation) seems to be different
(Khrenov see slides 19-22)

J. DI PAOLA et al. ReFacto and Advate: a single-dose, randomized, two-period crossover pharmacokinetics study in subjects with haemophilia A. Haemophilia (2007), 13, 124–130

Khrenov AV et al. (2006) Role of the B domain in proteolytic inactivation of activated coagulation factor VIII by activated protein C and activated factor X. Blood Coagulation and Fibrinolysis 17(5):379-388

FF 023/HC and Hot Topic # 29

CLINICAL EFFICACY

- % of bleeds solved with 1 infusion
- Hemostasis ratings (excellent, good, fair, none) based on the speed to stop bleeding and pain relief

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Product	% Bleeds solved with 1 infusion	% Bleeds solved with 1 or 2 infusion	% Bleeds solved with 3 infusion	% Bleeds solved with >4 infusion
ADVATE[1]	81	93	3	4
Recombinate[2]				
Kogenate[3]	75.5 (82 /73.9)	90 (94)	5.6	4.5
Kogenate FS US[4]		95		
Kogenate FS EU[4]	71	91		
Kogenate FS Japan[7]	80.5	88.7		
ReFacto prophylaxis[5]		81.7		
ReFacto on demand[5]		95.2		
ReFacto[6] PTP study	73			

No significant differences between products

[1] Tarantino et al 2004 [2] White et al 1997 [3]Aygören-Pürsün 1997 or Seremetis 1999 or Schwartz 1990 [4]Kogenate Bayer EPAR
[5]Giangrande 2004 [6]Lusher 2005 [7]Yoshioka 2001

Product	% Bleeds rated excellent	% Bleeds rated good or excellent	% Bleeds rated fair	% Bleeds rated no response
ADVATE[1]		86	12	0 (1 case)
Recombinate[2]	36.2	91.2	7.2	0.89
Kogenate[3]		89.8	10.2	
Kogenate FS US[4]	23.9	83.0	15.8	0.8
Kogenate FS EU[4]	17.4	75.8	16.6	0.9
Kogenate FS Japan[8]		98		
ReFacto (Courter)[5]		92	7	1.0
ReFacto (prophylaxis)[6]	48	93		7.0
ReFacto PTP study[7]		95		

1. Tarantino et al 2004 2. White et al 1997 3. Aygören-Pürsün 1997 4. Kogenate Bayer EPAR
5. Courter & Bedrosian 2001 6. Giangrande 2004 7.Lusher 2005 8. Yoshioka 2001

4.4 Special warnings and special precautions for use

…Reports of lack of effect, mainly in prophylaxis patients, have been received in the clinical trials and in the post-marketing setting. The reported lack of effect has been described as bleeding into target joints, bleeding into new joints or a subjective feeling by the patient of new onset bleeding. When switching to ReFacto it is important to individually titrate and monitor each patient's dose in order to ensure an adequate therapeutic response.

ReFacto European Public Assessment Report

http://www.emea.europa.eu/humandocs/Humans/EPAR/refacto/refacto.htm

Data that have contributed to the ReFacto label change

Time period: April 13, 1999 to April 12, 2003 (4 years update)

Patients treated: approximately 5,800 (± 1000) patients used ReFacto in the post-marketing setting

AE reports: 85 post-marketing reports of less than desired therapeutic effect and/or low recovery with ReFacto, for which concurrent inhibitor data is negative or not provided have been received from 81 patients (1.2-1.7% of patients)

Compared to ADVATE PV: 15 reports after 3566 patients treated (0.4% or 0.14% after exclusion of Brackmann)

Events reported

- bleeding into target joints or into new joints
- other bleeding or subjective feelings by patients of new onset bleeding
- low FVIII recovery or bleeding despite the absence of inhibitors
- increased bruising or sensation of bleeding into target joints
- required higher than anticipated doses for bleed resolution.
- increased bleeding after switching therapy to ReFacto.

Reporting rates determined on the basis of spontaneously reported post-marketing adverse events are generally presumed to underestimate the risks associated with drug treatments.

