(参考資料3) バイエル薬品(株)提出資料

バイエル薬品の遺伝子組換え第 VIII 因子製剤　インヒビターの調査結果報告
(2006 年 7 月作成)

1. バイエル薬品の遺伝子組換え第 VIII 因子製剤

バイエル薬品の遺伝子組換え第 VIII 因子製剤であるコージネイト FS（商品名）は，従来のコージネイトで安定剤として精製工程及び最終製品に添加していたヒト血清アルブミンに代えて，最終製品にショ糖を添加した製剤であり，2002 年 7 月から販売を開始して，現在に至っている（**表 1**）。

なお，コージネイトは現在販売していない。

表 1　バイエル薬品の遺伝子組み換え第 VIII 因子製剤

製品名	販売開始時期	備　　考
コージネイト	500IU：　1993 年　9 月 1000IU：1993 年 11 月 250IU：　1994 年　6 月	・　弊社，最初の遺伝子組換え第 VIII 因子製剤 ・　現在，コージネイトは販売していない。
コージネイト FS	1000IU：2002 年　7 月 500IU：　2002 年　9 月 250IU：　2002 年 11 月	・　従来のコージネイトに使用されていた安定剤のヒト血清アルブミンに代えて，ショ糖を添加。 ・　その後，凍結乾燥条件の変更，最終バルクのポリソルベート 80（界面活性剤）添加，精製カラム用モノクローナル抗体の非ヒト・動物原料への変更などを施したもの

2. コージネイト FS におけるインヒビター調査結果

2.1. これまでに実施した国内外におけるインヒビター調査結果の概要

これまでに実施した国内外におけるインヒビター調査結果を**表 2** に示す。なお，国内外の臨床試験におけるインヒビター検査は，所定の集中検査施設で実施された。

表 2 コージネイト FS / インヒビター調査結果一覧表

	調査の種類	対　象	調査実施期間	評価例数【重症】*	ｲﾝﾋﾋﾞﾀｰ発現例数【重症】*	検査最高値 (BU)			インヒビター検査
						低力価		高力価	
						0.6～≦1.0BU	1.0＜～＜5BU	5BU≦	
国内	臨床試験 [1]	PTPs	1997～ 1998	20 【15】	0	0	0	0	定期的に実施
国内	市販後の使用成績調査	PTPs PUPs	2003～2005	631 【426】	7 (1.1) 【7(1.6)】	1 (0.2) 【1】	4 (0.6) 【4】	2 (0.3) 【2】	日常診療下で実施
国内						5 (0.8)			
国外	臨床試験 [2]	PTPs	1996～1998	71 【71】	0	0	0	0	定期的に実施
国外	臨床試験 [3]**	PUPs MTPs	1997～2001	60 【60】	9 (15.0) 【9(15.0)】	0	3 (5.0) 【3】	6 (10.0) 【6】	定期的に実施
国外	市販後の調査 [4]	PTPs PUPs	2002～2005	202 【202】	2 (1.0) 【2(1.0)】	0	1 (0.5) 【1】	1 (0.5) 【1】	日常診療下で実施

PTPs: 過去に治療歴のある患者　　( )内はパーセント　BU：ベセスダ単位

PUPs: 過去に治療歴のない患者

MTPs：Minimally treated Patients（4 実投与日数以下の患者として定義した）

＊：重症血友病 A：国内調査では FVIII C<1%，国外調査では FVIII C<2%

＊＊：国外 PUPs 臨床試験ではナイメーヘン変法ベセスダ測定法（pH による誤差を除くために緩衝液を加えたもの）でインヒビターを測定

2.2. インヒビターの発現が報告された調査・試験について

2.2.1. 国内市販後の使用成績調査（PTP 及び PUP を含む）（データ最終確認中）

- 評価症例 631 例のうち，インヒビター発現例 7 例（1.1%）は，いずれも PTP で，このうち，2 例（0.3%）は高力価例（5 BU 以上）であった。PUP（17 例）ではインヒビターの発現は認められなかった。
- インヒビター発現例 7 例のうち，2 例は再発例（観察開始直前ではインヒビター陰性）で，実投与日数が 150 日以上の症例であった。4 例（実投与日数 150 日未満）は新規にインヒビターが発現した症例であり，残りの 1 例（実投与日数 150 日未満）については新規発現例か再発例かを現在調査中である。

2.2.2. 国外臨床試験/治療歴のない患者（PUP）及び 4 実投与日以下の患者（MTP）

- 本試験の評価例 60 例中，9 例（15.0%）にインヒビターが認められ，このうち，高力価例（5 BU 以上）は 6 例（10.0%）で，残り 3 例は低力価例（5.0%）であった（**表 3**）。
- 低力価例 3 例のうち，2 例（症例 11 及び 291）のインヒビターは一過性で，もう 1 例（症例 122）は免疫寛容療法が奏功した。

表 3　コージネイト FS /国外臨床試験（PUP）/インヒビター発現例一覧表

症例番号	タイプ	試験開始前 FⅧ値＊	年齢	インヒビター値（BU）	
				最高値	最終測定値
5067003	PUP	＜ 1%	0.82 ヵ月	249	25
5479003	PUP	＜ 1%	4.2 ヵ月	312	75
5579001	MTP	＜ 1%	5.5 ヵ月	154	60
5582002	PUP	＜ 1%	1.6 ヵ月	110	110
5583001	MTP	＜ 1%	19.2 ヵ月	23	19
11	MTP	＜ 1%	18.7 ヵ月	1.9	<0.6
71	PUP	＜ 1%	19.1 ヵ月	13	3.9
122	PUP	＜ 1%	13.1 ヵ月	4.0	<0.6
291	MTP	＜ 1%	11.9 ヵ月	1.3	<0.6

＊：正常値に対するパーセント　0.6 BU 以上をインヒビターと見なした。

2.2.3. 国外市販後の調査（PTP 及び PUP を含む）（中間集計）

- 評価例 202 例中に 2 例（1.0%）にインヒビターが認められた。これら 2 例は過去の治療日数 20 日未満の患者であった。
- インヒビター発現例 2 例のうち，1 例（0.5%）は高力価例（20 BU）で，もう 1 例は低力価例（2.2 BU）であった。

2.3. 国内市販後のインヒビター発現例（自発報告例も含む）

コージネイト FS は国内で 2002 年に発売して以来，延べ約 5,200 名（注）の患者に使用されており，2006 年 6 月末時点でインヒビターは自発報告も含めて 16 名の患者に報告されている。

注）推定投与患者数の算出方法；国内の第 VIII 因子製剤の全使用量（年間）÷血友病 A 患者数＝平均使用量

コージネイト又はコージネイト FS の全出荷数量÷平均使用量＝推定患者数（延べ人数）

3. 遺伝子組換え第 VIII 因子製剤のインヒビター発生に関するバイエル薬品の考え

弊社では，先頃，使用成績調査において631例のデータ収集を完了しており，その結果を医療関係者及び患者の皆様に対して情報提供する予定です。

血漿由来第VIII因子製剤と遺伝子組換え第VIII因子製剤のプロスペクティブの比較試験については，薬剤割付を無作為化するため，被験者が薬剤を選べないことから試験参加の同意取得が難しく，実際上困難であると予想されます。

一方，弊社では日常診療において確認されたインヒビター発現の報告を受けた場合には，そのすべての報告に対して担当医師に詳細調査への協力を求め，安全性情報を積極的に収集するよう努めております。このような安全性情報を集積することで，インヒビター発生要因の解明に寄与したいと考えております。

＜引用文献リスト＞

1) Safety and efficacy a new recombinant FVIII formulated with sucrose (rFVIII-FS) in patients with haemophilia A: a long-term, multicentre clinical study in Japan. A. Yoshioka et al: Haemophilia, VOL:7 No:3, 242－249, 2001.

2) Sucrose Formulated Recombinant Human Antihemophilic Factor VIII Is Safe and Efficacious for Treatment of Hemophilia A in Home Therapy. T. C. Abshire et al: Thromb Haemost, VOL:83, 811-816, 2000.

3) Full-length sucrose-formulated recombinant factor VIII for treatment of previously untreated or minimally treated young children with severe haemophilia A. Wolfhart Kreuz et al: Thromb Haemost, VOL:93, 457-467, 2005.

4) Post-Marketing Surveillance Study of KOGENATE® Bayer (Kogenate® FS) in 202 Patients with Severe Hemophilia A. Roberto Musso et al: Presentation at Hemophilia 2006 World Congress Vancouver, Canada, May 21-25, 2006.

コージネイトにおけるインヒビター調査結果
（コージネイトは現在販売していない）

1. 過去に実施した国内外におけるインヒビター調査結果の概要

過去に実施した国内外におけるインヒビター調査結果を**表 1** に示す。なお，いずれの試験・調査においてもインヒビター検査は各施設で実施された。

表 1 コージネイト / インヒビター調査結果一覧表

<table>
<tr><th rowspan="3"></th><th rowspan="3">調査の種類</th><th rowspan="3">対象</th><th rowspan="3">調査実施期間</th><th rowspan="3">評価例数
【重症】*</th><th rowspan="3">インヒビター発現例数
【重症】*</th><th colspan="3">検　査　最　高　値（BU）</th><th rowspan="3">インヒビター検査</th></tr>
<tr><th colspan="2">低力価</th><th>高力価</th></tr>
<tr><th>0.5～≦1.0BU</th><th>1.0＜～＜5BU</th><th>5BU≦</th></tr>
<tr><td rowspan="4">国内</td><td>臨床試験 1)</td><td>PTPs</td><td>1988～1991</td><td>19
【15】</td><td>0</td><td>0</td><td>0</td><td>0</td><td>定期的に実施</td></tr>
<tr><td rowspan="2">市販後特別調査</td><td>PUPs 2)</td><td>1993～1999</td><td>43
【31】</td><td>15（34.9）
【13（41.9）】</td><td>5（11.6）
【3】</td><td>4（9.3）
【4】</td><td>6（14.0）
【6】</td><td>定期的に実施</td></tr>
<tr><td colspan="2">9（20.9）</td><td>定期的に実施</td></tr>
<tr><td rowspan="2">PTPs 3)</td><td rowspan="2">1993～1999</td><td rowspan="2">115
【77】</td><td rowspan="2">8（7.0）
【5(6.5)】</td><td>4（3.5）
【2】</td><td>3（2.6）
【3】</td><td rowspan="2">1（0.9）</td><td rowspan="2">定期的に実施</td></tr>
<tr><td colspan="2">7（6.1）</td></tr>
<tr><td>市販後使用成績調査</td><td>PTPs
PUPs</td><td>1998～1999</td><td>578
【285】</td><td>6（1.0）**
【5(1.8)】</td><td>0</td><td>1（0.2）</td><td>4（0.7）
【4】</td><td>日常診療下で実施</td></tr>
<tr><td rowspan="2">国外</td><td>臨床試験 4)</td><td>PTPs</td><td>1988～1990</td><td>86
【-】</td><td>2（2.3）
【2】</td><td>0</td><td>0</td><td>2（2.3）
【2】</td><td>定期的に実施</td></tr>
<tr><td>臨床試験 5)</td><td>PUPs</td><td>1989～1992</td><td>81
【49】</td><td>16（19.8）
【14(28.6)】</td><td>0</td><td>4（4.9）
【3】</td><td>12（14.8）
【11】</td><td>定期的に実施</td></tr>
</table>

（ ）内はパーセント　BU：ベセスダ単位

PTPs：過去に治療歴のある患者

PUPs：過去に治療歴のない患者

＊　：重症血友病 A：国内調査では FVIII C<1%，国外調査では FVIII C<2%

＊＊：1 例測定値不明（重症症例）

2. インヒビターの発現が報告された調査・試験について

2.1. 国内市販後の特別調査/過去に治療歴のない患者（PUP）

- 本調査（1993 年から 1999 年に実施）において評価対象とした過去に治療歴のない患者 43 症例中，インヒビター発現例は 15 例であり，このうち，高力価例（5 BU 以上）は 6 例，残り 9 例は低力価であった（**表 2**）。
- 低力価のうち 5 例におけるインヒビター値はいずれも 1BU 以下であった。
- インヒビター発現例 15 例のうち，8 例のインヒビターは，観察期間中に消失することはなかったが，残りの 7 例のインヒビターは一過性であった。
- 1 例（症例 2）を除いて本剤治療による止血効果が得られ，治療が継続された。

表 2 コージネイト /国内特別調査（PUP）/インヒビター発現例一覧表

インヒビター反応	症例番号	年齢	血友病 A 重症度	インヒビター最高値（BU）	一過性か否か	観察期間（月）	コージネイト治療
高力価（5 BU 以上）	1	6 ヵ月	重症	975	－	54	継続
	2	9 ヵ月	重症	102	－	55	中止
	3	5 ヵ月	重症	53	－	43	継続
	4	13 ヵ月	重症	50	－	23	継続
	5	9 ヵ月	重症	13.1	－	33	継続
	10	19 ヵ月	重症	7.2	一過性	42	継続
低力価（5 BU 未満）	6	13 ヵ月	重症	3.3	－	47	継続
	7	15 ヵ月	重症	2	－	59	継続
	8	23 ヵ月	重症	1	一過性	38	継続
	9	6 ヵ月	重症	2.3	－	23	継続
	11	21 ヵ月	重症	4.6	一過性	80	継続
	12	23 ヵ月	中等症	0.7	一過性	80	継続
	13	3 ヵ月	中等症	0.5	一過性	56	継続
	14	83 ヵ月	重症	0.5	一過性	38	継続
	15	26 ヵ月	重症	0.5	一過性	35	継続

