

The B-Natural study—The outcome of immune tolerance induction therapy in patients with severe haemophilia B

Jan Astermark^{1,2}  | Katharina Holstein³ | Yasmina L. Abajas⁴ | Susan Kearney⁵ |
 Stacy E. Croteau⁶  | Riana Liesner⁷  | Eva Funding⁸ | Christine L. Kempton⁹  |
 Suchitra Acharya¹⁰ | Stefan Lethagen¹¹ | Petra LeBeau¹² | Joel Bowen¹³ |
 Erik Berntorp¹⁴  | Amy D. Shapiro¹³ 

¹ Department of Translational Medicine, Lund University, Malmö, Sweden

² Department of Haematology, Oncology and Radiation Physics, Skåne University Hospital, Malmö, Sweden

³ Medical Department, Hemophilia Center, University Medical Center Hamburg-Eppendorf, Hamburg, Germany

⁴ Hemophilia and Thrombosis Centre, University of North Carolina, Chapel Hill, North Carolina, USA

⁵ Children's Minnesota Centre for Bleeding and Clotting Disorders, Minneapolis, Minnesota, USA

⁶ Boston Children's Hospital, Boston Haemophilia Centre, Boston, Massachusetts, USA

⁷ Great Ormond Street Hospital for Children, NHS Trust supported by NIHR, GOSH, BRC, London, UK

⁸ Department of Haematology, Institute of Clinical Medicine, University Hospital Rigshospitalet University of Copenhagen, Copenhagen, Denmark

⁹ Emory University School of Medicine, Emory University, Atlanta, Georgia, USA

¹⁰ Northwell Haemostasis and Thrombosis Centre, New York, New York, USA

¹¹ Sobi, Stockholm, Sweden

¹² Rho, Inc., Durham, North Carolina, USA

¹³ Indiana Haemophilia and Thrombosis Centre, Indianapolis, Indiana, USA

¹⁴ Department of Translational Medicine, Clinical Coagulation Research, Lund University, Malmö, Sweden

Correspondence

Jan Astermark, Department of Translational Medicine, Lund University and Department of Haematology, Oncology and Radiation Physics, Skåne University Hospital, Malmö, Sweden.
 Email: Jan.astermark@med.lu.se

Abstract

Introduction: Inhibitors develop less frequently in haemophilia B (HB) than haemophilia A (HA). However, when present, the success of tolerization by immune tolerance induction (ITI) therapy is lower and the risk of complications higher.

Aim: To evaluate the use and outcome of ITI in patients with HB and inhibitors.

Methods: Subjects include singletons or siblings with a current/history of inhibitors enrolled in B-Natural—an observational study designed to increase understanding of clinical management of patients with HB. Patients were followed for 6 months and information on demographics, medical and social history, and treatment were recorded.

Results: Twenty-nine patients with severe HB and inhibitors were enrolled in 24 centres. Twenty-two underwent one or more courses of ITI with or without immune suppression. Eight patients (36.4%) were successfully tolerized after the first course of ITI.

One of these successes (12.5%) experienced allergic manifestations, whereas the corresponding number for the 10 treatment failures was five (50%). One of seven (14.2%) patients with large deletions and three of eight (37.5%) with nonsense mutations were tolerized at the first attempt, and all patients experiencing nephrosis either failed or were on-going. At study end, 11 (50%) were considered successfully tolerized after one or more ITI courses, three were unsuccessful, and eight were still undergoing treatment.

Conclusion: Our data underscore the possibilities and difficulties of achieving tolerization in patients with HB with inhibitors. The type of mutation and complications appear to correlate with ITI outcome, but more accurate definitions of successful ITI are warranted.

KEYWORDS

allergy, factor IX deficiency, haemophilia B, immune tolerance induction, inhibitors, nephrotic syndrome

1 | INTRODUCTION

Haemophilia B (HB) is a congenital bleeding disorder characterized by a deficiency of coagulation factor IX (FIX).¹ The phenotype and clinical management of this disease will in many ways be similar to that of factor VIII (FVIII) deficiency (haemophilia A [HA]). However, significant differences exist including the lower risk of developing neutralizing antibodies (inhibitors) towards the infused exogenous FIX than FVIII.² Inhibitors towards FIX are primarily seen in the severe subgroup of patients with large gene deletions and nonsense mutations in the *F9* gene. Moreover, the FIX antibodies appear to be more difficult to eradicate by immune tolerance induction (ITI) therapy. All available estimates of ITI success in HB are based on small cohorts and case series, but the reported success rates are with few exceptions approximately 50% or less.^{4,5} Further, FIX inhibitors are more often associated with allergic reactions and nephrotic syndrome, major concerns for both patients and health care providers.⁵ In fact, the prospect of these unwelcome occurrences explains, in part, why many patients with FIX inhibitors will never be offered ITI therapy, thereby foregoing an opportunity for tolerization and the ability to use prophylaxis for optimal bleed prevention.^{6,7} The underlying mechanism(s) of the allergic reactions and nephrotic syndrome is not well understood, but complete gene deletions and, to a lesser degree, partial deletions and nonsense mutations seem to significantly increase this risk.⁸⁻¹¹

