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Rituximab for Treatment of Inhibitors in Haemophilia A: A Phase II Study

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Conflict 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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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 ≥5 Bethesda Units/mL (BU) following a FVIII challenge infusion received rituximab 375 mg/m^2 weekly for weeks 1 through 4. Post-rituximab inhibitor titres were measured monthly from week 6 through week 22 to assess treatment response. Of sixteen 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

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 neutralize 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 Factor VIII, such as prothrombin complex or recombinant Factor VIIa concentrates. However, neither of these therapies leads to the predictable and effective hemostasis provided by Factor VIII replacement therapy. Patients with persistent inhibitors thus suffer consequences of serious, poorly controlled bleeding, which often leads to restrictive joint disease, prolonged hospitalizations, 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^2 weekly × 4 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 6 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 4 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 Hemophilia 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). 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 ≥5 Bethesda Units/mL (BU). The first detection of an inhibitor titre ≥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 7 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 normalized 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 humanized antibodies.

Study Design

The RICH study was a single-arm, open-label, phase II clinical trial of 4 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 Factor VIII inhibitor titre sample was drawn after 5–7 days; if this titre was ≥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 ≥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^2 intravenously once per week for 4 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 rituximab 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 Factor VIII 50 IU/kg, and an inhibitor titre sample was measured 5–7 days after this Factor VIII 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. Factor VIII 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 definition 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 ≥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-challenge with FVIII, the null hypothesis was that the true probability that a subject would meet the criteria for major response was ≤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, Utah. 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: SAS Institute; 2002–2010). In addition to the primary analysis of the primary outcome, two-sided exact binomial confidence intervals 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.

RESULTS

Study enrolment began in August 2006. The DSMB recommended in November 2010 that the study close to new enrolment due to 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 Factor VIII. 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 initial 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 ≥5 BU and qualified for the rituximab treatment phase of the study. Post-challenge peak inhibitor titres for the four non-qualifying subjects ranged between 1.8 and 2.5 (Supplemental Figures 1A–D). Characteristics for the 16 subjects with post-challenge titres qualifying them for the Treatment Phase and the 4 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 1

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 Factor VIII. 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 3 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 4 patients who had either a major or minor response (25.00%, 95% exact binomial confidence interval 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 Figures 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 (Figures 3B and 3D) were reported to have received FVIII prophylactically beginning approximately 1 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 7 months after achieving his response. The third subject (Figure 3C) known to have used Factor VIII 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 Supplementary Figures 2A–L. Supplemental Figure 2D corresponds to the subject who qualified for the re-challenge but did not receive it.

Characteristics for the 4 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 4 subjects who achieved 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 9 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 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 3 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 Factor VIII 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 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 without 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 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 Factor VIII 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.

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 4 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 3 of the 4 had historical inhibitor peak titres >50 BU and all were being managed as chronic high-responder inhibitor patients. Although 2 of the 4 had a known history of undergoing ITI in the past, they had been deemed ITI failures; all 4 were being treated with bypassing therapy for bleeding. Nevertheless, all 4 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 longstanding 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-randomized trial which was developed as a pilot study, due to the difficulty in conducting large scale randomized 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.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

1. Wight J, Paisley S. The epidemiology of inhibitors in haemophilia A: a systematic review. Haemophilia. 2003; 9:418–35. [PubMed: 12828678]
2. 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; 368:231–9. [PubMed: 23323899]
3. Hay CR, Ludlam CA, Colvin BT, et al. Factor VIII inhibitors in mild and moderate-severity haemophilia A. UK Haemophilia Centre Directors Organisation. Thromb Haemost. 1998; 79:762–6. [PubMed: 9569189]
4. Ewenstein BM, Gomperts ED, Pearson S, et al. Inhibitor development in patients receiving recombinant factor VIII (Recombinate rAHF/Bioclate): a prospective pharmacovigilance study. Haemophilia. 2004; 10:491–8. [PubMed: 15357776]
5. Gringeri A, Mantovani LG, Scalone L, et al. Cost of care and quality of life for patients with hemophilia complicated by inhibitors: the COCIS Study Group. Blood. 2003; 102:2358–63. [PubMed: 12816859]
6. Morfini M, Haya S, Tagariello G, et al. European study on orthopaedic status of haemophilia patients with inhibitors. Haemophilia. 2007; 13:606–612. [PubMed: 17880451]
7. Soucie JM, Cianfrini C, Janco RL, et al. Joint range-of-motion limitations among young males with hemophilia: prevalence and risk factors. Blood. 2004; 103:2467–73. [PubMed: 14615381]
8. Hay CRM, DiMichele DM. The principal results of the International Immune Tolerance Study: a randomized dose comparison. Blood. 2012; 119:1335–1344. [PubMed: 22101900]
9. Mariani G, Kroner B. International immune tolerance registry, 1997 update. Vox Sang. 1999; 77(Suppl 1):25–7. [PubMed: 10529682]
10. DiMichele DM, Kroner BL, The North American Immune Tolerance Study Group. The North American Immune Tolerance Registry: practices, outcomes, outcome predictors. Thromb Haemost. 2002; 87:52–7. [PubMed: 11848456]
11. Lenk H. The German Registry of immune tolerance treatment in hemophilia–1999 update. Haematologica. 2000; 85(10 Suppl):45–7. [PubMed: 11187870]
12. Haya S, López MF, Aznar JA, et al. Immune tolerance treatment in haemophilia patients with inhibitors: the Spanish Registry. Haemophilia. 2001; 7:154–9. [PubMed: 11260274]
13. McLaughlin P, Grillo-López AJ, Link BK, et al. Rituximab chimeric anti-CD20 monoclonal antibody therapy for relapsed indolent lymphoma: half of patients respond to a four doses treatment program. J Clin Oncol. 1998; 16:2825–33. [PubMed: 9704735]

