Received: 16 December 2022 | Revised: 12 May 2023 | Accepted: 21 May 2023

DOI: 10.1111/hae.14808

Haemophilia WFH WILEY

ORIGINAL ARTICLE

Clinical haemophilia

No immunological changes after factor VIII product switch: An in depth analysis in haemophilia A patients

Sarah J. Schep[1,2] | Kathelijn Fischer[1] | Marianne Boes[2,3] | Roger E. G. Schutgens[1]

[1]Center for Benign Haematology, Thrombosis and Haemostasis, Van Creveldkliniek, University Medical Center Utrecht, Utrecht University, Utrecht, Netherlands

[2]Center for Translational Immunology (CTI), University Medical Center Utrecht, Utrecht University, Utrecht, Netherlands

[3]Pediatrics Department, University Medical Center Utrecht, Utrecht University, Utrecht, Netherlands

Correspondence
Roger E. G. Schutgens, Van Creveldkliniek, University Medical Center Utrecht/Utrecht University, Huispost C01.428, Postbus 85500, 3508 GA Utrecht, Netherlands.
Email: r.schutgens@umcutrecht.nl

Funding information
NovoNordisk

Abstract

Background: A challenging complication in the treatment of haemophilia A is the formation of neutralizing anti-FVIII antibodies (inhibitors). There is ongoing debate on the effect of FVIII product and inhibitor risk, rendering patients and physicians reluctant to switch FVIII-products.

Aim: This study aimed to evaluate changes in the immune profile of haemophilia A patients after switching FVIII products and their possible relation to inhibitor development. Secondary, FVIII efficacy after switching were assessed.

Methods: Patients, who switched FVIII-products between 2017–2019, were included in this single centre cohort study. Prospective comparison of immunoregulatory cells and markers by flow-cytometry before and after the switch was performed in a subgroup. For the total cohort clinical data regarding inhibitor development and FVIII efficacy 1 year before and after switching were retrospectively collected.

Results: One-hundred patients (including 39 with prospective immunological assessment) were analyzed, of which 31% switched from plasma-derived (pdFVIII) to recombinant standard half-life FVIII (SHL-rFVIII), 47% between different SHL-rFVIII, and 22% from pdFVIII/SHL-rFVIII to rFVIII-Fc. No remarkable changes in immunoregulatory cell functions were observed after switching, regardless the type of switch. None of the patients developed an inhibitor. FVIII efficacy, that is, FVIII usage, half-life and annual bleeding rate (ABR), was similar before and after switch for the SHL products, whereas rFVIII-Fc associated with a longer half-life (13.1 vs. 15.0 h) and lower ABR (3.0 vs. 1.0).

Conclusions: Switching to a different FVIII product was not associated with inhibitor development, nor with differences in the immune profile. Switching to rFVIII-Fc lead to lower ABR.

KEYWORDS
anti-FVIII antibodies, extended half-life factor VIII, factor VIII, haemophilia A, inhibitor, rFVIII-Fc

1 | INTRODUCTION

The first descriptions of the severe X-linked bleeding disorder haemophilia A date back to the 2nd century AD.[1] Replacement therapy of the missing clotting factor is still the hallmark of treatment in many countries. Development of neutralizing FVIII-antibodies, 'inhibitors', are still a challenging complication.

Pivotal in inhibitor development is the lack of immune tolerance to FVIII, dependent on many immune mechanisms. Heavily debated external contributing factors are differences in the immunogenicity of plasma-derived FVIII (pdFVIII) and recombinant FVIII (rFVIII) products, including the protective role of Von Willebrand Factor (VWF) in pd-products or the different posttranslational modification of rFVIII.[2–5] Moreover the introduction of the extended half-life product rFVIII-Fc has added another component to the discussion, as fusion of FVIII to the Fc-region of IgG may have immunomodulatory consequences, including introduction of regulatory T-cells (Tregs).[6,7]

In light of this discussion, we asked whether switching to a different FVIII product should be considered a risk factor for inhibitor development. Since reports of increased inhibitor in previously treated patients (PTPs) after exposure to an intermediate-purity pasteurized concentrate and to a double virus-inactivated pdFVIII concentrate in the 1990s,[8,9] subsequent studies failed to confirm FVIII product switching concerns.[8–13] However, many patients and physicians are reported to believe that switching product raises the probability of inhibitor development in PTPs.[14,15] To unequivocally address this issue, we performed a cohort study in patients with haemophilia A (PWHA) who switched FVIII products as a result of national contracting. We performed an in-depth immunological analysis and assessed the rate of inhibitor development.[14,16] By this combined immunological and clinical evaluation in patients who changed FVIII products, we aimed to fully explore the immunogenicity and risks of FVIII product switching in PWHA.

2 | METHODS

2.1 | Objective

The primary objective is to evaluate changes in the immunoregulatory white blood cell profile after switching to a different FVIII product and its possible relation to inhibitor development in PWHA.

Secondary objectives are to determine differences in efficacy after switching FVIII product and to evaluate differences in the conventional standard half-life (SHL) FVIII and the extended half-life (EHL) FVIII, that is, rFVIII-Fc with regard to immunological changes as well as efficacy.

2.2 | Study design and patient selection

This is a combined prospective and retrospective single-centre cohort study performed at the Van Creveldkliniek, the haemophilia treatment centre of the University Medical Center Utrecht in the Netherlands. As a result of national contracting all PWHA treated at this centre were switched from their current FVIII product to either turoctocog alfa (NovoEight®) or efmoroctocog alfa (Elocta®) during 2017–2019. This switch was used to evaluate if changing products would result in differences in the immune profile and development of inhibitors and differences in the efficacy of the replacement therapy.

All patients with moderate (FVIII activity 1%–5%) or severe (FVIII activity < 1%) haemophilia A, aged 6 years and older, who switched to a different factor VIII product between 2017 and 2019, were eligible. Exclusion criteria were presence of an inhibitor and/or <50 Exposure Days (EDs).

The study was approved by the local medical ethics committee and informed consent was obtained from all patients.