重症: FVIII:C< 1%，中等症：FVIII:C 1～5%，0.5 BU 以上をインヒビターと見なした。

2.2. 国内市販後の特別調査/過去に治療歴のある患者（PTP）

- 本調査（1993年から1999年に実施）で評価対象とした過去に治療歴のある患者115例のうち，12例は調査開始前から既にインヒビターを発現しており，103例はインヒビターの発現は見られなかった（インヒビター値不明1例を含む）。
- 調査開始前にインヒビターを発現していなかった103例のち，7例で本剤による治療後に新規インヒビターの発現が報告された。いずれも低力価例で，臨床的に意味があるとされた1 BU以上の症例は3例（1.2，1.8 及び2.1 BU）で，その他4例では1 BU未満（0.5，0.7，0.8及び0.9 BU）であった（0.9BUの症例は調査前のインヒビター値不明例）（**表3**）。
- 調査開始前からインヒビターを発現していた12例の患者のうち，1症例（累積投与日数が100日未満）においてインヒビター値が10BUへ上昇した。残りの11例（いずれも低力価，最高値1.8 BU）のうち，5例では観察期間中にインヒビター値が減少，残り6例ではインヒビター値が安定して推移していることから，これら11症例は，**表1**および**表3**には含めなかった。

表 3　コージネイト /国内特別調査（PTP）/インヒビター例一覧表

	症例番号	年齢（歳）	血友病 A 重症度	インヒビター最高値（BU）		一過性か否か
				治療前	治療後	
新規発現例（7 例）	2	30	重症	陰性	2.1	一過性
	17	56	重症	陰性	1.8	－
	16	35	重症	陰性	1.2	－
	18	55	中等症	不明	0.9	一過性
	14	37	重症	陰性	0.8	－
	3	32	重症	陰性	0.7	－
	6	31	中等症	陰性	0.5	一過性
本剤治療後増悪例*	5	61	軽症	0.8	10	一過性

*：本剤治療開始前からインヒビターを有していた患者
0.5 BU 以上をインヒビターと見なした。

2.3. 国内市販後の使用成績調査（PTP 及び PUP を含む）

- 本調査（1998 年から 1999 年に実施）の評価症例 578 例のうち，6 例（1.0%）にインヒビターが認められ，このうち，5 例は PTP，1 例は PUP であった。
- これら 6 例のうち，4 例（0.7%）は高力価例（それぞれ，20，36，67，180 BU）で，1 例（0.2%）が低力価例（2 BU）であり，残り 1 例の力価は不明であった。

2.4. 国外臨床試験/過去に治療歴のある患者（PTP）

- 本試験（1988 年から 1990 年に実施）で評価対象とした過去に治療歴のある患者 86 例のうち，2 例（2.3%）にインヒビターが発現した。
- インヒビター発現例の 1 例は本剤治療開始後に新規に発現した症例（最高値 13.5 BU）で，もう 1 例は本剤治療前から存在したインヒビターが治療後に更に上昇した症例（最高値 28 BU）であった。

2.5. 国外臨床試験/過去に治療歴のない患者（PUP）

- 本試験（1989 から 1992 に実施）で評価対象とした過去に治療歴のない患者 81 例のうち，16 例（19.8%）でインヒビターの発現が認められ，このうち，高力価例（5 BU 以上）は 12 例（14.8%）で，残り 4 例（4.9%）は低力価例であった（**表 4**）。
- 4 例（症例 2，3，7 及び 8）のインヒビターは観察期間中に消失した。
- また，別の 4 例（症例 5，9，11 及び 12）では本剤高用量による免疫寛容療法が実施され，このうち，3 例（症例 5，9 及び 11）はインヒビターが消失した。
- 1 例（症例 4）を除いて本剤による治療が継続された。

表 4　コージネイト /国外臨床試験（PUP）/インヒビター発現例一覧表

インヒビター反応	症例番号	年齢	試験開始前 FⅧ値＊	インヒビター値（BU）		コージネイト治療
				最高値	最終測定値	
高力価（5 BU 以上）	1	5 ヵ月	＜ 1%	15	1.2	継続
	3	13.5 ヵ月	＜ 1%	9.8	0	継続
	4	25 ヵ月	0%	419	132	中止
	5	8 ヵ月	2%	19	0	継続
	6	8 ヵ月	0%	5.8	1.5	継続
	9	11 ヵ月	＜ 1%	19.5	0	継続
	10	14 ヵ月	＜ 1%	6.8	1.6	NA
	11	5.4 ヵ月	＜ 1%	34	0	継続
	12	13 ヵ月	＜ 1%	487	717	継続
	13	8.4 月	＜ 1%	8.2	4.8	継続
	15	13 ヵ月	＜ 1%	5**	5**	継続
	16	10 ヵ月	＜ 1%	131	131	NA
低力価（5 BU 以下）	2	4 ヵ月	＜ 1%	3.6	0	継続
	7	6 ヵ月	＜ 2%	3.4	0	継続
	8	13 ヵ月	＜ 1%	1.3	0.3	継続
	14	13 ヵ月	4.6%	2.8	0.8	継続

＊：正常値に対するパーセント（<2%:重症, 2-5%:中等症),

＊＊：Malmsð unit: 1 Malmsð unit は 3 BU に相当

NA：試験終了時までは，更なる止血治療は必要なかった。

0.6 BU 以上をインヒビターと見なした。

＜コージネイト 引用文献リスト＞

1) 遺伝子組換えヒト第Ⅷ因子製剤 BAY w 6240 の多施設共同による臨床的検討（長期投与試験）, 福井弘 他: 日本輸血学会雑誌, VOL:37 No:5, 593-604, 1991.

2) Clinical Evaluation of a Recombinant Factor VIII Preparation (Kogenate) in Previously Untreated Patients with Hemophilia A. A. Yoshioka et al: International Journal of Hematology, VOL:78 No:5, 467-474, 2003.

3) Clinical Evaluation of Recombinant Factor VIII Preparation (Kogenate®) in Previously Treated Patients with Hemophilia A. A. Yoshioka et al: International Journal of Hematology (in press).

4) HUMAN RECOMBINANT DNA-DERIVED ANTIHEMOPHILIC FACTOR (FACTOR VIII) IN THE TREATMENT OF HEMOPHILIA A. RICHARD S. SCHWARTZ et al: New England Journal of Medicine, VOL:323 No:26, 1800-1805, 1990.

5) RECOMBINANT FACTOR VIII FOR THE TREATMENT OF PREVIOUSLY UNTREATED PATIENTS WITH HEMOPHILIA A. JEANNE M. LUSHER et al: New England Journal of Medicine, VOL:328 No:7, 453-459, 1993.

An observational study of sucrose-formulated recombinant factor VIII for Japanese patients with haemophilia A

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Summary
The safety and efficacy of sucrose-formulated recombinant factor VIII (rFVIII-FS; Kogenate® FS) under usual clinical practice were evaluated for 12 months in an observational, postmarketing surveillance study conducted at 214 treatment centres throughout Japan. The study included 631 patients with haemophilia A, 80% of whom had severe or moderately-severe disease (≤2% FVIII:C). Most patients (n=477; 75.6%) had >100 prior exposure days (EDs), but the study also included 62 (9.8%) patients with <20 EDs who were at high risk for inhibitor development. A total of 71,240 infusions were administered during the observation (mean, 113 ± 108 per patient). Physicians rated efficacy and tolerability of rFVIII-FS as "very good" or "good" in >99% of patients. FVIII inhibitors were observed in seven patients (5 *de novo*; 1 persistent/fluctuating; 1 recurrent). The overall *de novo* inhibitor incidence was 0.8% (5/631; or 5/599 among the subgroup of patients with negative baseline titre and no known inhibitor history). *De novo* cases represented 3.2% (2/62) of patients with <20 EDs at enrollment (2/57 in the no inhibitor subgroup) and 0.2 % (1/477) of patients pretreated with >100 EDs (1/452 in the no inhibitor subgroup) at enrollment. The results of this large observational study demonstrate that rFVIII-FS is both safe and efficacious as used in the usual clinical setting for the treatment of Japanese patients with mild to severe haemophilia A. This study supports the efficacy of rFVIII-FS with an incidence of inhibitor formation no greater than in a comparable European study or previous phase III clinical studies.

Keywords
Haemophilia A, Kogenate, recombinant factor VIII, inhibitors

Thromb Haemost 2008; 100: 32–37

Introduction

Haemophilia A is a blood coagulation disorder characterized by a deficiency of functional blood coagulation FVIII. Treatment consists of conventional FVIII replacement therapy, which originally relied on pooled donor plasma as a source of FVIII (1). Advances in recombinant gene technology and protein purification techniques have minimized the risk of blood-borne pathogen transmission, permitting the development of highly purified recombinant factor products (2).

Recombinant FVIII-FS is a full-length recombinant FVIII (rFVIII) formulated with sucrose as a stabilizer in place of added human albumin and includes viral inactivation steps during manufacture (3, 4). The efficacy and safety of rFVIII-FS was demonstrated by clinical studies with previously treated patients (PTPs) and with previously untreated (PUPs) or minimally treated patients (MTPs) in North America and Europe (3, 5, 6). In these studies, haemostasis was satisfactorily achieved in approximately 90% of bleeding episodes after one or two infusions of rFVIII-FS, and rFVIII-FS had an excellent safety profile. Clinical studies have also demonstrated the efficacy and safety of rFVIII-FS in haemophilic patients during surgical procedures, both as bolus or continuous infusion (7, 8).

A potentially serious complication of haemophilia A treatment is the development of inhibitory antibodies to replacement FVIII. Inhibitor formation generally affects 20%-30% of PUPs and 1%-3% of PTPs treated with other rFVIII products (9–11). These antibodies usually develop following therapy onset after a median of 10–15 exposure days (EDs) (12). Patients of African or Hispanic ethnic backgrounds are known to be more susceptible than other ethnic groups to inhibitor antibody formation (13). Specific genetic variants within the major histocompatibility complex (14, 15) or of genes involved in the immune response (e.g. interleukin [IL]-10) (16) are also associated with increased risk for inhibitor formation.

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Financial support:
This observational study was sponsored by Bayer HealthCare Pharmaceuticals.

Received December 6, 2007
Accepted after major revision April 27, 2008

Prepublished online June 11, 2008
doi:10.1160/TH07-12-0724

In previous clinical evaluations of rFVIII-FS, the incidence of *de novo* inhibitor formation was zero among 71 PTPs and low (15%) in 60 PUPs/MTPs with severe haemophilia A (3, 5). However, these trials used North American and European study populations and enrolled only small numbers of patients, as is typical for a rare bleeding disorder such as haemophilia A. We report the results of a postmarketing surveillance (PMS) study designed to evaluate the efficacy and safety of rFVIII-FS as used in routine clinical practice for a 12-month observation period in a large Japanese haemophilia A patient population.

Materials and methods

Patient selection

The study enrolled patients of any age with mild, moderate, or severe haemophilia A seen at 214 participating centres throughout Japan. These are mostly local hospitals and practices where general physicians implement treatment strategies for patients that were designed in consultation with a haemophilia specialty centre. Aside from the contraindications described in the product information for rFVIII-FS (17), there were no restrictions on enrolling patients with additional underlying diseases or chronic infections.

Study design

This observational study, conducted as a Drug Use Investigation according to the definition of the Pharmaceutical Affairs Law of Japan, was aimed at collecting efficacy and safety data in the clinical practice setting. It was designed as a prospective, non-interventional, uncontrolled multi-centre PMS study. The planned observation period for each patient was 12 months and included an initial visit at the start of the observation period and a final safety and efficacy assessment. The treatment dose and regimen were selected by the treating physician. Regular prophylaxis was defined as ≥2 prophylactic infusions per week for at least 80% of the observation period. Throughout the study, rFVIII-FS (Kogenate® FS; Bayer HealthCare Pharmaceuticals, Hematology/Cardiology, Berkeley, CA, USA) was used as the sole source of FVIII for prophylaxis and on-demand treatment for all patients. Data were collected in case report forms (CRFs) and whenever possible, follow-up information subsequent to the period covered by the CRF was also collected.

The efficacy analysis was based on summarized data on infusions (number of infusions with average daily dosage by reasons) as well as a general efficacy assessment by the physician at the end of the observation period. The safety analysis comprised demographic data, clinical history, adverse events (AEs) during the study period, and drug tolerability as assessed by the physician at the end of the study period. An AE was defined as any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medical treatment or procedure that may or may not be considered related to the medical treatment or procedure. An AE that resulted in any positive detection of FVIII inhibitor titre was systematically handled as a causally associated serious adverse event (SAE), irrespective of the titre or presence/absence of any clinical symptom.

Data analysis

All patients with at least one documented infusion were included in the data analysis. Descriptive analysis of the data was performed using summary statistics for categorical and quantitative data. Continuous data were described by mean, standard deviation (SD) minimum, 1, 5, 25, 75, 95, and 99 percent quantiles, median, maximum, and number of non-missing values. Moreover, continuous data were categorized in a clinically significant way, including categories of continuous data presented in frequency tables.

The incidence rates of AEs, adverse drug reactions (ADRs), SAEs, and serious adverse drug reactions (SADRs) were calculated and defined as the number of events divided by the number of patients at risk, where number of events equals the number of patients reporting the event and the number at risk equals all patients valid for safety analyses. For multiple occurrences of an event within a patient, the event was counted only once.