14. Barcellini W, Zaja F, Zaninoni A, et al. Low-dose rituximab in adult patients with idiopathic autoimmune hemolytic anemia: clinical efficacy and biologic studies. Blood. 2012; 119:3691–7. [PubMed: 22267606]
15. Froissart A, Buffet M, Veyradier A, et al. Efficacy and safety of first-line rituximab in severe, acquired thrombotic thrombocytopenic purpura with a suboptimal response to plasma exchange. Experience of the French Thrombotic Microangiopathies Reference Center. Crit Care Med. 2012; 40:104–11. [PubMed: 21926591]
16. Stasi R, Del Poeta G, Stipa E, et al. Response to B-cell depleting therapy with rituximab reverts the abnormalities of T-cell subsets in patients with idiopathic thrombocytopenic purpura. Blood. 2007; 110:2924–30. [PubMed: 17548576]
17. Ahmed AR, Spigelman Z, Cavacini LA, et al. Treatment of pemphigus vulgaris with rituximab and intravenous immune globulin. N Engl J Med. 2006; 355:1772–9. [PubMed: 17065638]
18. Smith KG, Jones RB, Burns SM, et al. Long-term comparison of rituximab treatment for refractory systemic lupus erythematosus and vasculitis: Remission, relapse, and re-treatment. Arthritis Rheum. 2006; 54:2970–82. [PubMed: 16947528]
19. Franchini M. Rituximab in the treatment of adult acquired hemophilia A: a systematic review. Crit Rev Oncol Hematol. 2007; 63:47–52. [PubMed: 17236786]
20. Huth-Kühne A, Baudo F, Collins P, et al. International recommendations on the diagnosis and treatment of patients with acquired hemophilia A. Haematologica. 2009; 94:566–75. [PubMed: 19336751]
21. Boles JC, Key NS, Kasthuri R, et al. Single-center experience with rituximab as first-line immunosuppression for acquired hemophilia. J Thromb Haemost. 2011; 9:1429–31. [PubMed: 21575131]
22. Collins P, Baudo F, Knoebl P, et al. Immunosuppression for acquired hemophilia A: results from the European Acquired Haemophilia Registry (EACH2). Blood. 2012; 120:47–55. [PubMed: 22517903]
23. Linde R, Escuriola Ettinghausen C, Voigt B, et al. First successful inhibitor elimination with a new protocol in a high responding hemophilia A – patient after failure of various immune tolerance induction regimens. Blood. 2001; 98:534a.
24. Medeiros BC, Geraghty S, Stabler SP. Improved response to immune tolerance therapy for factor VIII inhibitor after rituximab therapy. Blood. 2002; 100:103b.
25. Pruthi RK, Schmidt KA, Slaby JA, et al. Rituximab in treatment of high titer FVIII inhibitor in congenital hemophilia A. Blood. 2002; 100:104b.
26. Fox RA, Neufeld EJ, Bennett CM. Rituximab for adolescents with haemophilia and high titre inhibitors. Haemophilia. 2006; 12:218–22. [PubMed: 16643204]
27. Mateo J, Badell I, Forner R, et al. Successful suppression using Rituximab of a factor VIII inhibitor in a boy with severe congenital haemophilia: an example of a significant decrease of treatment costs. Thromb Haemost. 2006; 95:386–7. [PubMed: 16493502]
28. Moschovi M, Aronis S, Trimis G, et al. Rituximab in the treatment of high responding inhibitors in severe haemophilia A. Haemophilia. 2006; 12:95–9. [PubMed: 16409183]
29. Carcao M, St Louis J, Poon MC, et al. Rituximab for congenital haemophiliacs with inhibitors: a Canadian experience. Haemophilia. 2006; 12:7–18. [PubMed: 16409170]
30. Mathias M, Khair K, Hann I, et al. Rituximab in the treatment of alloimmune factor VIII and IX antibodies in two children with severe haemophilia. Br J Haematol. 2004; 125:366–8. [PubMed: 15086418]
31. Collins P, Mathias M, Hanley J, et al. Rituximab and immune tolerance in severe hemophilia A: a consecutive national cohort. J Thromb Haemost. 2009; 7:787–94. [PubMed: 19320828]
32. Franchini M, Mengoli C, Lippi G, et al. Immune tolerance with rituximab in congenital haemophilia with inhibitors: a systematic literature review based on individual patients' analysis. Haemophilia. 2008; 14:903–12. [PubMed: 18671801]
33. Chuansumrit A, Husapadol S, Wongwerawattanakoon P, et al. Rituximab as an adjuvant therapy to immune tolerance in a haemophilia A boy with high inhibitor titre. Haemophilia. 2007; 13:108–10. [PubMed: 17212736]