By time of study approval part of included patients had already switched FVIII product. Therefore, periodic blood sample collection before and after the switch for immunological analysis could only be performed in patients who switched FVIII products after study approval ('prospective cohort'). Data regarding inhibitor development and efficacy of the FVIII product for equivalent periods of time (1 year) pre- and post-switch were extracted from the electronic patient files for both the prospective cohort, and patients who had already switched products at the time of study approval ('retrospective cohort').

2.3 | Prospective immunological analysis (prospective cohort)

In the prospective cohort blood samples were withdrawn just before (T = 0) and 6 months (T = 1) and 1 year (T = 2) after the switch for evaluation of changes in the immunoregulatory cell profile by flow cytometry.

Immunological evaluation included assessment of the frequency of regulatory B-cells (Bregs), regulatory T-cells (Tregs), myeloid-derived suppressor cells (MDSCs) and the expression of activation markers HLA-DR and PD-L1 on monocytes and CTLA4, ICOS and PD1 on $CD4^+$ T-cells.

These tests were performed in isolated peripheral blood mononuclear cells (PBMCs) stored in liquid nitrogen until use as previously described.[17]

For evaluation of the immune profile three different flow cytometry panels were used (1×10^6 cells for each panel): (1) B-cell panel for Breg evaluation; (2) Monocyte panel for MDSCs and expression of HLA-DR and PD-L1 on monocytes; (3) T-cell panel for Treg evaluation and expression of CTLA4, ICOS and PD1 on T-cells. We refer to our previously published study for details regarding the labelled antibodies and procedure of immunostaining.[17]

Bregs were defined as $CD19^+CD24^{high}CD38^{high}$ cells[18–20]; the frequency/proportion of Bregs as the percentage of Bregs/$CD19^+$ B-cells. Monocytes were defined as $CD3^-CD19^-CD56^-(Lin^-)CD14^+$ cells. MDSCs were defined as $Lin^-HLA\text{-}DR^{low}CD11b^+CD33^+$; the frequency of MDSCs is expressed as MDSCs/total PBMCs. HLA-DR and PD-L1 expression on monocytes was presented as mean fluorescence intensity (MFI).

Tregs were defined as $CD4^+CD25^+FoxP3^+$ cells; the frequency of Tregs Tregs/total $CD4^+$ T-cells. Expression of CTLA4, ICOS and PD1 was presented as MFI.FACS acquisition was performed on an LSR Fortessa flow cytometer (BD). Only samples with cell viability ≥ 95% were included. In order to minimalize inter-test variability, all available serial samples from one patient were measured simultaneously. For data analysis FlowJo software (LLC, version 10, Ashland, OR) was used.

2.4 | Inhibitor development and FVIII efficacy (complete cohort)

For each participant, clinical data during 1-year pre-switch and 1-year post-switch were extracted from the electronic patient files. The following parameters were collected: patient demographics (age, bodyweight, disease severity, number of EDs at the time of switching, history of inhibitors), FVIII treatment details (type, name and dose of FVIII product, treatment regimen and factor consumption data) and information regarding efficacy and safety of the product (FVIII half-life (T½), annual bleeding rate (ABR), adverse events/intolerance and inhibitor development).

FVIII activity was measured by the 1-stage activity assay and expressed in IU/dL. Inhibitor titre was assessed with the Nijmegen modification of the Bethesda assay and expressed in Bethesda Units (BU). FVIII recovery was defined as the difference in pre- and post-infusion level of FVIII. In patients with serial blood samples withdrawn after FVIII infusion, a FVIII T½ was calculated using the web-application WAPPS-Hemo (wapps-hemo.org, version 5.3 (2020-11-02), 2021 McMaster University, Canada).[21] ABR was based on self-reported episodes of bleeding events retrieved from patient bleeding diaries, verified and supplemented by review of clinical medical records. Factor VIII consumption was based on the total amount of FVIII prescribed in a defined time period (at least 1 year) and was expressed as units/week and units/kg/week.

2.5 | Statistical analysis

Continuous data are expressed as median with interquartile range (IQR). All continuous outcome parameters were tested for normality. For analyses of the immunological parameters, which included three serial measurements per patient, general linear modelling (GLM) repeated measures analysis or the Friedman test was used as appropriate. Comparisons of clinical outcomes pre- and post-switch were performed using paired-sample T-tests or non-parametric Wilcoxon signed-rank tests for continuous data with normal or skewed distributions, and Fisher's exact test for categorical data. In case of statistically significant results, a post-hoc analysis was performed by the Bonferroni correction. A p-value $< .05$ was considered as statistically significant.

Analyses were performed for the complete cohort, as well as subanalysis according to product type (SHL FVIII vs. rFVIII-Fc) and the type of switch (pdFVIII to rFVIII, rFVIII to rFVIII, pdFVIII to rFVIII-Fc and rFVIII to rFVIII-Fc). Both pdFVIII and rFVIII were analyzed as SHL FVIII products, rFVIII-Fc is the only used EHL FVIII product. Age was used as a covariate in the analyses.

For interpretation of the immunological data of the prospective cohort results were related to data obtained from our recent study in 20 inhibitor and 45 non-inhibitor patients, which showed that levels of Bregs and CTLA4 were 2–4 times lower in inhibitor patients compared to non-inhibitor patients and that these parameters significantly increased during successful immune tolerance induction (ITI).[17]

All analyses were performed with IBM SPSS Statistics for Windows, version 25.2 (Armonk, NY: IBM Corp). Graphs were produced with GraphPad Prism, version 8.3.0 for Windows (GraphPad Software, La Jolla, CA).

3 | RESULTS

A total of 100 PWHA were included. A selection of 39 subsequent patients of the complete cohort were included in the prospective cohort. In one patient blood was not withdrawn at T = 1; data collection was complete for all other patients of the prospective cohort.

Median age of the complete cohort was 39 years (IQR 22–54 years) and most patients had severe haemophilia A (97/100, 97%), Table 1. All patients had more than 100 EDs. Fifteen patients (15/100, 15.0%) had an inhibitor in the past.