The B-Natural study cohort was established to shed more light on the disease and optimal clinical management of patients with HB, with or without antibodies.¹² In the current paper, the use and outcome of ITI, including complications, is described and discussed. Our observations underscore the impact of the causative mutation on ITI outcome, but also the unpredictability and difficulties, as well as possibilities for the clinical management of patients with HB and inhibitors.

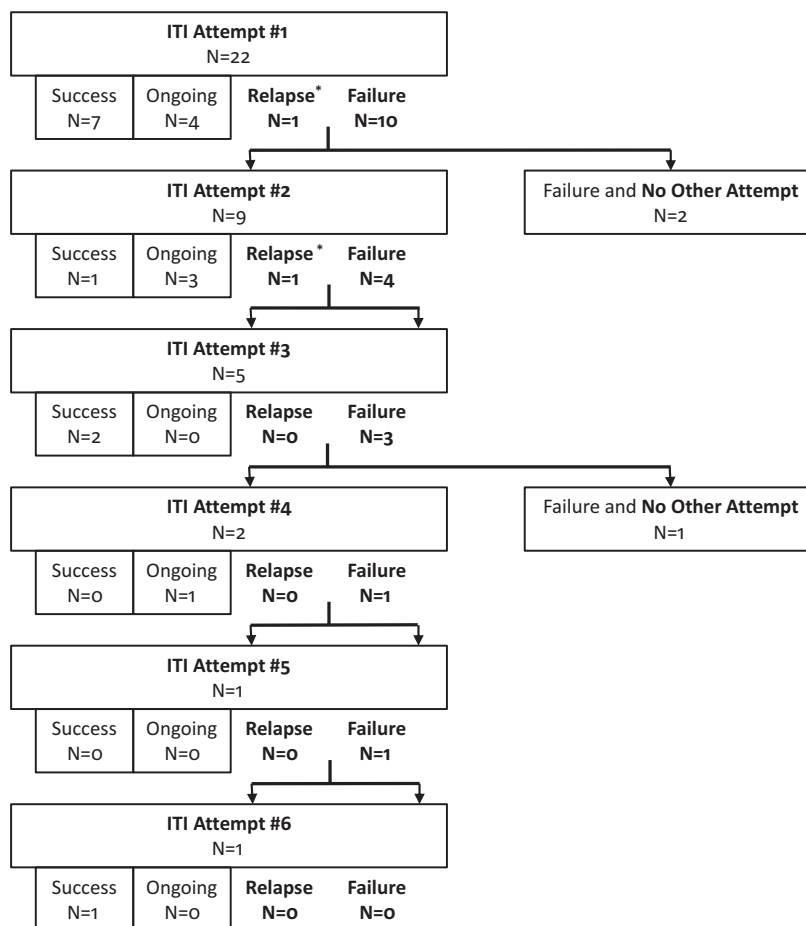
2 | METHODS

2.1 | Design and eligibility

B-Natural is a multi-centre observational study including both retro- and prospectively collected data as recently described.¹² In summary, subjects were eligible to participate if they had FIX deficiency, any level of severity, and were part of an affected sibling pair/group; and/or had a current or history of inhibitor, defined as >0.6 Bethesda units (BU) determined by the local laboratory. Participants were followed for at least 6 months and factors related to early treatment and product use were recorded. Medical and family history was obtained including information related to allergies and renal disease. Allergic symptoms included wheezing in the chest when breathing, sneezing or a running, blocked or itchy nose not caused by the flu, atopic dermatitis or eczema, hay fever, or asthma/bronchitis with no other cause. Renal disease included glomerulonephritis, tubulointerstitial nephritis, nephrosis or any other specified renal disease. During ITI, allergic reactions and nephrosis were not further defined, but defined and diagnosed at the discretion of the investigator. Standard methods for determination of the *F9* gene mutation were used.¹³

If the participant had a history of an inhibitor, additional information including characteristics of the inhibitor, high- (>5 BU) versus low-responding (≤5 BU), was recorded. Data were collected to describe the use of ITI, the outcome of each attempt, date and age at initiation, peak titre prior to and titre at start of ITI, type of concentrate used, dose and dose interval of the most intense treatment, use of immunomodulating agent(s), ITI end date, peak titre during ITI, whether the course of ITI was considered successful, and if yes, how success was measured. Any relapse was recorded, as well as the occurrence of allergic reaction or nephrosis during ITI.

