

# Rituximab for treatment of inhibitors in haemophilia A

## A Phase II Study

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### Summary

The development of antibodies against infused factor VIII (FVIII) in patients with haemophilia A is a serious complication leading to poorly controlled bleeding and increased morbidity. No treatment has been proven to reduce high titre antibodies in patients who fail immune tolerance induction or are not candidates for it. The Rituximab for the Treatment of Inhibitors in Congenital Hemophilia A (RICH) study was a phase II trial to assess whether rituximab can reduce anamnestic FVIII antibody (inhibitor) titres. Male subjects with severe congenital haemophilia A and an inhibitor titre  $\geq 5$  Bethesda Units/ml (BU) following a FVIII challenge infusion received rituximab 375 mg/m<sup>2</sup> weekly for weeks 1 through 4. Post-rituximab inhibitor titres were measured monthly from week 6 through week 22 to assess treatment response.

Of 16 subjects who received at least one dose of rituximab, three (18.8%) met the criteria for a major response, defined as a fall in inhibitor titre to  $< 5$  BU, persisting after FVIII re-challenge. One subject had a minor response, defined as a fall in inhibitor titre to  $< 5$  BU, increasing to 5–10 BU after FVIII re-challenge, but  $< 50\%$  of the original peak inhibitor titre. Rituximab is useful in lowering inhibitor levels in patients, but its effect as a solo treatment strategy is modest. Future studies are indicated to determine the role of rituximab as an adjunctive therapy in immune tolerisation strategies.

### Keywords

Anti-CD20, antibodies, monoclonal, murine-derived, blood coagulation inhibitor, CD20 antibody, haemophilia A

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## Introduction

For many patients with congenital haemophilia A, the genetic absence or dysfunction of the factor VIII (FVIII) protein causes an immune response to infused FVIII replacement therapy. High titre alloreactive FVIII antibodies, or inhibitors, that neutralise the function of infused FVIII develop in as many as 30–40% of patients with severe haemophilia A (1, 2), and up to 13% of those with mild or moderate haemophilia A (3).

Treatment of bleeding in patients with high titre inhibitors is difficult. The major therapeutic modalities consist of agents that

bypass the need for FVIII, such as prothrombin complex or recombinant FVIIa concentrates. However, neither of these therapies leads to the predictable and effective haemostasis provided by FVIII replacement therapy. Patients with persistent inhibitors thus suffer consequences of serious, poorly controlled bleeding, which often leads to restrictive joint disease, prolonged hospitalisations, and in some cases, early death (4–7).

The only approach that has been shown to eradicate inhibitors in patients with congenital haemophilia is immune tolerance induction (ITI) therapy which involves regular (usually daily) exposure to FVIII concentrates to promote immunologic acceptance of

the FVIII protein (8). ITI is almost always indicated as first line treatment in patients who have had an inhibitor for less than a year. However, a significant portion (up to 50%) of patients will fail ITI, resulting in the presence of permanent inhibitors (9–12). In addition, patients with long standing inhibitors are not usually considered candidates for ITI because inhibitors present for longer than a year are typically refractory to ITI, and because older age at ITI initiation reduces ITI success (10). Ultimately, as many as 15–20% of these patients have permanent life-long inhibitors.

Rituximab (Rituxan®) is a genetically engineered chimeric murine/human monoclonal antibody directed against the CD20 antigen. At a dose of 375mg/m<sup>2</sup> weekly for four weeks, circulating B cells are depleted within the first one to three doses with sustained depletion for up to 6–9 months. B-cell recovery begins at approximately six months following completion of treatment. Median B-cell levels return to normal by 12 months following completion of treatment (13). Due to its profound effect on circulating B lymphocytes, rituximab has been used to successfully treat a variety of autoimmune disorders (14–18), including acquired haemophilia due to autoantibodies directed against FVIII (19–22). Anecdotal reports have suggested that rituximab may also have benefit in the management of FVIII alloantibodies (inhibitors) in patients with congenital haemophilia A (23–25).

The purpose of the RICH study was to determine if rituximab given in four weekly doses could reduce the titre and anamnestic response of FVIII inhibitors following exposure to infused FVIII in patients with severe congenital haemophilia A who have high-responding inhibitors.

## Materials and methods

### Study development and oversight

The Rituximab for the Treatment of Inhibitors in Congenital Haemophilia A (RICH) Study was designed by members of the Haemophilia Subcommittee of the Transfusion Medicine/Hemostasis (TMH) Clinical Trials Network as a proof-of-concept trial to determine whether four weeks of rituximab treatment could significantly reduce or eliminate high titre inhibitors in patients with severe haemophilia A. The protocol, which was registered at ClinicalTrials.gov as NCT00331006 and under IND BB-12417 for off-label study of rituximab, was opened at 13 sites in the United States, each of which obtained approval from an Institutional Review Board. All adults enrolled provided written informed consent; consent for children was obtained from a parent or legal guardian. Assent was provided by children where required by local policy. Central laboratory testing for inhibitor titres was performed at Orthopaedic Hospital Special Coagulation Laboratory (Los Angeles, CA, USA). Central data coordination was performed by the TMH Network Data Coordinating Center (DCC) at New England Research Institutes. TMH was funded by grants from the National Heart, Lung, and Blood Institute (NHLBI). A data and safety monitoring board (DSMB), established by NHLBI, regularly reviewed the data including accrual, study endpoints, and adverse events.

### Study participants

Patients were eligible for inclusion in the study if they had severe congenital haemophilia A, were at least 18 months of age, and had a documented historical FVIII inhibitor titre  $\geq 5$  Bethesda Units/ml (BU). The first detection of an inhibitor titre  $\geq 5$  BU had to be at least 12 months prior to the initial screening visit unless the patient had failed ITI. Patients were excluded from the study if they were undergoing ITI; had received FVIII concentrate within seven days prior to the initial screening visit or were expected to use FVIII concentrate for the treatment of bleeds; had received immunomodulatory drugs within 30 days prior to the initial screening visit; had previously received rituximab; or were currently participating in trials of investigational therapies for haemophilia. Patients were also excluded if they were HIV positive or had any of the following: an immune deficiency disorder; liver disease defined by serum alanine transaminase (ALT) or aspartate aminotransferase (AST) greater than three times the upper limit of normal, albumin  $< 2.5$  g/dl and/or international normalised ratio (INR)  $> 1.7$ ; evidence of hepatitis B infection; a history of cardiac arrhythmias, renal insufficiency, or pulmonary infiltrates; active febrile illness; or known allergies to murine or humanised antibodies.

### Study design

The RICH study was a single-arm, open-label, phase II clinical trial of four weekly doses of rituximab. The study had four phases: Screening phase, Treatment phase, Follow-up phase I, and Follow-up phase II (► Figure 1).