34. Chang H, Sher GD, Blanchette VS, et al. The impact of inhibitors on the cost of clotting factor replacement therapy in Haemophilia A in Canada. Haemophilia. 1999; 5:247–52. [PubMed: 10469178]

35. Goudemand J. Pharmaco-economic aspects of inhibitor treatment. Eur J Haematol Suppl. 1998; 63:24–7. [PubMed: 9882929]

36. Globe DR, Cunningham WE, Andersen R, et al. The Hemophilia Utilization Group Study (HUGS): determinants of costs of care in persons with haemophilia A. Haemophilia. 2003; 9:325–31. [PubMed: 12694525]

37. Tony HP, Burmester G, Schulze-Koops H, et al. Safety and clinical outcomes of rituximab therapy in patients with different autoimmune diseases: experience from a national registry (GRAID). Arthritis Res Ther. 2011; 13:R75. [PubMed: 21569519]

38. Gea-Banacloche JC. Rituximab-associated infections. Semin Hematol. 2010; 47:187–9. [PubMed: 20350666]

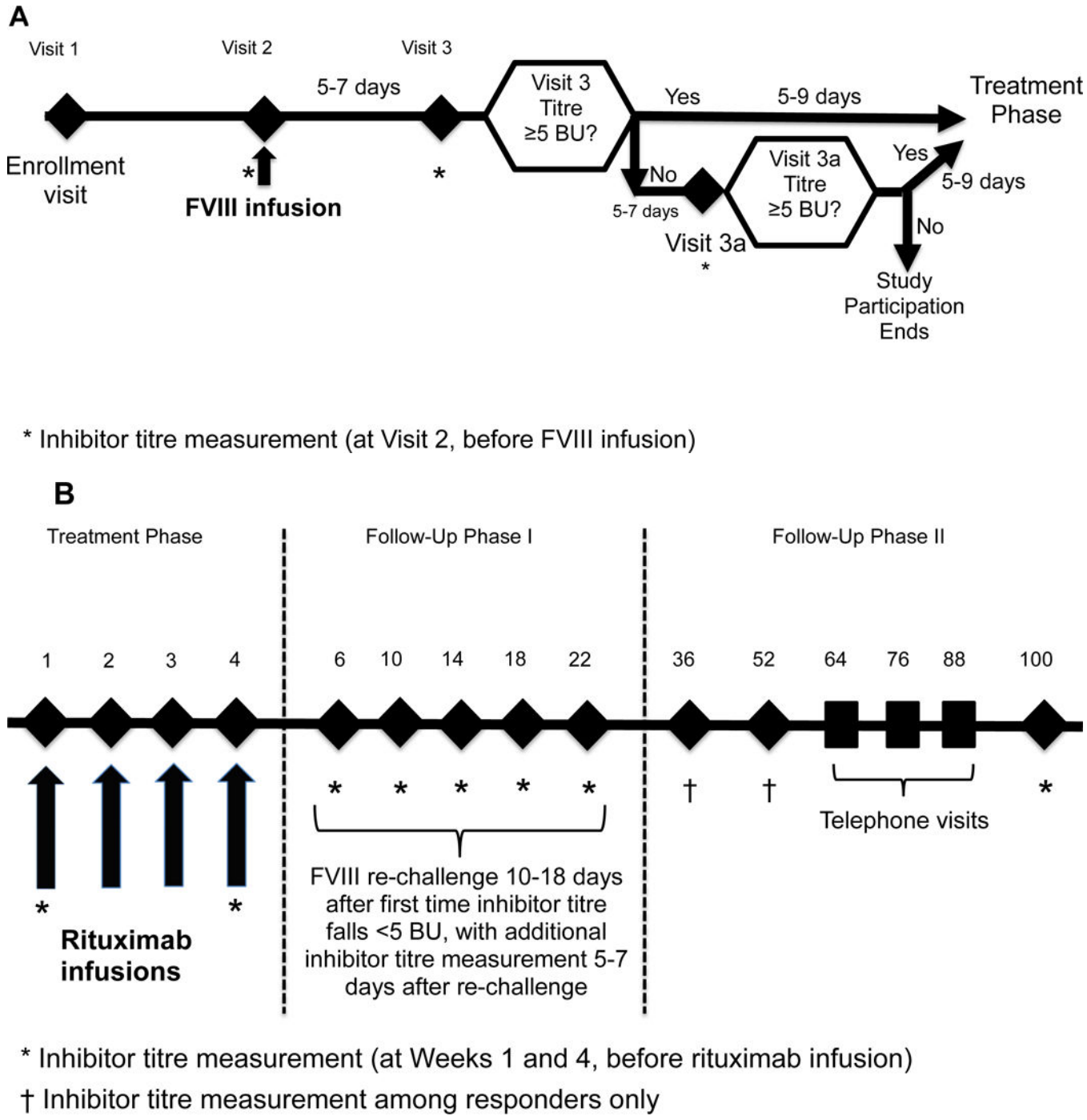


Figure 1. RICH Study Schema

A) Screening phase, B) Treatment and follow-up phase (by week on study following screening phase).