http://www.hc-sc.gc.ca/hpfb-dgpsa/tpd-dpt/refacto_hpc_e.html

Patient: 28-year-old male with severe hemophilia A

switched from Recombinate to ReFacto for prophylaxis

Result: despite administered ReFacto every 48h, daily injections of 28 IU/kg

were necessary to treat 3 new hemarthroses

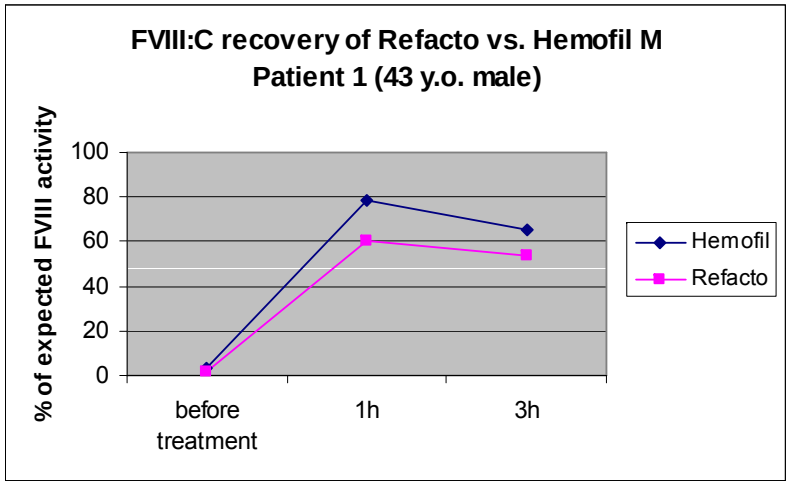
rFVIII recovery was 1.68 %/IU/kg and half life just 6h

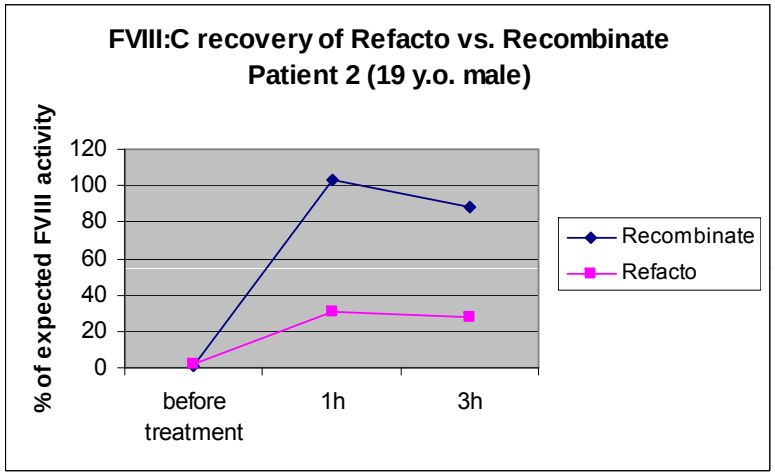
Patient wanted to be switched back to Recombinate

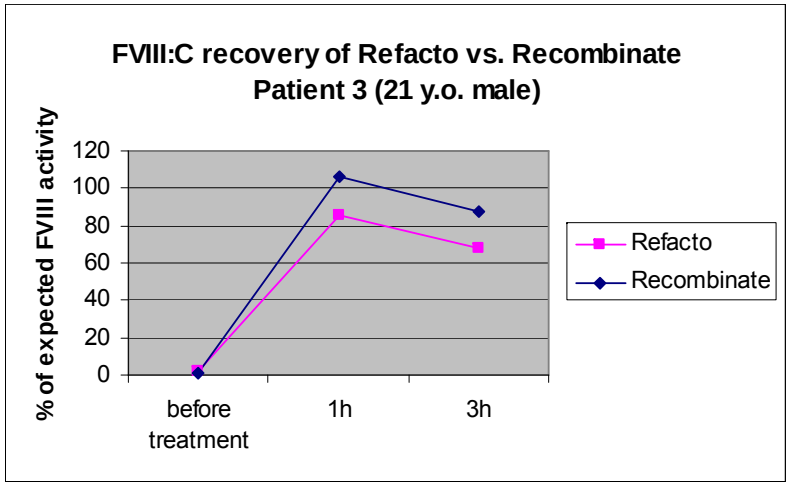
Prophylactic treatment every 48h cleared up joint bleedings in 10 days

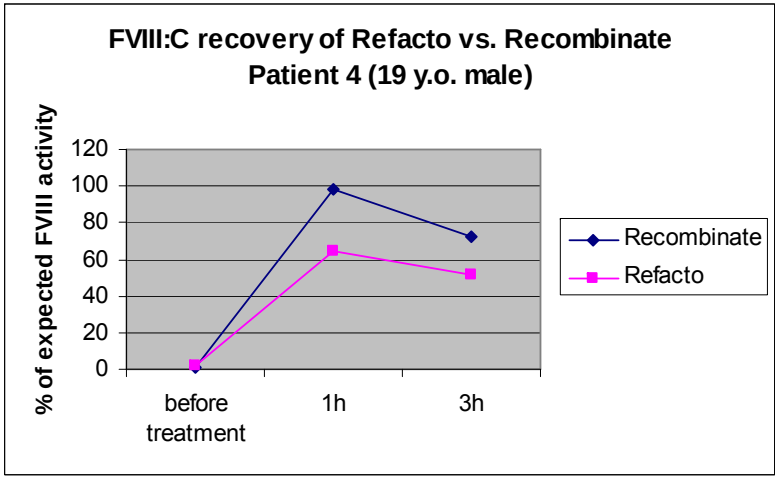
rFVIII recovery was 2%/IU/kg and half life was 12 hours

