CLINICAL TRIALS AND OBSERVATIONS

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

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Key Points

- A currently marketed rFVIII product is associated with a higher risk of inhibitor development in boys with severe hemophilia A.
- This result, validated by extensive sensitivity analyses, confirms a recently published study and cannot be explained by identified biases.

Six recombinant factor VIII (rFVIII) products have been marketed worldwide. In 2013, the Research of Determinants of Inhibitor Development (RODIN) study group reported an unexpectedly high risk of inhibitor development with a second-generation full-length rFVIII (Product D) in previously untreated patients (PUPs) with severe hemophilia A (HA). In 1994, French public health authorities established a prospective cohort to monitor hemophilia treatment safety. A PUP subgroup was designed to investigate inhibitor risk factors. We analyzed this subcohort in view of the RODIN findings. After excluding 50 patients who participated in the RODIN study, the primary analysis focused on 303 boys with severe HA first treated with a rFVIII product. A clinically significant inhibitor was detected in 114 boys (37.6%). The inhibitor incidence was higher with Product D vs the most widely used rFVIII product (adjusted hazard ratio [aHR], 1.55; 95% confidence interval [CI], 0.97-2.49). Similar results were found for high-titer inhibitors and in 10 sensitivity analyses. No heterogeneity was observed between RODIN and our results. Combined aHRs were 1.58 (95% CI, 1.17-2.14) for all inhibitors and 1.70 (95% CI, 1.15-2.52) for high-titer inhibitors. Our results confirm the higher immunogenicity of Product D vs other rFVIII products in PUPs with severe HA. (*Blood*. 2014;124(23):3398-3408)

Introduction

Hemophilia A (HA) is a hereditary coagulation disorder resulting from factor VIII (FVIII) deficiency.[1] Treatment consists of infusions of FVIII concentrates prepared from human plasma or by genetic engineering.[2] Some patients develop neutralizing antibodies against these products, mostly within the first 50 exposure days (EDs). These so-called inhibitors may jeopardize the patient's life[3] and make therapeutic management more complex[4,5] and costly.[6] Inhibitors arise in 15% to 35% of children with severe HA.[7,8] Several genetic and nongenetic risk factors for inhibitor development have been described. The main nongenetic risk factors are related to the modalities and circumstances of replacement therapy, such as age at treatment initiation, the FVIII product used, treatment intensity, prophylaxis regimen, major bleeds, and surgical procedures.[9-11] Research into inhibitor development has focused on the FVIII source—recombinant (r) vs plasma-derived (pd) products—with mixed results.[12,13] In 2004, a cohort study was launched in 29 hemophilia treatment centers (HTCs) in Europe, Israel, and Canada by the Research of Determinants of Inhibitor Development (RODIN) study group, to identify risk factors for inhibitor development in children with severe HA born between 2000 and 2009. The first article describing associations between FVIII products and inhibitor development in 574 consecutive patients was published in January 2013.[14] The only significant result was an unexpectedly higher risk of inhibitor development with a second-generation full-length

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rFVIII product (Product D) compared with the most widely used third-generation rFVIII product. This result perplexed prescribers and hemophilia patients worldwide.[15,16] The European Medicines Agency quickly launched a review of the RODIN results.[17] In December 2013, European Medicines Agency concluded that the available data did not support a higher inhibitor incidence with Product D than with other products. However, no new data challenged the RODIN findings, and it thus remained to be shown whether the observed difference was real or simply due to bias or chance (sampling fluctuations). In France, a prospective cohort was created in 1994 by the public health authorities to monitor hemophilia treatment safety.[18] A subcohort of previously untreated patients (PUPs) was established to investigate risk factors for inhibitor development. Here we compared inhibitor incidence rates across rFVIII products in these PUPs with severe HA.

Patients and methods

Patients

Almost all hemophilia patients in France have been included in the Réseau FranceCoag (hereafter, FranceCoag) national pharmacosurveillance network, which is based on voluntary participation of all French HTCs without support from pharmaceutical companies. Children included in the PUP cohort are closely monitored until age 18 years. Children born before 2000 were eligible if they had fewer than 4 EDs to FVIII, whereas those born from 2000 onward are included at diagnosis or as early as possible, provided their first infusions are traceable. The research into the differences between inhibitor development incidences in rFVIII and pdFVIII products is still relevant. However, the RODIN findings prioritized a comparison of rFVIII product immunogenicities to determine whether or not this class is homogeneous. We therefore selected boys with FVIII activity below 0.01 IU/mL enrolled in the FranceCoag PUP cohort who were first treated with rFVIII products. To facilitate the interpretation of the results of both studies, patients who also participated in the RODIN study were excluded from our analyses. The protocol for our observational cohort was approved by the French data protection watchdog. Parents or legal guardians received detailed written information about the objectives and modalities of the follow-up and were asked to approve their child's enrollment in accordance with the Declaration of Helsinki.

Data collection

Data were collected on electronic case report forms. Baseline data comprised demographic characteristics, HA-related medical history from birth, the *F8* gene defect, family history of hemophilia and inhibitors, and ethnic origin. At each follow-up visit, major events having occurred since the last visit were collected (severe bleeds, hemarthrosis, surgical procedures, vaccinations) along with detailed hemophilia treatments. The results of FVIII and inhibitor assays performed during follow-up were also recorded. The collected data were automatically checked and repeatedly monitored for completeness and inconsistencies by 3 dedicated clinical research assistants in the HTCs. Independently from the FranceCoag Network, the French Hemophilia Association publishes a customized booklet for collecting the adhesive labels of all injected FVIII or FIX products to obtain details of home treatment. For several decades, this tool has been distributed to hemophilia patients in all French HTCs. In addition to data collected during hospitalization, this booklet allowed us to record the first 75 EDs in the same way as in the RODIN study, including the infusion dates, FVIII product brands and doses, indications, bleeding events, and types of surgery. These additional data were collected by a fourth clinical research assistant, independently of the basic data collection. Thus, any discrepancy between the 2 recording systems (regarding the date of the first infusion, FVIII products received, number of EDs at the date of inhibitor detection, initiation of regular prophylaxis, surgical procedures, and severe bleeding episodes) triggered extensive investigations based on the original files.