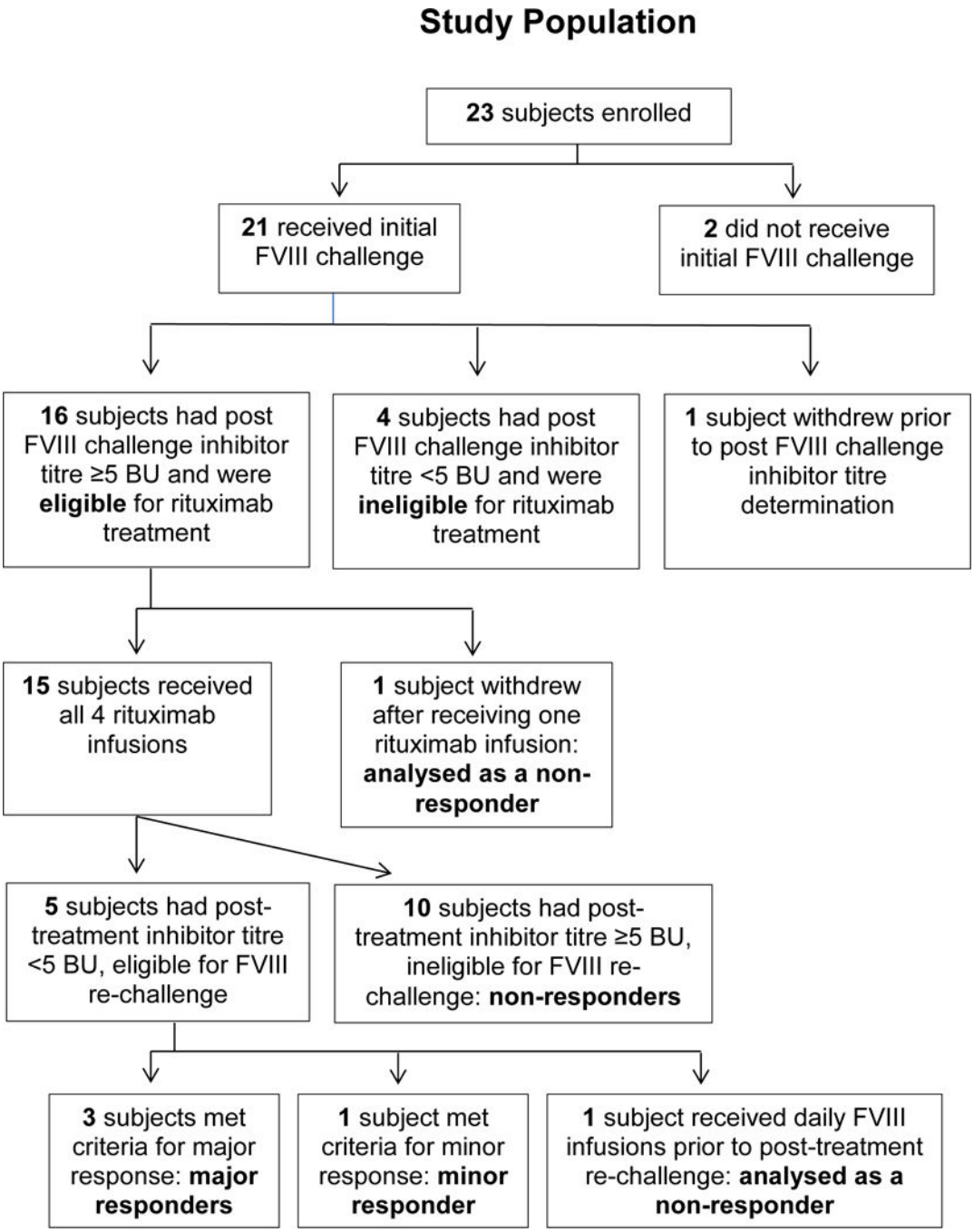