### Screening phase

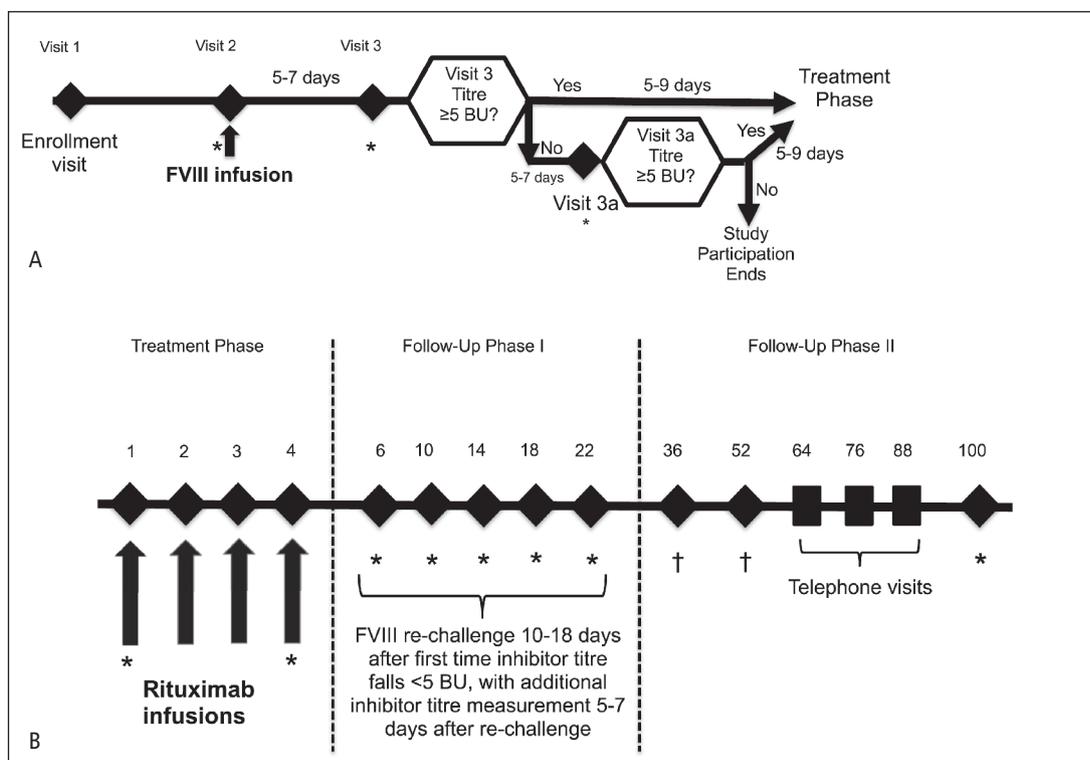
Patients meeting the eligibility criteria received a challenge infusion of FVIII at a dose of 50 IU/kg. A post-challenge FVIII inhibitor titre sample was drawn after 5–7 days; if this titre was  $\geq 5$  BU, the patient was eligible to enter the treatment phase of study. If the titre was  $< 5$  BU, another sample was drawn after an additional 5–7 days, and if the second post-challenge inhibitor titre was  $\geq 5$  BU, the patient entered the treatment phase of the study. If the inhibitor titre remained  $< 5$  BU, the patient was deemed ineligible to proceed to the treatment phase and study participation ended.

### Treatment phase

Rituximab treatment was initiated 5–9 days following the day that the treatment-qualifying post-challenge inhibitor titre sample was drawn. Patients received rituximab 375 mg/m<sup>2</sup> intravenously once per week for four weeks. Inhibitor titre samples were obtained immediately prior to the first and fourth infusion of rituximab.

### Follow-up phase I (Efficacy phase)

During Follow-up phase I, inhibitor titre samples were obtained every four weeks, beginning at week 6 (2 weeks after the fourth ri-



**Figure 1: RICH study schema.** A) Screening phase, \*Inhibitor titre measurement (at visit 2, before FVIII infusion). B) Treatment and follow-up phase (by week on study following screening phase). \*Inhibitor titre measurement (at weeks 1 and 4, before rituximab infusion). †Inhibitor titre measurement among responders only.

tuximab dose) and continuing through week 22. The first time an inhibitor titre was found to be  $< 5$  BU during Follow-up phase I, the subject was re-challenged with FVIII 50 IU/kg, and an inhibitor titre sample was measured 5–7 days after this FVIII re-challenge.

### Follow-Up phase II (Safety phase)

Patients continued to be followed beyond week 22 for long-term safety. Patients were monitored clinically at weeks 36, 52 and 100. FVIII inhibitor titres were determined at the week 36 and week 52 study visits for patients whose titre fell below 5 BU during Follow-up phase I, and at the week 100 study visit from all subjects. During weeks 64, 76 and 88, patients were contacted via telephone questionnaire to obtain information on infections, adverse events, and concomitant medications.

### Study outcomes

Because RICH was designed to investigate the possible efficacy and safety of rituximab, all study outcomes were to be assessed in subjects who received at least one dose of rituximab. All study measurements of inhibitor titres were carried out at the central laboratory.

RICH was designed as a proof-of-concept study. The primary efficacy outcome was the proportion of subjects who had a major response. A major response was defined to occur when a patient's FVIII inhibitor titre fell to  $< 5$  BU during Follow-up phase I and remained  $< 5$  BU following the re-challenge with FVIII. The defini-

tion of a major response did not require a complete eradication of the inhibitor. However, if rituximab was shown to be successful in reducing inhibitor titres and anamnestic response to FVIII, it is possible that some patients who previously could not be treated with FVIII might be able to receive FVIII treatment or prophylaxis.

The secondary efficacy outcome was the proportion of subjects who had either a major or minor response. A minor response was defined to occur when a patient's inhibitor titre fell to  $< 5$  BU during Follow-up phase I, and the inhibitor titre obtained 5–7 days following the re-challenge with FVIII was both between 5–10 BU and less than 50% of the treatment-qualifying inhibitor titre measured following the initial FVIII challenge.

Patients who did not achieve a FVIII inhibitor titre of  $< 5$  BU during Follow-up phase I were deemed to be non-responders. In addition, patients who achieved a FVIII inhibitor titre of  $< 5$  BU during Follow-up phase I, but who on re-challenge with FVIII had an inhibitor titre that was either  $> 10$  BU or  $\geq 50\%$  of their treatment-qualifying inhibitor titre or both, were also deemed to be non-responders. The protocol specified that all subjects who received at least one dose of rituximab and did not have data to determine whether a major or minor response occurred were considered non-responders.

### Statistical considerations

**Sample size:** Because it is very unlikely that an immunocompetent subject with a high titre inhibitor would have a spontaneous fall in inhibitor titre below 5 BU, and remain below 5 BU after re-chal-

allenge with FVIII, the null hypothesis was that the true probability that a subject would meet the criteria for major response was  $\leq 5\%$  (i.e.  $P_0=0.05$ ). The primary analysis of the primary outcome was to determine the probability of achieving at least the observed number of major responses, if the null hypothesis were true. If this probability was  $<0.05$ , the null hypothesis would be rejected. Because even a modest probability of major response could indicate that rituximab was a promising approach to inhibitor treatment, the study was designed to have 90% power to reject the null hypothesis if the true probability of a major response was at least 20% (i.e.  $P_1=0.20$ ). Assuming that up to 10% of subjects who received at least one dose of rituximab would be lost to follow-up before endpoint data were obtained, and considering those lost to follow-up as not having a major response, for purposes of sample size calculation  $P_0$  and  $P_1$  were reduced to 0.045 and 0.18, respectively. To achieve 90% power, the study planned to enrol 43 subjects who received at least one dose of rituximab (Hintze J. PASS 2008. NCSS, LLC. Kaysville, UT, USA. [www.ncss.com](http://www.ncss.com)). It was estimated that approximately 50 subjects would need to be enrolled to obtain 43 subjects who qualified for rituximab treatment.