Follow-up and outcomes

Patients enrolled in the FranceCoag Network are followed indefinitely, but only the first 75 EDs were considered for this analysis. The cutoff date was May 19, 2014. For patients who developed an inhibitor, follow-up ended on the last ED before its detection. The follow-up was censored after the last rFVIII infusion if the patient switched to a pdFVIII before ED75 or if the patient had not reached 75 EDs at the last clinical visit. The inhibitor assays were performed in the laboratory of each HTC. The primary outcome measure was inhibitor development during the first 75 EDs, defined as a positive assay result (>0.6 Bethesda units (BU)/mL) on any 2 dates. A secondary outcome measure was inhibitor development during the first 75 EDs with a peak titer of at least 5 BU/mL at any time.[19] A third outcome measure was added to reflect the therapeutic impact of inhibitors, namely inhibitor development during the first 75 EDs treated at any time with a bypassing agent and/or an immune tolerance induction (ITI). All clinically significant cases were validated by an ad hoc clinical committee using a standardized procedure (supplemental Methods, available on the *Blood* Web site).

rFVIII products studied

The main risk factor that was investigated was the rFVIII product received during the first 75 EDs. Six rFVIII products (A to F) have been marketed worldwide (Table 1). Products A to C were withdrawn from the European Union market and were replaced with new-generation rFVIII products (D to F) (supplemental Figure 1). Because patients could be switched from one product to another during the first 75 EDs, the rFVIII product was analyzed as a time-varying factor. As in the RODIN study, Product E served as reference.

Primary analysis

We constructed a Cox proportional hazards model with ED as the observational time unit. The best-acknowledged fixed risk factors—the *F8* gene defect, family history of hemophilia and inhibitors, ethnic origin, and age at first rFVIII infusion—were systematically included in the multivariate models, regardless of their statistical association with inhibitor development in this analysis. The following time-varying risk factors were also considered: calendar period, regular prophylaxis, treatment intensity markers as used in the RODIN study (interval between EDs and rFVIII dose calculated over the last 5 EDs, and peak treatment episodes), history of surgery,[14] and history of severe bleeding. Time-varying risk factors associated with the all inhibitors outcome with a P value $<.2$ were retained in the final multivariate models. Crude and adjusted hazard ratios (aHRs) are reported for the association between a given rFVIII product and clinically significant inhibitor development. Associations were considered significant if $P < .05$. Cofactor definitions, grouping, and missing data procedures are described in the supplemental Methods.

Sensitivity analyses

We performed 10 sensitivity analyses. In the first 8 analyses, the study population was rendered more homogeneous by excluding (1) patients who participated in an rFVIII product clinical trial; (2) or by selecting patients with an intron-22 inversion; (3) patients with no family history of inhibitors; (4) patients with white parents; (5) patients who fulfilled criteria 2, 3, and 4; (6) patients born between 2000 and 2009 (as in the RODIN population); (7) only EDs in the 2004-2014 period during which products D and E were both marketed; and (8) patients recruited by the most contributory HTCs to adjust for HTC in the multivariate analysis. In the ninth analysis, only the rFVIII product received at the first infusion (fixed factor) was considered. Finally, the main analysis was repeated by means of pooled logistic regression with the cumulative number of EDs as the time variable, which corresponded to the statistical method used in the RODIN study.[20]

Meta-analysis

We combined HRs for Product D compared with Product E (D/E) from the RODIN study and our study using indirect log HR and variance estimation[21]

Table 1. Characteristics of the 6 recombinant FVIII products commercialized worldwide

Product	Manufacturer	Distributor	Brand name	Generation	Production cell line	Protein length	Human or animal proteins in fermentation process	Albumin as stabilizer	Marketing authorization dates in EU
A	Baxter Bioscience	Baxter Healthcare Aventis Behring	Recombinate Bioclate	1	CHO	Full	Yes	Yes	June 17, 1993
B	Bayer HealthCare	Bayer HealthCare Aventis Behring	Kogenate Helixate	1	BHK	Full	Yes	Yes	June 15, 1994
C	Wyeth Pharmaceuticals	Wyeth Pharmaceuticals	ReFacto	2	CHO	B-domain deleted	Yes	No	April 13, 1999
D	Bayer HealthCare	Bayer HealthCare CSL Behring	Kogenate FS/Bayer Helixate NexGen	2	BHK	Full	Yes	No	August 4, 2000
E	Baxter Bioscience	Baxter Healthcare	Advate	3	CHO	Full	No	No	March 2, 2004
F	Pfizer	Pfizer	ReFacto AF Xyntha (used outside the EU)	3	CHO	B-domain deleted	No	No	April 15, 2009

EU, European Union.

for all inhibitors and high-titer inhibitors outcomes. The degree of inconsistency across both studies was estimated by the I^2 statistic.[22]

Role of the funding source

The French hemophilia surveillance system (Suivi thérapeutique National des Hémophiles, known as Réseau FranceCoag from 2003 onward) has been fully supported by the public health authorities since 1994. The additional data collection for the first 75 EDs was partially supported by the French National Clinical Research Program in 2009 and by Assistance Publique-Hôpitaux de Marseille. No public health authority representatives, except authors from the French Institute for Public Health Surveillance (V.G. and V.H.), had a role in the study design, data collection, data analysis, data interpretation, or report writing.

Results

Patient selection and characteristics

In all, 741 boys with severe HA born between 1991 and 2013 were recruited to the FranceCoag Network by 37 HTCs. The PUP cohort criteria were met by 492 of these boys, 52 (17.6%) of those born from 1991 to 1999 (n = 296), and 440 (98.9%) of those born from 2000 to 2013 (n = 445). Among these 492 eligible patients, 189 were excluded from the analyses: 12 had not started treatment at the last clinical visit, 110 received pdFVIII at the first infusion, 17 had insufficient data for the first EDs, and 50 also participated in the RODIN study. No patients were excluded because of their follow-up in a particular HTC. Finally, 303 patients followed in 33 HTCs were included in the analyses (Figure 1). Among the 6 rFVIII products marketed in France during the study period (1993 to 2014), those most frequently received for the first infusion were Products D (36.6%), E (32.0%), A (15.8%), and C (8.9%). The last 2 products (B and F) were received as first product by only 10 and 10 boys, respectively, and are therefore not presented individually in the Results section. Among the boys' fixed characteristics (Table 2), only the calendar period at the first infusion was significantly associated with the first rFVIII product received ($P < .001$), owing to the staggered market release dates of the products. Among the time-varying risk factors, a regular prophylaxis regimen was initiated within the first 50 EDs in 47.9% of patients. At least 1 peak treatment episode lasting at least 3, 5, and 10 consecutive days was experienced by 69.3%, 39.9%, and 13.5% of patients, respectively. Similarly, at least 1 surgical procedure and at least 1 severe bleeding episode were experienced by 12.5% and 11.6% of patients, respectively. Only the initiation of regular prophylaxis within the first 50 EDs was significantly associated with the first rFVIII product received ($P = .047$ for global test).

