Published in final edited form as:

Expert Rev Hematol. 2014 December ; 7(6): 747–755. doi:10.1586/17474086.2014.963550.

Design of the INHIBIT trial: preventing inhibitors by avoiding ‘danger’, prolonging half-life and promoting tolerance

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Abstract

Inhibitor formation is among the most serious complications of hemophilia treatment. With the US FDA licensure of the novel long-lasting recombinant factor VIII (FVIII) Fc fusion protein, *Eloctate*, which prolongs FVIII half-life, we propose an innovative approach to *prevent* inhibitor formation. In this paper, we describe a multicenter, Phase II, single-arm, 48-week trial, the INHIBIT trial, to determine if *Eloctate*, begun *before* a bleed and continued as once weekly prophylaxis, will reduce inhibitor formation in children with hemophilia A. We *hypothesize* that avoiding ‘danger,’ that is, immune activation by a bleed at first factor exposure and prolonging FVIII half-life will prevent inhibitors and promote FVIII-specific T-cell tolerance. If successful, this approach will suggest a new paradigm in clinical practice.

Keywords

coagulation factor VIII; hemophilia A; inhibitor formation; long-lasting factor VIII; Tregs; tolerance

Hemophilia A is a rare X-linked bleeding disorder, occurring in 1 in 5000 male births, resulting from deficiency of factor VIII (FVIII). It is characterized by bleeding into joints, muscles and body cavities, such as the CNS. Despite treatment of bleeds with clotting factor concentrate, significant morbidity, pain and orthopedic disability may result. Among the most serious complications of hemophilia treatment is the formation of inhibitor antibodies, estimated to occur in 25–30% of those with hemophilia A [1–4], overall 28% among previously untreated patients (PUPs) (TABLE 1) [5–14]. Inhibitor formation is a T-cell response to foreign infused FVIII [1,15,16] that renders lifesaving factor treatment

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Financial & competing interests disclosure: MV Ragni receives research funding from Baxter Bioscience, Bayer, Biogen Idec, CSL Behring, Merck, Novartis, Novo-Nordisk, SPARK and Vascular Medicine Institute. LM Malec has received research funding from Baxter Bioscience. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed. No writing assistance was utilized in the production of this manuscript.

ineffective and results in poorly controlled bleeding, with twice the hospitalizations [17] and 10-times the cost [18] of non-inhibitor patients. Treatment is difficult as the use of bypass agents, factor VIIa or activated IX is unpredictable and suboptimal. Immune tolerance induction, a program of regular FVIII infusions over a prolonged period of time to eradicate inhibitors, is inconvenient, costly and ineffective in up to 20% of patients [19]. Thus, preventing inhibitors *before* they occur would promote better health outcomes for children with hemophilia and would be practice-changing.

There is a ‘window of opportunity’ to promote tolerance to FVIII, specifically in the first 20–30 exposure days during which children with hemophilia are vulnerable to inhibitor formation. While the mechanism by which inhibitor formation occurs and the means by which it can be prevented remain elusive, several lines of evidence suggest that two concepts may be important in achieving tolerance to infused FVIII and reducing inhibitor formation: *reducing ‘danger,’* that is, avoiding immune activation at the time of first FVIII exposure and *prolonging FVIII half-life*. The ‘danger’ hypothesis suggests that to achieve tolerance to infused FVIII, ‘danger’ signals (bleeds, infection, trauma, vaccination) which may activate the immune system must be minimized at the time of first FVIII exposure. Emerging data suggest that *prolonging FVIII half-life* to achieve and sustain FVIII above 0.01 U/ml (1%) may be important in reducing FVIII immune response (inhibitor formation). We shall provide supporting evidence that an approach that combines two concepts: *minimizing ‘danger’* and *prolonging FVIII half-life* may reduce inhibitors. The problem of inhibitor formation and approach to its prevention is compelling, and if successful, will be practice-changing and promote better health outcomes for children with hemophilia.