Peynet J et al (2002) Clinical and Biological Failure of Prophylactic Treatment with B-Domain Deleted Recombinant FVIII in a Severe Haemophilia A Patient without Inhibitor. Presented at Deauville – Maladies hemorragiques hemostase au feminin; May 2002 and abstract presented at the 44th Annual congress of the American Society of Hematology 2002, published in Blood 100 (11) (abstract nr.:3904)









Hanna & Zimmerman (2001) Lack of Adequate Bleed Prevention and FVIII Activity Recovery with Refacto: A Series of Case Studies in Hemophilia A Patients. Blood 98 (Suppl 1): 531a, 2001

Product	Baxter ADVATE	Baxter Recombinate	Bayer & CSL Behring Kogenate FS Helixate NexGen	Wyeth ReFacto	VWF containing Pd-FVIII
Clinical Efficacy	93% bleeds resolved with 1-2 infusions Half live: 9.7 +/-1.9 (children) 11. 8 +/- 3.1 (adults)	91% rated excellent or good (PTPs) Half live: 14.7 +/-4.9 (adults)	95% bleeds resolved with 1-2 infusions Half live: 13.25 +/-1.56 CI licensed	93% bleeds resolved with 1-3 infusions Half live: 14.8+/- 5.6 Warning statement Lack of effect in prophylaxis	Half live: 12-14 h

Disadvantage for ReFacto due to B-depletion

- Lack of effect in prophylaxis (PI warning statement);

Wyeth‘s rebuttal:

- Development of ReFacto specific concentrate standard to overcome FVIII assay issues (Hot Topic#16)
- Increase of FVIII in each vial by 20% (Hot Topic#16)
- PK cross-over study demonstrated bioequivalency with Hemofil M and ADVATE (Hot Topic#16)

Advantage for Kogenate and Helixate: licensure of continuous infusion – is this a real advantage?

CLINICAL SAFETY

- Inhibitors in PUPs

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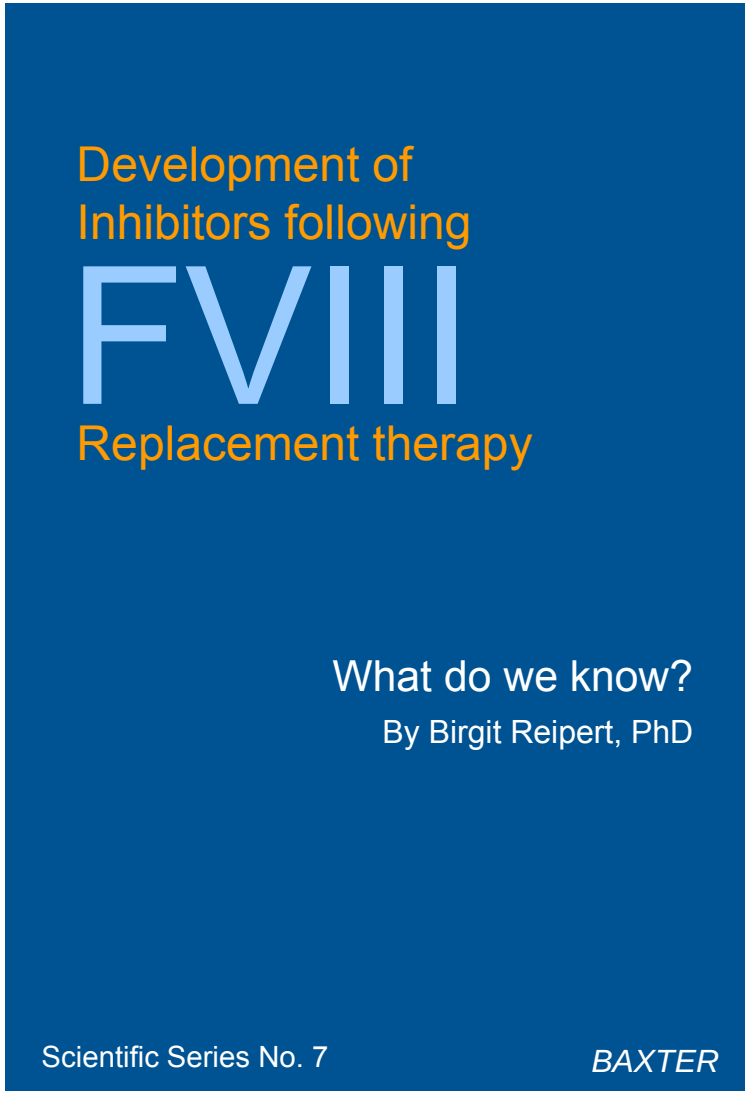

Scientific Series No. 7
Fast Fact 035/HC

Can be ordered via the GCMA website:

http://httpeur.europe.baxter.com/bioscience/NewBrochure/ListBook.asp?GroupID=50

Reasons why KOGENATE FS is not safer than ADVATE and RECOMBINATE

- PUPs are not a suitable population to study product-related immunogenicity (EMEA clinical guidelines)
- Due to significant variations in study design PUP studies cannot be directly compared
- The Kogenate FS PUP study included less PUPs with African origin, a patient group with a well-known increased inhibitor risk
- More PUPs in the Kogenate FS study (70%) were set on prophylaxis, a treatment-related factor known to reduce inhibitor risk. Interestingly all inhibitors occured in the on demand group.
- The Kogenate FS study monitored only the first 20 FVIII exposure days. Inhibitors that developed after that were not counted.