Follow-up and exposure

The observation period totaled 478 person-years and 14 044 EDs. Follow-up data are shown in supplemental Table 2 according to the first product received. In total, 274 boys (90.4%) received a single rFVIII product throughout follow-up. The other 29 boys switched at least once to another rFVIII product (supplemental Table 3). Most switches were from a first-generation rFVIII product to a second- or third-generation rFVIII product of the same brand. Five of these boys developed an inhibitor. The contributions in EDs according to the rFVIII product received during the follow-up and the 7 studied time-varying risk factors are shown in supplemental Table 4.

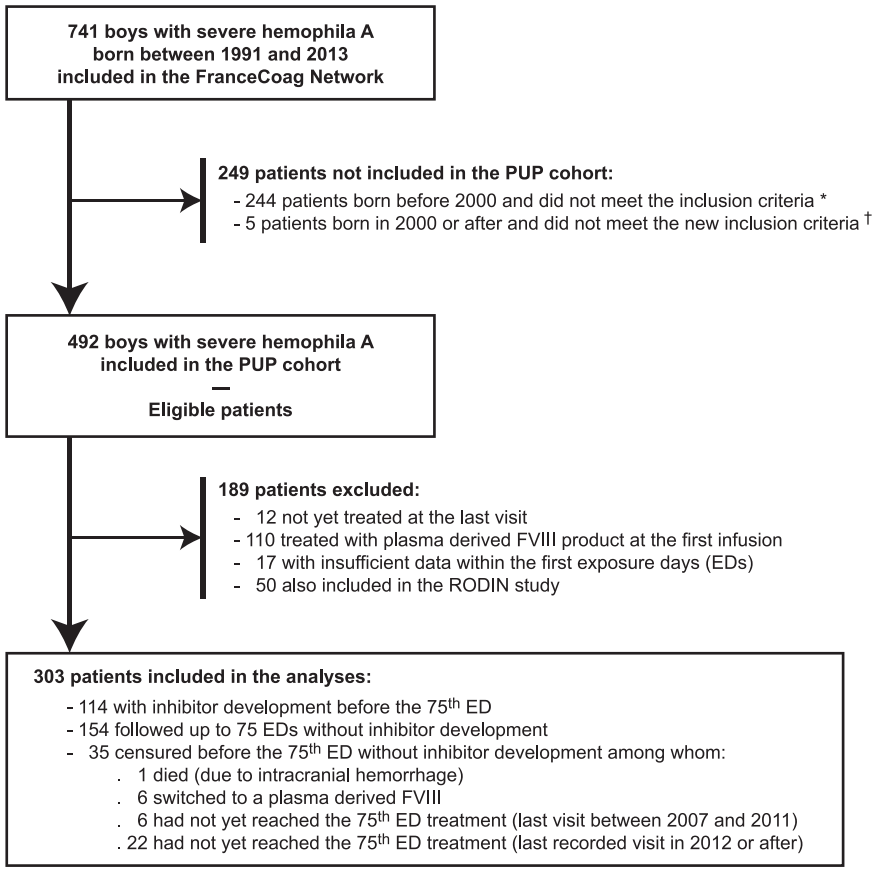
Inhibitor characteristics

In all, 1417 inhibitor assay results were recorded. On average, these assays were performed every 6.3 EDs during the first 25 EDs and every 9.9 EDs during the overall follow-up period. The assay frequency was very similar across the rFVIII products (supplemental Table 5). A clinically significant inhibitor was diagnosed in 114 boys (37.6%) after a median of 13 EDs (interquartile range [IQR], 8-19 EDs) and at a median age of 15.2 months (IQR, 11.1-22.8 months) (Table 3). Among the boys with inhibitors, 95 (83.3%) were subsequently treated with a bypassing agent and/or ITI at any time during FranceCoag follow-up. The cumulative incidence at ED75 was 40.2% (95% confidence interval [CI], 34.8%-46.2%). A high-titer inhibitor was diagnosed in 63 boys (20.8%), with a cumulative incidence of 23.9% (95% CI, 19.1%-29.6%) at ED75 (supplemental Figure 3). A clinically significant inhibitor was diagnosed in only 3 boys between ED50 and ED75, and no cases were diagnosed between ED75 and ED100 among the 303 selected boys.

Primary analysis

The risk of inhibitor development was different across the 4 studied rFVIII products for the all inhibitors outcome ($P = .025$) and also for the treated inhibitors outcome ($P = .019$) (Table 4

Figure 1. Patient selection process.



Exposure day (ED): Day during which one or more infusions of FVIII were given.
* *Less than 4 EDs upon inclusion into FranceCoag Network.*
† *Complete replacement therapy history upon inclusion into FranceCoag.*

and supplemental Figure 4). Specifically, the inhibitor risk was significantly higher with Product D than with Product E ($P = .031$ and $P = .046$ for both outcomes, respectively). Similar tendencies were observed in the multivariate analyses: D/E aHR was 1.55 (95% CI, 0.97-2.49) for all inhibitors, D/E aHR was 1.56 (95% CI, 0.82-2.98) for high-titer inhibitors, and D/E aHR was 1.58 (95% CI, 0.94-2.64) for treated inhibitors ($P < .2$). No significant difference or stable trend was observed for Product A or C vs Product E.

Sensitivity analyses

Detailed results of the 10 sensitivity analyses are shown in supplemental Tables 6-15. Estimated aHRs were between 1.52 and 2.59 for all inhibitors outcomes across the primary analysis and the 10 sensitivity analyses, between 1.29 and 2.00 for the high-titer inhibitors outcomes, and between 1.47 and 2.39 for the treated inhibitors outcomes (Figure 2). Wide HR CIs were observed for Products A and C, particularly in sensitivity analyses with reduced populations, and no stable trend was detected. Finally, the higher risk associated with Product D vs Product E was stable at approximately 60% for the 3 outcomes. *P* values were lower than .2 for all inhibitors and treated inhibitors outcomes.

Meta-analysis

The RODIN-FranceCoag combined D/E aHR was 1.58 (95% CI, 1.17-2.14) for all inhibitors and 1.70 (95% CI, 1.15-2.52) for high-titer inhibitors (Figure 3). No heterogeneity was observed between the RODIN and the FranceCoag studies for both outcomes ($I^2 = 0\%$).

Discussion

Main findings

A publicly funded pharmacosurveillance system for antihemophilia drugs has existed in France since 1994. We took advantage of this data set to challenge the unexpected and disturbing finding of the RODIN study.[14] Both RODIN and FranceCoag cohort studies precisely recorded the first EDs of replacement therapy under real-life conditions. The cumulative incidence rates of inhibitor development by ED75 were 32.4% and 40.2% for all inhibitors, and 22.4% and 23.9% for high-titer inhibitors in the RODIN study and our study, respectively.