Background

FVIII immune response

Inhibitor formation is a T-cell-dependent immune response [15,20–22] directed against infused FVIII, in which alloantibody binds to FVIII, primarily the heavy chain (A2 domain) and/or light chain (C2 domain) [23]; inhibits FVIII function and disrupts normal hemostasis. For an affected patient, this results in uncontrolled bleeding and significant morbidity. CDC studies indicate that hemophilia inhibitor patients are twice as likely to require hospitalization [17] and sustain 10-times the cost of those without inhibitors [18] or about several million dollars annually for a 70 kg inhibitor patient. Inhibitors also complicate the current standard of care, three-times weekly FVIII prophylaxis (preventive FVIII) to prevent joint bleeds and arthropathy [15] is recommended by the Medical and Scientific Advisory Committee of the National Hemophilia Foundation [24]. A recent survey, not surprisingly, has found that, despite the benefits of prophylaxis, only 46% of hemophilia treatment centers (HTCs) use the recommended prophylaxis regimen [25]. Inhibitors also complicate the placement of central lines required to infuse standard prophylaxis [26,27] and may complicate gene transfer, if directed at FVIII expressed by the transgene. Current *treatment* of inhibitors is difficult, as bypass agents, for example, factor VIIa or IX complex, are suboptimal and somewhat unpredictable. *Eradication* of inhibitors by immune tolerance induction, a program of regular FVIII infusions, is inconvenient, expensive and ineffective in 20% of patients [2,19]. *Prevention* of inhibitor formation, therefore, is a compelling

approach and supported by the NHLBI Hemostasis Thrombosis State of the Science Symposium.

Risk factors for inhibitor formation

Although risk factors for inhibitor formation have been well established, it is difficult to identify those at risk early enough to target prevention efforts. Furthermore, it is not known how to prevent inhibitor formation. Risk factors include patient-related (genetic) factors, that is, *race* (common in African Americans), *family history* (common if familial) and *FVIII genotype* (common with large gene deletions) [4,27] and *treatment-related factors*, that is, high-intensity factor exposure, especially if given at an early age [4,14,26,28]. Among children treated in the first month of life, 41% develop inhibitors, compared with only 18% after 18 months of age, which may be in part related to *treatment intensity*, as the difference disappears after adjustment for treatment intensity [26]. The FVIII *treatment regimen* may also influence inhibitor development: high intensity regimens, that is, *multiple exposures over a short period of time*, are associated with an inhibitor rate of 28% [27], whereas lower intensity regimens, including continuous infusion [29], enhanced episodic treatment (with intense treatment for bleeds) [30] and once-weekly prophylaxis with dose escalation for bleeds [31], are associated with rates of 4.6–6.2%. Yet, whether prophylaxis reduces inhibitor risk remains controversial [32].

Concept 1: minimizing ‘danger’

Immunologists have suggested that to achieve tolerance to foreign antigens, ‘danger’ signals at the time of first exposure must be reduced. The ‘danger’ theory holds that when the immune system is activated by events that cause inflammation and tissue damage, the so-called ‘danger signals,’ the innate immune system is activated and upregulates antibody response [33–35]. In the setting of ‘danger signals,’ that is, bleeds, infection, trauma and vaccination, in children with hemophilia who are receiving FVIII infusion, especially in the vulnerable first 20–30 exposure days [36], the immune system is activated and may upregulate antibody response (inhibitor formation) to the foreign infused FVIII. Evidence supporting this theory includes the relationship of inhibitor risk to *intensity of treatment* at the time of major bleeds or surgeries, as these may cause tissue damage and inflammation, the so-called ‘danger’ signals [14,33–35]. In the CANAL study, compared with factor given to *prevent a potential bleed* (regular prophylaxis), factor given to *treat* an existing bleed (on-demand) resulted in a 60% increase in inhibitor risk [26]. When initial FVIII was given at the time of surgery or hemorrhage, there was a 2.0 hazard ratio for inhibitor formation [14]. For those initially treated for a *surgical procedure*, if treatment lasted for 5 or more days, the relative inhibitor risk increased from 2.4 to 3.3 [26]. While the method of infusion is a risk factor, for example, via *central line placement*, especially in those who bleed with the procedure [26,29], by contrast, the type of product, recombinant versus plasma derived, full length versus B-domain deleted or von Willebrand factor content does not appear to be a risk [28]. Neither is *circumcision*, a procedure associated with bleeding in as many as 35% of children with hemophilia, associated with inhibitor risk [36,37]. In Israel, where nearly all male infants undergo the procedure, the inhibitor rate is 30% [KENET G, PERS. COMM.], not different from rates in the USA, where 87% of infants with bleeding disorders are circumcised. In South Africa, where circumcision rates are substantially lower, the inhibitor