Results

Patients

A total of 701 patients from 214 Japanese haemophilia centres were enrolled and observed for 12 months. The study lasted from July 22, 2002, through September 28, 2005. Seventy patients were excluded from the study analysis for the following reasons: no drug administered (n = 46); lost to follow-up (n = 21); and double enrollment (n = 3). Thus, 631 eligible patients (mean age, 23.7 years) were included in the analysis. FVIII activity was <1% in 426 (67.5%) patients, 1%-2% in 79 (12.5%) patients, >2%-5% in 64 (10.1%) patients, and >5% in 56 (8.9%) patients; no information was available for six (1.0%) patients. A target joint was specified for 365 (57.8%) patients; the knee was the most frequently affected joint (n = 108). Information for all types of infusions was available for 570 (90.3%) patients, and 583 (92.4%) patients had reports about prophylactic infusions. Table 1 shows the demographics and baseline characteristics of the study population.

The majority of patients with available infusion data (n = 477, 75.6%) had been extensively treated in the past, with >100 previous EDs accumulated before study entry. An additional 59 (9.4%) patients had 20–100 previous EDs, 62 (9.8%) had <20 previous EDs (of whom 17 were considered PUPs), the number of previous EDs could not be determined for 12 (1.9%). No information was available for 21 (3.3%) patients. Of the 593 patients with at least one documented prior exposure to any kind of FVIII, 355 (59.9%) had previously been treated with one or more rFVIII products and 194 (32.7%) with a plasma-derived FVIII product. Information for the remaining 44 (7.4%) patients was incomplete. A history of inhibitors to FVIII was reported in 32 (5.1%) patients enrolled in the study.

Infusion and consumption summary

Patients were observed over a median of 401 days (range 16–893 days). During this period, a total of 71,240 infusions were administered for any reason to 631 patients with a mean of 113 (± 108) infusions per patient. Patients received a mean (± SD) of 72,800 ± 79,000 IU rFVIII-FS during the year (median 54,800 IU; range <1,000–777,900 IU). Reasons given for rFVIII-FS infusions, in

Table 1: Patient demographics and baseline characteristics (n = 631).

Mean age (SD), years[a]	23.7 (16.6)
Gender, n (%)	
Male	628 (99.5)
Female	1 (0.2)
No information	2 (0.3)
FVIII activity, n (%)	
<1%	426 (67.5)
1%-2%	79 (12.5)
>2%-5%	64 (10.1)
>5%	56 (8.9)
No information	6 (1.0)
Exposure days (EDs) prior to study, n (%)	
0 (PUPs)	17 (2.7)
1–19	45 (7.1)
20–100	59 (9.4)
>100	477 (75.6)
No information	33 (5.2)
Previous treatment product, n (%)[b]	
Recombinant FVIII	355 (59.9)
Plasma-derived FVIII	194 (32.7)
Non-FVIII product	2 (0.3)
Missing	42 (7.1)
History of inhibitors, n (%)	
Positive history (total)	32 (5.1)
Peak level ≤5 BU	19 (3.0)
Peak level >5 BU	11 (1.7)
No peak-level information	2 (0.3)
Inhibitors at baseline, n (%)	
Positive at baseline (total)	8 (1.3)
Low titre (<5 BU)	4 (0.6)
High titre (≥5 BU)	2 (0.3)
No titre information	2 (0.3)
Known seropositive status, n (%)	
Hepatitis A	9 (1.4)
Hepatitis B	112 (17.7)
Hepatitis C	311 (49.3)
HIV	86 (13.6)
Target joint specified, n (%)	365 (57.8)

SD, standard deviation; BU, Bethesda units; PUPs, previously untreated patients. [a]n = 629, [b]n = 593.

order of mean frequency per patient per year, were prophylaxis, joint bleeds, other bleeds, or surgery (Table 2). The mean number of infusions and mean total consumption of rFVIII-FS per patient for each of these reasons, for the total population as well as for the 111 (17.6%) subjects who received regular prophylaxis, are summarized in Table 2.

Bleeding events

On average, the mean number of follow-up treatments (± SD) required to manage joint bleeding episodes was 1.1 (± 1.5) and for other bleeding episodes, 1.2 (± 3.3). The results of follow-up treatments of bleeding episodes for patients on regular prophylaxis did not significantly differ from those of the total sample.

Efficacy assessment

At the end of the observation period, the efficacy of rFVIII-FS was globally evaluated for each patient by the physician; assessment data was available for 630 of 631 evaluable patients. Efficacy of treatment was rated "very good" in 409 (64.8%) patients, "good" in 219 (34.7%) patients, and "sufficient" in two (0.3%) patients. No cases were rated "insufficient"; an efficacy assessment was not available for one (0.2%) patient. Overall, the efficacy of rFVIII-FS was rated "very good" or "good" in 99.5% (628/631) of evaluable patients.

Safety evaluation

The safety analysis included data for the 631 eligible patients. Fifteen AEs were reported in 15 different patients. These included seven cases of inhibitor development, one non-serious case of a drug-related allergy, and seven serious non-drug-related SAEs. Four deaths occurred during the observation period due to lymphoma, cerebral haemorrhage, hepatic embolization, and ruptured liver carcinoma, respectively. All patient deaths were assessed as unrelated to rFVIII-FS by the investigator.

Inhibitor development

Positive inhibitor tests were detected and reported for seven patients during the study: five with *de novo* inhibitor formation, one with a persistent (fluctuating) inhibitor, and one with recurrent inhibitor development (Table 3).

Overall, there were five patients with *de novo* inhibitors in the total study population, giving an incidence of 0.8% (5/631). Data for 33 patients was insufficient to classify them among a particular pre-treatment group. Among all patients with <20 EDs at enrollment who were considered to be at highest risk for new inhibitor development, the *de novo* inhibitor rate was two in 62 (3.2%). Both cases occurred in severe haemophilia A patients (n = 35; 5.7% among patients with <1% FVIII:C) who were minimally treated at enrollment. No positive inhibitor titre was reported in any of the 17 PUPs. Among patients with 20–100 EDs at enrollment who are still at risk for inhibitor formation, two in 59 (3.4%) developed a *de novo* inhibitor (the inhibitors were transient in both patients). Both patients had severe haemophilia, making the rate in this subgroup two in 29 (6.9%). Among patients with >100 EDs at enrollment who are considered to be at low risk for new inhibitor development, *de novo* inhibitors were observed in one of 477 patients (0.2%) overall, and one in 344 (0.3%) of the severe patient subset.

Limiting the analysis to only the 599 patients with no known history of inhibitors and negative titre at baseline, the incidence is 0.8% (5/599) overall and 1.3% (5/399) among patients with <1% baseline FVIII:C. Within the no previous inhibitor group, the *de novo* inhibitor rate among the highest risk patients (<20 EDs at enrollment) was 3.5% (2/57) for all patients and 6.7% (2/30) excluding those with ≥1% FVIII:C. Among all patients

Table 2: Extent of exposure to rFVIII-FS during the study for the total population (n = 631).

	Total population (n = 631)	Prophylaxis population[a] (n = 111)
Mean no. of observation days (SD)	460 (142)	n/d
Mean no. of bleeds, surgeries, and prophylactic infusions (SD) per patient per year		
Prophylactic infusions	53.1 (60.4)[b]	148.9 (49.3)
Joint bleeds	10.5 (18.0)	3.1 (8.4)
Other bleeds	4.1 (16.5)	1.9 (4.3)
Surgeries	0.1 (0.8)	0.1 (0.3)
Mean consumption (SD) per patient per year, by reason (IU/kg)		
Prophylaxis[a]	1029 (1390)	2898 (1644)
Joint bleeds	551 (1020)	189 (384)
Other bleeds	252 (1102)	109 (209)
Surgery	24 (136)	21 (134)

SD, standard deviation; n/d = not determined. [a]Defined as regular treatment ≥2 prophylactic injections per week. [b]Patients who received treatment for any prophylactic reason, not limited to the subgroup of patients who received ≥2 injections per week.

with 20–100 EDs at enrollment, two in 57 (3.5%) developed a *de novo* inhibitor (2/28 [7.1%] patients with severe disease), and among patients with >100 EDs at enrollment, *de novo* inhibitors were observed in one of 452 (0.2%) patients (1/323 [0.3%] patients with severe haemophilia). The 447 extensively pretreated patients with no present or historical inhibitor titre were observed during this study for a sum total of 572 years, yielding a rate of 1.75 inhibitor cases per 1,000 person-years of observation. Among only the 323 extensively pretreated severe haemophilia A patients, there were 409 person-years of observation, yielding a rate of 2.44 cases per 1,000 person-years.

When considering the total number of EDs accumulated by the day of first inhibitor detection, all *de novo* FVIII inhibitors except for one (2 BU/ml) were detected in patients with <150 cumulative EDs to any FVIII preparation. High-titre inhibitors were detected in two patients, one with <20 EDs and the other with <40 EDs in total on the day of first detection. The overall rate of recurrent inhibitor formation was one in 32 (3.1%) patients with a history of inhibitors. No positive inhibitor titre was detected in the study in any patient with a documented switch from plasma-derived FVIII (pdFVIII) to rFVIII-FS. However, although not documented, one cannot definitely exclude that the 27-year-old patient who experienced inhibitor recurrence during this study may have received pdFVIII at some point in the past.

Tolerability assessment

At the conclusion of the observation period, the tolerability of rFVIII-FS was globally evaluated for each patient by the physician. The tolerability of rFVIII-FS treatment was rated "very good" or "good" in 627 of 631 evaluable patients (99.4%) with available assessment data; tolerability was rated as "sufficient" for three (0.5%) patients, and for one (0.2%) patient there was no available assessment of tolerability; no patient received a rating of "insufficient tolerability". Physicians recorded patient ratings of their acceptance of the treatment during the observation period. A total of 619 of 631 evaluable patients (98.1%) rated their acceptance of the treatment as "very good" or "good." Of the remaining 12 patients, eight (1.3%) rated their acceptance as "sufficient", three (0.5%) as "insufficient", and one (0.2%) patient had no assessment available.

Discussion

This non-interventional study was designed to evaluate the safety and efficacy of full-length rFVIII-FS, as used in routine clinical practice, during a 12-month observation period in a Japanese haemophilia A patient population. With over 700 patients enrolled, this trial is one of the largest studies performed in haemophilic patients. Furthermore, the design of this Japanese study was similar to that of another large, recently completed PMS study of full-length rFVIII-FS that enrolled over 230 European patients (18). The results of both studies support the very good safety and efficacy profile of rFVIII-FS for the treatment and prevention of bleeding episodes in routine clinical practice.

On average, joint bleeding episodes in this study required 1.1 follow-up infusions of rFVIII-FS to achieve adequate haemostasis, and other (non-joint) bleeding episodes required 1.2 follow-

Table 3: Patients with positive inhibitor tests during the study (n = 7).

Inhibitor type	Patient age, years	Disease severity[a]	No. of EDs prior to enrollment	No. of cumulative EDs prior to detection	Titre at first detection (BU)	Peak level during study (BU)	Titre at end of study (BU)
De novo	1	Severe	<20	<20	16	27	27
De novo	1	Severe	<20	<40	46.1	183[b]	103.4[c]
De novo	0.1	Severe	20–100	27	2	2	1
De novo	2	Severe	20–100	100–150	1	2	2
De novo	1	Severe	>100	150–200	2	2	0
Persistent (fluctuating)	8	Severe	>100	>150	1	3	3
Recurrent	27	Severe	>100	>150	1	3	2

EDs, exposure days; BU, Bethesda units; ND, no data available. [a]Disease severity defined by baseline FVIII:C levels as follows: severe (<1%), moderately severe (1–2%), moderate (>2–5%), or mild (>5%). [b]After initiating immune tolerance therapy. [c]Titre decreased to 7.8 BU/ml on last follow-up data available after completion of study.

up infusions. The efficacy of rFVIII-FS was rated by physicians as "very good" or "good" in 99.5% of patients. No treatment with rFVIII-FS was rated "insufficient". These findings are very similar to those observed in the European study, where 85.4% of haemorrhages were controlled using one or two infusions of rFVIII-FS, and 98.7% of physicians assessed efficacy as "very good" or "good" (18). By comparison, in a recently published interim analysis of an ongoing observational study of a B-domain-deleted rFVIII product in Germany, the overall physician assessment of efficacy was "very good" or "good" in 77.0% of treated cases (19).

When considering the extent of rFVIII-FS exposure, an average of 6,066 (± 6,583) IU were administered per patient per month in the current study (including patients on prophylaxis). Interestingly, patients in the European observational study consumed more than twice the amount of FVIII (mean 14,000 IU per patient per month) (18). The comparatively lower rFVIII consumption in this Japanese study may be related to the slightly smaller proportion of severe and moderately severe haemophilia A patients (<2% FVIII:C) enrolled (80.0% of patients) compared to 99.5% of patients in the European rFVIII-FS surveillance studies. The disparity between consumption rates may also be indicative of differences in body weight, culture, and/or medical practices between Japan and Europe, which would emphasize the importance of performing confirmatory studies in a Japanese patient population.