Statistical analyses were performed using SAS (SAS/STAT Software, version 9.3. Cary, NC, USA: SAS Institute; 2002–2010). In

addition to the primary analysis of the primary outcome, two-sided exact binomial confidence intervals (CIs) for the proportion of subjects with a major response, and the proportion of subjects with either a major or minor response, were calculated. To assess safety, data on the number and types of bleeding events, and other serious adverse events (SAEs) and non-serious adverse events (AEs) were tabulated. Comparisons between subjects whose post-challenge inhibitor titres were at least 5 BU vs less than 5 BU were made using Fisher's Exact test for binary or categorical variables, and the non-parametric Wilcoxon rank sum test for continuous variables. Similar analyses compared rituximab-treated subjects who achieved at least a minor response vs rituximab-treated subjects who were considered non-responders. For each such comparison, all subjects with available data were analysed.

Stopping guidelines are detailed in the Supplementary Appendix (available online at [www.thrombosis-online.com](http://www.thrombosis-online.com)).

## Results

Study enrolment began in August 2006. The DSMB recommended in November 2010 that the study close to new enrolment due to

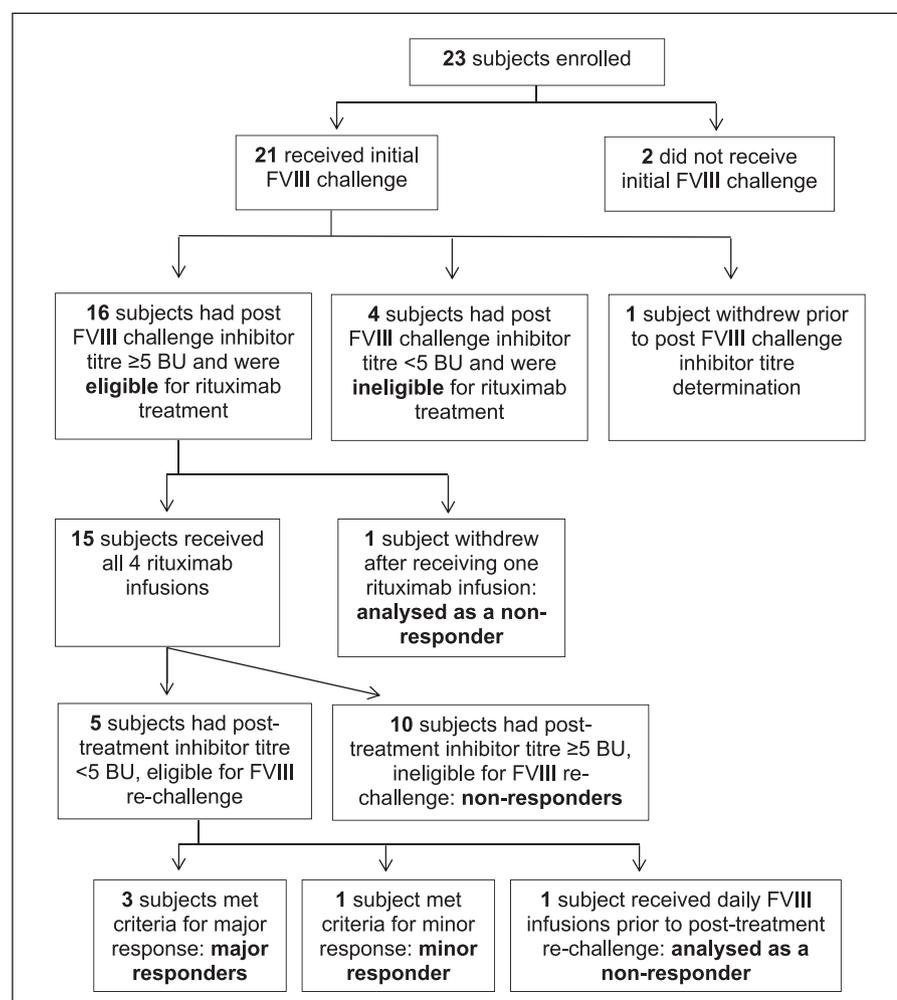


Figure 2: RICH study population.

the low accrual rate. ► Figure 2 shows the flow of subjects through the study. Twenty-three male patients were enrolled. The median age of enrolled subjects was 13.7 years (range 2.9 – 60.2 years). Of the 23 enrolled patients, 21 received the initial challenge with FVIII. The two patients who were not challenged included one patient who was lost to follow-up prior to receiving the initial FVIII challenge and a second patient who was unable to receive the in-

itial challenge because the study was closed before he received the FVIII challenge. One additional patient withdrew from the study after receiving the initial FVIII challenge but before eligibility for the treatment phase was determined. Of the 20 patients who received an initial FVIII challenge and subsequent inhibitor titre determination, 16 (80%) had a post-challenge titre  $\geq 5$  BU and qualified for the rituximab treatment phase of the study. Post-challenge

**Table 1: Comparison of selected variables between subjects who qualified for rituximab treatment and those who did not qualify for rituximab treatment.**

Baseline Demographic/Laboratory Test	Qualifiers N = 16	Non-Qualifiers N = 4	P value
	Median (Range)	Median (Range)	
Age (years)	14.2 (4.2 – 38.2)	14.4 (10.6 – 18.0)	0.82
Weight (kg)	48.2 (15.6 – 78.1)	64.2 (48.4 – 99.7)	0.16
Height (cm)	158.0 (102.0 – 184.0)	164.5 (145.5 – 169.0)	0.93
Ethnic origin			
Hispanic	4 (25%)	0 (0%)	0.54
Race			
Black/African American	3 (19%)	2 (50%)	0.25
White	13 (81%)	2 (50%)	
Age at first exposure to FVIII (months) <sup>a</sup>	1.5 (0.0 – 43.0)	7.0 (7.0 – 7.0)	0.34
Age inhibitor first detected (years) <sup>b</sup>	2.3 (0.3 – 11.0)	0.9 (0.5 – 1.4)	0.20
Duration of inhibitor (years) <sup>b</sup>	10.3 (0.6 – 34.4)	14.6 (11.6 – 17.6)	0.37
Historical peak inhibitor titre (BU) <sup>c</sup>	235.0 (33.3 – 4096.0)	115.0 (55.0 – 288.0)	0.35
History of immune tolerance induction <sup>c</sup>	13 (81%)	2 (67%)	0.53
Duration of immune tolerance induction (years) <sup>d</sup>	1.0 (0.01 – 2.9)	5.2 (5.2 – 5.2)	0.19
Baseline inhibitor titre (BU) measured prior to initial FVIII infusion	9.6 (0.5 – 730.0)	1.8 (0.8 – 4.0)	0.04
ALT (units/l)	25.0 (6.0 – 49.0)	19.0 (17.0 – 24.0)	0.61
AST (units/l)	23.5 (15.0 – 52.0)	29.5 (20.0 – 33.0)	0.68
WBC ( $10^3/\mu\text{l}$ )	6.5 (3.3 – 11.1)	5.7 (4.6 – 5.9)	0.16
Absolute neutrophil count ( $10^3/\mu\text{l}$ ) <sup>e</sup>	4.1 (1.1 – 8.8)	2.4 (1.5 – 3.0)	0.18
Absolute lymphocyte count ( $10^3/\mu\text{l}$ )	2.1 (1.1 – 4.2)	2.2 (2.0 – 2.6)	0.61
CD3 ( $10^3/\text{ml}$ )	1407 (661 – 3221)	1602 (1436 – 1706)	0.61
CD4 ( $10^3/\text{ml}$ )	916 (410 – 1915)	965 (754 – 1056)	0.74
CD8 ( $10^3/\text{ml}$ )	539 (182 – 1059)	496 (366 – 551)	0.64
CD19 ( $10^3/\text{ml}$ ) <sup>f</sup>	353 (64 – 748)	457 (412 – 963)	0.27
CD20 ( $10^3/\text{ml}$ ) <sup>f</sup>	316 (8 – 861)	457 (407 – 960)	0.14
Immunoglobulin G (mg/dl)	1060 (847 – 1740)	1122 (891 – 1188)	0.74
Immunoglobulin M (mg/dl)	120 (53 – 162)	89 (73 – 115)	0.12
Post-baseline laboratory test			
Peak inhibitor titre following initial FVIII challenge (BU) <sup>g</sup>	12.2 (5.5 – 1380.0)	2.0 (1.8 – 2.5)	N/A

