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.

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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.