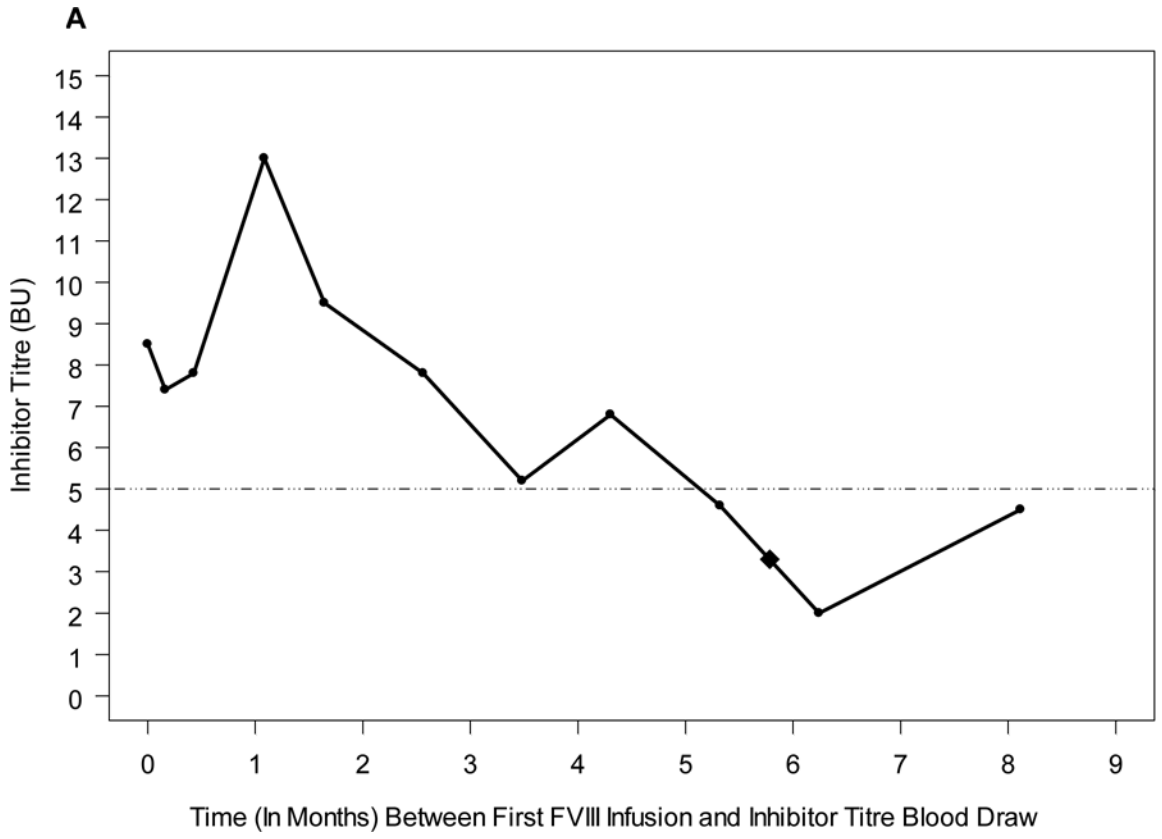
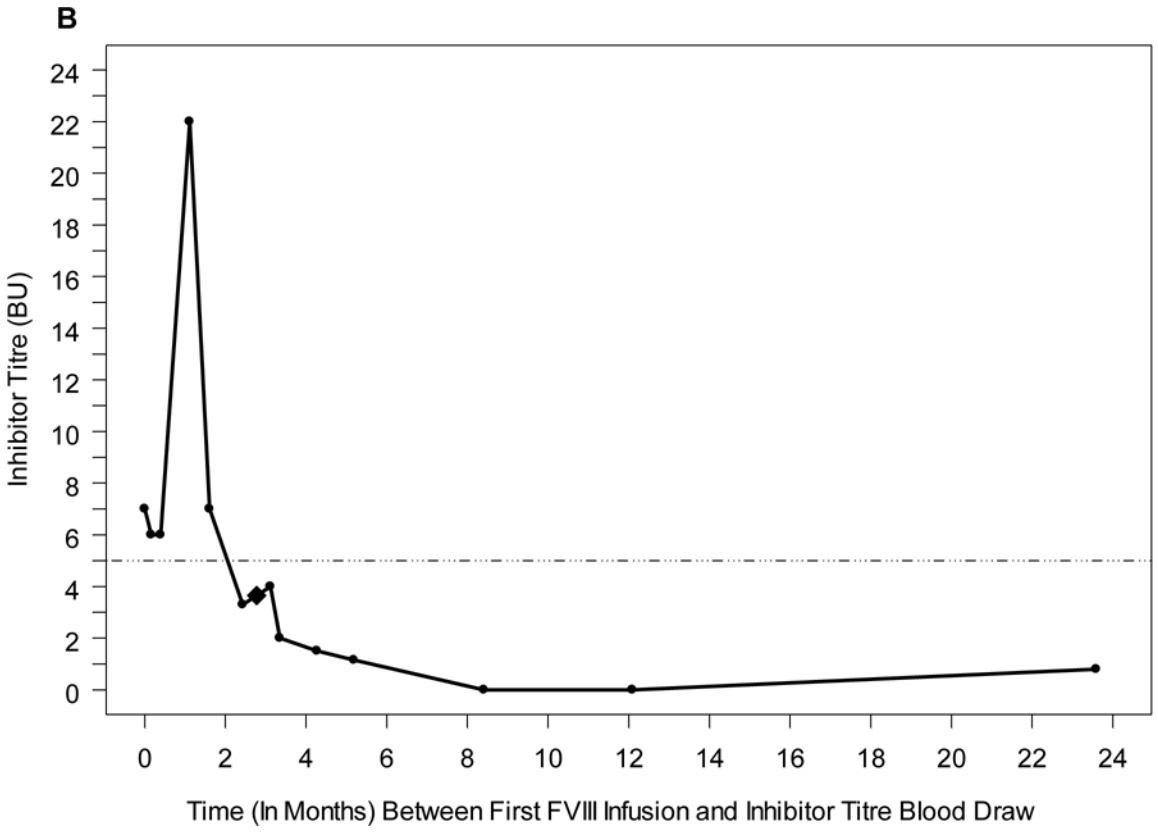