FIGURE 1 Flow diagram showing the number of subjects per immune tolerance induction (ITI) attempt and the outcome



The procedures followed were approved by the ethical committees in each participating centre. B-Natural is registered at ClinicalTrials.gov (NCT02502409).

successfully tolerized after 15 to 805 days (Figure 1). No hospitalizations for central venous line infections and/or thrombotic events were reported.

3 | RESULTS

3.1 | Characteristics and general outcome

Two hundred and 24 individuals ranging in age from 1 to 73 from 107 families from 24 centres were enrolled. The median age at enrolment was 16.5 years. Of the 224 participants, 29 had a current or history of an inhibitor and had severe disease (FIX activity <1%): 13 had peak titres ≤ 5 BU, 13 had peak titres >5 BU, and for three the peak titre was unknown. The most frequent causative *F9* gene mutations were large deletions, identified in seven participants, and nonsense mutations in eight.

At study start, eight patients with inhibitors had never been exposed to ITI. The reason(s) for this included: risk of allergic reactions and/or nephrosis (N = 3), low-titre with no indication for ITI according to the investigator (N = 1), starting phase of ITI, that is, not yet initiated (N = 1), no reason provided (N = 3). Twenty-two underwent one or more courses of ITI prior to enrolment (N = 21) or during follow-up (N = 1) (Table 1); two of these subjects were siblings from the same family (subjects 16 and 22). At the study end, 11 were considered

3.2 | First ITI attempt

The outcome of the first ITI attempt in all study subjects including the ongoing procedures at study end stratified by the treatment provided are summarized in Table 1 and Figures 2 and 3. Eight patients (36.3%) were considered successfully tolerized after the first attempt defined by a negative titre and/or normal response to FIX therapy and in two patients also normal recovery and half-life. Fourteen patients either failed the attempt (n = 10) or were on-going since 52 to 1020 days (n = 4) at study end. One of the successes had a later relapse with a measurable titre despite having a negative titre, normal recovery and half-life. The number of successfully treated patients by the type of underlying *F9* gene mutations included 3 of 8 (37.5%) nonsense mutations, 1 of 7 (14.2%) large deletion, 1 of 3 (33.3%) small deletion with frame shift, the one patient with a complex mutation consisting of both a small deletion and insertion, and both patients with missense mutations.

The peak titres prior to start of ITI in the eight patients tolerized were between 0.7 and 1380 BU and, at start of ITI, ranged from <0.4 to 4.4 BU (Table 1). Four of these patients were tolerized in less than

**TABLE 1** Subject level details for ITI attempt 1

Patient ID	Age at inhibitor (years) ^a	Age at ITI Start (years) ^a	Mutation type and location (HGVS_cDNA)	Peak titer prior to ITI Start ^a	Titer at ITI start ^a	Type of FIX concentrate (Brand) ^b	FIX dosing (units/kg + interval) ^c	Immunosuppression	Allergic symptoms	Nephrotic syndrome	Successful (time in days to outcome) ^a	Age at end of study (years)
1	1	18	Large deletion (c.-29-?_1386+?del)	0.9	0.4	Recombinant SHL (Benefix)	90 BID	Rituximab, Mycophenolate mofetil,	Yes	No	Yes (805)	24
2	52	52	Missense (c.84T > G)	5.0	1.8	Recombinant SHL (Benefix)	50 Daily		No	No	Yes (607)	59
3	1	1	Missense (c.423C > G)	0.7	0.7	Recombinant SHL (Benefix)	83 Daily		No	No	Yes (53)	10
4	26	53	Nonsense (C.719G > A)	1380	0.6	Pd Monoclonal Antibody-purified (Mononine)	103 Daily	Cyclophosphamide	No	No	Yes (39)	74
5	29	39	Nonsense (c.880C > T)	MD	MD	Non-monoclonal Antibody (Preonativ)	36 TID	Cyclophosphamide, IVIG	No	No	Yes (15)	74
6	3	10	Nonsense (c.880C > T)	5.1	<0.4	Recombinant SHL (Benefix)	109 Every other day		No	No	Yes (43)	13
7	1	1	Small deletion (c.571delC)	4.4	4.4	Recombinant SHL (Benefix)	100 Daily	Cyclophosphamide, IVIG, Hydrocortisone	No	No	Yes (375)	7
8	5	11	Complex (c.142_147delinsG)	6.2	<0.4	Pd Non-monoclonal Antibody-purified (Immunine)	200 Daily	Rituximab, Mycophenolate mofetil, IVIG, Dexamethasone	No	No	Yes, then relapsed (421)	24
9	4	10	Small deletion/Frameshift (c.969_975delACCCTTA)	10.0	0.2	Pd Non-monoclonal Antibody-purified (Preonativ)	Missing		No	No	No (24)	45
10	MD	13	Large deletion (c.89-?-723+?del)	10.0	9.0	Recombinant SHL (Benefix)	140 Daily	IVIG, Cyclophosphamide	No	Yes	No (1207)	31