Before the switch four different FVIII-products were used; 33% of PWHA (33/100) used a pdFVIII-product and the remainder (67/100, 67.0%) used one of 3 rFVIII-products (third generation full-length rFVIII octocog alfa 61/100, single-chain B-domain-truncated (BDT) rFVIII lonoctocog 5/100 and BDT-rFVIII turoctocog alfa 1/100).

The majority of patients (78/100, 78.0%) switched to turoctocog alfa, NovoEight®); 22/100 patients (22.0%) switched from SHL FVIII to rFVIII-Fc (efmoroctocog alfa, Elocta®).

3.1 | Prospective cohort: Immunological changes and inhibitor development after switching FVIII products

Prospective data for immunological analysis were available in 39 PWHA. Baseline characteristics of this subgroup were similar to the complete cohort (Table 1). Median age was 39 years (IQR 25–54), 94.9% (37/39) had severe haemophilia A and 7.7% (3/39) had an inhibitor in the past. Most patients (20/39, 51.3%) switched between SHL rFVIII products, whereas 30.8% (12/39) switched from pdFVIII to SHL rFVIII and 17.9% (7/39) from SHL rFVIII to EHL rFVIII-Fc.

None of the patients in the prospective cohort developed an inhibitor.

As shown in Figure 1 and Supplemental Table S1 frequency of Bregs, Tregs, expression of PD-L1 and HLA-DR on monocytes and the expression of PD1 and ICOS on T-cells did not change following product switching. The percentage of MDSC slightly increased (median percentage on T = 0, T = 1 and T = 2 respectively .5%, .5%, and .6%,

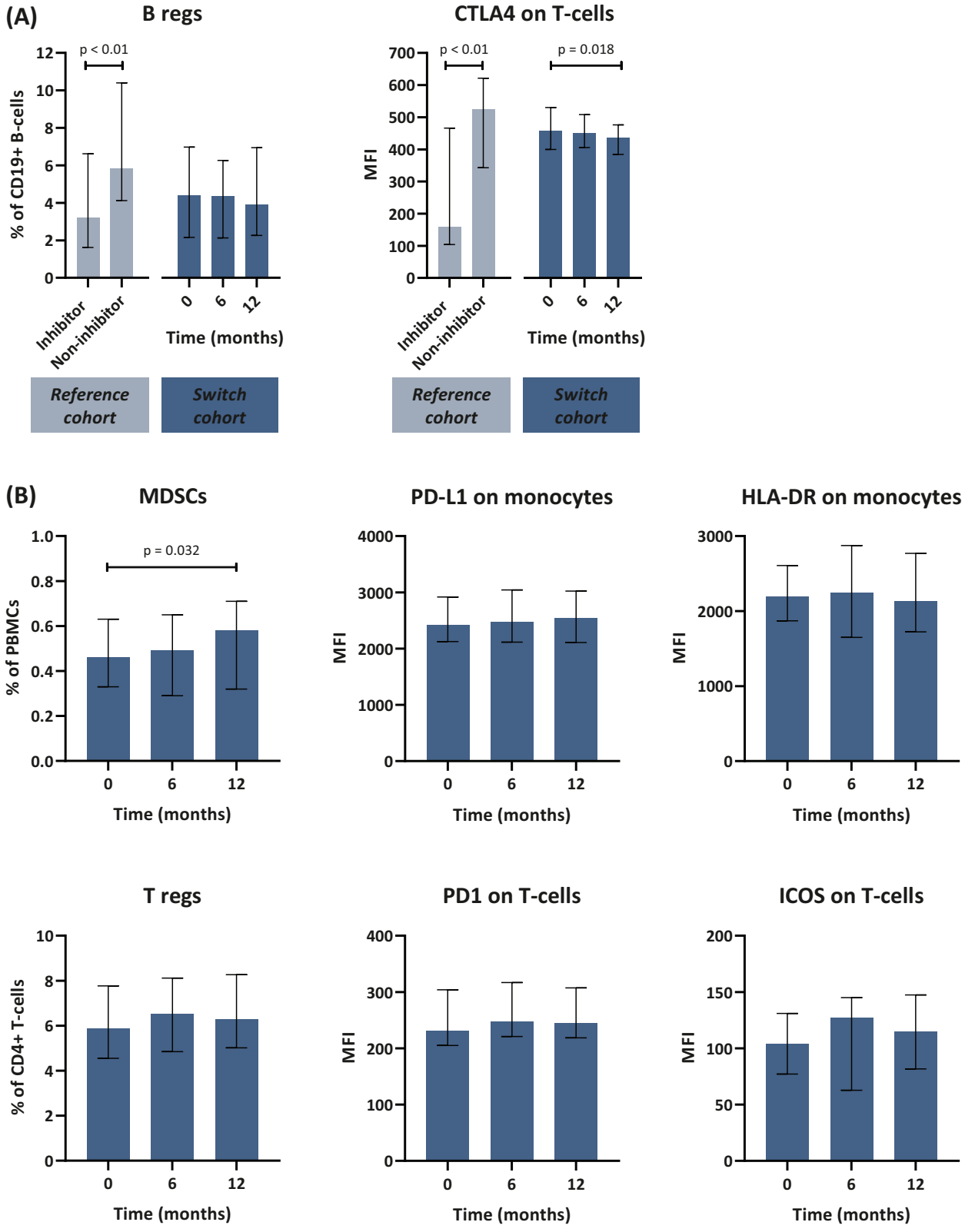

FIGURE 1 Immune profile characteristics before and after switching FVIII product Frequencies of several immunoregulatory cells and markers were determined in 39 patients with haemophilia A (PWHA) who switched to a different factor VIII (FVIII) product. Panel A: For better interpretation of clinical relevance, results of the switch cohort were compared with a previous study in PWHA, in which inhibitor patients were compared with ex-inhibitor and non-inhibitor patients. For clarity purposes only results of the inhibitor and non-inhibitor group are shown (reference cohort).[36] In this study mainly Bregs and CTLA4 were significantly decreased in patients with a current inhibitor. Panel B: Remaining immuno-regulatory parameters of the switch cohort.Compared to changes observed in inhibitor versus no-inhibitor patients, the observed significant changes in CTLA4 and MDSCs in the switch cohort are negligible. Bregs: regulatory B-cells; MDSCs: myeloid derived suppressor cells; PBMCs: peripheral blood mononuclear cells; MFI: mean fluorescence intensity; Tregs: regulatory T-cells. T-cells represent the CD4+ T-cell fraction.