In the safety evaluation, seven cases of FVIII inhibitor formation accounted for all AEs considered related to treatment (by definition, inhibitors were to be considered drug-related). Because they interfere with the haemostatic efficacy of infused FVIII, inhibitor development is a serious concern for the management of patients with haemophilia. The risk of inhibitor formation is related to numerous endogenous factors (e.g. FVIII gene mutation, severity of haemophilia) and exogenous factors (e.g. intensity of treatment, surgeries, on-demand treatment versus prophylaxis) (20, 21). The risk for inhibitor development decreases with additional exposure to infused FVIII; therefore young patients with a limited number of previous EDs are at highest risk. In this study, the rate of *de novo* inhibitor formation in high-risk patients (<20 previous EDs at enrollment) was 2/62 (3.2%). This figure compares favourably to the rate reported in the European observational study (2/25; 8.0%) (18), although the difference in the incidence rates may be related to the greater number of mild and moderate haemophilia patients included in the Japanese cohort, as these patients are at lower risk compared to severe haemophiliacs. In the subgroup of only severe haemophilia A patients at high risk in our study, the inhibitor rate was 5.7% (2/35). The findings of both these PMS studies are supportive of the inhibitor incidence reported in a phase III clinical trial with rFVIII-FS in PUPs and MTPs with severe haemophilia A (9/60; 15%) (5). Because the incidence of inhibitor development among previously untreated severe patients is generally considered to be in the range of 20%-30% (10), these findings suggest that full-length rFVIII-FS has a low incidence of inhibitor formation in these patients. Moreover, reports of patients with positive inhibitor tests suggest a positive correlation between the number of EDs prior to and after enrollment before the onset of inhibitor development.

In contrast to high inhibitor risk patients, PTPs (those with at least 100 or 150 EDs to infused FVIII), are generally considered to be at low risk for inhibitor formation. This makes pretreated patients the ideal population in which to assess the immunogenicity of new FVIII products (11, 22). In the Japanese cohort studied here, the rate of *de novo* inhibitor formation in patients with >100 EDs at enrollment was 0.21% (1/477), which is consistent with reported rates in the European observational study (0/181, or 0%) (18) and a phase III study of patients with >150 previous EDs at enrollment (0/71, or 0%) (3). Notably, there were no reports of inhibitor formation in patients with a documented switch from a pdFVIII concentrate as a previous therapy to rFVIII-FS in this study. A retrospective study of a cohort of 838 PTPs with haemophilia A in the US determined an incidence of 2.14 inhibitor cases per 1,000 person-years assessed (2.26 cases per 1,000 person-years among only those with severe disease) (23). In our population of extensively pretreated (>100 EDs at enrollment) Japanese patients with no evidence of prior or current inhibitor, we calculated a rate of 1.75 inhibitor cases per 1,000 person-years of observation (2.44 cases per 1,000 person-years among only those with severe disease). Although these rates appear comparable, the US report excludes patients without a confirmation inhibitor test, which was not done in our observational study. One would expect this methodological difference to bias the incidence rates in the Japanese study higher relative to that of the US study. Collectively, the inhibitor safety findings in our study suggest that rFVIII-FS may have a low immunogenic potential.

There are a number of caveats to the interpretation of inhibitor incidence within the context of a surveillance study such as the one described here. One is that FVIII genotyping could not be specified within the design of the study; therefore, the proportion of high inhibitor risk (e.g. large deletion) compared to low inhibitor risk (e.g. single nucleotide substitution) subjects who were included in the analyses is not known. Two, the frequency of inhibitor testing also could not be specified by study protocol. Since in normal clinical practice routine inhibitor testing may only occur once or twice annually, unless an inhibitor is suspected, it must be considered that transient inhibitors and low-titer inhibitors that do not have a clinical impact may be missed. This would lead to a lower inhibitor incidence in a surveillance study compared to an interventional trial. Third, the lack of centralised inhibitor testing during this study leaves open the possibility for variation in the quality of testing performed at individual centres that may either lead to false positive or, more critically, false negative results that could depress the determined incidence. For these reasons, surveillance studies are best compared to other non-interventional, observational trials, and the comparability of the findings in our study to those of the large epidemiological study in the US (23) described above, suggests that the results are valid when such caveats are taken into consideration. Surveillance studies do, however, provide critical insight into the use of a product within the usual practice setting. The surveillance study described here would be expected to identify the occurrence of clinically relevant inhibitors (i.e. inhibitors that would require medical intervention) within the Japanese haemophilia population studied, and therefore would be of relevance to treating physicians.

In this study, there was no indication of blood-borne pathogen transmission from use of rFVIII-FS, which was a concern for plasma-derived concentrates in the past (24–28). Patients rated their own acceptance of rFVIII-FS treatment as "very good" or "good" in 98.1% of cases, indicating that the therapy was well tolerated.

In summary, this observational PMS study demonstrates a very good efficacy, safety, and tolerability profile for rFVIII-FS in a large population of Japanese patients with mild to severe haemophilia A, with no indication of pathogen transmission and a low rate of inhibitor formation. These results confirm those obtained in a similar European observational study of rFVIII-FS. Together, the results of these observational trials add substantial additional evidence of the safety, tolerability, and efficacy to the profile of rFVIII-FS determined in pre-licensure studies.

Acknowledgements

The authors thank the 214 haemophilia treatment centres throughout Japan that participated in this study. In addition, the authors would like to acknowledge the contributions of Yasuhiko Kai, Yasuhiko Hirata, Akihiko Donkai, and Yukiko Hanemoto (Bayer Yakuhin Ltd., Osaka, Japan); Hiroyuki Okano, Miyabi Kinoshita, and Atsuhito Tomotsugu (Bayer Yakuhin Ltd., Osaka, Japan) for haemophilia centre monitoring support; Siegfried Freytag and Ira Kurz (TNS Healthcare GmbH, Munich, Germany) for statistical support and data management; and Kenyon Ogburn and Bless Castro for editorial support in the preparation of this manuscript.

References

1. Pool JG, Gershgold EJ, Pappenhagen AR. High-potency antihaemophilic factor concentrate prepared from cryoglobulin precipitate. Nature 1964; 203: 312.
2. Tuddenham EG. In search of the eighth factor: a personal reminiscence. J Thromb Haemost 2003; 1: 403–409.
3. Abshire TC, Brackmann HH, Scharrer I, et al. Sucrose formulated recombinant human antihemophilic factor VIII is safe and efficacious for treatment of hemophilia A in home therapy. Thromb Haemost 2000; 83: 811–816.
4. Lee DC, Miller JL, Petteway SR, Jr. Pathogen safety of manufacturing processes for biological products: special emphasis on KOGENATE Bayer. Haemophilia 2002; 8 (Suppl 2): 6–9.
5. Kreuz W, Gill JC, Rothschild C, et al. Full-length sucrose-formulated recombinant factor VIII for treatment of previously untreated or minimally treated young children with severe haemophilia A: results of an international clinical investigation. Thromb Haemost 2005; 93: 457–467.
6. Oldenburg J, Ivaskevicius V, Schroder J, et al. Genetic background and inhibitors in previously untreated or minimally treated young patients with severe haemophilia A treated with sucrose-formulated recombinant factor VIII. Thromb Haemost 2006; 95: 903–905.
7. Luboshitz J, Lubetsky A, Maas-Enriquez M, et al. Clinical evaluation of continuously infused sucrose-formulated recombinant factor VIII during surgery. Haemophilia 2006; 12 (Suppl 2): 05PO102.
8. Scharrer I, Brackmann HH, Sultan Y, et al. Efficacy of a sucrose-formulated recombinant factor VIII used for 22 surgical procedures in patients with severe haemophilia A. Haemophilia 2000; 6: 614–618.
9. Lusher JM. First and second generation recombinant factor VIII concentrates in previously untreated patients: recovery, safety, efficacy, and inhibitor development. Semin Thromb Hemost 2002; 28: 273–276.
10. Peerlinck K, Hermans C. Epidemiology of inhibitor formation with recombinant factor VIII replacement therapy. Haemophilia 2006; 12: 579–590.
11. White GC, DiMichele D, Mertens K, et al. Utilization of previously treated patients (PTPs), noninfected patients (NIPs), and previously untreated patients (PUPs) in the evaluation of new factor VIII and factor IX concentrates. Recommendation of the Scientific Subcommittee on Factor VIII and Factor IX of the Scientific and Standardization Committee of the International Society on Thrombosis and Haemostasis. Thromb Haemost 1999; 81: 462.
12. Kreuz W, Ettingshausen CE, Zyschka A, et al. Inhibitor development in previously untreated patients with hemophilia A: a prospective long-term follow-up comparing plasma-derived and recombinant products. Semin Thromb Hemost 2002; 28: 285–290.
13. Oldenburg J, Schroder J, Brackmann HH, et al. Environmental and genetic factors influencing inhibitor development. Semin Hematol 2004; 41: 82–88.
14. Hay CR, Ollier W, Pepper L, et al. HLA class II profile: a weak determinant of factor VIII inhibitor development in severe haemophilia A. Thromb Haemost 1997; 77: 234–237.
15. Oldenburg J, Picard JK, Schwaab R, et al. HLA genotype of patients with severe haemophilia A due to intron 22 inversion with and without inhibitors of factor VIII. Thromb Haemost 1997; 77: 238–242.
16. Astermark J, Oldenburg J, Carlson J, et al. Polymorphisms in the TNFA gene and the risk of inhibitor development in patients with hemophilia A. Blood 2006; 108: 3739–3745.
17. Antihemophilic Factor (Recombinant) Kogenate FS Formulated with Sucrose [prescribing information]. Bayer HealthCare, Tarrytown, NY; 2007.
18. Musso R, Santagostino E, Faradji A, et al. Safety and efficacy of sucrose-formulated full-length recombinant factor VIII: Experience in the standard clinical setting. Thromb Haemost 2008; 99: 52–58.
19. Pollmann H, Externest D, Ganser A, et al. Efficacy, safety and tolerability of recombinant factor VIII (REFACTO) in patients with haemophilia A: interim data from a postmarketing surveillance study in Germany and Austria. Haemophilia 2007; 13: 131–143.
20. Gouw SC, van der Bom JG, Marijke van den Berg H. Treatment-related risk factors of inhibitor development in previously untreated patients with hemophilia A: the CANAL cohort study. Blood 2007; 109: 4648–4654.
21. Oldenburg J, Brackmann HH, Schwaab R. Risk factors for inhibitor development in hemophilia A. Haematologica 2000; 85: 7–14.
22. Neugebauer B, Drai C, Haase M, et al. Factor VIII products and inhibitor development: concepts for revision of European regulatory guidelines. Haemophilia 2008; 14: 142–144.
23. Kempton CL, Soucie JM, Abshire TC. Incidence of inhibitors in a cohort of 838 males with hemophilia A previously treated with factor VIII concentrates. J Thromb Haemost 2006; 4: 2576–2581.
24. Cho YK, Foley BT, Sung H, et al. Molecular epidemiologic study of a human immunodeficiency virus 1 outbreak in haemophiliacs B infected through clotting factor 9 after 1990. Vox Sang 2007; 92: 113–120.
25. Jee YM, Go U, Cheon D, et al. Detection of hepatitis A virus from clotting factors implicated as a source of HAV infection among haemophilia patients in Korea. Epidemiol Infect 2006; 134: 87–93.
26. Kernoff PB, Lee CA, Karayiannis P, et al. High risk of non-A non-B hepatitis after a first exposure to volunteer or commercial clotting factor concentrates: effects of prophylactic immune serum globulin. Br J Haematol 1985; 60: 469–479.
27. Lee CA, Phillips A, Elford J, et al. The natural history of human immunodeficiency virus infection in a haemophilic cohort. Br J Haematol 1989; 73: 228–234.
28. Yee TT, Lee CA. Transfusion-transmitted infection in hemophilia in developing countries. Semin Thromb Hemost 2005; 31: 527–537.

Safety and efficacy of sucrose-formulated full-length recombinant factor VIII: Experience in the standard clinical setting

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Summary

The safety of full-length sucrose-formulated recombinant factor VIII (rFVIII-FS; Kogenate® FS) for up to 24 months of use was evaluated in a postmarketing observational study in Europe. Long-term safety and efficacy data were available for 212 patients with severe haemophilia A, including 13 previously untreated patients (PUPs) and 12 patients with 1–19 exposure days (EDs). Patients accumulated a mean (± SD) of 187 (121) EDs to rFVIII-FS and received a total of 39,627 infusions, mainly for prophylaxis and for the treatment of 4,283 spontaneous or trauma-related bleeds during an average observation time of 710 (136) days. Of these bleeding episodes, 85.4% were successfully treated with one or two infusions of rFVIII-FS. Haemostasis was also evaluated during 46 minor to major surgical procedures, and the response to infusion was "excellent" or "good" in all cases. FVIII inhibitor formation was observed in six patients (two *de novo*; four persistent or recurrent). The *de novo* cases represent 8.0% (2 of 25) of patients who reported 0–19 previous EDs at study entry. Four of the five patients who reported possible drug-related adverse effects developed inhibitors. The results of this observational study demonstrate the efficacy and safety of rFVIII-FS during normal clinical use in the treatment of patients with severe haemophilia A. Furthermore, these findings are consistent with those of previous phase III clinical studies with rFVIII-FS, particularly with regard to its efficacy and low incidence of inhibitor formation.

Keywords

Haemophilia, recombinant factor VIII, Kogenate, inhibitors, prophylaxis

Thromb Haemost 2008; 99: 52–58

Introduction

Factor VIII (FVIII) replacement therapy for haemophilia A once relied solely on clotting factor concentrated or purified from the plasma cryoprecipitate of donor blood (1). The advent of FVIII production via recombinant DNA technology was a milestone in haemophilia treatment because FVIII concentrate became more widely available, reducing the need for human plasma-derived products that may carry a risk for transmission of blood-borne infections. Recombinant FVIII-FS (rFVIII-FS; Kogenate® FS in North America; KOGENATE® Bayer in Europe; Bayer HealthCare Pharmaceuticals) is a full-length rFVIII product formulated with sucrose, instead of human albumin, as a stabilizer. The production process for rFVIII-FS was designed to eliminate human-derived proteins from the final formulation and purification steps of the product and to reduce the likelihood of pathogen transmission (2). Clinical studies to date have reported no pathogen transmission with rFVIII-FS (3–7).