(Continues)



TABLE 1 (Continued)

Patient ID	Age at inhibitor (years) ^a	Age at ITI Start (years) ^a	Mutation type and location (HGVS_cDNA)	Peak titer prior to ITI Start ^a	Titer at ITI start ^a	Type of FIX concentrate (Brand) ^b	FIX dosing (units/kg + interval) ^c	Immunosuppression	Allergic symptoms	Nephrotic syndrome	Successful (time in days to outcome) ^a	Age at end of study (years)
11	1	1	Large deletion (c.-29-?_1386+?del)	8.0	2.0	Recombinant SHL (Benefix)	100 QID	Rituximab, Mycophenolate mofetil, IVIG, Dexamethasone	No	No	No (73)	6
12	<1	<1	Large deletion (c.-29-?_1386+?del)	0.7	<0.4	Recombinant SHL (Benefix)	100 Daily	Cyclophosphamide, Hydrocortisone	No	No	No (42)	11
13	MD	6	Large deletion (c.-29-?_1386+?del)	160	MD	Pd Monoclonal Antibody-purified (AlphaNine) + Recombinant SHL (Benefix)	200 Daily	Cyclosporine, Steroids,	Yes	Yes	No (MD)	32
14	<1	1	Nonsense (c.967G > T)	1.0	<0.4	Recombinant SHL (Benefix)	100 BID	Rituximab, Mycophenolate mofetil IVIG, Dexamethasone	Yes	No	No (252)	3
15	1	1	Nonsense (c.892C > T)	0.5	0.5	Recombinant SHL (Benefix)	Missing	Rituximab, Mycophenolate mofetil, IVIG, Dexamethasone	Yes	No	No (MD)	2
16	1	2	Nonsense (c.223C > T)	3.1	<0.4	Pd Non-monoclonal Antibody-purified (AlphaNine)	200 Daily	Rituximab, Mycophenolate mofetil	No	Yes	No (1216)	11
17	MD	MD	Nonsense (c.223C > T)	MD	MD	Pd Monoclonal Antibody-purified (Mononine)	200 Daily	Cyclophosphamide, Cyclosporine, Prednisolone	Yes	Yes	No (MD)	25
18	1	2	Splice-site mutation (c.967G > T)	3.0	<0.4	Recombinant SHL (Benefix)	120 Daily		Yes	No	No (60)	8

(Continues)



TABLE 1 (Continued)

Patient ID	Age at inhibitor (years) ^a	Age at ITI Start (years) ^a	Mutation type and location (HGVS_cDNA)	Peak titer prior to ITI Start ^a	Titer at ITI start ^a	Type of FIX concentrate (Brand) ^b	FIX dosing (units/kg + interval) ^c	Immunosuppression	Allergic symptoms	Nephrotic syndrome	Successful (time in days to outcome) ^a	Age at end of study (years)
19	1	1	Small deletion/Frameshift (c.196delG)	14.0	14.0	Recombinant SHL (Benefix)	40 Daily		No	No	Ongoing (867)	3
20	MD	MD	Large deletion (c.-29-?_1386+?del)	558	MD	Recombinant SHL (Benefix)	40 Daily	Sirolimus, Mycophenolate mofetil	No	Yes	Ongoing (MD)	23
21	<1	<1	Large deletion (c.-29-?_1386+?del)	0.6	0.6	Pd Monoclonal Antibody-purified (Mononine)	300 Daily	Rituximab	Yes	No	Ongoing (1020)	3
22	<1	2	Nonsense (c.223C>T)	27.6	<0.4	Pd Non-monoclonal Antibody-purified (AlphaNine)	100 BID	Rituximab	Yes	No	Ongoing (52)	2

^aMD: Missing data.^bPd: Plasma-derived, SHL: Standard half-life.^cBID: twice a day, TID: three times a day, QID: four times a day.