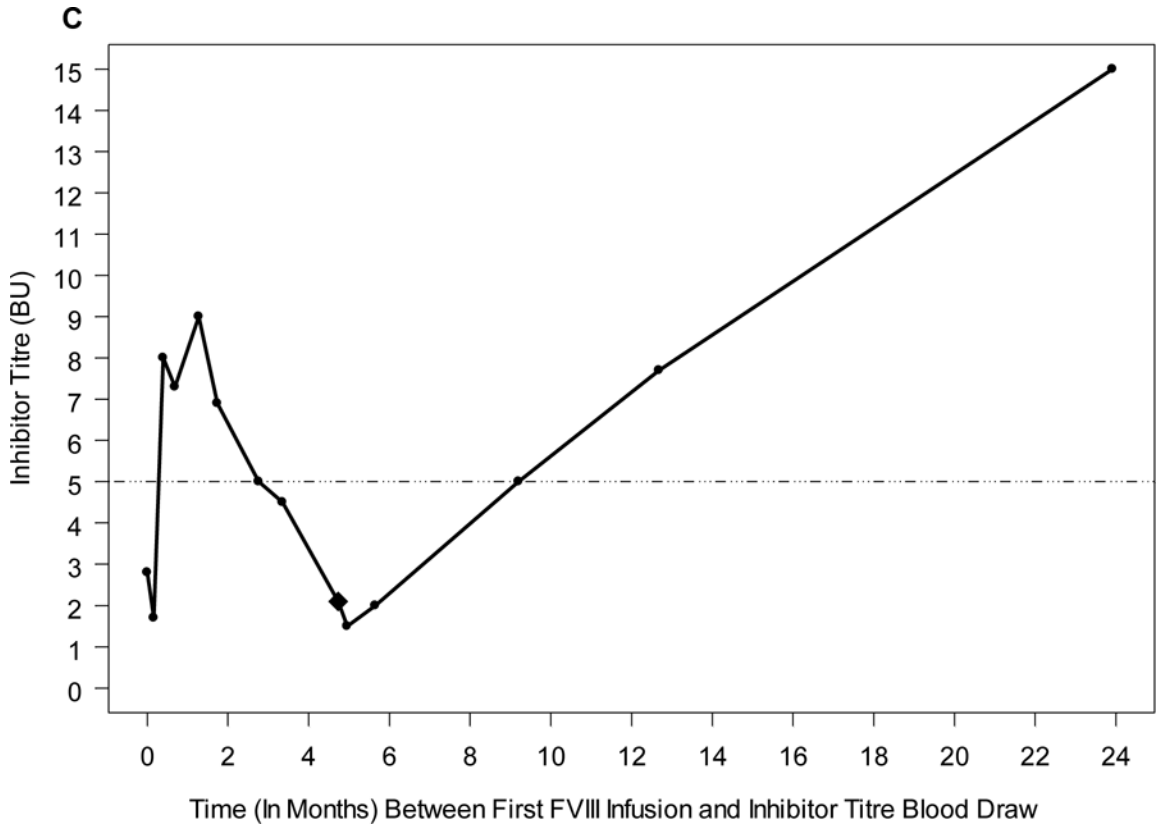