TABLE 1 Baseline characteristics of haemophilia A patients switching FVIII product.

Variable	Prospective cohort (*N* = 39)	Complete cohort (*N* = 100)
Age, years	38.6 (25-54)	39.1 (22-54)
- Age < 18 years	8 (20.5%)	21 (21.0%)
Haemophilia severity		
- Mild	0 (.0%)	0 (.0%)
- Moderate	2 (5.1%)	3 (3.0%)
- Severe	37 (94.9%)	97 (97.0%)
Inhibitor in the past	3 (7.7%)	15 (15.0%)
Any comorbid disorder	34 (87.2%)	67 (67.0%)
HCV	20 (51.3%)	51 (51.0%)
- Untreated, stable viral load	5 (12.8%)	7 (7.0%)
- Successfully treated[a]	15 (38.5%)	44 (44.0%)
HIV	2 (5.2%)	14 (14.0%)
- Untreated (CD4 > 400/μL)	1 (2.6%)	1 (1.0%)
- Successfully treated[a]	1 (2.6%)	13 (13.0%)
FVIII product type pre-switch		
- pdFVIII	12 (30.8%)	33 (33.0%)
- rFVIII	27 (69.2%)	67 (67.0%)
FVIII product type post-switch		
- rFVIII (turoctocog)	32 (82.1%)	78 (78.0%)
- rFVIII-Fc (efmoroctocog)	7 (17.9%)	22 (22.0%)
Type of switch		
- pdFVIII→ rFVIII	12 (30.8%)	31 (31.0%)
- rFVIII→ rFVIII	20 (51.3%)	47 (47.0%)
- pdFVIII→ FVIII-Fc	0(.0%)	2 (2.0%)
- rFVIII→ rFVIII-Fc	7 (17.9%)	20 (20.0%)
Inhibitor development	0 (.0%)	0 (.0%)

Note: Categorical variables are shown as cases/total (percentage, %); continuous variables are shown as median (interquartile range; IQR).
Abbreviations: FVIII, factor VIII; HCV, Hepatitis C Virus infection; HIV, Human Immunodeficiency Virus infection; na, not applicable; pdFVIII, plasma-derived FVIII; rFVIII, recombinant FVIII; rFVIII-Fc, rFVIII-Fc fusion product (extended half-life product).
[a]Successfully treated is defined as an undetectable viral load and, in case of HIV, a CD4 count > 400 per μL.

$p = .032$). The expression of CTLA4 on CD4+ T-cells showed a significant decrease from T = 0 to 1-year post-switch (CTLA4 on all CD4+ T-cells from MFI 457 pre-switch to MFI 436 post-switch, $p = .018$).

Next, we analyzed whether dynamics of the immunoregulatory parameters were different according to the product type (switch to SHL FVIII vs. rFVIII-Fc) and the type of switch (pdFVIII to rFVIII vs. rFVIII to rFVIII vs. rFVIII to rFVIII-Fc). Comparison of patients switching to SHL FVIII (32/39, 82.1%) with patients switching to rFVIII-Fc (7/39, 17.9%) showed no changes in the distributions of any of the immune cells and markers studied (Figure 2/Supplemental Table S2). Similar findings were seen in the sub-analysis comparing the three different types of switches. Dynamics of immunoregulatory parameters after the switch were similar across for patients switching from pdFVIII to rFVIII (*N* = 12, 30.8%), between SHL rFVIII products (*N* = 20, 51.3%) or from SHL rFVIII to rFVIII-Fc (*N* = 7, 17.9%), Figure 2/Supplemental Table S3.

3.2 | Complete cohort: Efficacy and safety after switching FVIII products

Details regarding treatment regimen and FVIII efficacy and safety are shown in Tables 2 and 3.

The use of prophylaxis (96.0% and 95.0%, respectively) and FVIII consumption were similar before and after the switch. Patients who switched to rFVIII-Fc were significantly younger and had a lower bodyweight compared to patients who switched to SHL FVIII. After switching to rFVIII-Fc, both FVIII consumption as well as the number of weekly injections remained stable.

The FVIII T½ could be calculated in 62 patients pre-switch and in 70 patients post-switch. Pre-switch the median overall T½ was 13.1 h (IQR 10.2–16.8). Post-switch T½ was 13.4 h (IQR 10.6–17.7) for SHL FVIII and 15.0 h (12.9–18.3) for rFVIII-Fc respectively, showing a significant increase in T½ in patients who switched to rFVIII-Fc.