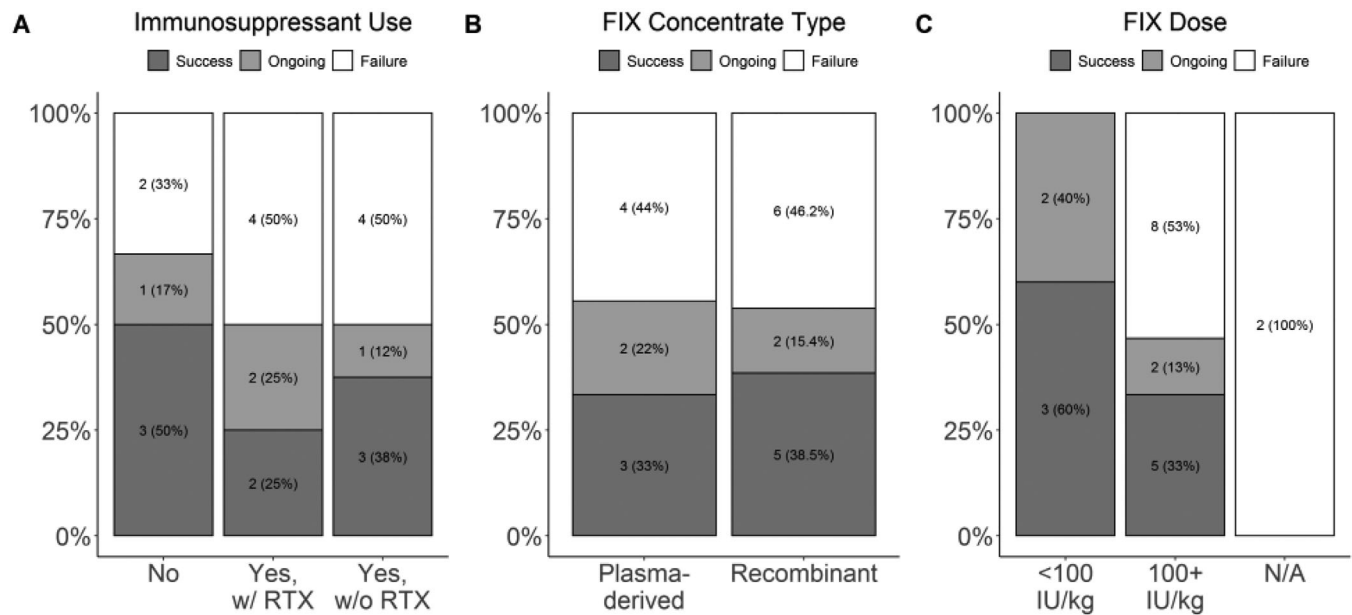


FIGURE 2 Summary of immune tolerance induction (ITI) attempt one showing success of tolerization grouped by the use of immunosuppressants (A), type of factor IX (FIX) concentrate (B) and FIX dosage (C). The numbers inside each of the categories of a bar indicate the number of subjects in that category as well as the associated percentage within the bar

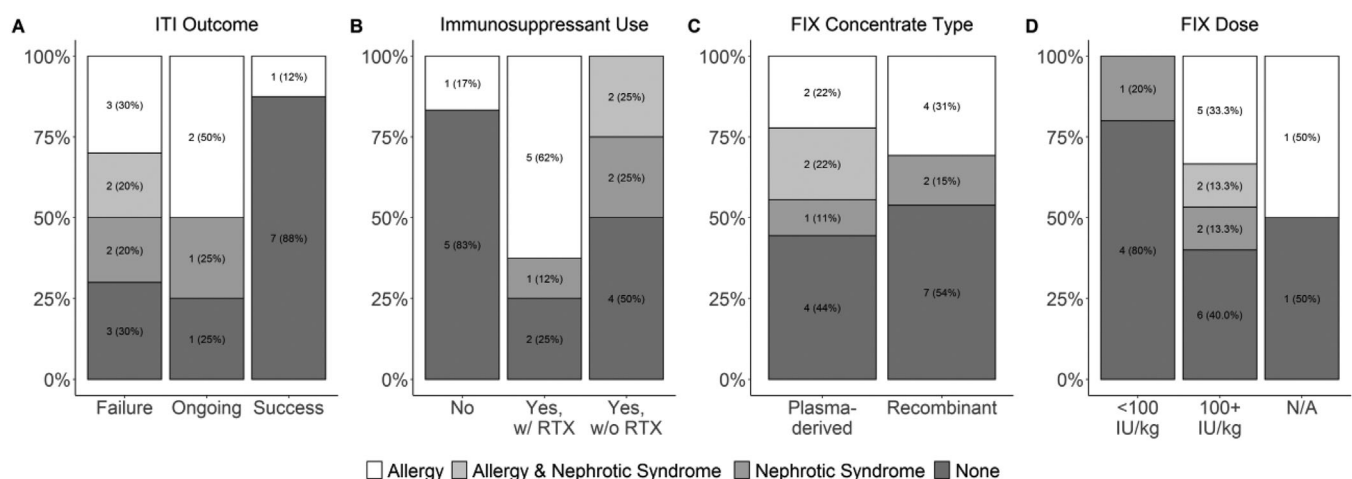


FIGURE 3 Summary of immune tolerance induction (ITI) attempt one showing success of tolerization (A), use of immunosuppressants (B), type of factor IX (FIX) concentrate (C) and FIX dosage (D) grouped by type of experienced complications during ITI attempt one. The numbers inside each of the categories of a bar indicate the number of subjects in that category as well as the associated percentage within the bar