For more details please refer to brochure Scientific Series # 7 (by B. Reipert)

The most recent PUP study had a larger number of patients who switched to prophylaxis during study

	Kogenate FS	Kogenate	Recombinate
Study Period	1997-2001	1989-1997	1990-1998
No. (%) of prophy. patient at study end	43 (70%)	N/A	23 (32%)*
No. (%) of inhibitor patients on an on demand regimen	9 (100%)	N/A	20 (91%)

* 6/73 (8%) switched prior to 20 EDs; 19/73 (26%) switched prior to 50 EDs

Kreuz et al (2005) Thromb Haemost 93:457-467; Lusher JM et al (1993) n Engl J Med 328:453-459;
Bray GL et al(1994) Blood 83 (9):2428-2435

Patients are generally at highest risk for inhibitor development within the first 50 EDs;

most, *but not all*, develop within the first 20 EDs

	Kogenate FS	Kogenate	Recombinate
Observation Period	2 years or 20 EDs	5 years or 100 EDs	5 years or 100 EDs
EDs prior to inhibitor development: range	(3 - *18*)	(3 - *54*)	(3 – *69*)
No. (%) of patients w/out 20 EDs	8 (13%)	N/A	3 (4%)

Kreuz et al (2005) Thromb Haemost 93:457-467; Lusher JM et al (1993) n Engl J Med 328:453-459;

Bray GL et al(1994) Blood 83 (9):2428-2435

Blacks and Hispanics are at highest risk for inhibitor development

	Kogenate FS	Kogenate	Recombinate
No. (%) of blacks and hispanics	5 (8%)	14 (22%)	19 (26%)
No. (%) of blacks and hispanics w/ inhibitors	2 (40%)	6 (43%)	8 (42%)

Kreuz et al (2005) Thromb Haemost 93:457-467; Lusher JM et al (1993) n Engl J Med 328:453-459;
Bray GL et al(1994) Blood 83 (9):2428-2435

CLINICAL SAFETY

- Inhibitors in PTPs

ReFacto

The risk of developing inhibitors is correlated to the exposure to antihaemophilic factor VIII, this risk being highest within the first 20 exposure days. In the postmarketing setting, high and low titre inhibitors have been observed in previously treated patients.

Cases of recurrence of inhibitors (low titre) have been observed after switching from one recombinant factor VIII product to another in previously treated patients with more than 100 exposure days who have a history of inhibitor development. Patients treated with recombinant coagulation factor VIII should be carefully monitored for the development of inhibitors by appropriate clinical observations and laboratory tests.…

4.8. …Also there have been spontaneous postmarketing reports of high titre inhibitors involving previously treated patients.

ADVATE, Kogenate Bayer, and Helixate NexGen

Rarely, inhibitors may develop after the first 100 exposure days.

Cases of recurrence of inhibitors (low titre) have been observed after switching from one recombinant Factor VIII product to another in previously treated patients with more than 100 exposure days who have a history of inhibitor development. Patients treated with recombinant coagulation Factor VIII should be carefully monitored for the development of inhibitors by appropriate clinical observations and laboratory tests.

European Public Assessment Reports on the EMEA website:
http://www.emea. europa.eu/humandocs/Humans/EPAR/advate/advate.htm
http://www.emea.europa.eu/humandocs/Humans/EPAR/kogenatebayer/kogenatebayer.htm
http://www.emea.europa.eu/humandocs/Humans/EPAR/refacto/refacto.htm
As of February 27, 2008

Data that have contributed to the ReFacto label change

Factor VIII inhibitor detected in 4 hemophilic subjects
on ReFacto

Patients: 3 PTPs aged 27, 33 and 66 with severe hemophilia A
1 PTP aged 46 with mild hemophilia A
Inhibitor was detected 28, 19, 63 and 14 days after exposure to ReFacto
Inhibitor titers were 0.9 BU, 5.3 BU, 40 BU and 1.8 BU

For 2 patients bypassing agents were needed
the other 2 were treated with elevated ReFacto doses.