<sup>a</sup>Data missing for one qualifier. Data unknown for five qualifiers and two non-qualifiers. <sup>b</sup>Data unknown for two non-qualifiers. <sup>c</sup>Data unknown for one non-qualifier. <sup>d</sup>Among those with known history of ITI. Data unknown for two qualifiers and one non-qualifier. Data missing for one qualifier. <sup>e</sup>Additional lab test required by the study beginning April 6, 2007. Not required for five qualifiers and one non-qualifier. <sup>f</sup>Data missing for one qualifier. <sup>g</sup>Computed as the maximum inhibitor titre obtained at Visit 3 or 3A. No p-value computed because this titre was used to determine whether a subject was a qualifier.

Subjects with major or minor response	Type of response <sup>2</sup>	Peak inhibitor titre after baseline challenge	Inhibitor titre after post-treatment challenge	Change in BU <sup>3</sup>	Percentage change in BU
Subject A	Major	7.4	2.0	-5.4	-73.0%
Subject B	Major	6.0	4.0	-2.0	-33.3%
Subject C	Major	8.0	1.5	-6.5	-81.3%
Subject D	Minor	12.4	5.5	-6.9	-55.6%

<sup>1</sup>Subjects with BU ≤5 during Follow-up Phase I. <sup>2</sup>A major response was defined as occurring when a patient's inhibitor titre fell to <5 BU and remained <5 BU following a re-challenge with FVIII. A minor response was defined to occur when a patient's inhibitor titre fell to <5 BU during Follow-Up Phase I, and the inhibitor titre obtained 5–7 days following the re-challenge with FVIII was both between 5–10 BU and less than 50% of the treatment-qualifying inhibitor titre measured following the initial FVIII challenge. <sup>3</sup>(BU after post-treatment Factor VIII challenge) – (BU after baseline Factor VIII challenge).

**Table 2: Changes in inhibitor titre among those who were re-challenged.<sup>1</sup>**

peak inhibitor titres for the four non-qualifying subjects ranged between 1.8 and 2.5 (Suppl. Figure 1A-D, available online at [www.thrombosis-online.com](http://www.thrombosis-online.com)). Characteristics for the 16 subjects with post-challenge titres qualifying them for the Treatment phase and the four subjects with non-qualifying post-challenge titres are shown in ► Table 1. The 16 subjects who qualified for treatment had significantly higher pre-challenge inhibitor titre than the 4 subjects who did not qualify for treatment (median 9.6 BU vs 1.8 BU for qualifiers and non-qualifiers, respectively;  $p=0.04$ ).

All 16 patients who qualified for the rituximab treatment phase of the study received at least one rituximab treatment and are included in the analyses of efficacy and safety. One subject received only one partial rituximab infusion, which was stopped due to hypotension. The patient subsequently withdrew from the study and did not receive any further doses of rituximab. Of the remaining 15 patients, 14 patients received four complete infusions and one received three complete infusions and one partial infusion. Among these 15 patients, one subject was lost to follow-up after the Week 18 visit, one was lost to follow-up after the Week 36 visit and two subjects were lost to follow-up after the Week 52 visit.

## Efficacy

Five patients (31.25% of those treated with rituximab) had an inhibitor titre <5 BU during Follow-up phase I. Four of these patients received a re-challenge with FVIII. The fifth patient's clinical team began treating him with daily infusions of FVIII (200 IU/kg/day) following the week 22 visit but before the central laboratory inhibitor titre result for week 22 (4.5 BU) was known. Therefore, a post-treatment challenge was deemed clinically inappropriate for this patient, and he was considered a non-responder per protocol guidelines.

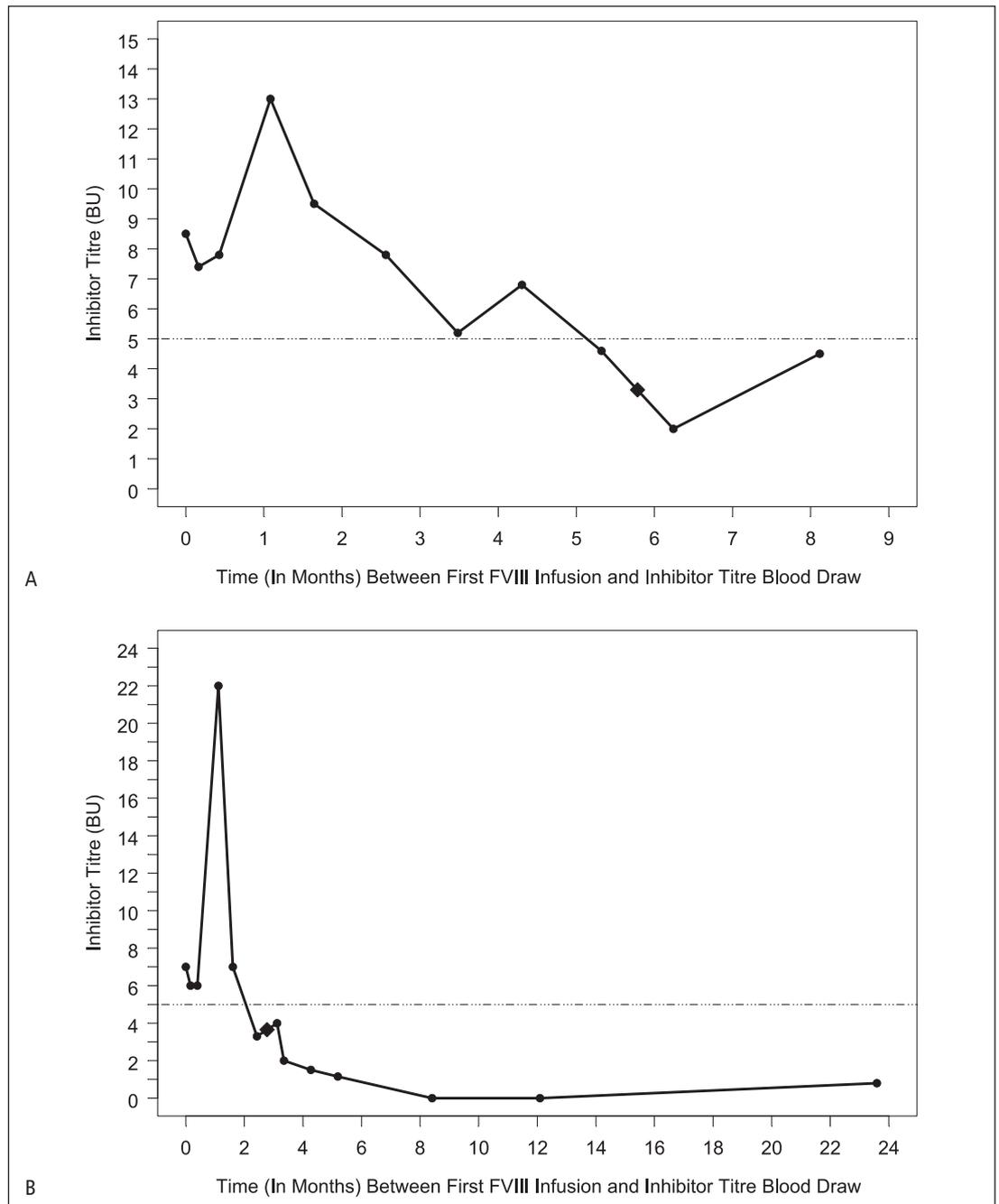
Three patients (18.75% of those treated with rituximab) had a major response. The exact binomial probability of observing at least three major responders in 16 subjects, if the true major response rate is 5%, is 0.043, therefore rejecting the null hypothesis that the major response rate is ≤5%. Due to the small sample size, the two-sided 95% exact binomial confidence interval for this percentage is wide, 4.05% – 45.65%.