2 months, whereas more than a year of ITI treatment were required in the others. The type of products used for these successes were, in five cases, recombinant standard half-life (rSHL) FIX (Benefix) in a daily dose of 50-180 IU/kg in all but one case (109 IU/kg every other day). Plasma-derived non-monoclonal products (Immunine and Preconativ) were used in two cases in a daily dose of 108-200 IU/kg, whereas in the remaining case, a monoclonal product (Mononine) was used in a dose of 103 IU/kg/d. Immunosuppression (IS) was used in five of these cases, two of whom were tolerized within a few weeks: cyclophosphamide (N = 3); rituximab (RTX) (N = 2); IVIG (N = 3); mycophenolate mofetil

(N = 2); steroids (N = 2). Interestingly, none of the patients with missense mutations and successful tolerization at first attempt received IS (Table 1). Only one patient with an underlying large deletion, experienced an allergic reaction and no cases of nephrotic syndrome were reported in the successfully tolerized cases (Figure 3). As noted above, one patient considered tolerant after achieving a negative titre and normal recovery and half-life had a relapse.

The 10 subjects who failed the first ITI attempt had peak titres prior to ITI of 0.5 to 160 BU and titres at onset of ITI ranging from <0.4 to 9.0 BU (Table 1). Seven patients were treated with rSHL FIX (Benefix),

TABLE 2 Subject level details for ITI attempt 2

Patient ID	Age at ITI Start (years)	Peak titer prior to ITI Start ^a	Titer at ITI Start	Type of FIX concentrate (<i>Brand</i>) ^b	FIX dosing (units/kg + interval) ^c	Immunosuppression	Allergic symptoms	Nephrotic syndrome	Successful (time in days to outcome)
17	22	MD	<0.4	Pd Non-monoclonal Antibody purified (<i>Haemonine</i>)	50 Daily	Rituximab, Prednisolone	No	No	Yes (311)
8	18	2.6	2.2	Pd Non-monoclonal Antibody purified (<i>Immunine</i>)	200 Daily	Rituximab, Mycophenolate mofetil, IVIG, Dexamethasone	No	No	Yes, then relapsed (216)
9	10	65.0	65.0	Pd Non-monoclonal Antibody purified (<i>Preconativ</i>)	150 Daily	IVIG, steroids	No	No	No (8)
11	3	4.0	<0.4	Pd Monoclonal Antibody purified (<i>Mononine</i>)	125 Daily	Rituximab, Mycophenolate mofetil, IVIG, Dexamethasone	Yes	Yes	No (48)
12	1	0.9	0.9	Recombinant SHL (<i>Benefix</i>)	100 Daily	Rituximab	No	No	No (243)
18	4	1.6	<0.4	Recombinant SHL (<i>Benefix</i>)	120 Daily		Yes	No	No (184)
13	30	160.0	<0.4	Recombinant SHL (<i>Benefix</i>)	200 Daily	Rituximab, Mycophenolate mofetil, IVIG, Dexamethasone	Yes	No	Ongoing (693)
14	2	3.0	2.0	Recombinant SHL (<i>Benefix</i>)	100 BID	Rituximab, Mycophenolate mofetil, IVIG, Dexamethasone	Yes	No	Ongoing (485)
15	2	4.0	2.0	Recombinant SHL (<i>Benefix</i>)	MD	Rituximab, Mycophenolate mofetil, IVIG, Dexamethasone	No	No	Ongoing (184)

^aMD: Missing data.
^bPd: Plasma-derived, SHL: Standard half-life.
^cBID: twice a day, MD: Missing data.

one with a plasma-derived non-monoclonal purified product (Alpha-Nine), and one with a monoclonal product (Mononine) (Figure 2). Type of concentrate was not provided for the remaining attempt. All patients with known treatment used 100-300 IU/kg daily. Eight patients also received IS with different agents and combinations. No immunosuppressive agent(s) were used without FIX for ITI. Rituximab (RTX) was used in four cases, steroids in six, mycophenolate mofetil/cyclosporine in six, IVIG in four and cyclophosphamide in three patients. As seen in Table 1 and Figure 3, of the 10 failures, three had no adverse reactions, three experienced allergic reactions, two nephrotic syndromes, and two had allergic reactions as well as nephrotic syndrome. No desensitization procedures were reported. Interestingly, both siblings carrying a nonsense mutation and not tolerized by the end of study follow-up, also experienced adverse events despite the use of RTX.