Evaluation of rFVIII-FS in several clinical studies showed a positive safety and efficacy profile. In clinical studies involving previously treated patients (PTPs; n = 71) and previously untreated or minimally treated patients (PUPs/MTPs; n = 61) from

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Received June 13, 2007
Accepted after revision October 28, 2007

Prepublished online December 5, 2007
doi:10.1160/TH07-06-0409

North America and Europe (3, 4), bleeding episodes were successfully treated with one or two infusions of rFVIII-FS in 80.5% (5) and 89% (6) of cases. Moreover, less than 1% of infusions were associated with adverse events (AEs) that were considered possibly drug related. In addition, the efficacy and safety of rFVIII-FS have been evaluated for use during a total of 37 surgical procedures in clinical studies, including its administration by continuous infusion (7, 8). In all cases, haemostatic outcomes for patients receiving rFVIII-FS during surgery were rated "good" or "excellent." Overall, rFVIII-FS has been well tolerated and effective in controlling bleeding in patients with severe haemophilia A in the clinical setting.

The formation of inhibitory antibodies to FVIII is a potentially serious complication of haemophilia A treatment. Patients at increased risk of inhibitor formation are those who suffer from severe disease (9), have certain genetic mutations in the FVIII gene (10) or possess variants in specific genes that constitute the major histocompatibility complex (11, 12) or are involved in immune response (e.g. interleukin [IL]-10) (13), are PUPs or MTPs, or are of African-American or Hispanic ethnicity (14). Inhibitors occur in approximately 20%–30% of PUPs and in 1%–3% of PTPs treated with other recombinant FVIII products (15–17). Phase III clinical trials on rFVIII-FS reported no *de novo* inhibitor formation in PTPs and inhibitors occurring in 15% of PUPs/MTPs (4). Here we report the results of a post-licensure observational study designed to evaluate the safety and efficacy of rFVIII-FS as used in clinical practice for up to 24 months in a large (>200 patients), unselected haemophilia A patient population.

Materials and methods

Patient selection

The study enrolled males with severe haemophilia A (<2% FVIII:C at baseline) of any age. There were no restrictions in enrolling patients with additional underlying diseases or chronic infections, aside from the contraindications for Kogenate® FS – i.e. known intolerance, allergy, or hypersensitivity to mouse or hamster proteins or other constituents of the preparation (Bayer HealthCare Pharmaceuticals, Berkeley, CA, USA).

Ethical conduct and confidentiality

The study protocol was approved by the appropriate ethics committees as required by local law in Denmark, Italy, Spain, and Sweden; this was not required in the other participating countries (Austria, Belgium, France, Greece, Netherlands, and Switzerland).

The study was carried out in accordance with the approved SmPC (Summary of Product Characteristics), EMEA (European Agency for the Evaluation of Medical Products) guidelines, and applicable local laws and regulations.

Only data collected during regular therapy was documented; no intervention into the investigators' decisions were required or performed, and no additional diagnostic or monitoring procedures were to be applied. Therefore, the patients' informed consent was not necessary. All records were kept confidential; only patient number, initials, and date of birth, but not patient names, were supplied to the sponsor.

Study design

This study was designed as a prospective, open-label, multinational (all-European) postmarketing surveillance study to collect safety and efficacy data over a 24-month period for rFVIII-FS used to treat patients with severe haemophilia A in a clinical setting or in home therapy. During the observation period, patients were treated solely with rFVIII-FS for prophylaxis and for on-demand treatment of spontaneous bleeding, trauma-related bleeding, surgery, or immune tolerance induction (ITI). Regular prophylaxis was defined as ≥2 prophylactic infusions per week for ≥80% of the observation time. The treatment dose and regimen were decided by the treating physician. Data were collected in case report forms, which included data obtained from patient treatment diaries (infusion reports).

The efficacy analysis was based on observations documented in the case report forms (number of infusions with dosage, reason for infusion, bleeding site, and assessment of response) and on a general efficacy assessment performed by the attending physician at the end of the observation period. The safety analysis comprised FVIII recovery data, inhibitor assay results, maintenance of haemostasis during surgery, laboratory examinations, and AEs recorded during the observation period as well as a drug tolerability assessment by the physician at the end of the study period. An AE was defined as any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medical treatment or procedure that may or may not be considered related to the medical treatment or procedure (18). An AE that resulted in any of the following was considered a serious AE (SAE): death, life-threatening condition, hospitalization or prolongation of existing hospitalization, or persistent or significant disability/incapacity. An AE was classified as an adverse drug reaction (ADR; or serious ADR, if appropriate) if considered by the physician to be possibly related to the study drug or its administration (19).

Data analysis

At the end of the observation period, the efficacy of the therapy was evaluated globally for each patient by the physician; the biometric evaluation was primarily descriptive and exploratory, using summary statistics for categorical and quantitative data. Patients who received at least one infusion were included in the analysis; patients with missing data were presented as a separate category. Percentages were calculated as a proportion of each category, including the category for missing values. In some subgroup analyses, percentages were calculated based on available figures (adjusted frequencies).

The incidence rates of adverse events and drug reactions were calculated and defined as the number of events divided by the number of patients at risk, where number of events equals the number of patients reporting the event and the number at risk equals all patients valid for safety analyses.

Results

Patients

A total of 231 male patients from 54 haemophilia treatment centres in ten European countries were enrolled and observed in the study from December 3, 2002, through December 31, 2005;

Table 1: Patient baseline characteristics and demographics (N = 220).

Patient characteristics	N	%
Total population	220	100
Age, years		
<2	14	6.4
2 to <12	54	24.5
≥12 to <18	24	10.9
≥18	128	58.2
Ethnicity		
White	180	81.8
Black	3	1.4
Asian	2	0.9
Other	8	3.6
Not reported	27	12.3
Factor VIII:C		
<1%	197	89.5
1%–2%	22	10
>2%	1	0.4
History of haemophilia		
Familial (inherited)	129	58.6
New mutation	57	25.9
Not known	34	15.5
EDs prior to trial		
Previously untreated	13	5.9
1–19	12	5.5
20–100	14	6.4
>100	181	82.3
History of inhibitors		
Positive history (total)	33	15.0
≥5 BU	20	9.1
<5 BU	11	5.0
Titre not available	2	0.9
Seropositive status		
HIV	43	19.5
Hepatitis A	112	50.9
Hepatitis B	179	81.4
Hepatitis C	116	52.7
Patients with target joints	84	38.2

BU, Bethesda units; EDs, exposure days; HIV, human immunodeficiency virus.

however, 11 patients either received no infusions (n = 6) or were lost to follow-up (n = 5). Thus, 220 eligible patients (mean age, 23.6 years; range, <0.1–71 years) were included in the analysis. FVIII activity was <1% in 197 (89.5%) patients, 1%–2% in 22 (10.0%) patients, and >2% in 1 (0.5%) patient. A target joint was specified for 84 (38.2%) patients, and the most frequently affected joint was the knee (n = 27). Infusion reports were available for 212 (96.4%) patients, and 210 (95.5%) patients had reports that detailed all infusions.

Most of the patients with available infusion data (n = 181, 82.3%) had been heavily treated in the past, with >100 previous exposure days (EDs) accumulated before study entry. Another 14 (6.4%) patients had 20–100 previous EDs, 12 (5.5%) had 1–19 EDs, and 13 (5.9%) were previously untreated patients (PUPs). Of the 207 previously exposed patients, 108 (52.2%) patients had previously been treated with one or more recombinant FVIII products and 92 (44.4%) with a plasma-derived FVIII product; the remaining seven (3.4%) patients received either an alternate, non-FVIII product or an unknown product. Of the 108 patients who had previously received recombinant FVIII, 42 (38.9%) had used human albumin-stabilized Kogenate® (Bayer HealthCare), the predecessor product of the sucrose-stabilized KOGENATE® Bayer (Bayer HealthCare).

A history of inhibitors to FVIII was reported in 33 (15.0%) patients enrolled in the study. Table 1 summarizes the baseline characteristics and demographics of the study population.

Infusion and consumption summary

Patients were observed over a mean (± SD) of 710 (± 136) days, during which they accumulated a mean of 187 (± 121) EDs. Observation times ≥1 year were achieved for 214 (97.3%) patients. A total of 39,627 infusions were administered to 212 patients with available infusion reports, with a mean of 188 (± 121) infusions per patient. Patients were infused with rFVIII-FS for prophylaxis, spontaneous bleeds, trauma-related bleeds, ITI therapy, surgery, or other reasons (Table 2). The overall mean infusion dose was 31.4 (± 14.9) IU/kg for all patients excluding those who received ITI therapy. A higher mean dose was administered to patients undergoing surgery (52.2 [± 28.6] IU/kg) or ITI therapy (90.5 [± 21] IU/kg). The mean dose for prophylactic infusion was 29.5 (± 14.5) IU/kg, slightly lower than that administered for the treatment of trauma-related bleeding (33.9 [± 15.8] IU/kg) or spontaneous bleeding (33.3 [± 15.6] IU/kg).

On average, each patient received a mean of 147,000 (± 122,000) IU rFVIII yearly (median 118,000 IU, range 2,000–744,000 IU). Median consumption for patients with complete data was 4,400 IU/kg/year in the prophylaxis group and 1,600 IU/kg/year in the non-prophylaxis group. Patients who received ITI (n = 8) had higher factor utilization (634,000 [± 1,106,000] IU per patient per year). Excluding patients undergoing ITI, the mean consumption for patients with at least 50 weeks of data was 4,600 (± 2,100) IU/kg/year in the prophylaxis group (n = 68) and 2,000 (± 1,500) IU/kg/year in the non-prophylaxis group (n = 130).

Bleeding events

During the study, a total of 4,283 bleeding events were documented in patients for whom infusion reports were available (n = 210). Of these, 138 patients reported 2,487 spontaneous bleeds, and 156 patients experienced 1,796 bleeds related to trauma (Table 3). The most commonly reported bleeding sites were the joints (71.9%); other bleeding sites included muscle (15.2%), head (6.3%), internal organs (1.1%), and other sites (5.9%). A total of 33 (15.7%) patients reported no bleeding events during the course of the study, including six of 70 (8.6%) patients re-

Table 2: Infusion summary (n = 212).

Total no. of infusions	39,627
Mean (± SD) infusions per patient	188 (121)
No. of infusions by reason, n (%)	
Prophylaxis	28,896 (72.9)
Spontaneous bleeding	4,048 (10.2)
Trauma-related bleeding	3,334 (8.4)
ITI	2,062 (5.2)
Surgery	487 (1.2)
Missing or other	800 (2.0)
Mean (± SD) infusion dose by reason, IU/kg	
All patients (excluding ITI)	31.4 (14.9)
ITI	90.5 (21.0)
Surgery	52.2 (28.6)
Trauma-related bleeding	33.9 (15.8)
Spontaneous bleeding	33.3 (15.6)
Prophylaxis	29.5 (14.5)
Other	33.3 (13.5)
No. of patients on regular prophylaxis[a] (%)	70 (31.8)
No. of infusions for patients on regular prophylaxis	21,340
No. of infusions by reason for patients on regular prophylaxis, n (%)	
Prophylaxis	19,732 (92.5)
Trauma-related bleeding	705 (3.3)
Spontaneous bleeding	563 (2.6)
Surgery	181 (0.8)
Missing or other	159 (0.7)

ITI, immune tolerance induction; SD, standard deviation. [a]Includes only patients who received ≥ 2 infusions for prophylaxis per week for ≥ 80% of the study period (not for ITI).

Table 3: Bleeding summary (n = 210).

No. of patients with bleeds, n (%)	
Total	177 (84.3)
Spontaneous bleeds	138 (65.7)
Trauma-related bleeds	156 (74.3)
No. of bleeds, n (%)	
Total	4,283 (100)
Spontaneous bleeds	2,487 (58.1)
Trauma-related bleeds	1,796 (41.9)
Mean (± SD) no. of bleeds per patient per year (n = 204)[a]	
All bleeds	10.4 (13.6)
Spontaneous bleeds	6.1 (10.5)
Trauma-related bleeds	4.3 (7.1)
Mean (± SD) no. of infusions for bleeds per patient per month	
All bleeds	1.51 (1.78)
Spontaneous bleeds	0.80 (1.29)
Trauma-related bleeds	0.71 (1.11)
No. of bleeds for patients on regular prophylaxis (n = 68), n (%)	
All bleeds	656 (100)
Spontaneous bleeds	294 (44.8)
Trauma-related bleeds	362 (55.2)
Mean (± SD) no. of bleeds per patient on regular prophylaxis per year (n = 68)[a]	
All bleeds	4.8 (5.0)
Spontaneous bleeds	2.2 (3.6)
Trauma-related bleeds	2.6 (3.6)
Mean (± SD) no. of infusions for bleeds per patient on regular prophylaxis per month	
All bleeds	0.75 (0.84)
Spontaneous bleeds	0.34 (0.65)
Trauma-related bleeds	0.41 (0.59)

SD, standard deviation. [a]For patients with ≥ 350 observation days on the study.

ceiving regular prophylaxis therapy. In patients who had ≥350 observation days on the study (n = 204), a mean of 10.4 (± 13.6) bleeds per year was reported overall. The mean number of bleeds per patient per month was 0.9 (± 1.1) (range, 0–6.2 bleeds) for patients with detailed infusion reports.