Roussel-Robert V et al (2003) Factor VIII inhibitors development following introduction of B-domain-deleted recombinant factors VIII in four hemophilia A previously treated patients.
Presented at Deauville – Maladies hemorragiques hemostase au feminin, May 2002; published in J Thromb Haemost 1: 11, 2450-2451

Product	Baxter ADVATE	Baxter Recombinate	Bayer & CSL Behring Kogenate FS Helixate NexGen	Wyeth ReFacto	VWF containing Pd-FVIII
Clinical Safety (Inhibitors)	PTPs: 1/145 (0.7%) PUPs: 5/25 (20%)	PTPs: 0/69 (0%) PUPs: 22/73 (30%)	PTPs: 1 recurrent/71 PUPs: 9/60 (15%)	PTPs: 1/113 (0.9%) PUPs: 32/101 (32%) Warning statement EMEA workshop outcome	PTPs: most 0 PUPs: 4-26%

Discussion

Disadvantage for ReFacto: High titer inhibitors in PTPs (warning statement and EMEA workshop data)

Claimed advantage for Kogenate and Helixate: Bayer claims for Kogenate the lowest inhibitor rate (15%) in PUPs from all rFVIII concentrates.

Claimed advantage for pd VWF containing FVIII concentrates: Pd VWF-containing FVIII have been discussed as less immunogenic in PUPs (Goudemand (FF022/HC and FF025/HC) and Chalmers (FF029/HC) versus CANAL study; (FF026HC and FF028HC; Hot Topic#31)) and more successful in ITI.

Our view on inhibitor development is summarized in Scientific Series #7

and Fast Fact 035/HC

CONVENIENCE

evropský sociální fond v ČR
EVROPSKÁ UNIE
MINISTERSTVO ŠKOLSTVÍ, MLÁDEŽE A TĚLOVÝCHOVY
OP Vzdělávání pro konkurenceschopnost
INVESTICE DO ROZVOJE VZDĚLÁVÁNÍ

Product	Baxter ADVATE	Baxter Recombinate	Bayer & CSL Behring Kogenate FS Helixate NexGen	Wyeth ReFacto	VWF containing Pd-FVIII
Convenience • Infusion volume • Potency range • RT storage • Reconstitution devices	5 ml 250-3000 IU [1] 24 m / 2 m RT BaxJect II	10 ml 250-1000 IU 24 m / 24-36 m RT BaxJect II	2.5 ml/5ml 250-2000 IU 30m / 3 m RT Bioset/Mix2vial	4 ml 250-2000 IU 24m / 3 m RT Pre-filled syringe	5-10ml 250-1000 IU 0 to 3 years RT (Fanhdi/Grifols) Needle systems

- Preferred infusion volume is customer specific (Nordic versus US) What is better? 5 ml is best balance between infusion rate/time and loss of product.
  ADVATE and Recombinate have the fastest infusion time (10ml/minute)
- Wide potency range (250-3000 IU) is seen as advantage – increase of flexibility; reduction of infusion time
- RT the longer the better - increase of flexibility (travel)
- Preference for devices customer specific – different surveys give different results. Prefilled syringes are made of glass (disadvantage). But Baxter‘s BaxJect II is preferred by many patients and nurses (market survey)

[1] 2000 IU and 3000 IU approved in the US, expected for Europe May 2008

	Submission	Approval
2000 and 3000 IU potencies		Q2/2008
6 months RT		Q4/2008
CI indication (15 prospective patients)	Q4/2008	Q2/2009
ITI indication	Q2/2009	Q4/2009

Reconstitution devices

- All in one syringe: Lyoject – for those who use only one vial was good
- PressMix device (pre-assembled BaxJect II) „one shot“ was overwhelmingly preferred

expected Q1/2010

2ml diluent choice for pediatric use

expected 2011

COMPANY REPUTATION

Leadership is..

..to have a vision for the future hemophilia treatment (research, innovation)

..to develop product improvements (longer half life etc)

..to show responsibility for pricing policy (social responsibility)

..to be a reliable company (research study data)

..to provide continuous funding of research studies, patient and physicians education and support (committment to the community, service)

BAXTER

evropský sociální fond v ČR
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Baxter has a tradition in developing advanced FVIII treatment

with “firsts” in each product category

1) Hemofil M (pdFVIII) (first mab purified, first SD treated pdFVIII)

2) Recombinate (first rFVIII) (also claimed by Wyeth as Recombinate was developed by Genetic Institutes, which today is part of Wyeth)

3) ADVATE (first plasma & albumin free rFVIII)
Wyeth had the first plasma/albumin free rFIX (BeneFIX)

1) rVWF for the treatment of VWD (by-product of the ADVATE production)
PK study in humans to be started in 2008

2) rFVIII with improved PK properties (4 programs: pegylated rFVIII, pegylated rVWF, rFVIII or rVWF with different glycosylation)
Clinical studies to be started 2008 probably with >1 product