Figure 2. RICH Study Population







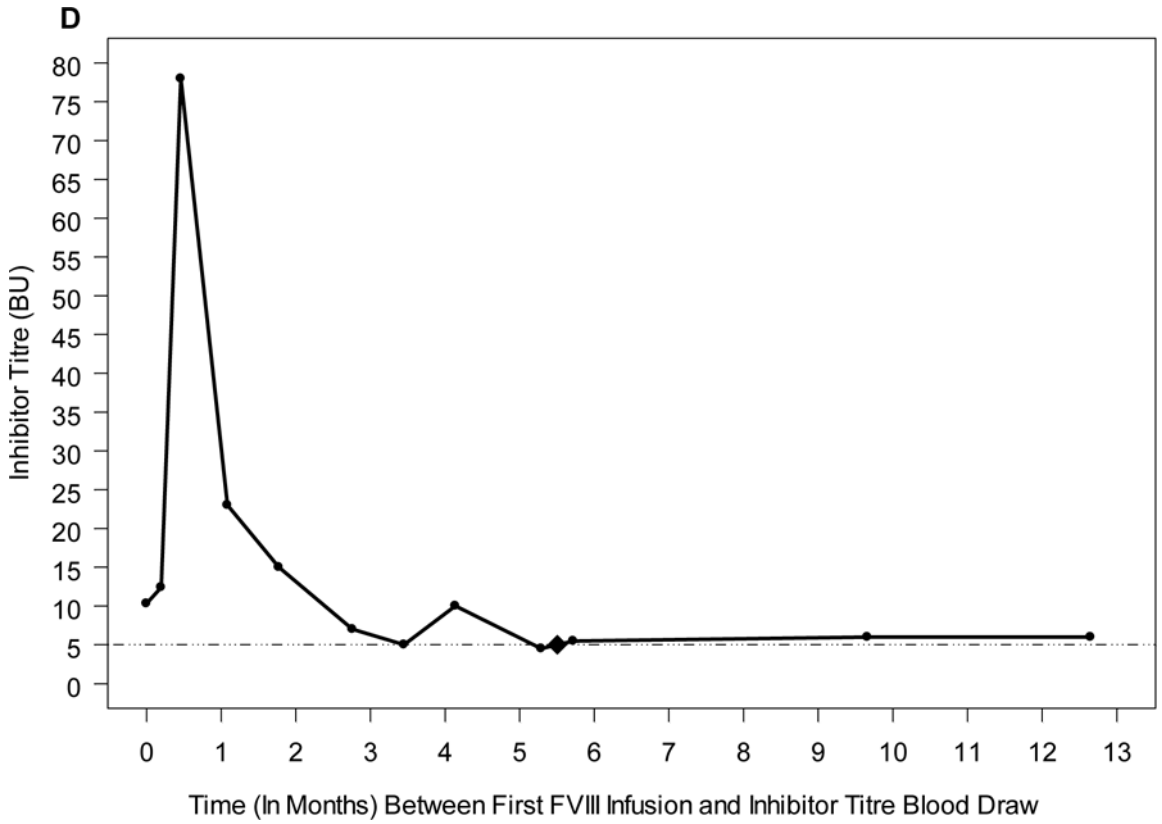


Figure 3. Inhibitor titre results among subjects who responded, by subject

Solid diamond represents date of FVIII re-challenge. A) major responder 1, B) major responder 2, C) major responder 3, D) minor responder 1.

Table 1

Comparison of Selected Variables Between Subjects Who Qualified For Rituximab Treatment and Those Who Did Not Qualify For Rituximab Treatment