3.3 | Second ITI attempt

Nine of the 10 failures at the first ITI attempt had a second ITI treatment (see Table 2). Two patients carrying a nonsense or complex mutation were successfully treated according to the investigator: both had negative titre and normal response to FIX therapy, and one also normal recovery and half-life, however had a later relapsing low-titre inhibitor. Three patients had an on-going procedure for 184 to 693 days at study end. The titres at start of ITI were ≤ 2.2 BU in all but one failure case (65 BU). In five patients, all failures or on-going, recombinant FIX (Benefix) was used and plasma-derived agents were used in the remaining four, two of whom were successfully treated. All but one patient (classified as a treatment failure) also received IS. The two successful cases received 50 or 200 IU/kg daily. Four patients—all ITI failures or still under treatment—experienced allergic reactions during this

second attempt and one, with no reported adverse events during the first course despite high dose FIX and a similar immunosuppressive regimen, also suffered from a nephrotic syndrome (Table 2).

3.4 | Third ITI attempt

A third course of ITI was performed in five patients (data not shown). Two of these patients, that is, patient no. eight with a complex mutation and previous relapse and patient no. 11 carrying a large deletion, were considered successfully tolerized after this third attempt: both had a negative titre, and no. 11 also normal recovery and half-life. All known titres at start of ITI were low (≤ 1.6 BU). Four of the patients received plasma-derived products for this course including the two successes and one received rSHL FIX (Benefix). In all cases, the FIX was combined with IS. The successfully treated patients received a FIX dose of 100 IU/kg/d or 125 IU/kg twice daily and, in both cases, RTX and corticosteroids were used alone or in combination with IVIG, and other immunosuppressive agents (cyclophosphamide/mycophenolate mofetil). For two of the three failures, the FIX dosing was similar (100 IU/kg/d) and combined with RTX, either alone or in combination with mycophenolate mofetil. In the remaining case, the FIX dosing regimen using Preconativ was not recorded, but was combined with cyclophosphamide, IVIG and corticosteroids. One of the treatment failures carrying a large gene deletion experienced an allergy, for the first time, during this third ITI course.

3.5 | More than three ITI attempts

Two patients had more than three attempts as defined by a measurable inhibitor titre above cut off using plasma-derived FIX together with a combination of various immunosuppressive agents. One patient was finally considered tolerant after the sixth attempt using Preconativ (75 IU/kg/d) combined with cyclophosphamide, IVIG and corticosteroids. The success was defined by a negative titre and normal response to treatment.

4 | DISCUSSION

The B-Natural study cohort is the second largest published cohort of patients with HB and inhibitors exposed to ITI therapy and, at study end, 11 of our 22 (50%) patients were deemed successfully treated. This rate of success is higher than that of 31% in the North American IT Registry, and 16% in the ISTH-SSC International FIX Inhibitor Registry.^{5,14} In this context it is worth pointing out, that three of our patients were tolerized after more than one attempt indicating that tolerance may be achieved despite initial failures. Regarding the potential impact of the mutation type on the outcome, only 1 of 7 (14.2%) patients with large deletions and 3 of 8 (37.5%) with nonsense mutations were tolerized at the first attempt. Importantly, three patients with ongoing ITI courses at study end had large deletions. These cases

may be considered as having a poor prognosis based on the age of 23 years and an ongoing ITI therapy for almost 3 years without success. In comparison, both patients with missense mutations were tolerized after 53 and 605 days without relapse. In at least two of the three cases with small deletions, frame shift and subsequent stop codons were induced, which makes the evaluation of this mutation type difficult. Moreover, previous data have indicated an association between poor outcome of ITI and an allergic phenotype. In the North American registry, eight of 11 (73%) failures had adverse reactions including four registered as allergic reactions.¹⁴ In agreement with this notion, only one of the eight (12.5%) successes at the first attempt in our study experienced allergic manifestations, whereas the corresponding number for treatment failures was five of 10 (50%). In addition, all patients experiencing nephrosis, during the first attempt carried a large deletion or a nonsense mutation, and either failed or were still ongoing at the end of the study. Overall, nine of our patients (40.9%), all carrying large deletions, nonsense or splice site inducing a frame shift, experienced allergic reactions during ITI, which is a lower figure than that of 60% reported in the ISTH-SSC International FIX Inhibitor registry.⁵

Altogether, however, our data support the potential negative impact of the underlying mutation in terms of large deletions and/or nonsense on ITI outcome, in particular, when associated with allergy and nephrosis.

Several of our tolerized patients received a relatively high daily dose of FIX together with immune suppression in various combinations. This is consistent with literature reports of different immunosuppressive agents used successfully for ITI in HB.^{15–24} Among the options and combinations, the original Malmö protocol using cyclophosphamide, IVIG and corticosteroids and the more recent German approach using rituximab, mycophenolate mofetil, IVIG and corticosteroids has been of particular interest and focus.^{16,17,20} The addition of IS should at least be considered in patients with allergic manifestations and/or nephrotic syndrome. To avoid potential drawbacks of chemotoxic agents, the German protocol may be a first option to consider. Importantly, however, although just two cases, the two unrelated subjects with missense mutations involving cysteines became easily tolerant without IS suggesting that this, based on the underlying gene mutation, could initially be avoided. It is also interesting to point out that the only brother pair enrolled behaved similarly—both had side-effects and were not tolerized by the end of the study. Therefore, although not possible to conclude based on our limited dataset, this may suggest that inheritance is of relevance for outcome.