3) Mimetic proteins
Low MW peptides that are able to mimic FVIII function in clotting cascade
(Partner Jerini AG, Germany)

4) rADAMTS 13 for treatment of TTP (thrombotic thrombocytopenic purpurea)
= super orphan drug status
We currently look into potential additional indications

Presented at Study Update Meetings in Portugal, at WFH 2006 and ISTH 2007

Latest update at HRSU in Rome (April 2008)

BAYER

1) Projects for FVIII with prolonged half-life

Goal: effective prophylaxis with significantly reduced FVIII application

- Clearance receptor binding mutations (principle: weaker binding to receptors results in slower clearance) – LRP, HSPG
- Activity mutations of FVIII
  - S-S bridge between A2 and A3 domains slows down deactivation of FVIII (Radtke 2007. Thromb Haemost 5:102)
  - Site specific pegylation (steric hindrance of ligand binding); PK in mice and rabbits showed 2-fold increase of half-life
  - Unspecific pegylation (PegLip Kogenate FS = BAY 79-4980) partnering with Recoly)

2) Gene therapy (partnering with AskBios)

- FIX
- Biological nano particles (BNP, capside chimers)
- Projects to increase efficiency of gene transfer

Presented by Dr. Lemm (Bayer Berkely) at GTH 2007

Powell JS et al. Safety and pharmacokinetics of a recombinant factor VIII with pegylated liposomes in severe hemophilia A. Pre-published on-line: doi:10.1111/j.1538-7836.2007.02856.x ; accepted for J Thromb Haemost 2007.

Fast Fact 033/HC

Spira J, Plyushch OP, Andreeva TA, Andreev Y. Prolonged bleeding-free period following prophylactic infusion of recombinant factor VIII (Kogenate FS) reconstituted with pegylated liposomes. *Blood* 108 (12): 3668-3673; 2006;

Fast Fact 001/KC&BY

- PEGLip Kogenate FS still based on a 2nd generation rFVIII = not plasma/albumin free
- The mechanism of action (MoA) for the clinical effect (prolonged bleed free time after prophylactic dose) observed in the study is unknown.
  PK parameters (recovery and half-life not improved in humans)
- There was a statistically significant increase in LDL cholesterol levels following the 2nd infusion of Kog-FS PEGLip. This raises an important safety question regarding what the cumulative effect of a 1X week prophylaxis regimen of Kog-FS PEGLip would be when used over an extended period of time.
- Infusion via pumps over 20-30 minutes – not very convenient
- Patient population not comparable with W-EU standards. One inclusion criterion for patients in this study was at least 4 bleeds/months which corresponds to 48 bleeds per year. Patients with this high bleeding frequency are not available in developed countries in Western Europe. This shows that patients are obviously extremely undertreated when they started the study. Therefore any treatment should reduce the number of bleeds and thereby prolonging the bleed free period.

Planned: 250 patients from 63 centers in 14 countries

Study arm 1: 35 IU/kg BAY 80 once a week

Study arm 2: 25 IU/kg Kogenate FS 3 times a week

Bleeds are treated with BAY 80 or Kogenate FS respectively

Randomized and double blind

Challenges: milky (BAY 80) versus clear (Kogenate FS) solution

35 versus 25 IU/kg

1x versus 3 infusions per week

Solution: Use Kogenate FS in liposomes without PEG (milky)

Use liposome buffer without FVIII as mock infusions 2 times a week

Bayer hemophilia Award program (launched in 2002)

http://www.bayer-hemophilia-awards.com/about_the_program.cfm

The Bayer Hemophilia Awards Program supports basic and clinical research and education in hemophilia. Through grants provided to early career investigators, fellows in training, and other hemophilia care professionals, the program seeks to support the next steps for the next generation of care and treatment options for people with hemophilia worldwide.

Spending: $2.7 mil per year for

- Special project awards
- Early career investigator awared
- Clinical training award
- Hemophilia caregivers edication award

WYETH

In 2003 the FDA set as an upper limit for inhibitor formation of < 6.8% which translates into maximal 1 inhibitor in n=80 study subjects.

ReFacto AF Pivotal Trial (Protocol 306) was considered a failed trial by the FDA for exceeding the proscribed safety criterion. The ReFacto AF trial demonstrated 3 inhibitor cases in ~90 patients.

(BASS XII, Savannah, Georgia, Nov 8-11, 2005; annual Biopharmaceutical applied statistics symposium; http://bass.georgiasouthern.edu/presentations2005.html. Presentation by David Huang (Wyeth)

Wyeth triggered discussions about patient- and product-related inhibitor risk factors and proposed a different methodology for the assessment of inhibitor development in PTP studies (current method: confidence interval approach; proposed method: Bayesian approach).

Main argument: with the CI approach currently licensed and safe products such as Recombinate and Kogenate FS would not meet safety requirements. With Bayesian approach all licensed products would meet requirements.