We observed a significant association between the rFVIII product received and the all inhibitors outcome. More specifically, after taking known major genetic and nongenetic cofactors into account, the D/E aHR was 1.55 (95% CI, 0.97-2.49). The D/E aHR was 1.56 (95% CI, 0.82-2.98) for high-titer inhibitors and 1.58 (95% CI, 0.94-2.64) for treated inhibitors. The events number in our study is lower than that in the RODIN study and leads to a lower analysis power. However, the difference between inhibitor incidence with

Table 2. Patient characteristics according to the first recombinant VIII product received

	First recombinant FVIII product received																			
	Product E (n = 97)				Product D (n = 111)				Product A (n = 48)				Product C (n = 27)				All rFVIII products* (n = 303)			
Characteristics	**No.**	**%**	**M**	**IQR**	**No.**	**%**	**M**	**IQR**	**No.**	**%**	**M**	**IQR**	**No.**	**%**	**M**	**IQR**	**No.**	**%**	**M**	**IQR**
Fixed risk factors																				
F8 gene defect																				
Low risk	30	30.9			19	17.1			11	22.9			6	22.2			68	22.4		
High risk †	62	63.9			85	76.6			35	72.9			21	77.8			214	70.6		
Undetermined (eg, untested, unidentified)	5	5.2			7	6.3			2	4.2			0	0.0			21	6.9		
Family history																				
Hemophilia without inhibitor	45	46.4			37	33.3			13	27.1			9	33.3			110	36.3		
Hemophilia with inhibitor	9	9.3			15	13.5			6	12.5			3	11.1			36	11.9		
No family history of hemophilia	43	44.3			59	53.2			29	60.4			15	55.6			157	51.8		
Ethnic origin‡																				
White only (both parents)	74	76.3			85	76.6			37	77.1			20	74.1			233	76.9		
Others not African or African-American	17	17.5			23	20.7			7	14.6			6	22.2			55	18.2		
African or African-American (at least one grandparent)	6	6.2			3	2.7			4	8.3			1	3.7			15	5.0		
Calendar period of first exposure to rFVIII																				
Before 2000	0	0.0			19	17.1			35	72.9			19	70.4			83	27.4		
2000-2003	24	24.7			31	27.9			12	25.0			7	25.9			74	24.4		
2004-2007	35	36.1			31	27.9			1	2.1			1	3.7			69	22.8		
2008 and after	38	39.2			30	27.0			0	0.0			0	0.0			77	25.4		
Age at first exposure to rFVIII, months			9.5	3.6-13.3			11.6	7.4-14.5			10.1	5.3-16.8			10.6	4.7-16.7			10.4	5.6-14.3
<6	30	30.9			23	20.7			14	29.2			7	25.9			78	25.7		
6-11	35	36.1			35	31.5			15	31.3			8	29.6			102	33.7		
≥12	32	33.0			53	47.7			19	39.6			12	44.4			123	40.6		
Time-varying risk factors																				
Initiation of regular prophylaxis within first 50 EDs§	56	57.7			49	44.1			19	39.6			9	33.3			145	47.9		
Cumulative EDs at start of prophylaxis			15	7-26			20	10-33			22	16-47			30	20-54			20	11-33
Peak treatment episode at first exposure, consecutive EDs																				
≥3	31	32.0			35	31.5			12	25.0			5	18.5			91	30.0		
≥5	14	14.4			21	18.9			8	16.7			3	11.1			50	16.5		
≥10	8	8.2			11	9.9			2	4.2			1	3.7			23	7.6		

M, median.

*Product B (n = 10) and Product F (n = 10) are included in all rFVIII products.

†High-risk gene defects include large deletions (≥1 exon), intron 1 and 22 inversions, small deletions/insertions with stop codon (out of A-run), and nonsense mutations.

‡Up to four ethnic origins per patient could be recorded (one for each grandparent).

§The initiation of regular prophylaxis was defined as the moment at which at least 3 consecutive prophylactic infusions of rFVIII were given within a period of at least 15 days (RODIN definition).[14]

Table 2. (continued)

Characteristics	First recombinant FVIII product received																			
	Product E (n = 97)				Product D (n = 111)				Product A (n = 48)				Product C (n = 27)				All rFVIII products* (n = 303)			
	No.	%	M	IQR	No.	%	M	IQR	No.	%	M	IQR	No.	%	M	IQR	No.	%	M	IQR
History of peak treatment episodes (≥1 during follow-up), consecutive EDs																				
≥3	63	64.9			75	67.6			37	77.1			21	77.8			210	69.3		
≥5	35	36.1			44	39.6			22	45.8			12	44.4			121	39.9		
≥10	14	14.4			17	15.3			5	10.4			4	14.8			41	13.5		
First exposure linked to surgical procedure (with ≥3 EDs)	2	2.1			1	0.9			0	0.0			0	0.0			5	1.7		
History of surgical procedures (with ≥3 EDs) during follow-up	10	10.3			8	7.2			10	20.8			5	18.5			38	12.5		
First exposure linked to severe bleeding episode	10	10.3			10	9.0			2	4.2			0	0.0			22	7.3		
History of severe bleeding episodes during follow-up	13	13.4			17	15.3			3	6.3			2	7.4			35	11.6		
Outcomes																				
All inhibitors	33	34.0			55	49.5			13	27.1			7	25.9			114	37.6		
High-titer inhibitors	20	20.6			28	25.2			7	14.6			5	18.5			63	20.8		
Inhibitors occurring during the first 75 EDs and treated at any time during FranceCoag follow-up																				
Cases treated with bypassing agents	24	24.7			38	34.2			6	12.5			6	22.2			77	25.4		
Cases treated with ITI	23	23.7			40	36.0			7	14.6			5	18.5			79	26.1		
Cases treated with bypassing agents and/or ITI	29	29.9			47	42.3			8	16.7			7	25.9			95	31.4		

M, median.

*Product B (n = 10) and Product F (n = 10) are included in all rFVIII products.

†High-risk gene defects include large deletions (≥1 exon), intron 1 and 22 inversions, small deletions/insertions with stop codon (out of A-run), and nonsense mutations.

‡Up to four ethnic origins per patient could be recorded (one for each grandparent).