There were four patients who had either a major or minor response (25.00%, 95% exact binomial CI 7.27% – 52.38%). Inhibitor titres measured after the initial and post-treatment FVIII challenges for the four subjects who achieved a major or minor response are shown in ► Table 2 and ► Figure 3A-D. After the initial FVIII challenge, peak inhibitor titres for these subjects ranged between 6.0 and 12.4 BU, and after the post-treatment challenge, titres ranged between 1.5 and 5.5 BU. The average absolute decrease was 5.2 BU and the average percentage change in BU was a decrease of 60.8%.

Although the RICH study did not collect systematic data on the use of FVIII after a subject had a response, three of the four subjects who met the criteria for a major or minor response are known to have received at least some FVIII after achieving a response. Two subjects (► Figure 3B and D) were reported to have received FVIII prophylactically beginning approximately one month after the FVIII re-challenge. Inhibitor titres for the major responder in ► Figure 3B remained below 5 BU through his month 24 visit, approximately 21 months after achieving his response. The minor responder (► Figure 3D) had inhibitor titres that remained just over 5 BU throughout his remaining time on study, approximately seven months after achieving his response. The third subject (► Figure 3C) known to have used FVIII prophylactically after achieving a response experienced a steady rise in inhibitor titres after the FVIII post-treatment challenge, rising to approximately 15 BU by the month 24 visit. This subject was reported to have received FVIII prophylactically beginning six weeks after the FVIII re-challenge. In addition, this subject also received FVIII for the treatment of bleeding events reported during the last study visit.

Inhibitor titres over time for the 12 subjects who qualified for rituximab treatment but were not classified as having a major or minor response are shown in Suppl. Figure 2A-L (available online at [www.thrombosis-online.com](http://www.thrombosis-online.com)). Suppl. Figure 2D (available online at [www.thrombosis-online.com](http://www.thrombosis-online.com)) corresponds to the subject who qualified for the re-challenge but did not receive it.

Characteristics for the four subjects who had either a major or minor response to rituximab and the 12 subjects who were non-responders are shown in ► Table 3. The four subjects who achieved



**Figure 3: Inhibitor titre results among subjects who responded, by subject.** Solid diamond represents date of FVIII re-challenge. A) major responder 1, B) major responder 2, C) major responder 3, D) minor responder 1.

at least a minor response were significantly older when the inhibitor to FVIII was first detected than the 12 subjects who were non-responders (median 7.2 years vs 1.5 years for responders and non-responders, respectively;  $p=0.03$ ).

## Safety

► Table 4 shows the types and frequencies of the SAEs and other AEs that were reported during the study. Among the 16 subjects who received at least one dose of rituximab, 574 adverse events (both serious and non-serious) were reported, and 15 subjects

(94%) had at least one adverse event. Among all adverse events, 47 events (8%) met SAE criteria and 11 subjects (69%) experienced at least one SAE. An additional 527 AEs did not meet SAE criteria. These 527 AEs occurred in 15 subjects.

Of all adverse events, 459 (80%) events occurring among 15 subjects were classified as bleeding events. Among these bleeding adverse events, 31 (7%) met SAE criteria, and nine subjects experienced at least one such bleeding SAE.

The remaining 115 (20%) adverse events were classified as non-bleeding adverse events and were reported among 13 subjects. Sixteen (14%) of the non-bleeding adverse events met SAE criteria