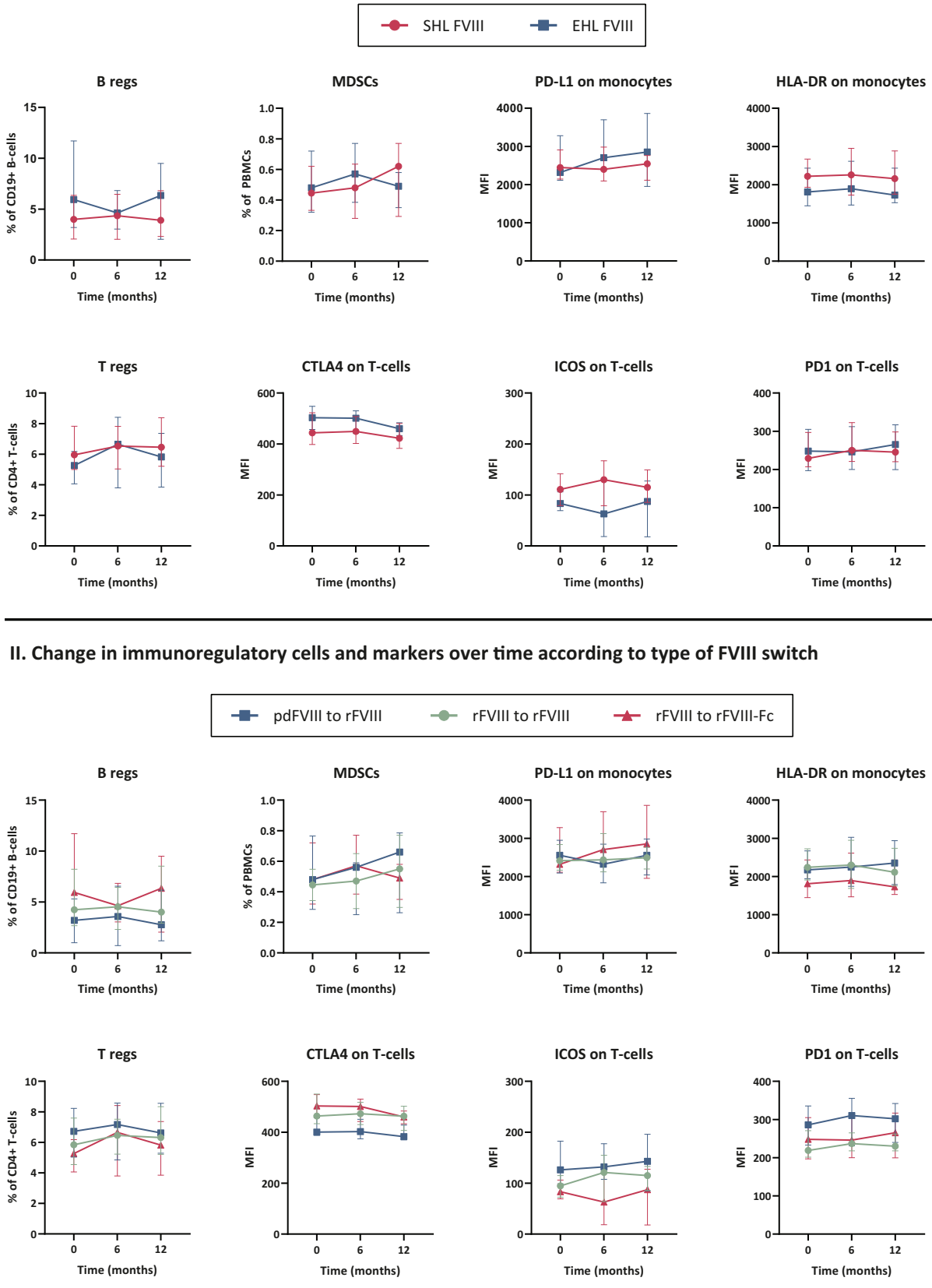

FIGURE 2 No changes in immune profile after switching FVIII according to FVIII product and type of switch Frequencies of several immunregulatory cells and markers were determined in 39 PWHA who switched to a different factor VIII (FVIII) product, stratified by FVIII product (panel I) and type of switch (panel II); analyses were performed using age as covariate. SHL: standard half-life; EHL: extended half-life;

TABLE 2 FVIII efficacy and safety before and after switching FVIII product.

	Pre-switch	*Post-switch*	*p*
Treatment regimen			1.000
- On demand	4/100 (4.0%)	5/100 (5.0%)	
- Prophylaxis	96/100 (96%)	95/100 (95.0%)	
FVIII prescription			
- Dose (IU) per infusion	1000 (1000-1000)	1000 (1000-1000)	.092
- Frequency/week	3 (3.0-3.5)	3 (3.0-3.5)	.897
- Dose (IU)/kg/week	39.6 (30.0-51.0)	40.9 (33.7-52.3)	.713
FVIII consumption			
- IU/week	2722 (1849-3498)	2838 (1929-3669)	.470
- IU/kg/week	37.6 (24.8-50.0)	35.9 (26.5-52.3)	.768
Intra-articular bleeding[a]	101/177 (57.1%)	81/134 (60.4%)	.563
Inhibitor development	0/100 (.0%)	0/100 (.0%)	1.000
Intolerance/adverse events	0/100 (.0%)	14/100 (14.0%)	<.001
- Subjective higher bleeding tendency		10/10 (10.0%)	
- Gastro-intestinal symptoms (no bleeding)		2/100 (2.0%)	
- Diaphoresis		1/100 (1.0%)	
- Pruritus		1/100 (1.0%)	
2nd switch because of intolerance	n.a.	9/100 (9.0%)	–

Note: Categorical variables are shown as cases/total (percentage, %); continuous variables are shown as median (interquartile range; IQR).
Abbreviations: FVIII, factor VIII; IU, international units; n.a., not applicable.
aIn case of intra-articular bleeding the number of cases per total number of reported bleeding episodes is shown.

The median ABR remained similar for patients who switched from one SHL FVIII to another SHL FVIII (ABR pre- and post-switch respectively 2.0 (IQR 1.0–4.0) and 1.0 (IQR .0–3.0), $p = .099$). On the other side, patients, switching to rFVIII-Fc, showed a significant decrease in the median ABR (ABR pre- and post-switch 3.0 (IQR 1.0–5.5) and 1.0 (IQR .0–3.0). For the whole cohort, intra-articular or severe bleeding frequencies were similar pre- and post-switch.

No inhibitors developed in the year after changing FVIII product (.0%, 95% confidence interval 0-.295%). Only one ex-inhibitor patient experienced recurrence of an inhibitor, but this occurred 1.5 years after the switch. This was a 16-year-old patient, who developed a high-titre antibody (maximum titre 67 BU) by the age of 1 year after 4 EDs, for which he received successful treatment with ITI. Since the inhibitor recurred 1.5 year after the switch, we consider it unlikely that this was provoked by the switch itself.