§The initiation of regular prophylaxis was defined as the moment at which at least 3 consecutive prophylactic infusions of rFVIII were given within a period of at least 15 days (RODIN definition).[14]

Table 3. Characteristics of clinically significant inhibitors

	All inhibitors (n = 114)				High-titer inhibitors* (n = 63)				Low-titer inhibitors (n = 51)			
Characteristic	**No.**	**%**	**M**	**IQR**	**No.**	**%**	**M**	**IQR**	**No.**	**%**	**M**	**IQR**
No. of EDs at inhibitor detection			13	8-19			11	7-16			17	8-22
Age at inhibitor detection, mo			15.2	11.1-22.8			12.8	8.7-18.8			18.6	14.8-30.3
Duration between first ED and inhibitor detection, mo			4.3	2.0-9.6			3.0	1.5-6.7			7.3	2.8-18.2
Maximal inhibitor titer, BU/mL			7.5	2.1-69.0			53.0	13.0-220.0			2.0	1.0-3.2
Treatments received at any time during FranceCoag follow-up												
Bypassing agents	77	67.5			55	87.3			22	43.1		
ITI	79	69.3			52	82.5			27	52.9		
Bypassing agents and/or ITI	95	83.3			59	93.7			36	70.6		

*High-titer inhibitor defined as peak titer ≥5 BU/mL at any time during FranceCoag follow-up.

Products D and E persisted in 10 sensitivity analyses based on more homogeneous subgroups and different statistical approaches. Meta-analysis of the RODIN and FranceCoag results showed a concordant and significant 60% higher risk with Product D compared with Product E for the all inhibitors and high-titer inhibitors outcomes. In contrast, in the literature only 9 (15%) of 60 PUPs and minimally treated patients enrolled in registration trials of Product D developed inhibitors,[23,24] and postmarketing studies showed even lower rates (supplemental Table 21).[25,26] One key difference with cohort studies is that patients are not selected, whereas clinical trials exclude some patients at an increased risk of inhibitor development, such as those requiring intensive initial treatment because of early severe bleeding.

Strengths and potential biases

The prevalence of HA at birth in France from 1991 to 2008 was estimated at 23.3 cases per 100 000 male live births (supplemental Table 1). This result, which represents one of the highest rates observed in an industrialized country,[27] supports the exhaustiveness of the FranceCoag Network. All but 5 of the boys with severe HA born since 2000 met the enrollment criteria for the PUP cohort. Among the 370 PUPs with severe HA treated with rFVIII, only 17 (4.6%) were excluded because of insufficient data. Our findings are thus representative of PUPs with severe HA treated in France. More than half the boys (51.5%) were enrolled after a few EDs, regardless of whether or not an inhibitor had already been discovered. Their clinical history, including treatments, was fully recorded from birth. Thus, these boys do not correspond to minimally treated patients, and their inclusion does not constitute a selection bias. Another potential selection bias is that patients with known and identifiable risk factors for inhibitor development at treatment outset might have received Product D preferentially. This prescription bias is conceivable from 2002 and 2005, years in which 2 articles reported a notably low rate of inhibitor development (15%) in PUPs treated with Product D.[23,24] During this period only the main genetic risk factors for inhibitor development were identified in PUPs. In our study, slightly higher proportions of patients with a high-risk *F8* gene defect and a family history of inhibitors were observed among PUPs treated with

Table 4. Inhibitor risk according to the type of recombinant FVIII (rFVIII) product (primary analysis)

		Unadjusted analysis			Multivariate analysis		
	No. of EDs	**Crude HR**	**95% CI**	***P***	**Adjusted HR**	**95% CI**	***P***
All inhibitors				.025*			.221*
Product E	4995	1.00			1.00		
Product D	4749	1.61	1.04-2.47	.031	1.55	0.97-2.49	.069
Product A	2074	0.69	0.34-1.40	.300	0.97	0.40-2.37	.952
Product C	1412	0.93	0.43-2.02	.864	1.20	0.47-3.08	.705
High-titer inhibitors				.489*			.547*
Product E	4995	1.00			1.00		
Product D	4749	1.42	0.79-2.52	.240	1.56	0.82-2.98	.177
Product A	2074	0.83	0.35-1.97	.673	1.87	0.59-5.89	.286
Product C	1412	1.02	0.38-2.74	.963	1.94	0.54-6.91	.307
Inhibitors subsequently treated with a bypassing agent and/or ITI				.019*			.165*
Product E	4995	1.00			1.00		
Product D	4749	1.61	1.01-2.56	.046	1.58	0.94-2.64	.082
Product A	2074	0.49	0.20-1.17	.108	0.81	0.28-2.35	.705
Product C	1412	1.11	0.50-2.43	.799	1.67	0.62-4.51	.311

Population: boys with severe HA (FVIII activity <0.01 IU/mL) first treated with rFVIII and treated with Products A, C, D, or E within the first 75 EDs (n = 287). Products B and F were used only in 10 and 10 boys and 331 and 483 EDs, respectively, so their effect on inhibitor development was not studied. Main studied factor: type of rFVIII product received during the first 75 EDs (time-varying factor). Statistical method: Cox proportional hazards model with ED as observational time. aHR took into account the following cofactors: 4 fixed factors (*F8* gene defect, family history of hemophilia and inhibitor, ethnic origin, and age at first infusion of rFVIII) and 7 time-varying factors (calendar period, regular prophylaxis initiation, interval between EDs calculated over the last 5 EDs, mean dose of rFVIII product calculated over the last 5 EDs, history of peak treatment episodes ≥5 consecutive EDs, history of peak treatment episodes ≥10 consecutive EDs, and history of severe bleeding episodes).