Study name: ReFacto AF (albumin free)
Brand name in the US: XYNTHA (licensed in Feb 2008)

Is a plasma/albumin free 3rd generation rFVIII product similar to ADVATE

B-chain depleted rFVIII produced in CHO cells without addition of human or animal-derived proteins and without the use of mouse mab (ligand technology); stabilized by sucrose.
Addition of a nanofiltration step(only suitable for b-depleted FVIII molecules)

Global Q&A available (please contact Paul Woo from Global Marketing);
Hot Topic Training planned

ReFacto FS = XYNTHA

..from the Q&A

Comment: Please do not use the brand name XYNTHA in order not to help Wyeth diverting from the safety and efficacy issues experienced with ReFacto.

ReFacto AF might be the brand name in EU

- Cooperation with France‘s Nautilus for a longer half life FIX (by changes in aa sequence in order to slow down breakdown of protein and improve duration of the drug (The Associated Press ; February 5, 2007)
- „Wyeth – World‘s 4th largest biopharmaceutical company – builds on committment to innovation in hemophilia“ (DrugNewswire; Feb. 5, 2007)
- Wyeth sign research agreement with MediVas LLC (San Diego, Ca) to develop haemophilia treatments (AFX News; Feb. 5, 2007)
- MediVas: unique polymer-based drug delivery system to develop advanced delivery methods (non IV) for rec. Hemophilia products and improve patient convenience through the creation of a longer half-life for these proteins (Earthtimes.org; Feb. 5, 2007)

Not much details published yet

OCTAPHARMA

Octapharma's R&D offers exciting new therapeutic approach to management of haemophilia

OCTAGENE GmbH Biomedical Laboratories (OCTAGENE GmbH) is a privately owned biotechnology company specializing in the development of new treatments against haemophilia. It was founded in 1997 by Dr. Charlotte Hauser as a joint venture with Octapharma. Dr. Hauser, the managing director of OCTAGENE GmbH has long experience in the research of recombinant proteins and gene therapy. The strategic alliance provides OCTAGENE with state-of-the-art technologies and enables an international exchange of know-how. The company is located in Martinsried near Munich, Germany, a place that has already acquired a world-wide reputation as a leading biotechnology research centre.

OCTAGENE GmbH is a product-oriented R&D company whose main focus is the production of novel recombinant clotting factors and gene therapy in order to discover alternatives to the customary substitution therapy.

Recombinant clotting factors are manufactured biosynthetically. This eliminates any risk of viral contamination. OCTAGENE is focusing on the improvement of recombinant clotting factors with respect to human compatibility. Unlike all other recombinant clotting factors that are based on rodent cell lines OCTAGENE´s recombinant factor VIII has been developed on the basis of a human cell line. Preliminary studies in animals have demonstrated that this product (Octagene SF®) displays stability and clearance kinetics comparable to those of a licensed recombinant FVIII, indicating that the protein is physiologically active in vivo and providing strong support for its therapeutic potential in humans.. It is anticipated that OCTAGENE SF will begin clinical development within the next one or two years.

In parallel, OCTAGENE is pursuing an alternative strategy: gene therapy as a completely new form of treatment.

OCTAGENE´s concept is based on the fact that specific hormones which are given orally can penetrate the cell membrane. The hormone binds onto a hormone receptor which in turn is coupled with the DNA that defines the genetic blueprint for the production of the clotting factor in the cell.

Oral application of gene therapy offers enormous benefits to patients, not least of which will be the avoidance of repeated painful, time-consuming infusions.

H. Sandberg (Stockholm): “A novel rFVIII expressed in a human cell line”

The rational to have rFVIII produced in human cells is that the glycosylation pattern of the protein would be more natural and this could improve the function. rFVIII produced in hamster cell lines are known to differ slightly in their glycosylation from human FVIII.

Details on the new rFVIII:

- B-domain depleted
- Expressed in the human embryotic kidney cell line HEK 293 F that was established 10 years ago and is also used for production of activated protein C (XIGRIS by Ely Lilly).
- Cell culture medium free of animal-sourced material
- No animal-sourced protein used

} what about human albumin?

- Purification via 5 chromatography steps (IA, IE, size exclusion)
- 2 virus inactivation steps (SD, nanofiltration)
- Specific activity: 10,000 IU/mg protein
- rFVIII is fully sialyated and does not express Neu5Gc such as the hamster cell produced rFVIII (“more natural gylcosylation pattern”).
- New rFVIII has a higher affinity to VWF whereas PL binding, thrombin generation, 1-stage and chromogenic assays are comparable with other rFVIII. This was discussed as a potential disadvantage that can cause a reduced efficacy.
- Pre-clinical studies have demonstrated no toxicity of the new rFVIII (acute tox in rats, local tolerance in rabbits, repeat dose tox studies in cynomolgus monkeys)
- Infusion of the new rFVIII into hemophilic dogs resulted in normal FVIII recovery and half life.
- Product developed in cooperation with Octagene Munich, Octapharma Stockholm, Sweden and David Lillicrap, Kingston, Canada.