Adverse events and/or dissatisfaction with the new FVIII product were observed in 14 patients, including 10/14 patients who switched from pdFVIII to rFVIII, 2/14 who switched from rFVIII to another rFVIII and 2/14 who switched from rFVIII to rFVIII-Fc (Table 2). Most reported was a subjective experience of higher bleeding tendency, even though registered ABR did not significantly increase in these patients (median ABR pre-switch and post-switch 4.0 and 3.0 respectively, $p = .422$). Eventually patients' dissatisfaction resulted in discontinuation of the new FVIII product in 8 patients. Of note, dissatisfaction and discontinuation occurred significantly more frequent in patients who were originally using pdFVIII (dissatisfaction: pdFVIII 30.3% (10/33) vs. rFVIII 6.0% (4/67), $p = .002$; discontinuation: pdFVIII 18.2% (6/33) vs. rFVIII 3.0% (2/67), $p = .005$).

4 | DISCUSSION

In this cohort study, we demonstrate that switching of FVIII product is not accompanied by clinically significant changes in immune profiles, nor the development of FVIII inhibitors. This finding was consistent in patients switching between SHL rFVIII concentrates as well as in those switching from pdFVIII to rFVIII or from SHL rFVIII to rFVIII-Fc.

Our findings are in line with previous reports demonstrating that switching of FVIII product is not associated with an increased risk of inhibitor development nor significant changes in the efficacy of the FVIII product.[12,13,22–24]

This is one of the first studies performing an in-depth immunological analysis in PWHA as they switch to a different FVIII product. We demonstrated that no significant differences were found in the

FVIII: factor VIII; pdFVIII: plasma-derived FVIII; rFVIII: recombinant FVIII; rFVIII-Fc: rFVIII-Fc fusion product (extended half-life product); Bregs: regulatory B-cells; MDSCs: myeloid derived suppressor cells; PBMCs: peripheral blood mononuclear cells; MFI: mean fluorescence intensity; Tregs: regulatory T-cells. T-cells represent the CD4+ T-cell fraction.

TABLE 3 Sub-analysis of FVIII efficacy and safety according to type of switch.

	SHL FVIII → SHL FVIII (N = 78 / 78.0%)	*SHL FVIII → rFVIII-Fc (N = 22 / 22.0%)*	*P**
Baseline characteristics			
Age, years	40.5 (26.0-54.0)	25.0 (12.0-56.8)	.027
- Age < 18 years	−11/78 (14.1%)	−10/22 (45.5%)	−.003
Weight, kg	82.0 (68.0-89.0)	71.0 (50.0-83.8)	.033
Severe haemophilia	75/78 (96.2%)	22/22 (100.0%)	1.000
Inhibitor in the past	9/78 (11.5%)	6/22 (27.3%)	.091
Clinical outcomes			
FVIII injections/week			
- Pre-switch	3.0 (2.9-3.5)	3.3 (3.0-3.5)	
- Post-switch	3.0 (3.0-3.5)	3.3 (3.0 −3.5)	.230
*P***	*.094*	*.101*	
FVIII consumption (IU/week)			
- Pre-switch	2561 (1815-3355)	3186 (2053-4598)	
- Post-switch	2828 (1855-3611)	2872 (1977-4048)	.107
*P***	*.155*	*.157*	
T½ FVIII, hours			
- Pre-switch	14.0 (11.0-19.6)	10.5 (9.2-13.8)	
- Post-switch	13.4 (10.6-17.7)	15.0 (12.9-18.3)	<.001
*P***	*.140*	*< .001*	
ABR			
- Pre-switch	2.0 (1.0-4.0)	3.0 (1.0-5.5)	
- Post-switch	1.0 (.0-3.0)	1.0 (.0-3.0)	.006
*P***	*.099*	*.002*	
ABR = 0			
- Pre-switch	11/77 (14.3%)	3/21 (14.3%)	
- Post-switch	19/75 (25.3%)	8/22(36.4%)	.417
*P***	*.073*	*.162*	

Note: Categorical variables are shown as cases/total (percentage, %); continuous variables are shown as median (interquartile range; IQR).
Abbreviations: ABR, annual bleeding rate; FVIII, factor VIII; IU, international units; rFVIII-Fc, extended half-life FVIII product; SHL, standard half-life FVIII product; T½: half-life time.
P*-value 1 (*P): Analysis whether the change in pre- and post-switch outcome was significantly different between the 2 groups (i.e., between patients switching from SHL FVIII to SHL FVIII and patients switching from SHL FVIII to EHL FVIII).
P*-value 2 (*P): Analysis whether there was a significant change in outcome pre- and post-switch within the two different types of switch (i.e., SHL to SHL and SHL to EHL).

frequency of most of the immunoregulatory cells and markers before and after the switch. The detected changes in MDSCs and CTLA4 on CD4+ T-cells were very small and are difficult to interpret. MDSCs and the marker CTLA4 both have regulatory/immunosuppressive functions, since we observed an increase in MDSCs but a decrease in CTLA4, these results are contradictory and therefore do not seem to shift the balance to either tolerance or intolerance/immunity. Together with the small changes we observed, especially when comparing these results to the differences between inhibitor and non-inhibitor patients, we consider the results in the switch cohort not clinically relevant.

These findings provide an important support to results from earlier studies, showing that switching to a different FVIII product is not associated with increased immunogenicity and the risk of inhibitor formation.[10–13,22,25] Highly debated in this regard is the role of the FVIII product type, that is, pdFVIII versus rFVIII and rFVIII-Fc. In this study a sub-analysis was performed to evaluate if the change in immunoregulatory parameters was different according to the product type and type of switch. We found no differences in the dynamics of the immune profile between patients who switched between similar or different FVIII concentrate types. This is especially relevant for the discussion about differences between pdFVIII and rFVIII. For rFVIII-Fc numbers were too small to draw proper conclusions. Earlier, preclinical and clinical reports demonstrated a potential tolerogenic effect of rFVII-Fc, including upregulation of Tregs and anti-inflammatory cytokines.[6,7,15,26] Although these results are promising, the risk of inhibitor development with rFVIII-Fc does not seem to be lower

compared to SHL FVIII products.[27] More pre- and clinical data is needed to establish a potential beneficial tolerogenic role of rFVIII-Fc.