**P* value for global test.

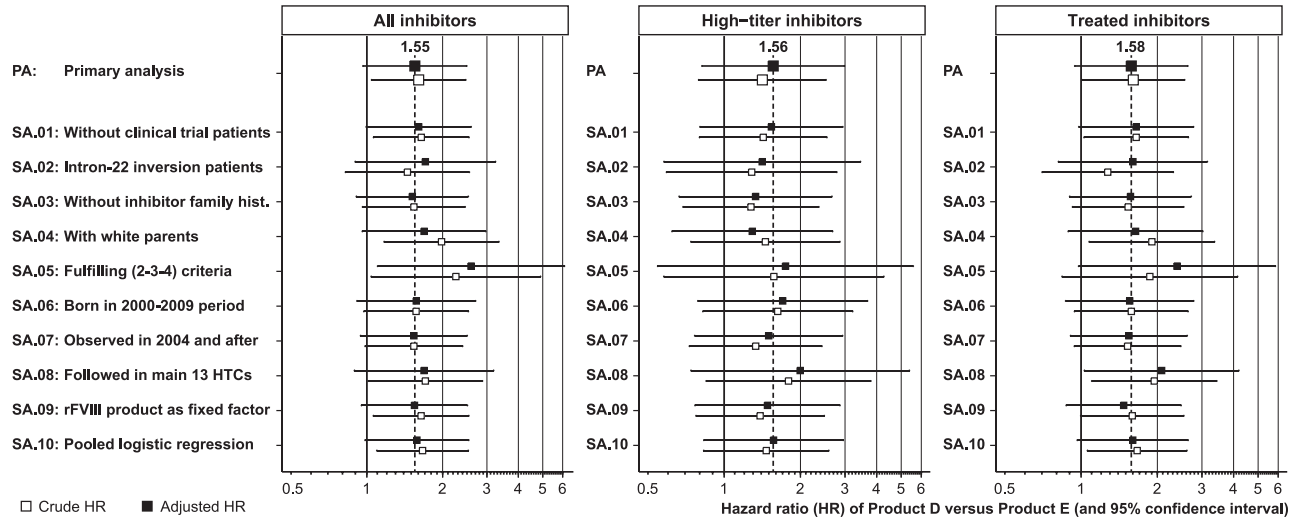


Figure 2. Crude and adjusted HRs and 95% CIs for Product D vs Product E in the primary analysis (PA) and 10 sensitivity analyses (SAs). Three outcomes are shown: all inhibitors, high-titer inhibitors, and inhibitors subsequently treated with a bypassing agent and/or ITI (detailed results are presented in supplemental Tables 6-15).

Product D than among those treated with Product E, whereas the opposite was observed in the RODIN study.[14] Moreover, owing to the high proportion of sporadic cases among patients with severe HA, the *F8* gene defect is often unknown when choosing the FVIII product for initial treatment.[28] It is therefore unlikely that such a prescription bias occurred in either cohort. Furthermore, the 3 main genetic risk factors were included in both multivariate analyses. High-titer inhibitors are usually diagnosed after hemorrhagic events or ineffective replacement treatment, and any underreporting would preferentially concern low-titer inhibitors. Such a bias might be envisaged if the frequency and/or sensitivity of inhibitor screening assays varied according to the rFVIII product received. Because the calendar period clearly influenced the choice of product and might have affected the outcome assessment,[7,15] we considered it in multivariate analyses. Furthermore, Products D and E were used during concurrent time periods (supplemental Figure 1). Differences in practices across HTCs with respect to the choice of rFVIII products and outcome assessments are also conceivable, but such variations would have been limited because our study took place in a single country. Furthermore, no difference in inhibitor assay frequency was observed between Product D and Product E (supplemental Table 5). We obtained similar results when we restricted the analysis to patients treated in the 13 most contributory HTCs and when we included the HTC effect in multivariate analysis (supplemental Table 13). Ultimately, similar results were observed after successively removing the data for each of the 13 most contributory HTCs and in the 20 less contributory HTCs (supplemental Table 20). We addressed potential genetic confounding factors by taking most of them into account in multivariate analyses. Although greater precision would be desirable,[16,29] we could compare only 2 classes of *F8* gene defects because of limited subgroup sizes. Again, similar results were obtained in patients belonging to the largest homogeneous class (intron-22 inversion) (supplemental Table 7). Other highlighted genetic inhibitor risk factors were HLA genotype[30-32]

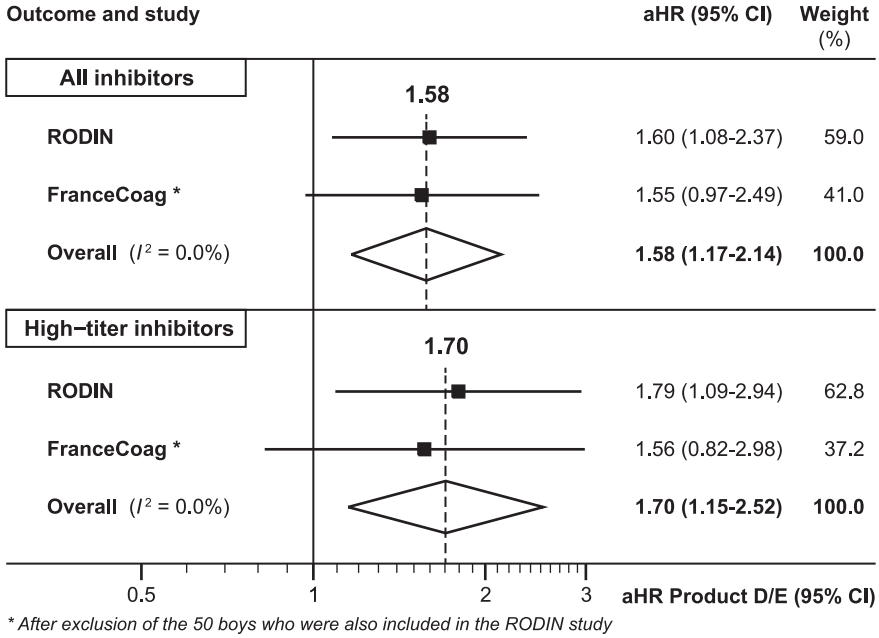


Figure 3. aHRs and 95% CIs for Product D vs Product E of the RODIN and the FranceCoag studies and combined aHRs between both studies for all inhibitors and high-titer inhibitors outcomes.

and polymorphisms in immune regulatory genes.[33,34] In clinical practice, these genetic markers are rarely investigated, and there is no argument to support their possible influence in the choice of FVIII product. Genetic risk factors are thus unlikely to have played a confounding role. The best-known potential nongenetic confounding factors—replacement treatment intensity[35] and related conditions (surgical procedures and severe bleeds)—were factored into our multivariate analyses in addition to regular prophylaxis.

Possible biological explanations and implications

The main difference between Product D and Product E is that Product D is produced in baby hamster kidney (BHK) cells and Product E is produced in Chinese hamster ovary (CHO) cells. Changes in BHK manufacturing practices between Product B and Product D might have led to increased immunogenicity,[36] but the number of patients treated with Product B in both the RODIN study and in our cohort was too small for comparison with Product D. rFVIII molecules produced by BHK and CHO cells differ in several respects, such as the degree of tyrosine residue sulphation and type of glycosylation.[37] The presence of specific glycan chains might affect dendritic cell uptake and thereby modify rFVIII immunogenicity.[38] The amino acid sequence of the BHK rFVIII products (B and D) corresponds to the most frequent haplotype (H1) in the white population.[39] Full-length CHO rFVIII products (A and E) have an amino acid difference at B-domain position 1241, but no substantial interaction was found between the patient's *F8* haplotype, rFVIII product received, and inhibitor risk.[40] Furthermore, these products also differ in their biological activities.[41] In fact, the mechanism of the difference in inhibitor incidence between rFVIII remains unclear and has not been elucidated by this study. Nonclinical studies will thus be necessary to identify the mechanism underlying the difference in immunogenicity between Products D and E. However, it is conceivable that a slight difference in immunogenicity among rFVIII products could lead to a moderate increase in the risk of inhibitor development.[42] Unfortunately, few if any other HA PUP cohorts exist worldwide, ruling out further epidemiologic results. The consistency between our findings and those of the RODIN study suggests (but does not prove) that the observed association between rFVIII products and the risk of inhibitor development is causal. The potential impact of the higher risk of inhibitor development associated with Product D could be estimated to 1 or 2 extra cases annually in France (supplemental Results). Thus, we think that these results concerning a major adverse effect are sufficiently convincing to warrant consideration in the choice of rFVIII products for PUPs with severe HA in France and in other countries.