The titre at start of ITI therapy has been a debated prognostic factor for outcome in HA.^{25,26} In our HB cohort, all reported titres at start were relatively low. Therefore, the potential impact of the pre-ITI titre could not be evaluated. Whether this reflects treaters' concern about ITI and an initial wait-and-see approach and/or indicates a different natural course of the immune response toward FIX compared to FVIII remains to be elucidated.

With respect to the type of factor concentrate and dose, both plasma-derived and recombinant products were used with a similar outcome. Therefore, although the cases are few in number, our data do not suggest that the type of concentrate—as discussed for the



VWF-containing products in HA²⁷ – may be an issue. Importantly, however, the type of product available did not include extended half-life products, so we were not able to evaluate how these agents will perform in the ITI setting. More or less all patients in our cohort received daily treatment of FIX in relatively high doses (≥ 100 IU/kg). In one case, however, an every-other-day regimen was successfully used in combination with four doses of RTX, suggesting that lower doses could be considered in HB as well as for HA—at least in combination with immunosuppressive agents. Lower doses of FIX may be an attractive option as an initial regimen in patients with complications.

The retrospective design of our data collection has weaknesses with some missing data and not all treatment regimens were registered. The most intense treatment during each course of ITI was however captured, so some of our patients may actually have been treated with lower doses during treatment than that reported. We are also not able to specify when, and for what reason, changes to the treatment were made, and there may have been events during the course of ITI potentially affecting outcome. Another potential weakness may be the recruitment method: at each centre, only patients who were part of a sibling pair and/or had experienced an inhibitor were included, that is, singletons without inhibitor history were not eligible. However, based on the study objectives including the current analyses on ITI outcome, this should have no impact on the content and interpretation of data presented. Instead, the main weakness is the lack of well-defined criteria for success which resulted in inconsistencies among the treaters. Therefore, it is not clear that patients considered tolerant were actually fully tolerized according to specific pharmaco-kinetic criteria. Patients considered tolerant did, however, use regular replacement therapy without obvious concern and had no measurable inhibitor titre indicating at least partial success and a beneficial outcome of ITI.

In conclusion, the success rate of the initial ITI attempt in our patients with severe HB was approximately one-third, using primarily daily FIX concentrate combined with IS. Tolerance can be reached despite initial failure and complications, and this should be kept mind in the future management of patients. Although the study cohort is relatively small, the outcome appears not to be associated with the types of concentrate and/or specific immunosuppressive drugs. Large deletions and nonsense mutations with or without complications, for example, allergic reactions and nephrosis, seem to be poor prognostic factors for ITI outcome. There is clearly a need for further international collaboration to produce ITI guidelines and recommendations. This has been quite difficult to achieve,²⁸ but essential for optimizing the management of these patients in the future as we face new treatment options.¹

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CONFLICTS OF INTEREST

Jan Astermark: has received research grants from Sobi, CSL Behring, Takeda/Shire, and Bayer. Speakers fee and consultant for Octapharma, Novo Nordisk, Pfizer, Bayer, Sobi, CSL Behring, Takeda/Shire, BioMarin, Uniqure, and Spark Therapeutics. Katharina Holstein: received honoraria for Advisory Boards and/or speaker fees from Bayer, Biotest,

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AUTHOR'S CONTRIBUTIONS

Jan Astermark, Erik Berntorp and Amy D. Shapiro conceptualized and designed the study, acquired, analysed and interpreted the data, drafted and finalized the manuscript. Yasmina L. Abajas, Katharina Holstein, Stacy E. Croteau, Riana Liesner, Eva Funding, Susan Kearney, Christine L. Kempton, and Suchitra Acharya acquired the data, reviewed and participated in the critical review and final approval of the version to be submitted. Stefan Lethagen partly designed the study and participated in critical review and final approval. Petra LeBeau and Joel Berntorp analysed and interpreted the data, drafted and finalized the manuscript.

ORCID

Jan Astermark  <https://orcid.org/0000-0001-8500-2483>

Stacy E. Croteau  <https://orcid.org/0000-0001-6112-6166>

Riana Liesner  <https://orcid.org/0000-0001-5659-554X>

Christine L. Kempton  <https://orcid.org/0000-0002-2391-2016>

Erik Berntorp  <https://orcid.org/0000-0002-2888-4931>

Amy D. Shapiro  <https://orcid.org/0000-0003-2821-7159>

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