For patients receiving regular prophylaxis, 294 spontaneous bleeds and 362 trauma-related bleeds were documented. A mean of 4.8 (± 5.0) bleeds per year was reported for those with ≥ 350 observation days on a regular prophylaxis regimen during the study (n = 68). In contrast, all other non-ITI, non-prophylaxis patients (n = 132) reported a mean of 1.16 (± 1.29) bleeds per month, which corresponds to a mean of 13.9 bleeds per year during the observation period. The latter patient group includes on-demand patients and those on irregular prophylaxis regimens.

The majority of bleeding episodes (n = 3,658, 85.4%) were successfully treated with one or two infusions of rFVIII-FS. Overall, responses to rFVIII-FS treatment were rated by physicians as "very good" or "good" in 217 of 220 study subjects (98.6%) who were treated with rFVIII-FS in the study.

Surgical procedures

During the study, 37 patients underwent 46 minor or major surgical procedures, including 17 knee replacements or synovectomies; nine tooth extractions or dental implantations; six orthopedic surgeries involving the hip, ankle, elbow, spine, or Achilles tendon; six replacements, implantations, or removals of intravenous access devices; four skin biopsies or cyst ablations; two abdominal surgeries; one eye atheroma resection; and one cholecystectomy. Surgery accounted for 1.2% of all infusions administered during the study period, with a mean dose of 52.2 IU/kg (± 28.6) per infusion per patient. Haemostasis was assessed by study investigators as "excellent" in 28 cases or "good" in 16 cases. None of the patients who underwent surgery developed inhibitors.

Safety evaluation

All 220 patients were included in the safety analysis. Seventy (31.8%) patients reported 130 AEs, and 45 (20.5%) patients reported 72 SAEs. Of these, only 11 AEs that occurred in five (2.3%) patients were considered by physicians to be possibly related to the study drug or its administration (ADRs), which included eight events reported by four patients that were considered serious (SADRs) (Table 4). Four of these eight SADRs were related to inhibitor formation.

Four deaths occurred during the study. The causes of death were non-Hodgkin's lymphoma (n = 2) and intracranial haemorrhage (n = 2), neither of which was considered related to the study drug. For the study population overall, physicians considered the safety of rFVIII-FS to be "very good" or "good" in 99.1% of the cases treated.

Type of event	ADRs (n = 5)		SADRs (n = 4)	
	No. of patients[a]	No. of events	No. of patients[a]	No. of events
Factor VIII inhibition	4	5	3	4
Catheter placement complications	1	1	1	1
Haemarthrosis	1	3	1	3
Pain in extremity	1	1	0	0
Arthralgia	1	1	0	0
Total number of events		11		8

ADRs, adverse drug reactions; SADRs, serious adverse drug reactions. [a]Each patient may have experienced more than one type of event.

Table 4: Frequency of adverse drug reactions (ADRs) and serious adverse drug reactions (SADRs).

Table 5: Patients with positive inhibitor titres during the study (n = 6).

	Before study		During study				
Age, years	No. of EDs prior to study	Last titre (BU)	First titre (BU)	Peak titre (BU)	Last titre (BU)	Inhibitor description	Treatment notes
De novo inhibitors							
1	0	Negative	20.0[a]	272.0	108.0	*De novo*	rFVIII-FS discontinued
1	1–19	Negative	2.2[b]	2.2	Negative	*De novo*	Successful ITI
Recurrent or preexisting inhibitors							
2	1–19	2.0	2.0	2.0	2.0	Persistent low titre	NA
7	20–100	Missing	5.7	7.4	Negative	Recurrent[c]	NA
6	>100	11.0	13.6	13.6	3.0	Preexisting	Decreasing titre during ITI treatment
18	>100	1.5	154.0	315.0	250.0	Increase at start of ITI	rFVIII-FS discontinued

BU, Bethesda units; EDs, exposure days; ITI, immune tolerance induction; NA, not available. [a]*De novo* inhibitors detected after 15 ED. [b]*De novo* inhibitors detected after 9 ED. [c]Positive history of inhibitor.

Nine (4.1%) patients seroconverted from negative to positive after vaccination for hepatitis A or B during the study. There were no conversions for hepatitis C reported during the study.

Inhibitor formation

During the observation period, FVIII inhibitor assays were conducted in 175 (79.5%) patients. Between one and 20 inhibitor assays were conducted in each of these patients. Six patients (age range, 1–18 years) were found to have a positive inhibitor test during the course of the study, including three patients who had positive titres at the start of the study and one patient who had a positive inhibitor history but did not have a documented titre at the start of the observation period (Table 5). Of the six patients with inhibitors, two entered the study with >100 EDs, one with 20–100 EDs, two with 1–19 EDs, and one patient was previously untreated.

The six patients who presented with inhibitors during the study period included two cases of *de novo* inhibitors. The incidence of *de novo* inhibitors was 1/13 (7.7%, high-titre) in PUPs and 1/12 (8.3%, low-titre) in patients with 1–19 EDs prior to study entry. No *de novo* inhibitor was detected in patients with at least 20 previous treatments with FVIII (n = 195). Of the two patients with *de novo* inhibitors, the high-titre patient discontinued rFVIII-FS therapy altogether and the low-titre patient underwent successful ITI treatment. In addition, the latter patient reported a recurrent episode of the inhibitor (1 BU) six months after resolution of the initial episode.

In the three patients who had documented positive titres for inhibitors at the start of the study, the titre remained unchanged for one patient who did not receive ITI (2.0 BU), decreased from 13.6 BU to 3.0 BU for one patient who underwent ITI, and surged to a peak of 315.0 BU for one patient who initiated ITI (Table 5). The latter patient discontinued rFVIII-FS therapy altogether. The fourth patient, who had a history of inhibitors but no documented inhibitor test at study entry, developed inhibitor titres of 5.7 BU and 7.4 BU during the study, and eventually converted to negative by the end of the study. This patient was the only one of 33 patients with a history of inhibitors who developed a recurrent inhibitor after switching to rFVIII-FS from another product (he had previously received a B-domain–deleted [BDD] product).

Of the patients who underwent surgical procedures with intensive treatment during the study, four had a prior history of inhibitor formation. None of these patients developed inhibitors during surgery.

Discussion

Recombinant FVIII formulated with sucrose (rFVIII-FS) has been available for the treatment of haemophilia A since 2000. The pres-

ent study, a 24-month-long, multinational, postmarketing surveillance study, was designed to evaluate the safety and efficacy of rFVIII-FS during its use in the clinical and home therapy settings.

The results of this study are consistent with the results of the pre-licensure clinical trials and indicate that rFVIII-FS is well tolerated and efficacious for the treatment and prevention of bleeding episodes. There were no reports of pathogen transmission during the study. The final assessment by the physicians of the efficacy of rFVIII-FS was "very good" or "good" in 98.7% of the cases treated. The efficacy results of this study are comparable to those obtained from the licensure clinical trials in terms of the mean number of bleeds per patient per month for patients on prophylaxis (0.4 in this study vs. 0.64 in an international study of PTPs) and the percentage of bleeding episodes successfully treated with one or two infusions (85.4% in this study vs. 93.5% and 89.0% in an international study of PTPs and a study of PUPs/MTPs, respectively) (3, 4). A recently published postmarketing surveillance study of a BDD rFVIII product observed 217 patients with mild to severe hemophilia A who were treated for a mean of 24.7 months in treatment centres in Germany (20). Although differences in study design and definitions make it difficult to compare between studies, in the BDD rFVIII postmarketing surveillance study the final overall physician assessment of efficacy was "very good" or "good" in 77.0% of cases treated.

The development of inhibitors against replacement FVIII is a major concern associated with the treatment of haemophilia A. Factors such as particular FVIII gene mutations, particular genetic features, racial background, familial history, limited prior exposure to FVIII products, and even variations in the FVIII manufacturing process have all been implicated as potential risk factors that can influence inhibitor development in patients (10, 21–23). Clinical studies of other rFVIII products in PUPs have documented *de novo* inhibitor rates of about 30% (24). In contrast, a recent phase III clinical study of rFVIII-FS in PUPs and MTPs (≤4 EDs prior to study) found a lower rate of *de novo* inhibitor formation (9/60, or 15.0%) (4). The rate of *de novo* inhibitor formation in high-risk patients (<20 EDs at study entry) that was documented in this postmarketing surveillance study was 8.0% (2/25), and 7.7% (1/13) in PUPs.

Phase III evaluation of rFVIII-FS in PTPs with ≥100 EDs at study entry showed no *de novo* inhibitor formation among 71 patients studied (5). In the current observational study, *de novo* inhibitors were reported in 0.5% (1/207) of patients with ≥ 1 ED prior to entry. While inhibitor assays were performed in only 175/220 (79%) of all patients, this low incidence of *de novo* inhibitors may indicate a relatively low immunogenic potential for rFVIII-FS in PTPs, if confirmed in larger studies.

Because postmarketing surveillance studies evaluate "real-world" use of FVIII, inhibitor assays are not performed as frequently as in clinical studies. Thus, occurrences of transient or low-titre inhibitors without clinical relevance might be missed in these types of studies. Nonetheless, the rate of *de novo* inhibitors found in this study of rFVIII-FS is low and consistent with the rates observed in the rFVIII-FS phase III program.

In summary, this observational study has found that the use of rFVIII-FS in the normal clinical setting was safe and well tolerated, with no clinical or laboratory evidence of pathogen transmission, and a low rate of inhibitor formation. Furthermore, rFVIII-FS was shown to be efficacious for the treatment of bleeding episodes and for haemostatic control during surgical procedures. This observational study provides safety and efficacy data on "real-world" use of rFVIII-FS, with no restrictions on patient enrollment and obtained data, which support the results of the rFVIII-FS clinical study program.

Acknowledgements

The authors thank Verena Haupt, PhD, for assistance in study coordination and data management, and Anna Lau, PhD, and Blesila Castro, PhD, for editorial support for the development of this manuscript. This study was funded by Bayer HealthCare (Leverkusen, Germany).

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References

1. Pool JG, Gershgold EJ, Pappenhagen AR. High-potency antihaemophilic factor concentrate prepared from cryoglobulin precipitate. Nature 1964; 203: 312.
2. Lee DC, Miller JL, Petteway SR Jr. Pathogen safety of manufacturing processes for biological products: special emphasis on KOGENATE Bayer. Haemophilia 2002; 8 (Suppl 2): 6–9.
3. Aygoren-Pursun E, Scharrer I. A multicenter pharmacosurveillance study for the evaluation of the efficacy and safety of recombinant factor VIII in the treatment of patients with hemophilia A. German Kogenate Study Group. Thromb Haemost 1997; 78: 1352–1356.
4. Seremetis S, Lusher JM, Abildgaard CF, et al. Human recombinant DNA-derived antihaemophilic factor (factor VIII) in the treatment of haemophilia A: conclusions of a 5-year study of home therapy. The KOGENATE Study Group. Haemophilia 1999; 5: 9–16.
5. Abshire TC, Brackmann HH, Scharrer I, et al. Sucrose formulated recombinant human antihemophilic factor VIII is safe and efficacious for treatment of he-

mophilia A in home therapy. International Kogenate-FS Study Group. Thromb Haemost 2000; 83: 811–816.
6. Kreuz W, Gill JC, Rothschild C, et al. Full-length sucrose-formulated recombinant factor VIII for treatment of previously untreated or minimally treated young children with severe haemophilia A: results of an international clinical investigation. Thromb Haemost 2005; 93: 457–467.
7. Luboshitz A, Lubetsky A, Maas Enriquez M, et al. Clinical evaluation of continuously infused sucrose-formulated recombinant factor VIII during surgery. Poster presented at: Hemophilia 2006 World Congress; May 5–21, 2006; Vancouver, BC.
8. Scharrer I, Brackmann HH, Sultan Y, et al. Efficacy of a sucrose-formulated recombinant factor VIII used for 22 surgical procedures in patients with severe haemophilia A. Haemophilia 2000; 6: 614–618.
9. Lusher JM, Arkin S, Abildgaard CF, et al. Recombinant factor VIII for the treatment of previously untreated patients with hemophilia A. Safety, efficacy, and development of inhibitors. Kogenate Previously Untreated Patient Study Group. N Engl J Med 1993; 328: 453–459.
10. Schwaab R, Brackmann HH, Meyer C, et al. Haemophilia A: mutation type determines risk of inhibitor formation. Thromb Haemost 1995; 74: 1402–1406.
11. Hay CR, Ollier W, Pepper L, et al. HLA class II profile: a weak determinant of factor VIII inhibitor development in severe haemophilia A. UKHCDO Inhibitor Working Party. Thromb Haemost 1997; 77: 234–237.
12. Oldenburg J, Picard JK, Schwaab R, et al. HLA genotype of patients with severe haemophilia A due to intron 22 inversion with and without inhibitors of factor VIII. Thromb Haemost 1997; 77: 238–242.
13. Astermark J, Oldenburg J, Pavlova A, et al. Polymorphisms in the IL10 but not in the IL1beta and IL4 genes are associated with inhibitor development in patients with hemophilia A. Blood 2006; 107: 3167–3172.
14. Astermark J, Oldenburg J, Escobar M, et al. The Malmo International Brother Study (MIBS). Genetic defects and inhibitor development in siblings with severe hemophilia A. Haematologica 2005; 90: 924–931.
15. Lusher JM. First and second generation recombinant factor VIII concentrates in previously untreated patients: recovery, safety, efficacy, and inhibitor development. Semin Thromb Hemost 2002; 28: 273–276.
16. Peerlinck K, Hermans C. Epidemiology of inhibitor formation with recombinant factor VIII replacement therapy. Haemophilia 2006; 12: 579–590.
17. Wight J, Paisley S. The epidemiology of inhibitors in haemophilia A: a systematic review. Haemophilia 2003; 9: 418–435.
18. National Cancer Institute. Common Terminology Criteria for Adverse Events v3.0, August 2006. Available at: http://ctep.cancer.gov/forms/CTCAEv3.pdf.
19. Edwards IR, Aronson JK. Adverse drug reactions: definitions, diagnosis, and management. Lancet 2000; 356: 1255–1259.
20. Pollmann H, Externest D, Ganser A, et al. Efficacy, safety and tolerability of recombinant factor VIII (REFACTO) in patients with haemophilia A: interim data from a postmarketing surveillance study in Germany and Austria. Haemophilia 2007; 13: 131–143.
21. Mauser-Bunschoten EP, Rosendaal FR, Nieuwenhuis HK, et al. Clinical course of factor VIII inhibitors developed after exposure to a pasteurised Dutch concentrate compared to classic inhibitors in hemophilia A. Thromb Haemost 1994; 71: 703–706.
22. Peerlinck K, Arnout J, Gilles JG, et al. A higher than expected incidence of factor VIII inhibitors in multitransfused haemophilia A patients treated with an intermediate purity pasteurized factor VIII concentrate. Thromb Haemost 1993; 69: 115–118.
23. Rosendaal FR, Nieuwenhuis HK, Van den Berg HM, et al. A sudden increase in factor VIII inhibitor development in multitransfused hemophilia A patients in The Netherlands. Dutch Hemophilia Study Group. Blood 1993; 81: 2180–2186.
24. Astermark J. Overview of inhibitors. Semin Hematol 2006; 43 (2 Suppl 4): S3–S7.

