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 present 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.

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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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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

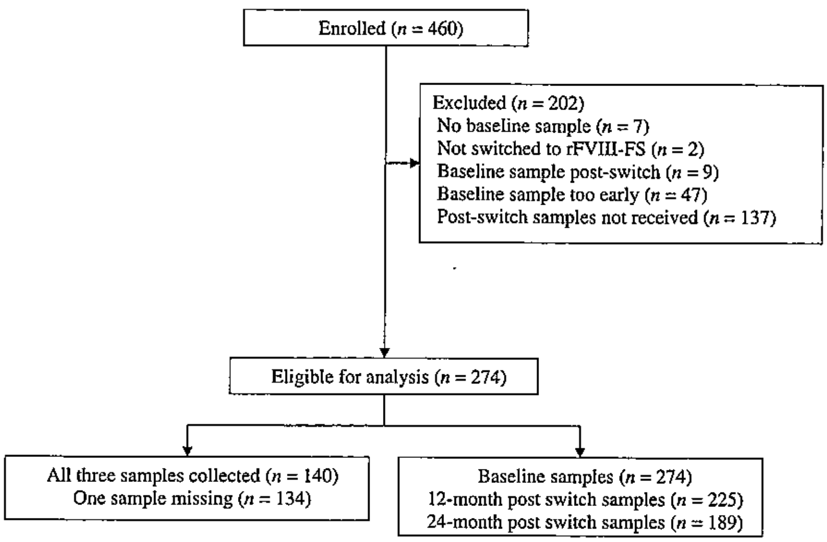

Fig. 1. Cohort of patients enrolled, excluded* and reason for exclusion, and patients eligible for analysis. *Patients have more than one exclusion criteria, which explains the discrepancy in the final number of patients eligible for analysis.