American and European medicine agencies recognize that registration trials with small numbers of selected patients may not be able to detect a moderate difference in FVIII immunogenicity. Several initiatives were implemented in the past decade to improve our knowledge of the association between FVIII products and inhibitor incidence. The ongoing Survey of Inhibitors in Plasma-Product Exposed Toddlers (SIPPET) trial was launched in 2009 to compare incidence of inhibitors with von Willebrand factor–containing pdFVIII products versus rFVIII products in PUPs with severe HA.[43] The expected number of patients in the rFVIII arm may be too small to demonstrate a difference within this group. To the best of our knowledge, no other randomized trials comparing FVIII products are planned, owing particularly to the rarity of HA and the very young age of the target population. In the United States, the Centers for Disease Control and Prevention began the Hemophilia Inhibitor Research Study in 2006 to determine the feasibility of using a public health surveillance system to collect key information about inhibitors.[44] This feasibility study has not yet produced results for PUPs with HA, but national implementation of this inhibitor surveillance system is being considered. The European Hemophilia Safety Surveillance System (EUHASS) was established concurrently.[45] Adverse events and data for exposed populations are periodically reported, but a stable number of patients treated with a particular product over at least a 2-year period is required to estimate the cumulative incidence of inhibitors (at ED50 in PUPs) with that product.[46] This could limit the precision of product comparisons in this pharmacovigilance program. Our findings and those of the RODIN study highlight the need to strengthen the power and responsiveness of postmarketing monitoring of hemophilia treatments worldwide. This is a major issue because several new FVIII products, including human cell–derived and long-acting rFVIII are reaching an advanced stage of clinical development and may be marketed soon in several countries. Given the heavy burden of inhibitors for individual patients and the high costs of bypassing agents and ITI, this mobilization would not only benefit patients but would also lead to substantial savings for national health care budgets.

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Authorship

Contribution: T.C., H.C., and J.G. designed the FranceCoag Network and wrote the manuscript; T.C. and V.H. performed the statistical analyses; R.d.O., S.C.-D., J.G., and V.R.-R. validated all inhibitor cases; all authors designed the analytical strategy, discussed the results, critically reviewed the manuscript, and had final responsibility for the decision to submit.

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A complete list of contributors to the FranceCoag Network appears in supplemental Material.

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References

1. Hoyer LW. Hemophilia A. *N Engl J Med.* 1994; 330(1):38-47.
2. Berntorp E, Shapiro AD. Modern haemophilia care. *Lancet.* 2012;379(9824):1447-1456.
3. Darby SC, Keeling DM, Spooner RJD, et al; UK Haemophilia Centre Doctors' Organisation. The incidence of factor VIII and factor IX inhibitors in the hemophilia population of the UK and their effect on subsequent mortality, 1977-99. *J Thromb Haemost.* 2004;2(7):1047-1054.
4. Hay CR, Baglin TP, Collins PW, Hill FG, Keeling DM. The diagnosis and management of factor VIII and IX inhibitors: a guideline from the UK Haemophilia Centre Doctors' Organization (UKHCDO). *Br J Haematol.* 2000;111(1):78-90.
5. Teitel JM, Sholzberg M. Current status and future prospects for the prophylactic management of hemophilia patients with inhibitor antibodies. *Blood Rev.* 2013;27(2):103-109.
6. Di Minno MN, Di Minno G, Di Capua M, Cerbone AM, Coppola A. Cost of care of haemophilia with inhibitors. *Haemophilia.* 2010;16(1):e190-e201.
7. Iorio A, Halimeh S, Holzhauer S, et al. Rate of inhibitor development in previously untreated hemophilia A patients treated with plasma-derived or recombinant factor VIII concentrates: a systematic review. *J Thromb Haemost.* 2010; 8(6):1256-1265.
8. Franchini M, Tagliaferri A, Mengoli C, Cruciani M. Cumulative inhibitor incidence in previously untreated patients with severe hemophilia A treated with plasma-derived versus recombinant factor VIII concentrates: a critical systematic review. *Crit Rev Oncol Hematol.* 2012;81(1): 82-93.
9. Astermark J, Altisent C, Batorova A, et al; European Haemophilia Therapy Standardisation Board. Non-genetic risk factors and the development of inhibitors in haemophilia: a comprehensive review and consensus report. *Haemophilia.* 2010;16(5):747-766.
10. Gouw SC, Fijnvandraat K. Identifying nongenetic risk factors for inhibitor development in severe hemophilia a. *Semin Thromb Hemost.* 2013;39(7): 740-751.
11. Gouw SC, van den Berg HM, Fischer K, et al; PedNet and Research of Determinants of INhibitor development (RODIN) Study Group. Intensity of factor VIII treatment and inhibitor development in children with severe hemophilia A: the RODIN study. *Blood.* 2013;121(20): 4046-4055.
12. Gouw SC, van der Bom JG, Auerswald G, Ettinghausen CE, Tedgård U, van den Berg HM. Recombinant versus plasma-derived factor VIII products and the development of inhibitors in previously untreated patients with severe hemophilia A: the CANAL cohort study. *Blood.* 2007;109(11):4693-4697.
13. Mancuso ME, Mannucci PM, Rocino A, Garagiola I, Tagliaferri A, Santagostino E. Source and purity of factor VIII products as risk factors for inhibitor development in patients with hemophilia A. *J Thromb Haemost.* 2012;10(5):781-790.
14. Gouw SC, van der Bom JG, Ljung R, et al; PedNet and RODIN Study Group. Factor VIII products and inhibitor development in severe hemophilia A. *N Engl J Med.* 2013;368(3): 231-239.
15. Iorio A, Skinner MW, Makris M. Factor VIII products and inhibitors in severe hemophilia A. *N Engl J Med.* 2013;368(15):1456.
16. Kessler CM, Iorio A. The Rodin (Research Of Determinants of INhibitor Development among PUPs with haemophilia) study: the clinical conundrum from the perspective of haemophilia treaters. *Haemophilia.* 2013;19(3):351-354.
17. European Medicines Agency. Kogenate Bayer and Helixate NexGen. http://www.ema.europa.eu/ema/index.jsp?curl=pages/medicines/human/referrals/Kogenate_Bayer_and_Helixate_NexGen/human_referral_prac_000022.jsp. Accessed July 1, 2014.
18. Calvez T, Biou M, Costagliola D, et al; Suivi Thérapeutique National des Hémophiles Group. The French haemophilia cohort: rationale and organization of a long-term national pharmacosurveillance system. *Haemophilia.* 2001;7(1):82-88.
19. Kempton CL, White GC II. How we treat a hemophilia A patient with a factor VIII inhibitor. *Blood.* 2009;113(1):11-17.
20. D'Agostino RB, Lee ML, Belanger AJ, Cupples LA, Anderson K, Kannel WB. Relation of pooled logistic regression to time dependent Cox regression analysis: the Framingham Heart Study. *Stat Med.* 1990;9(12):1501-1515.
21. Parmar MK, Torri V, Stewart L. Extracting summary statistics to perform meta-analyses of the published literature for survival endpoints. *Stat Med.* 1998;17(24):2815-2834.
22. Higgins JPT, Thompson SG, Deeks JJ, Altman DG. Measuring inconsistency in meta-analyses. *BMJ.* 2003;327(7414):557-560.
23. Giangrande PLF; KOGENATE Bayer Study Group. Safety and efficacy of KOGENATE Bayer in previously untreated patients (PUPs) and minimally treated patients (MTPs). *Haemophilia.* 2002;8(Suppl 2):19-22.
24. Kreuz W, Gill JC, Rothschild C, et al; International Kogenate-FS Study Group. 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(3):457-467.
25. Musso R, Santagostino E, Faradji A, et al; KOGENATE Bayer European PMS Study Group. Safety and efficacy of sucrose-formulated full-length recombinant factor VIII: experience in the standard clinical setting. *Thromb Haemost.* 2008;99(1):52-58.
26. Delumeau J-C, Ikegawa C, Yokoyama C, Haupt V. An observational study of sucrose-formulated recombinant factor VIII for Japanese patients with haemophilia A. *Thromb Haemost.* 2008; 100(1):32-37.
27. Stonebraker JS, Bolton-Maggs PHB, Soucie JM, Walker I, Brooker M. A study of variations in the reported haemophilia A prevalence around the world. *Haemophilia.* 2010;16(1):20-32.
28. Kasper CK, Lin JC. Prevalence of sporadic and familial haemophilia. *Haemophilia.* 2007;13(1): 90-92.
29. Gouw SC, van den Berg HM, Oldenburg J, et al. F8 gene mutation type and inhibitor development in patients with severe hemophilia A: systematic review and meta-analysis. *Blood.* 2012;119(12): 2922-2934.
30. Hay CR, Ollier W, Pepper L, et al; UKHCDO Inhibitor Working Party. HLA class II profile: a weak determinant of factor VIII inhibitor development in severe haemophilia A. *Thromb Haemost.* 1997;77(2):234-237.
31. Oldenburg J, Picard JK, Schwaab R, Brackmann HH, Tuddenham EG, Simpson E. HLA genotype of patients with severe haemophilia A due to intron 22 inversion with and without inhibitors of factor VIII. *Thromb Haemost.* 1997;77(2): 238-242.
32. Pavlova A, Delev D, Lacroix-Desmazes S, et al. Impact of polymorphisms of the major histocompatibility complex class II, interleukin-10, tumor necrosis factor-alpha and cytotoxic T-lymphocyte antigen-4 genes on inhibitor development in severe hemophilia A. *J Thromb Haemost.* 2009;7(12):2006-2015.
33. Lozier JN, Rosenberg PS, Goedert JJ, Menashe I. A case-control study reveals immunoregulatory gene haplotypes that influence inhibitor risk in severe haemophilia A. *Haemophilia.* 2011;17(4): 641-649.
34. Astermark J, Donfield SM, Gomperts ED, et al; Hemophilia Inhibitor Genetics Study (HIGS)