Haemophilia (2008), 14, 281–286 DOI: 10.1111/j.1365-2516.2007.01634.x

ORIGINAL ARTICLE *Inhibitors*

A prospective surveillance study of factor VIII inhibitor development in the Canadian haemophilia A population following the switch to a recombinant factor VIII product formulated with sucrose

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Summary. The introduction of new factor concentrates has, at times, resulted in an increase in inhibitor development; hence large systematic surveys of inhibitor development are necessary whenever new products are introduced. This study presents the results of a surveillance study conducted by the Inhibitor Subcommittee of the Association of Hemophilia Clinic Directors of Canada that evaluated inhibitor development in patients with haemophilia A following the switch to a second generation recombinant FVIII product (rFVIII-FS; Kogenate® Bayer). Four hundred and sixty haemophilia A paediatric and adults patients from 17 Canadian Comprehensive Hemophilia Care Centers were enrolled in the study. Of these, 274 patients had evaluable data. Blood samples collected at baseline (prior to the switch to rFVIII-FS), and at 12 and 24 months following conversion were tested for inhibitors by the Nijmegen-modified Bethesda assay. Four subjects had positive inhibitor titres at baseline, with values ranging from 3.3 to 160 BU. Of the 274 patients who had baseline samples collected, 225 had postswitch samples collected at 12 months and 189 subjects had samples collected at 24 months. Only patients with positive baseline inhibitor titres (n = 4) had positive inhibitor titres at either the 12- or 24-month postswitch time points; therefore no *de novo* inhibitors developed over the 2-year evaluation period in this patient population. The results of this surveillance study suggest that the altered formulation of this recombinant FVIII concentrate was not associated with an increased incidence of inhibitor formation.

Keywords: factor VIII, haemophilia, inhibitor, surveillance

Introduction

Haemophilia A is an inherited bleeding disorder caused by a deficiency of coagulation factor VIII (FVIII) that affects between 1/5000 to 1/10 000 males. The development of an inhibitor to FVIII (an antibody that neutralizes the coagulant activity of factor) following FVIII replacement therapy is the most serious treatment-related complication currently facing haemophilia patients; an inhibitor reduces the effectiveness of treatment, resulting in an increase in medical costs, and an increase in morbidity and

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Accepted after revision 28 November 2007

mortality [1]. Known or suspected risk factors for the development of inhibitors to FVIII include: the severity of disease, the genetic mutation responsible for haemophilia, family history of inhibitors, ethnicity, age of first exposure to FVIII, molecular modifications of the FVIII molecule and the number of exposure days to FVIII [2,3]. The incidence of inhibitors appears to vary among users of different FVIII concentrates, but there is no evidence to support the concern that switching from one product to another is itself a risk factor for inhibitor formation, independent of the FVIII product [4–8]. Recombinant FVIII products have the inherent safety benefit of eliminating the need for large pools of donor plasma, yet lingering concerns regarding the potential immunogenicity of recombinant products remain. That recombinant proteins can induce antibodies when given therapeutically is well illustrated by the occurrence of pure red cell aplasia induced by anti-erythropoietin antibodies following therapy with certain preparations of recombinant erythropoietin [9].

Formed in 1994, the Association of Hemophilia Clinic Directors of Canada (AHCDC) provides a structure through which Canadian haemophilia treaters, blood system regulators and operators can exchange information regarding product tracking, utilization, monitoring and surveillance for product efficacy and safety. Such monitoring is particularly important with the introduction of any new coagulation products. As reported in the study by Giles *et al.* [10], this organization initiated an inhibitor surveillance programme designed to address the theoretical concern that highly purified plasma-derived or recombinant FVIII products might be more immunogenic than earlier plasma derived products (this coincided with the conversion of most Canadian haemophilia A patients to either recombinant or affinity-purified plasma-derived preparations in 1994). An important element of the surveillance study design was the establishment of a central laboratory for the tracking and monitoring of inhibitors. The use of a central laboratory helped to ensure consistent methodology and standardized measurement for inhibitor detection, allowing evaluation and pooling of results across participating centres. In the study by Giles *et al.*, 478 patients switched from plasma-derived products to a first generation rFVIII product (Kogenate® Bayer, Bayer Healthcare, Berkeley, CA, USA) and inhibitor formation was then monitored for 1–2 years. This study found no evidence of increased inhibitor formation in these patients following the switch.

Similar to many other recombinant proteins, first generation rFVIII products, as studied by Giles *et al.* [10], were stabilized with human albumin in their final formulations. However, concerns regarding the therapeutic use of mammalian-derived protein, such as human albumin, prompted the Medical and Scientific Advisory Council of the National Hemophilia Foundation in the US to encourage manufacturers to remove albumin from products used in the treatment of haemophilia [11]. Subsequently, Bayer Inc. developed a full-length rFVIII (Kogenate® FS; Bayer) that contains sucrose rather than albumin in the final formulation (rFVIII-FS) [12].

We report here a continuation of the efforts of the Inhibitor Subcommittee of the AHCDC, specifically evaluating inhibitor development following the conversion of haemophilia A patients to rFVIII-FS.

Materials and methods

Eligible subjects were Canadian paediatric and adults patients with moderate or severe haemophilia A who were switched from FVIII to rFVIII-FS. The study was approved by the respective review board/ethics committees of participating centres. This study was funded by Canadian Blood Services and Héma-Quebec following a recommendation from the AHCDC. Participation in the study was not influenced by the factor VIII product used prior to the switch, or concentrate history over the year prior to conversion. In addition, patients were eligible irrespective of whether an inhibitor was detected at baseline. The characteristics of the 274 eligible patients are summarized in Table 1. Based on FVIII measurements at baseline, 72.3% of

Table 1. Patient characteristics*.

Age at switch to rFVIII-FS	
Mean age ± SD (years)	16.8 ± 10.2
Range (years)	0.9–40.8
Severity of haemophilia based on CRF data†	
Severe	220 (89)
Moderate	19 (8)
Mild	3 (1)
Severity not reported	4 (2)
Severity of haemophilia based on baseline FVIII measurement	
Severe (≤0.01 U mL^{-1})	198 (72)
Moderate (>0.01–0.05 U mL^{-1})	38 (14)
Mild (>0.05 U mL^{-1})	38 (14)
Family history of inhibitor†	
Yes	24 (10)
No	203 (83)
Unknown	19 (8)

Not all patients had completed CRFs; however, lab analyses were conducted on all samples collected, unless otherwise noted. Values are given as *n* (%).

*n = 274 evaluable patients.

†n = 246 evaluable patients with completed CRF.

patients were severe, 13.9% were moderate and 13.9% were mild. To be eligible for evaluation patients had to have baseline plasma samples collected within 3 months prior to the switch to rFVIII-FS and to have samples collected at 12 and 24 months following the switch to rFVIII-FS. All samples were drawn at least 48 h following any FVIII replacement therapy. Patients were withdrawn from the study if samples were not collected within 3 months of the 12- and 24-months postswitch time frame.

Blood samples were collected directly into vacuum-sealed tubes or indirectly via syringe and transferred into vacuum-sealed tubes. Platelet-poor plasma was obtained by centrifugation, and samples were frozen (−60°C or lower), and shipped to the Central Laboratory (Hemophilia Research Reference Laboratory, Kingston General Hospital, Kingston, ON) for analysis. All samples were tested for inhibitors by the Nijmegen-modification of the Bethesda method [13]. A positive inhibitor value was considered to be ≥0.5 BU.

Results

Four hundred and sixty haemophilia A patients from 17 Canadian comprehensive haemophilia care centres were enrolled. Of these, 274 met enrollment protocol requirements. During the time frame of this study, 28 August, 2000 until 28 September, 2003, an unanticipated disruption of rFVIII-FS production occurred (29 September 2001), and therefore some patients were switched from rFVIII-FS to other rFVIII products to manage the shortage. Data from such patients were included until the date they switched from rFVIII-FS to another product.

Study criteria were set out to include only moderate and severe haemophilia patients, but baseline factor measurements resulted in some patients being recategorized as mild (FVIII > 0.05 U mL^{-1}), in contrast to information on the case report forms (CRF) that categorized these patients differently. This discrepancy between baseline laboratory factor levels and the CRF may be explained by patients having received factor VIII 48–96 h prior to the baseline sample being taken or by simple imprecision of results from local laboratories; patients with levels of factor of 0.05–0.07 U mL^{-1} may at times be found to have levels of 0.03 or 0.04 U mL^{-1}. Because the goal of this study was an evaluation of inhibitor development (a safety endpoint), mild, moderate and severe haemophilia patients were included in the data analysis. For most patients (82.5%) there was no family history of a FVIII inhibitor.

Subjects were excluded from the study for the following: problems with obtaining baseline sample (sample not obtained, $n = 7$, sample obtained after switch to rFVIII-FS, $n = 9$, sample obtained more than 90 days prior to switch to rFVIII-FS, $n = 47$), and problems with obtaining postswitch samples (samples not obtained, $n = 137$). As well, two subjects were excluded as they did not switch to rFVIII-FS ($n = 2$). As some patients had more than one exclusion criteria the final number of eligible patients amounted to 274 (Fig. 1).

While the goal of the study was to follow all patients for at least 2 years following conversion to rFVIII-FS, data were not collected for all patients at each of the protocol designated sampling times, both for reasons of non-availability of rFVIII-FS and other reasons. Of the 274 patients who had baseline

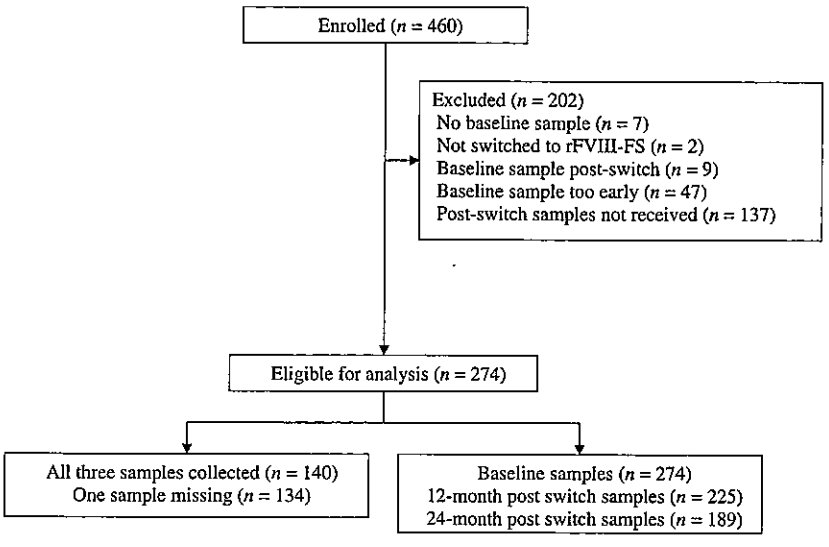

Fig. 1. Cohort of patients enrolled, excluded* and reason for exclusion, and patients eligible for analysis. *Patients have more than one exclusion criteria, which explains the discrepancy in the final number of patients eligible for analysis.

Table 2. Patients with positive inhibitor titres at baseline.

Patient	Baseline sample (BU)	12 months postswitch to rFVIII-FS (BU)	24 months postswitch to rFVIII-FS (BU)
1	3.3	1.2	NA
2	16	3.2	NA
3	160	33.8	27.7
4	4.5	4.6	5.8

BU, Bethesda units; NA, samples not collected for assay.

samples collected, 12-month postswitch samples were collected for 225 and 24-month postconversion samples were collected for 189 subjects. One hundred and forty patients completed all three samples; baseline, 12 and 24 months following conversion to rFVIII-FS. A slightly smaller group, 123 patients, completed all three samples, and used only rFVIII-FS replacement factor (without switching to another product) for the duration of the study period. Four subjects had positive inhibitor titres at baseline, with values ranging from 3.3 to 160 BU (Table 2). Of these, two patients had severe haemophilia, one had moderate haemophilia and for one patient the severity of haemophilia was unknown. Inhibitor assays for these four patients remained positive at 12 months following the switch to rFVIII-FS, with values ranging from 1.2 to 33.8 BU. At the 24-month postconversion time point, two of these patients tested positive for inhibitors (27.7 and 5.8 BU), while the remaining two subjects did not have 24-month samples collected. None of these patients received immune-tolerization.