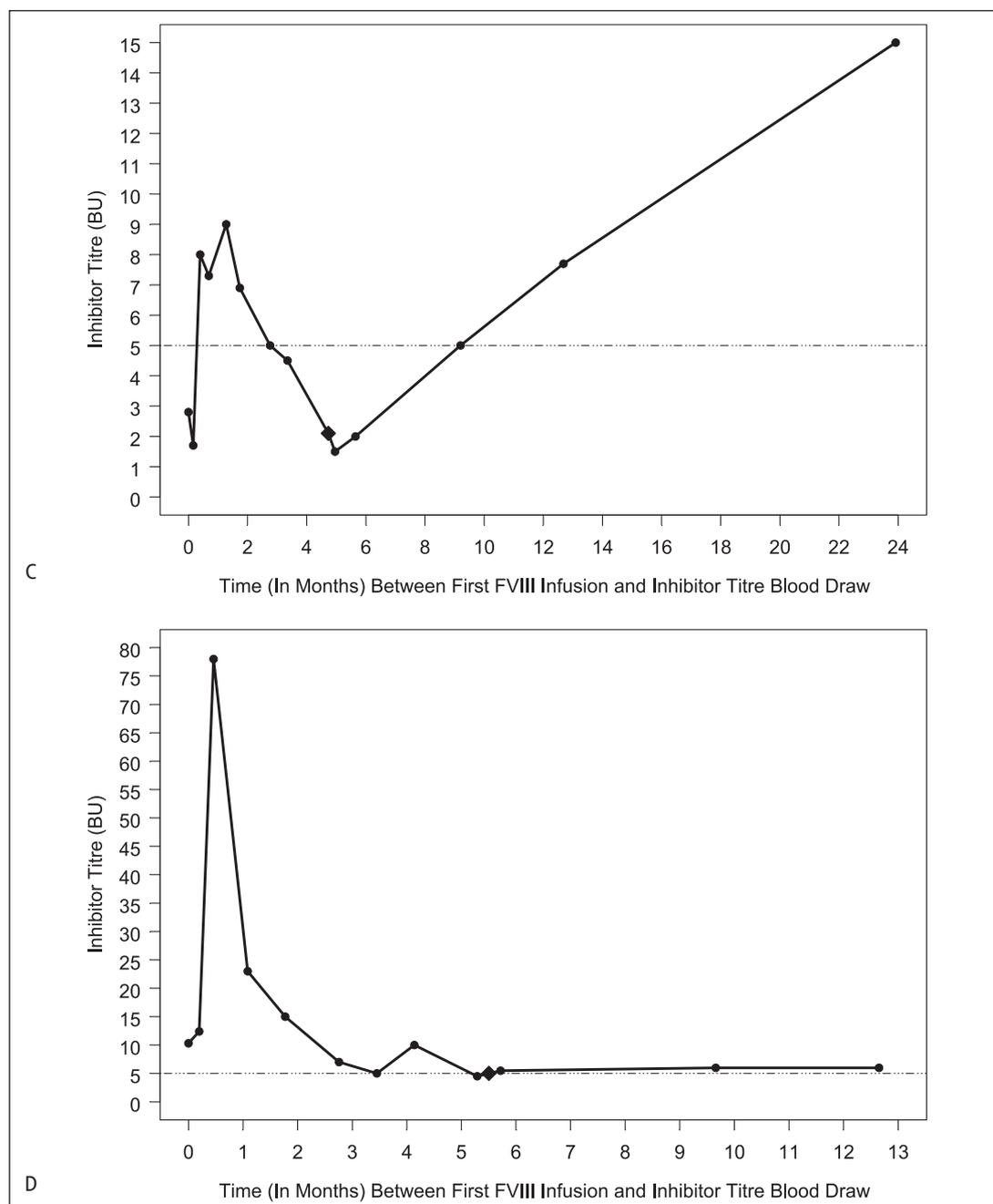


Figure 3: Continued

and 10 subjects experienced at least one such non-bleeding SAE. All non-bleeding SAEs resolved by the time the subject ended RICH participation (13 with no sequelae and 3 with sequelae). Nearly all the other non-bleeding AEs also resolved (94 without sequelae and 1 with sequelae), but three were ongoing at the end of study participation, and the final status of one non-bleeding AE was unknown. There were no deaths among study participants.

## Discussion

Rituximab has previously demonstrated efficacy in treating autoimmune disorders, leading to clinical benefit by reducing or eliminating pathological autoantibodies (14–18). However, its ability to suppress alloantibodies has not been clearly demonstrated. Numerous reports have indicated that rituximab is effective in eradicating FVIII autoantibodies and it is now considered a major treatment strategy for patients with acquired haemophilia A (19–22). Given the success in treating autoreactive FVIII inhibitors, there were early attempts to use rituximab as an adjunctive agent in ITI regimens that were designed to eradicate inhibitors in patients

Table 3: Comparison of selected variables between rituximab-treated subjects who achieved at least a minor response and rituximab-treated subjects who were non-responders.

Baseline Demographic/Laboratory Test	Responders N = 4	Non-responders N = 12	P value
	Median (Range)	Median (Range)	
Age (years)	15.2 (11.5 – 17.2)	13.2 (4.2 – 38.2)	0.68
Weight (kg)	68.6 (37.8 – 78.1)	36.2 (15.6 – 77.9)	0.12
Height (cm)	167.8 (144.5 – 184.0)	150.5 (102.0 – 175.0)	0.32
Ethnic origin			
Hispanic	0 (0%)	4 (33%)	0.52
Race			
Black/African American	2 (50%)	1 (8%)	0.14
White	2 (50%)	11 (92%)	
Age at first exposure to FVIII (months) <sup>a</sup>	3.5 (0.0 – 7.0)	1.5 (0.0 – 43.0)	1.00
Age inhibitor first detected (years)	7.2 (3.3 – 11.0)	1.5 (0.3 – 7.5)	0.03
Duration of inhibitor (years)	8.0 (0.6 – 13.9)	11.5 (2.8 – 34.4)	0.44
Historical peak inhibitor titre (BU)	216.5 (64.0 – 235.0)	661.0 (33.3 – 4096.0)	0.29
History of immune tolerance induction			
Duration of immune tolerance induction (years) <sup>b</sup>	0.4 (0.01 – 0.8)	1.7 (0.4 – 2.9)	0.18
Baseline inhibitor titre (BU) measured prior to initial FVIII infusion	7.8 (2.8 – 10.3)	11.9 (0.5 – 730.0)	0.27
ALT (units/l)	20.5 (13.0 – 34.0)	28.0 (6.0 – 49.0)	0.68
AST (units/l)	20.5 (18.0 – 34.0)	24.5 (15.0 – 52.0)	0.38
WBC (10 <sup>3</sup> /μl)	6.6 (3.3 – 7.0)	6.5 (3.8 – 11.1)	0.68
Absolute neutrophil count (10 <sup>3</sup> /μl) <sup>c</sup>	3.1 (1.1 – 5.2)	4.2 (1.4 – 8.8)	0.49
Absolute lymphocyte count (10 <sup>3</sup> /μl)	1.8 (1.1 – 2.8)	2.1 (1.6 – 4.2)	0.38
CD3 (10 <sup>3</sup> /ml)	1068 (661 – 1734)	1514 (949 – 3221)	0.15
CD4 (10 <sup>3</sup> /ml)	555 (410 – 929)	985 (432 – 1915)	0.08
CD8 (10 <sup>3</sup> /ml)	456 (182 – 565)	563 (347 – 1059)	0.32
CD19 (10 <sup>3</sup> /ml) <sup>d</sup>	446 (299 – 620)	332 (64 – 748)	0.48
CD20 (10 <sup>3</sup> /ml) <sup>d</sup>	165 (8 – 525)	338 (9 – 861)	0.29
Immunoglobulin G (mg/dl)	1134 (1000 – 1579)	1060 (847 – 1740)	0.91
Immunoglobulin M (mg/dl)	108 (59 – 111)	126 (53 – 162)	0.06
Post-baseline laboratory tests			
Peak inhibitor titre following initial FVIII challenge (BU) <sup>e</sup>	7.7 (6.0 – 12.4)	20.0 (5.5 – 1380.0)	0.24
Inhibitor titre (BU) at visit 12	3.3 (1.2 – 4.6)	23.5 (4.5 – 1577.0)	0.02
Inhibitor titre (BU) at visit 15	7.9 (0.8 – 15.0)	17.0 (0.0 – 325.0)	0.43
Nadir CD19 count (10 <sup>3</sup> /ml) between visits 7 and 12 (inclusive) <sup>f</sup>	0.000 (0.000, 0.000)	0.001 (0.000, 0.069)	0.11

<sup>a</sup>Data unknown for two responders and three non-responders, missing for one non-responder. <sup>b</sup>Among those with known history of ITI. Data unknown for two responders. Data missing for one non-responder. <sup>c</sup>Additional lab test required by the study beginning April 6, 2007. Not required for one responder and four non-responders. <sup>d</sup>Data missing for one non-responder. <sup>e</sup>Computed as the maximum inhibitor titre obtained at Visit 3 or 3A. <sup>f</sup>CD19 measurements were scheduled to occur at Visits, 7, 10 and 12 and immediately before the FVIII re-challenge, if any. One non-responder did not have any CD19 counts during this time frame because he withdrew after Visit 4. A second non-responder did not have a CD19 at Visit 12 because he was lost to follow-up after Visit 11. Four additional non-responders had a Visit 12 but did not have CD19 counts reported from the visit. One other non-responder did not have CD19 counts reported at Visits 10 or 12.