In the retrospective cohort we observed no differences in FVIII efficacy in patients switching from one SHL FVIII to another SHL FVIII, whereas a switch to rFVIII-Fc was associated with a longer T½ and lower ABR. Contrary to some studies, we did not found a decrease in the number of weekly injections in patients switching to an EHL product.[24,28,29] This is most reasonable explained by confounding by indication in our retrospective study. Patients who switched to rFVIII-Fc tend to be younger and were characterized with a lower FVIII T½, a higher FVIII consumption and a higher bleeding rate at baseline. Letting the FVIII regimen unchanged in these patients was a consciously decision in order to better prevent bleeding, which was successful, as shown by the decrease in ABR. Regardless the lower injection frequency with rFVIII-Fc, most reports show that the total weekly FVIII consumption remains similar (or even higher), which was also observed in this study.[28,29]

Despite the fact that the new FVIII product appeared to be non-inferior to the old FVIII product, the rate of dissatisfaction and/or discontinuation of the switched product was quite high, especially in patients originally using pdFVIII. Notably, a subjective higher bleeding tendency/lower efficacy was most frequently reported as reason for dissatisfaction, whereas no objective increase in ABR was recorded. We, therefore, hypothesize that, at least part of, the dissatisfaction results from the strong psychological link patients develop with their current FVIII product and the non-evidence-based reluctance to switch to a 'new' treatment.[30]

Some limitations of our study remain to be addressed. First of all, this study is limited by its partly retrospective design, which increased the risk of reporting bias, missing data and confounding by indication. The latter is especially relevant for patients that switched for a particular reason (such as bleeding symptoms) to rFVIII-Fc. Regarding missing data, the von Willebrand Factor antigen level was unknown in many patients. It is known that this level influences FVIII T½, but this could not be taken into account in our analysis.[28] Nor could we address the role of *F8* gene mutation, as these data were unavailable in this study cohort. Another limitation is the small number of included patients who switched to rFVIII-Fc in the prospective cohort. This hampered our conclusions in the evaluation of immunological differences between the FVIII product types and type of switch. Since none of the patients developed an inhibitor, we were unable to detect any immunological changes that could associate with inhibitor formation. It should be noted that the baseline risk of inhibitor formation in this cohort of extensively pretreated patients was also low and that our results might not apply for patients with a higher inhibitor risk, such as patients with less than 50 exposure days to FVIII. Finally, analyses were performed batch-wise in order to prevent intertest variability. As it is known that the viability of Tregs might be influenced by the duration of cryopreservation, this might have influenced our results.

In our study the FVIII one-stage assay was used. This assay is thought to underestimate FVIII activity and therefore also effect calculation of FVIII T½. However, since this was the only assay used during the study (before and after switch) we do not think this influenced the results.

A strength of this study is the detailed and extensive collection of clinical data during a relatively long period of time around the FVIII product switch. Moreover, this study is unique in performing an in-depth immunological analysis, which provides extra mechanistic insight to support the concept it is safe to switch FVIII products.

In conclusion, we performed a combined immunological and clinical study to analyze the effect of FVIII product switching. Switching to a different FVIII product was not associated with differences in the immune profile or efficacy of FVIII, nor with an increased inhibitor risk. Thereby this study contributes to reduce the concerns to switch to a different FVIII product, both in patients and their physicians.

AUTHOR CONTRIBUTIONS

Sarah J. Schep performed the experiments, analyzed the data and wrote the paper. Marianne Boes, Kathelijn Fischer and Roger E. G. Schutgens contributed to the set-up and implementation of the study and provided feedback on the manuscript.

ACKNOWLEDGEMENTS

The authors are thankful to all participants of this study and the staff of the Van Creveldkliniek and Center for Translational Immunology.

CONFLICTS OF INTEREST STATEMENT

This study was supported by funding from NovoNordisk. R.S. has received research support from Bayer, Baxter, CSL Behring, Pfizer and Sobi (funds to the institute). K.F. has received speaker's fees from CSL Behring, NovoNordisk; consultancy fees from Biogen, CSL-Behring,Freeline, NovoNordisk, Roche and SOBI; and research support from Bayer, Pfizer, and Novo Nordisk (all funds to the institute). S.S. and M.B. declare no other competing financial or commercial interests.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request

ETHICS STATEMENT

The study was approved by the local medical ethics committee and informed consent was obtained from all patients.

ORCID

Kathelijn Fischer https://orcid.org/0000-0001-7126-6613
Roger E. G. Schutgens https://orcid.org/0000-0002-2762-6033

REFERENCES

1. Rosner F. Hemophilia in the Talmud and rabbinic writings. *Ann Intern Med*. 1969;70(4):833-837.
2. Franchini M, Lippi G. Von Willebrand factor-containing factor VIII concentrates and inhibitors in haemophilia A: a critical literature review. *Thromb Haemost*. 2010;104(5):931-940. doi:10.1160/TH10-03-0151
3. Kannicht C, Ramström M, Kohla G, et al. Characterisation of the post-translational modifications of a novel, human cell line-derived