- rFVIII yield was too low in human cell line. Using a B-depleted FVIII concept is known to increase rFVIII release by cells.

- Production in human cells requires better virus inactivation or removal. The B-depleted FVIII molecule can be nano filtrated.

New approach to prolong half life of complex coagulation factors:
fusion of human albumin with other proteins to prolong their circulation half lives.

Rationale for the albumin fusion concept

- Albumin has a circulation half life of 20 days
- Albumin is the main protein in plasma (42g/l)
- The molecule structure of albumin is known
- Albumin-cytokine fusions have worked already

Results

- rFVIIa and albumin fusion is done on DNA level. The fusion protein is then produced in CHO cells
- PK studies in rats have demonstrated a 6-fold prolonged half life and a 2-fold increased recovery

	Half life in rats	In vivo recovery in rats
Albumin	620 min	106%
rFVIIa (NovoSeven)	40-45 min	20-35%
rAlbumin-rFVIIa fusion protein	262 min	47%

- Fusion protein has somewhat (70-90%) reduced activity which can be optimized by the length of the linker between albumin and rFVIIa.
- BT could be normalized in a ROTEM system when the fusion protein was added to a human inhibitor plasma. The reduced activity was automatically solved by the increased recovery.

Challenges by the audience

- Is it possible that the linker between albumin and FVIIa is immunogenic?
- Could the system also work with FVIII and would an albumin-FVIII fusion be able to bind VWF?

Product	Baxter	Bayer	CSL Behring	Wyeth	Plasma supplier
Company Image (leadership)	Large R&D program presented: • pegylated ADVATE (longer half life product) • rVWF • Mimetics (non IV)	Large R&D program presented: • Next gen Kogenate pegylated liposomes (longer half life) already in clinical studies • FVIII mutations (of clearance receptors to slow down clearance) • site-specific pegylation • Genetherapy (FIX)	R&D project presented at GTH 2008 in Germany: • rFVIIa/albumin fusion with 6x longer half life in rats • Leader in VWD	XYNTHA (3rd gen rFVIII licensed in the US) Cooperation with Nautilus (longer lasting mutant of rFIX; longer half-life and non IV projects)	B-domain depleted rFVIII (Octapharma) From a human cell line (HEK 293F embryotic kidney cell) Claim: natural glycosylation and better VWF binding affinity ; Preclinical studies in dogs done. (GTH presentation 2008)

- Disadvantage in leadership for plasma suppliers due to lack of latest rec. technology? However Octapharma is working on a B-depleted rFVIII produced by human cells
- Baxter and Bayer started to disclose R&D programs very early before product enters clinical studies – Question is how project failures are rated by customers?
- Potential advantage for Wyeth: only company with rFVIII and rFIX in their portfolio (could also be a disadvantage due to a fair share policy)

COMPANY CLAIMS

1) Baxter developed Hemofil M, the first mab purified SD treated pdFVIII

2) Baxter and Wyeth claim to have been the first with rFVIII (Recombinate)
(Fact is that Recombinate was developed by Genetic Institutes, which today is part of Wyeth, Baxter was the first who marketed Recombinate)

3) Bayer and Wyeth claim to have been the first with an albumin free formulated rFVIII (Kogenate FS and ReFacto)

4) Wyeth claims to be first with a b-domain depleted rFVIII, the concept of the future (because gene therapy projects all with b-depleted rFVIII).

5) Baxter had the first plasma/albumin free rFVIII (ADVATE)

6) Wyeth had the first plasma/albumin free rFIX (BeneFIX)

7) Bayer had the first rFVIII with longer half life in clinical studies.

….

	Baxter	Bayer	CSL Behring	Wyeth	Octapharma
Company Image (leadership)	Leader in Hemophilia	Leader in Hemophilia and in Hemostasiology	Leader in VWD (Humate/Hemate)	Leader in rec. technology and only company with full portfolio (rFVIII and rFIX)	Partner in plasma fractionation Leader in VWD (Wilate) and in ITI

General considerations

- All companies do claim leadership by presenting innovative products from their R&D pipeline
- Lack of the latest recombinant technology is seen as a disadvantage in leadership. That is why Octapharma and CSL Behring also started to present their recombinant products to come (B-depleted rFVIII produced by human cells and a rFVIIa with longer half life).
- The disclosure of new products before clinical study results are available bears two risk factors: a) how will customers rate project failures? and b) competitors can be motivated to start similar projects.
- The advantage to have a full recombinant hemophilia portfolio (rFVIII and rFIX like Wyeth) can also be a disadvantage when fair share policy plays a role
- Being a leader may not everywhere be perceived as a positive trait. Being a partner may be the higher valued feature.