Table 3 summarizes the inhibitor results from all valid patients for 12 and 24 months following conversion to rFVIII-FS. Only patients with positive baseline inhibitor titres (n = 4) had positive inhibitor titres at either the 12- or 24-month postswitch time points; therefore, no *de novo* inhibitors developed over the 2-year evaluation period in this patient population. Specifically, in patients in whom all sequential samples were collected, there was no evidence of inhibitor development over the course of the study (Table 4). Similarly the 123 patients who received only rFVIII-FS during the study and had all the required samples collected did not show any evidence of inhibitor formation.

Discussion

The results of this surveillance study suggest that the formulation of recombinant FVIII with sucrose (Kogenate®-FS; Bayer) rather than albumin did not result in an increased risk of inhibitor formation in previously treated haemophilia A patients. The first surveillance study of the Canadian haemophilia A population showed that there was no increase in the incidence of FVIII inhibitors when previously treated patients (PTPs) were converted to either a first generation rFVIII or high purity affinity-purified plasma-derived FVIII [10].

It is important to emphasize that this surveillance study differed from a more structured clinical trial with regard to sampling frequency for inhibitor detection. Several clinical studies evaluating inhibitor formation in both previously untreated patients (PUPs) and PTPs collected samples at 3-month intervals for inhibitor titres [14–19]. In fact, in one study of PUPs and minimally treated patients, the

Table 3. Inhibitor summary following conversion to rFVIII-FS*.

	12 months postswitch to rFVIII-FS			24 months postswitch to rFVIII-FS		
Baseline samples (preswitch)	Negative	Positive	Missing	Negative	Positive	Missing
Negative for inhibitors (n = 270)	221 (81%)	0	49 (18%)	185 (68%)	0	85 (31%)
Positive for inhibitors (n = 4)	0	4 (1.5%)	0	0	2 (0.7%)	2 (0.7%)†

*All samples, n = 274. A 'missing' sample can be a reflection of no sample collected at time point, or a patient switched to another product. †Twenty-four month postswitch samples were collected for only two patients, with the other two being not evaluable due to 'missing' samples. A positive FVIII inhibitor had a value ≥0.5 BU.

Table 4. Inhibitor summary following conversion to rFVIII-FS – patients completing the full surveillance protocol*.

	12 months postswitch to rFVIII-FS			24 months postswitch to rFVIII-FS		
Baseline sample (preswitch)	Negative	Positive	Missing	Negative	Positive	Missing
Negative for inhibitors (n = 138)	138 (99%)	0	0	136 (97%)	0	2 (1.4%)
Positive for inhibitors (n = 2)	0	2 (1.4%)	0	0	2 (1.4%)	0

*Defined as subjects in whom all three samples (baseline, 12 and 24 months following conversion to rFVIII-FS) were obtained, n = 140. A 'missing' sample can be a reflection of no sample collected at specific time point, or a patient switched to another product. A positive FVIII inhibitor had a value ≥0.5 BU.

frequency of sampling for inhibitor detection was even higher during the high-risk period [16]. With the less frequent sampling of every 12 months in this surveillance study, detection of transient inhibitors might have been missed. In addition, the study was not designed to match clinical evidence of an inhibitor (using FVIII recovery values or other clinical parameters) with laboratory detection, nor was it developed to detect non-neutralizing antibodies to FVIII. However, it is reasonable to suggest that the switch to rFVIII-FS from other recombinant FVIII formulations does not appear to lead to the development of new inhibitors of important clinical significance.

The relationship between FVIII product type and inhibitor risk is clearly an important issue for haemophilia patients and care givers, and the subject of ongoing debate [4–6]. An association between modification of the FVIII production (plasma-derived, pasteurized FVIII concentrates with either prior controlled-pore silica adsorption or solvent detergent treatment) and an increased incidence of inhibitors in PTPs was documented by Peerlinck *et al.* and Rosendaal *et al.* [20,21]. The pasteurization process may have produced epitope alterations in these preparations that resulted in the increased development of inhibitors. A recent study in France compared inhibitor incidence in PUPs treated with either a single recombinant or a single plasma-derived FVIII product [22]. These investigators noted a lower incidence of inhibitors associated with the use of the plasma-derived FVIII product compared with rFVIII. However, review of several studies with either plasma-derived or rFVIII products suggests that the recombinant products are not more immunogenic than FVIII preparations when the comparisons take into consideration the details of study design (including the frequency of inhibitor testing) and risk factors influencing inhibitor development [7,8]. *In toto* these data suggest that individual FVIII molecules may possess different inhibitor-inducing profiles but that amongst the many risk factors known to affect inhibitor development any one factor may be difficult to isolate. Also relevant to a discussion of inhibitor incidence is the number of exposure days (EDs), as patients with <20 EDs are still at high risk for inhibitor formation [7]. While the number of EDs was not documented in the present surveillance study, most of the patients enrolled in this study had received many more than 20 EDs. Additionally, it is important to note the recommendation of the Scientific Subcommittee of the International Society of Thrombosis and Haemostasis to use PTPs as the appropriate population in which to evaluate product immunogenicity [23].

Differences in study design, numerous risk factors for inhibitor development (severity of haemophilia, genetic mutation type, ethnicity, number of EDs, etc.) and the heterogeneity of the patient population complicate direct comparison of inhibitor incidence between studies. While it is difficult to prospectively assess one specific host or treatment-related risk factor, it is important to continually monitor new and existing FVIII replacement products for inhibitor development, and to identify significant deviations from the very low frequency 'background' immunogenicity of these products.

Acknowledgements

In addition to the author, the following Canadian Hemophilia Clinic Directors participated: Dr Dorothy Barnard (Halifax); Dr Victor Blanchette (Toronto); Dr Mason Bond (Vancouver); Dr Josée Brossard (Sherbrooke); Dr Manuel Caracao (Toronto); Dr Robert Card (Saskatoon); Dr Anthony K.C. Chan (Hamilton); Dr Stephanie Cloutier (Quebec); Dr Jeffrey Davis (Vancouver); Dr Michael Delorme (Kelowna); Dr Christine Demers (Quebec); Dr Sean Dolan (St John's); Dr Nancy Dower (Edmonton); Dr Bernadette Garvey (Toronto); Dr Kulwant Gill (Sudbury); Dr Jack Hand (St John's); Dr Rosemary Henderson (Charlottetown); Dr Donald S. Houston (Winnipeg); Dr Sara Israels (Winnipeg); Dr Lawrence Jardine (London); Dr Paula James (Kingston); Dr Robert Klassen (Ottawa); Dr Mariette Lepine-Martin (Sherbrooke); Dr David Lillicrap (Kingston); Dr Reinhard Lohmann (London); Dr Patricia J. McCusker (Winnipeg); Dr Man-Chiu Poon (Calgary); Dr Victoria Price (Halifax); Dr Margaret L. Rand (Toronto); Dr Bruce Ritchie (Edmonton); Dr Georges Rivard (Montreal); Dr Sue Robinson (Halifax); Dr Sheldon Rubin (Moncton); Dr Mary France Scully (St John's); Dr Sam Schulman (Hamilton); Dr Mariana Silva (Kingston); Dr Jean St -Louis (Montreal); Dr Jerome Teitel (Toronto); Dr Alan Timmouth (Ottawa); Dr Dimitrios Vergidis (Thunder Bay); Dr Linda Vickars (Vancouver); Dr Irwin Walker (Hamilton); Dr Margaret Warner (Montreal); Dr Phillip Wells (Ottawa); Dr Blair Whittemore (Montreal); Dr Rochelle Winikoff (Montreal); Dr John W.Y. Wu (Calgary); Dr John K. M. Wu (Vancouver); Dr Margaret Yhap (Halifax). I wish to thank Wendy Scales, PhD, Scientific Norton, LLL. for editorial contribution and Pamela Johnston, RN, for her diligence in data collection.

Disclosures

Dr. Teitel has acted as a paid consultant for Baxter Healthcare, Wyeth and Novo Nordisk. He is a recipient of research grant support from the Bayer Partnership fund. All other authors stated that they had no interests which might be perceived as posing a conflict or bias.

References

1 Key NS. Inhibitors in congenital coagulation disorders. *Br J Haematol* 2004; 127: 379–91.
2 Lusher J. Natural History of inhibitor development in children with severe hemophilia A treated with factor VIII products. In: Lee Blackwell C, Berntorp E, Hoots W, eds. *Textbook of Hemophilia*. Malden, Massachusetts: Publishing Ltd., 2005: 34–9.
3 Dimichele D, Rivard G, Hay C, Antunes S. Inhibitors in haemophilia: clinical aspects. *Haemophilia* 2004; 10(Suppl. 4): 140–5.
4 Aledort LM. Is the incidence and prevalence of inhibitors greater with recombinant products? Yes. *J Thromb Haemost* 2004; 2: 861–2.
5 Hoots WK, Lusher J. High-titer inhibitor development in hemophilia A: lack of product specificity. *J Thromb Haemost* 2004; 2: 358–9.
6 Lusher JM. Is the incidence and prevalence of inhibitors greater with recombinant products? No. *J Thromb Haemost* 2004; 2: 863–5.
7 Scharrer I, Bray GL, Neutzling O. Incidence of inhibitors in haemophilia A patients – a review of recent studies of recombinant and plasma-derived factor VIII concentrates. *Haemophilia* 1999; 5: 145–54.
8 Scharrer I, Ehrlich HJ. Lack of evidence for increased inhibitor incidence in patients switched from plasma-derived to recombinant factor VIII. *Haemophilia* 2001; 7: 346–8.
9 Boven K, Stryker S, Knight J *et al.* The increased incidence of pure red cell aplasia with an Eprex formulation in uncoated rubber stopper syringes. *Kidney Int* 2005; 67: 2346–53.
10 Giles AR, Rivard GE, Teitel J, Walker I. Surveillance for factor VIII inhibitor development in the Canadian Hemophilia A population following the widespread introduction of recombinant factor VIII replacement therapy. *Transfus Sci* 1998; 19: 139–48.
11 Medical Scientific and Advisory Council (MASAC). *Recommendation Regarding the Use of Recombinant Factor VIII in the Treatment of Hemophilia A*. New York: National Hemophilia Foundation, 1995, Medical Bulletin 232 (Chapter Advisory # 234).
12 Boedeker BG. Production processes of licensed recombinant factor VIII preparations. *Semin Thromb Hemost* 2001; 27: 385–94.
13 Verbruggen B, Novakova I, Wessels H, Boezeman J, van den Berg M, Mauser-Bunschoten E. The Nijmegen modification of the Bethesda assay for factor VIII:C inhibitors: improved specificity and reliability. *Thromb Haemost* 1995; 73: 247–51.
14 Abshire T, Brackmann H-H, Scharrer I *et al.* Sucrose-formulated recombinant human antihemophilic factor VIII is safe and efficacious for treatment of hemophilia A in home therapy: results of a multicenter, international, clinical investigation. *Thromb Haemost* 2000; 83: 811–6.
15 Bray GL, Gomperts ED, Courter S *et al.* A multicenter study of recombinant factor VIII (Recombinate): safety, efficacy, and inhibitor risk in previously untreated patients with hemophilia A. *Blood* 1994; 83: 2428–35.
16 Kreuz W, Gill JC, Rothschild C *et al.* Full-length sucrose-formulated recombinant factor VIII for treatment of previously untreated or minimally treated young children with severe haemophilia A. Results of an international clinical investigation. *Thromb Haemost* 2005; 93: 457–67.
17 Lusher JM, Lee CA, Kessler CM, Bedrosian CL. The safety and efficacy of B-domain deleted recombinant factor VIII concentrate in patients with severe haemophilia A. *Haemophilia* 2003; 9: 38–49.
18 Lusher J, Abildgaard C, Arkin S *et al.* Human recombinant DNA-derived antihemophilic factor in the treatment of previously untreated patients with hemophilia A: final report on a hallmark clinical investigation. *J Thromb Haemost* 2004; 2: 574–83.
19 White GC II, Courter S, Bray GL *et al.* A multicenter study of recombinant factor VIII (Recombinate) in previously treated patients with hemophilia A. The Recombinate Previously Treated Patient Study Group. *Thromb Haemost* 1997; 77: 660–7.
20 Peerlinck K, Arnout J, DiGiambattista M, Gilles J, Laub R, Jacquemin M. Factor VIII inhibitors in previously treated haemophilia A patients with a double virus-inactivated plasma derived Factor VIII concentrate. *Thromb Haemost* 1997; 77: 80–6.
21 Rosendaal FR, Nieuwenhuis HK, van den Berg HM *et al.* A sudden increase in factor VIII inhibitor development in multitransfused hemophilia A patients in The Netherlands. Dutch Hemophilia Study Group. *Blood* 1993; 81: 2180–6.
22 Goudemand J, Rothschild C, Demiguel V *et al.* Influence of the type of factor VIII concentrate on the incidence of factor VIII inhibitors in previously untreated patients with severe hemophilia A. *Blood* 2006; 107: 46–51.
23 White GC, DiMichele DM, Mertens K *et al.* Utilization of previously treated patients (PTPs), noninfected patients (NIPs), and previously untreated patients (PUPs) in the evaluation of new Factor VIII and Factor IX concentrates. *Thromb Haemost* 1999; 81: 462.