Combined Cohort. The polygenic nature of inhibitors in hemophilia A: results from the Hemophilia Inhibitor Genetics Study (HIGS) Combined Cohort. *Blood.* 2013;121(8): 1446-1454.

35. 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(11):4648-4654.
36. Lusher JM, Scharrer I. Evolution of recombinant factor VIII safety: KOGENATE and Kogenate FS/Bayer. *Int J Hematol.* 2009;90(4):446-454.
37. Preston RJS, Rawley O, Gleeson EM, O'Donnell JS. Elucidating the role of carbohydrate determinants in regulating hemostasis: insights and opportunities. *Blood.* 2013;121(19): 3801-3810.
38. Dasgupta S, Navarrete A-M, Bayry J, et al. A role for exposed mannosylations in presentation of human therapeutic self-proteins to CD4+ T lymphocytes. *Proc Natl Acad Sci USA.* 2007;104(21): 8965-8970.
39. Viel KR, Ameri A, Abshire TC, et al. Inhibitors of factor VIII in black patients with hemophilia. *N Engl J Med.* 2009;360(16):1618-1627.
40. Schwarz J, Astermark J, Menius ED, et al; Hemophilia Inhibitor Genetics Study Combined Cohort. F8 haplotype and inhibitor risk: results from the Hemophilia Inhibitor Genetics Study (HIGS) Combined Cohort. *Haemophilia.* 2013; 19(1):113-118.
41. Pahl S, Pavlova A, Driesen J, Müller J, Pötzsch B, Oldenburg J. In vitro characterization of recombinant factor VIII concentrates reveals significant differences in protein content, activity and thrombin activation profile. *Haemophilia.* 2013;19(3):392-398.
42. van der Bom JG, Gouw SC, Rosendaal FR. Second-generation recombinant factor VIII and inhibitor risk: interpretation of RODIN study findings and implications for patients with haemophilia A. *Haemophilia.* 2014;20(2): e171-e174.
43. Mannucci PM, Gringeri A, Peyvandi F, Santagostino E. Factor VIII products and inhibitor development: the SIPPET study (survey of inhibitors in plasma-product exposed toddlers). *Haemophilia.* 2007;13(Suppl 5):65-68.
44. Soucie JM, Miller CH, Kelly FM, et al; Haemophilia Inhibitor Research Study Investigators. A study of prospective surveillance for inhibitors among persons with haemophilia in the United States. *Haemophilia.* 2014;20(2):230-237.
45. Makris M, Calizzani G, Fischer K, et al. EUHASS: The European Haemophilia Safety Surveillance system. *Thromb Res.* 2011;127(Suppl 2):S22-S25.
46. Fischer K, Lewandowski D, Marijke van den Berg H, Janssen MP. Validity of assessing inhibitor development in haemophilia PUPs using registry data: the EUHASS project. *Haemophilia.* 2012; 18(3):e241-e246.