Table 4: Attributes of the serious adverse events (SAEs) and non-SAE adverse events\*, among the subjects who received at least one dose of rituximab.

	Serious Adverse Events		Adverse Events Other Than SAEs		Total	
	Number (%) of SAEs N = 47	Number (%) of Subjects With At Least One Such Event	Number (%) of AEs N = 527	Number (%) of Subjects With At Least One Such Event	Number (%) of Events N = 574	Number (%) of Subjects With At Least One Such Event
Bleeding Events	31 (66)	9 (56)	428 (81)	15 (94)	459 (80)	15 (94)
•Joint Bleeding	21 (45)	6 (38)	293 (56)	15 (94)	314 (55)	15 (94)
•Haematomas	7 (15)	5 (31)	105 (20)	14 (88)	112 (20)	14 (88)
•Subdural haematoma	2 (4)	1 (6)	0 (0)	0 (0)	2 (<1)	1 (6)
•Mallory Weiss tear	1 (2)	1 (6)	0 (0)	0 (0)	1 (<1)	1 (6)
•Haematuria	0 (0)	0 (0)	1 (<1)	1 (6)	1 (<1)	1 (6)
•Bleeding, Other	0 (0)	0 (0)	29 (6)	6 (38)	29 (5)	6 (38)
Non-Bleeding Events	16 (34)	10 (63)	99 (19)	11 (69)	115 (20)	13 (81)
Infection	5 (11)	4 (25)	5 (1)	3 (11)	10 (2)	5 (31)
•Central line infection	1 (2)	1 (6)	0 (0)	0 (0)	1 (<1)	1 (6)
•Conjunctivitis	0 (0)	0 (0)	1 (<1)	1 (6)	1 (<1)	1 (6)
•Herpes Zoster	1 (2)	1 (6)	0 (0)	0 (0)	1 (<1)	1 (6)
•Pulmonary infection	1 (2)	1 (6)	0 (0)	0 (0)	1 (<1)	1 (6)
•Sepsis, Septic Arthritis	1 (2)	1 (6)	1 (<1)	1 (6)	2 (<1)	1 (6)
•Sinusitis	0 (0)	0 (0)	1 (<1)	1 (6)	1 (<1)	1 (6)
•Streptococcus	0 (0)	0 (0)	2 (<1)	1 (6)	2 (<1)	1 (6)
•Viral meningitis	1 (2)	1 (6)	0 (0)	0 (0)	1 (<1)	1 (6)
Laboratory Abnormality	0 (0)	0 (0)	21 (4)	2 (13)	21 (4)	2 (13)
•Anaemia	0 (0)	0 (0)	3 (1)	1 (6)	3 (1)	1 (6)
•ALT abnormality	0 (0)	0 (0)	10 (2)	1 (6)	10 (2)	1 (6)
•WBC abnormality	0 (0)	0 (0)	7 (1)	2 (13)	7 (1)	2 (13)
•Other	0 (0)	0 (0)	1 (<1)	1 (6)	1 (<1)	1 (6)
Orthopaedic Events	0 (0)	0 (0)	6 (1)	3 (11)	6 (1)	3 (19)
•Joint pain	0 (0)	0 (0)	4 (1)	2 (13)	4 (1)	2 (13)
•Joint loss of motion	0 (0)	0 (0)	2 (<1)	2 (13)	2 (<1)	2 (13)
Surgery	4 (9)	4 (25)	0 (0)	0 (0)	4 (1)	4 (25)
•Port placement	2 (4)	2 (13)	0 (0)	0 (0)	2 (<1)	2 (13)
•Synovectomy	2 (4)	2 (13)	0 (0)	0 (0)	2 (<1)	2 (13)

with congenital haemophilia, particularly in patients who had failed to achieve tolerance with standard ITI (26-30). Several reports and one larger cohort study (31) suggested that rituximab augmented the efficacy of ITI in some patients and led to tolerisation in some individuals who appeared to be resistant to standard ITI. In addition there have been a few case reports of successful inhibitor eradication in patients who received rituximab with-

out concomitant ITI, although the follow-up of these patients was limited (32). In patients where tolerance is not achieved, lowering the inhibitor using rituximab may decrease bleeding frequency, especially in patients with mild or moderate haemophilia (33).

For patients who have failed ITI therapy or who are not candidates for ITI, persistence of inhibitors greatly increases morbidity and treatment costs (34-36). Given the disease burden, physical

Table 4: Continued

	Serious Adverse Events		Adverse Events Other Than SAEs		Total	
	Number (%) of SAEs N = 47	Number (%) of Subjects With At Least One Such Event	Number (%) of AEs N = 527	Number (%) of Subjects With At Least One Such Event	Number (%) of Events N = 574	Number (%) of Subjects With At Least One Such Event
Other	7 (15)	5 (31)	67 (13)	10 (63)	74 (13)	11 (69)
•Abdominal Pain	0 (0)	0 (0)	3 (1)	2 (13)	3 (1)	2 (13)
•Allergic reaction	1 (2)	1 (6)	1 (<1)	1 (6)	2 (<1)	2 (13)
•Anorexia, nausea, vomit	0 (0)	0 (0)	8 (2)	1 (6)	8 (1)	1 (6)
•Asthma	0 (0)	0 (0)	3 (1)	1 (6)	3 (1)	1 (6)
•Bell's Palsy	0 (0)	0 (0)	1 (<1)	1 (6)	1 (<1)	1 (6)
•Cold, Chills, Cough	0 (0)	0 (0)	15 (3)	6 (38)	15 (3)	6 (38)
•Cold Sore	0 (0)	0 (0)	1 (<1)	1 (6)	1 (<1)	1 (6)
•Diarrhoea	1 (2)	1 (6)	1 (<1)	1 (6)	2 (<1)	2 (13)
•Dyspepsia	0 (0)	0 (0)	1 (<1)	1 (6)	1 (<1)	1 (6)
•Dyspnoea	1 (2)	1 (6)	0 (0)	0 (0)	1 (<1)	1 (6)
•Fatigue, Lethargy	0 (0)	0 (0)	3 (1)	2 (13)	3 (1)	2 (13)
•Fever	1 (2)	1 (6)	4 (1)	2 (13)	5 (1)	2 (13)
•Fracture	1 (2)	1 (6)	0 (0)	0 (0)	1 (<1)	1 (6)
•Headache	1 (2)	1 (6)	2 (<1)	2 (13)	3 (1)	3 (19)
•Head Injury	0 (0)	0 (0)	2 (<1)	2 (13)	2 (<1)	2 (13)
•Hypertension	0 (0)	0 (0)	1 (<1)	1 (6)	1 (<1)	1 (6)
•Hypotension	1 (2)	1 (6)	4 (1)	1 (6)	5 (1)	2 (13)
•Lymphadenopathy	0 (0)	0 (0)	1 (<1)	1 (6)	1 (<1)	1 (6)
•Myalgia	0 (0)	0 (0)	2 (<1)	1 (6)	2 (<1)	1 (6)
•Pain	0 (0)	0 (0)	3 (1)	2 (13)	3 (1)	2 (13)
•Photophobia	0 (0)	0 (0)	1 (<1)	1 (6)	1 (<1)	1 (6)
•Pulmonary Hypertension	0 (0)	0 (0)	1 (<1)	1 (6)	1 (<1)	1 (6)
•Rash	0 (0)	0 (0)	3 (1)	2 (13)	3 (1)	2 (13)
•Sunburn	0 (0)	0 (0)	1 (<1)	1 (6)	1 (<1)	1 (6)
•Sweating	0 (0)	0 (0)	1 (<1)	1 (6)	1 (<1)	1 (6)
•Toothache	0 (0)	0 (0)	1 (<1)	1 (6)	1 (<1)	1 (6)
•Vaccination Site Reaction	0 (0)	0 (0)	1 (<1)	1 (6)	1 (<1)	1 (6)
•Wheezing	0 (0)	0 (0)	2 (<1)	1 (6)	2 (<1)	1 (6)