	Qualifiers N = 16	Non-Qualifiers N = 4	
Baseline Demographic/Laboratory Test	**Median (Range)**	**Median (Range)**	**P value**
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)[a]	1.5 (0.0 – 43.0)	7.0 (7.0 – 7.0)	0.34
AGE INHIBITOR FIRST DETECTED (years)[b]	2.3 (0.3 – 11.0)	0.9 (0.5 – 1.4)	0.20
DURATION OF INHIBITOR (years)[b]	10.3 (0.6 – 34.4)	14.6 (11.6 – 17.6)	0.37
HISTORICAL PEAK INHIBITOR TITRE (BU)[c]	235.0 (33.3 – 4096.0)	115.0 (55.0 – 288.0)	0.35
HISTORY OF IMMUNE TOLERANCE INDUCTION[c]	13 (81%)	2 (67%)	0.53
• DURATION OF IMMUNE TOLERANCE INDUCTION (years)[d]	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/μL)	6.5 (3.3 – 11.1)	5.7 (4.6 – 5.9)	0.16
ABSOLUTE NEUTROPHIL COUNT (10^3/μL)[e]	4.1 (1.1 – 8.8)	2.4 (1.5 – 3.0)	0.18
ABSOLUTE LYMPHOCYTE COUNT (10^3/μL)	2.1 (1.1 – 4.2)	2.2 (2.0 – 2.6)	0.61
• CD3 (10^3/mL)	1407 (661 – 3221)	1602 (1436 – 1706)	0.61
• CD4 (10^3/mL)	916 (410 – 1915)	965 (754 – 1056)	0.74
• CD8 (10^3/mL)	539 (182 – 1059)	496 (366 – 551)	0.64
• CD19 (10^3/mL) [f]	353 (64 – 748)	457 (412 – 963)	0.27
• CD20 (10^3/mL)[f]	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)[g]	12.2 (5.5 – 1380.0)	2.0 (1.8 – 2.5)	N/A

[a] Data missing for 1qualifier. Data unknown for 5 qualifiers and 2 non-qualifiers

[b] Data unknown for 2 non-qualifiers

[c] Data unknown for 1 non-qualifier

[d] Among those with known history of ITI. Data unknown for 2 qualifiers and 1 non-qualifier. Data missing for 1 qualifier.

[e] Additional lab test required by the study beginning April 6, 2007. Not required for 5 qualifiers and 1 non-qualifier

[f] Data missing for one qualifier.

[g] 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.

Table 2

Changes In Inhibitor Titre Among Responders Who Were Rechallenged[1]

SUBJECTS WITH MAJOR OR MINOR RESPONSE	TYPE OF RESPONSE	PEAK INHIBITOR TITRE AFTER BASELINE CHALLENGE	INHIBITOR TITRE AFTER POST-TREATMENT CHALLENGE	CHANGE IN BU[3]	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%

[1] Subjects with BU ≤5 during Follow-up Phase I

[2] 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

[3] (BU after post-treatment Factor VIII challenge) - (BU after baseline Factor VIII challenge)

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

	Responders N = 4	Non-responders N = 12	
Baseline Demographic/Laboratory Test	**Median (Range)**	**Median (Range)**	**P value**
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 • WHITE	2 (50%) 2 (50%)	1 (8%) 11 (92%)	0.14
AGE AT FIRST EXPOSURE TO FVIII (months)[a]	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	4 (100%)	9 (75%)	0.53
• DURATION OF IMMUNE TOLERANCE INDUCTION (years)[b]	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 (10^3/μL)	6.6 (3.3 – 7.0)	6.5 (3.8 – 11.1)	0.68
ABSOLUTE NEUTROPHIL COUNT (10^3/μL)[c]	3.1 (1.1 – 5.2)	4.2 (1.4 – 8.8)	0.49
ABSOLUTE LYMPHOCYTE COUNT (10^3/μL)	1.8 (1.1 – 2.8)	2.1 (1.6 – 4.2)	0.38
• CD3 (10^3/mL)	1068 (661 – 1734)	1514 (949 – 3221)	0.15
• CD4 (10^3/mL)	555 (410 – 929)	985 (432 – 1915)	0.08
• CD8 (10^3/mL)	456 (182 – 565)	563 (347 – 1059)	0.32
• CD19 (10^3/mL)[d]	446 (299 – 620)	332 (64 –– 748)	0.48
• CD20 (10^3/mL)[d]	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)[e]	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 (10^3/mL) BETWEEN VISITS 7 AND 12 (INCLUSIVE)[f]	0.000 (0.000, 0.000)	0.001 (0.000, 0.069)	0.11

[a] Data unknown for 2 responders and 3 non-responders, missing for 1 non-responder

[b] Among those with known history of ITI. Data unknown for 2 responders. Data missing for 1 non-responder.

[c] Additional lab test required by the study beginning April 6, 2007. Not required for 1 responder and 4 non-responders

[d] Data missing for one non-responder

[e] Computed as the maximum inhibitor titre obtained at Visit 3 or 3A.

[f] 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 47 serious adverse events (SAEs) and 527 non-SAE adverse events * occurring during the RICH study, among the 16 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)
• Hematomas	7 (15)	5 (31)	105 (20)	14 (88)	112 (20)	14 (88)
• Subdural hematoma	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)**

	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
• 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)
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)

	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
• 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 3 bleeding events with onset before date of consent but ongoing at baseline