recombinant human factor VIII. *Thromb Res*. 2013;131(1):78-88. doi:10.1016/j.thromres.2012.09.011
4. Peyvandi F, Mannucci PM, Garagiola I, et al. A randomized trial of factor VIII and neutralizing antibodies in hemophilia A. *N Engl J Med*. 2016;374(21):2054-2064. doi:10.1056/NEJMoa1516437
5. Hartholt RB, van Velzen AS, Peyron I, ten Brinke A, Fijnvandraat K, Voorberg J. To serve and protect: the modulatory role of von Willebrand factor on factor VIII immunogenicity. *Blood Rev*. 2017;31(5):339-347. doi:10.1016/j.blre.2017.07.001
6. de Groot AS, Moise L, McMurry JA, et al. Activation of natural regulatory T cells by IgG Fc–derived peptide "Tregitopes". *Blood*. 2008;112(8):3303-3311. doi:10.1182/blood-2008-02-138073.An
7. Krishnamoorthy S, Liu T, Drager D, et al. Recombinant factor VIII Fc (rFVIIIFc) fusion protein reduces immunogenicity and induces tolerance in hemophilia A mice. *Cell Immunol*. 2016;301:30-39. doi:10.1016/j.cellimm.2015.12.008
8. Rosendaal F, Nieuwenhuis H, Van Den Berg H, et al. A sudden increase in factor VIII inhibitor development in multitransfused hemophilia A patients in the Netherlands. *Blood*. 1993;81:2180-2186.
9. Peerlinck K, Arnout J, Di Giambattista M, et al. Factor VIII inhibitors in previously treated haemophilia A patients with a double virus-inactivated plasma derived factor VIII concentrate. *Thromb Haemost*. 1997;77:80-86.
10. Giles A, Rivard G, 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(2):139-148.
11. Singleton E, Smith J, Kavanagh M, Nolan B, White B. Low risk of inhibitor formation in haemophilia patients after a change in treatment from Chinese hamster ovary cell-produced to baby hamster kidney cell-produced recombinant factor VIII. *Thromb Haemost*. 2007;98(6):1188-1192. doi:10.1160/TH07-06-0408
12. Rubinger M, Lillicrap D, Rivard GE, et al. 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. *Haemophilia*. 2008;14(2):281-286. doi:10.1111/j.1365-2516.2007.01634.x
13. Rea C, Dunkerley A, Sørensen B, Rangarajan S. Pharmacokinetics coagulation factor consumption and clinical efficacy in patients being switched from full-length FVIII treatment to B-domain-deleted r-FVIII and back to full-length FVIII. *Haemophilia*. 2009;15(6):1237-1242. doi:10.1111/j.1365-2516.2009.02071.x
14. Santagostino E, Auerswald G, Benson G, et al. Switching treatments in haemophilia: is there a risk of inhibitor development? *Eur J Haematol*. 2015;94(4):284-289. doi:10.1111/ejh.12433
15. Liu T, Hoehn T, Patarroyo-White S, Pierce G, Jiang H. Evaluation of antibody responses to rFVIIIFc compared to Xyntha and Advate in hemophilia A mice. *Haemophilia*. 2012;18(3):41. Suppl.
16. Matino D, Iorio A. Answering relevant research questions via careful observation of clinical practice: a fresh look at the old way forward. *Haemophilia*. 2014;20(5):604-606. doi:10.1111/hae.12511
17. Schep SJ, Schutgens REG, Fischer K, Voorberg J, Boes M. Role of regulatory cells in immune tolerance induction in hemophilia A. *HemaSphere*. 2021;5(5):1-9.
18. Blair PA, Norena LY, Flores-Borja F, et al. CD19+CD24hiCD38hi B cells exhibit regulatory capacity in healthy individuals but are functionally impaired in systemic lupus erythematosus patients. *Immunity*. 2010;32(1):129-140. doi:10.1016/j.immuni.2009.11.009
19. Daien CI, Gailhac S, Mura T, et al. Regulatory B10 cells are decreased in patients with rheumatoid arthritis and are inversely correlated with disease activity. *Arthritis Rheumatol*. 2014;66(8):2037-2046. doi:10.1002/art.38666
20. Barsotti NS, Almeida RR, Costa PR, Barros T, Kalil J, Kokron CM. IL-10-producing regulatory B cells are decreased in patients with common variable immunodeficiency. *PLoS One*. 2016;11(3):1-17. doi:10.1371/journal.pone.0151761
21. Iorio A, Keepanasseril A, Foster G, et al. Development of a web-accessible population pharmacokinetic service—hemophilia (WAPPS-Hemo): study protocol. *JMIR Res Protoc*. 2016;5(4):e239. doi:10.2196/resprot.6558
22. Bacon CL, Singleton E, Brady B, et al. Low risk of inhibitor formation in haemophilia A patients following en masse switch in treatment to a third generation full length plasma and albumin-free recombinant factor VIII product (ADVATE®). *Haemophilia*. 2011;17(3):407-411. doi:10.1111/j.1365-2516.2010.02430.x
23. Aznar JA, Moret A, Ibáñez F, et al. Inhibitor development after switching of FVIII concentrate in multitransfused patients with severe haemophilia A. *Haemophilia*. 2014;20(5):624-629. doi:10.1111/hae.12439
24. Carcao M, Zunino L, Young NL, et al. Measuring the impact of changing from standard half-life (SHL) to extended half-life (EHL) FVIII prophylaxis on health-related quality of life (HRQoL) in boys with moderate/severe haemophilia A: lessons learned with the CHO-KLAT tool. *Haemophilia*. 2020;26(1):73-78. doi:10.1111/hae.13905
25. Gouw SC, van der Bom JG, Ljung R, et al. Factor VIII products and inhibitor development in severe hemophilia A. *N Engl J Med*. 2013;368(3):231-239. doi:10.1056/nejmoa1208024
26. Malec LM, Journeycake J, Ragni MV. Extended half-life factor VIII for immune tolerance induction in haemophilia. *Haemophilia*. 2016;22(6):552-554. doi:10.1111/hae.13064
27. Schiavoni M, Napolitano M, Giuffrida G, et al. Status of recombinant factor VIII concentrate treatment for hemophilia A in Italy: characteristics and clinical benefits. *Front Med*. 2019;6(December):1-13. doi:10.3389/fmed.2019.00261
28. Shapiro AD, Ragni MV, Kulkarni R, et al. Recombinant factor VIII Fc fusion protein: extended-interval dosing maintains low bleeding rates and correlates with von Willebrand factor levels. *J Thromb Haemost*. 2014;12(11):1788-1800. doi:10.1111/jth.12723
29. Brennan Y, Parikh S, McRae S, Tran H. The Australian experience with switching to extended half-life factor VIII and IX concentrates: on behalf of the Australian Haemophilia Centre Directors' Organisation. *Haemophilia*. 2020;26(3):529-535. doi:10.1111/hae.13970
30. Iorio A, Puccetti P, Makris M. Clotting factor concentrate switching and inhibitor development in hemophilia A. *Blood*. 2012;120(4):720-727. doi:10.1182/blood-2012-03-378927

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Schep SJ, Fischer K, Boes M, Schutgens REG. No immunological changes after factor VIII product switch: An in depth analysis in haemophilia A patients. *Haemophilia*. 2023;29:1039–1048. https://doi.org/10.1111/hae.14808