\* Includes three bleeding events with onset before date of consent but ongoing at baseline.

morbidity, psychosocial impact, and financial cost, there has been a rapid escalation of rituximab use as a potentially effective treatment modality, despite a lack of clear evidence of benefit. Rituximab has potential side-effects that include complications from immune suppression such as serious opportunistic infections (35, 36); in addition, the long-term effects on children are unclear.

The problem in understanding the potential benefit of rituximab in earlier reports is that it is difficult to assess whether its addition to ongoing ITI contributed to ITI success since it is possible that simply continuing ITI without rituximab may have resulted in the same good outcome. In those rare cases where lowering or eliminating a FVIII inhibitor was ascribed to rituximab as a single

agent (without concomitant ITI), it is unclear if this response was maintained after re-exposure to FVIII. High titre inhibitors in congenital haemophilia A are immunologically anamnestic and may disappear over time as long as there is no exposure to FVIII, but will usually reappear upon further exposure to FVIII. In the case reports of inhibitor eradication, it was often unclear whether the patient subsequently received re-exposure to FVIII and demonstrated that the anamnestic immunologic response was truly abated by rituximab therapy.

The RICH Study was the first prospective clinical trial designed to measure the effect of rituximab on reducing the anamnestic immune response against FVIII in patients with inhibitors, which we deemed a more definitive assessment of rituximab efficacy than simply observing decreasing inhibitor titres after its use. This phase II study was a proof-of-principle study to determine whether the safety and efficacy of rituximab would justify its inclusion in a larger, definitive study in which it would be combined with ITI for inhibitor eradication. Even though the definition of a major response used in RICH did not require the eradication of the inhibitor, three of the four patients who achieved at least a minor response were treated with FVIII prophylactically after achieving a response, per the discretion of the treating physician. Two of these subjects had titres that remained low after the initiation of FVIII prophylaxis. The RICH results indicate that there is evidence for a modest benefit of rituximab as a single agent to reduce the immune response to FVIII in some patients.

### What is known about this topic?

- Inhibitor formation to infused clotting factor is among the most common and clinically important complications of haemophilia A. These alloantibodies arise as a T-cell mediated B cell response.
- Immune tolerance induction (ITI) with repeated doses of factor VIII can eliminate inhibitors in some patients but is not universally effective. As many as 40–50% of inhibitor patients fail immune tolerance.
- Many patients who fail ITI are currently receiving salvage treatment with the CD20 antibody rituximab, based on anecdotal reports, without available data on response rates or adverse events.

### What does this paper add?

- Rituximab was administered in a prospective single-arm clinical trial to subjects with haemophilia A and high titre inhibitors who had failed immune tolerance induction or would not be expected to respond. Data were collected on inhibitor levels, anamnestic response and adverse events.
- Inhibitor titres and anamnestic response were reduced in 25% of subjects. Rituximab was generally well tolerated.
- This study provides data on response characteristics and adverse events that has been lacking; these results can inform future studies assessing whether rituximab in combination with other immunosuppressive drugs or in conjunction with ITI would be effective in a larger proportion of patients.

Rituximab reduced the anamnestic inhibitor rise in 4 of 16 subjects (25%), who were considered treatment responders. Responders, compared to non-responders, were older at the time of inhibitor development (7.2 compared to 1.5 years), but showed no difference in duration of inhibitor or historical peak inhibitor titre. A fifth subject, whose inhibitor declined to less than 5 BU, met the criteria for a FVIII re-challenge dose, but was placed on FVIII therapy without receiving the FVIII challenge for the primary study endpoint.

All responders had a baseline inhibitor level <12 BU, suggesting that rituximab may be more effective when inhibitor levels are not high at the time of treatment initiation. A similar observation has been made with respect to ITI where a lower baseline inhibitor titre at ITI initiation predicts a higher likelihood of achieving tolerance (10). Responders also had somewhat lower levels of CD4+ and CD20+ lymphocyte populations and Immunoglobulin M (IgM) at study baseline, but these comparisons did not reach statistical significance.

There were no major safety concerns with rituximab in this patient population. The majority of adverse events were bleeding events, as expected in this patient population. While there were some non-serious ALT elevations, no clinical liver disease was reported.

One unexpected observation was that four of the 20 subjects who received the initial FVIII challenge and had a subsequent inhibitor titre determination did not experience an anamnestic rise in their inhibitor titre after exposure to FVIII, and were thus ineligible to proceed to rituximab therapy. This group was similar to the group that did have a post-challenge titre >5 BU, except that their pre-challenge inhibitor titre was lower. At least three of the four had historical inhibitor peak titres >50 BU and all were being managed as chronic high-responder inhibitor patients. Although two of the four had a known history of undergoing ITI in the past, they had been deemed ITI failures; all four were being treated with bypassing therapy for bleeding. Nevertheless, all four behaved as low-titre, low-responder inhibitor patients, with inhibitor titres not going above 2.5 BU after FVIII exposure. This raises the possibility that some high responding inhibitor patients may, over time, lose immunologic anamnesis and be candidates for management with FVIII replacement therapy. These observations should perhaps prompt an occasional FVIII challenge for long-standing inhibitor patients whose current inhibitor titres are <5 BU, to identify those who may benefit from returning to FVIII replacement therapy.

The study had several limitations. The sample size was small due to the rarity of inhibitor patients who met the study criteria, and the difficulty in recruiting among this rare disease population. In addition, the study was a phase II, non-randomised trial which was developed as a pilot study, due to the difficulty in conducting large scale randomised clinical trials in this patient population. The duration of response to rituximab as a single agent is also a concern since it is clear that rituximab-induced reduction in circulating CD20 lymphocytes lasts 6–9 months, although longer-term clinical responses are seen in patients with autoimmune disorders. However, given the characteristics of our patient population, we

expect that these results may be generalizable to patients with a history of high titre inhibitors who have failed ITI or who are not candidates for ITI.

Despite its limitations, the RICH Study demonstrates that as many as 25% of patients with alloantibody inhibitors to FVIII achieved some benefit from rituximab as a single agent. While this benefit is modest, it nevertheless points to demonstrable efficacy and supports further study of rituximab as an adjunctive therapy in immune tolerance regimens.

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### Conflicts of interest

C. Leissinger reports receiving grant funding from Baxter, NovoNordisk and CSL Behring, and receiving honoraria for speaking and participation in advisory boards for Baxter, Bayer, CSL Behring, Kedrion, NovoNordisk, and Pfizer. J.C. Gill reports receiving honoraria for advisory board membership for Baxter, Bayer, CSL Behring and Octapharma.

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