rate (16.8%), while lower than in the USA and Israel, does not differ between children with hemophilia A who are circumcised versus those who are not [POTGIETER JC, PERS. COMM., MAHLANGU JN, PERS. COMM.] [38,39]. Thus, while FVIII exposure at the time of 'danger' (surgery, trauma, non-circumcision bleeding) increases inhibitor risk, whether avoiding 'danger' is sufficient to prevent inhibitor formation remains unresolved, as the EPIC study which tested this hypothesis by initiating *preemptive* weekly factor, included previously treated children and was not powered to answer this question [10,11].

Concept 2: prolonging FVIII half-life

More recently, human and animal studies suggest that prolonging FVIII half-life and area under the curve may *also* promote FVIII tolerance. Lines of evidence supporting this concept include the observation that inhibitor formation is lower (<5%) in mild or moderate hemophilia A (FVIII >1%) than in severe hemophilia A (FVIII <1%), 28%; and sustaining FVIII levels above 1% achieved by gene therapy given to the inhibitor-prone Queen's (exon 22 knockout) hemophilia A dog or to neonatal mice and cats sustains FVIII activity above 1% and prevents inhibitor formation [40–43].

A newly developed 'long-lasting' FVIII protein, recombinant factor VIII Fc fusion protein (rFVIII-Fc) or *Eloctate*, prolongs FVIII half-life and FVIII activity above 1% in the circulation. rFVIII-Fc is a fusion protein in which a single molecule of recombinant FVIII (rFVIII) is fused covalently with the Fc domain of IgG₁, which delays lysosomal degradation of IgG via the neonatal Fc receptor and endogenous IgG recycling pathway, thereby prolonging FVIII half-life 1.5-fold [44,45]. *Eloctate* is structurally and functionally indistinguishable from standard recombinant FVIII and may have immunoregulatory properties, as recognized with other Fc fusion proteins [46]. For example, IgG antibodies coupled to haptens can induce antigen-specific tolerance [47], and intravenous immunoglobulin induces immune-Tregs [48]. Importantly, when FVIII peptide epitopes or FVIII immunogenic regions are coupled to IgG Fc, they induce immune-Tregs [49,50], suggesting that, in addition to prolonging FVIII half-life, the FVIII Fc-fusion protein may be tolerogenic [46]. In preclinical studies, when *Eloctate* was given weekly at a dose of 50 or 100 U/kg to hemophilia mice, inhibitor rates, 10–30 versus 70–90%, and inhibitor titers were significantly lower, each $p < 0.05$, compared with mice given standard full length rFVIII or B-domain-deleted rFVIII [51]. In clinical trials, *Eloctate* was safe and effective in treating and preventing bleeds in adults and children, increasing half-life 1.5-fold, with fewer bleeds than with standard rFVIII, despite a FVIII level sustained >1% for 5 days in only half of the adults [52,53]. Thus far, however, no data have been published regarding inhibitor formation in PUPs receiving *Eloctate*.

It is important to recognize that the mechanism(s) by which prolonging half-life may reduce inhibitor formation is not established. Thus, while inhibitor risk is reduced in mild and moderate patients and animals receiving gene therapy, there may be other mechanisms by which inhibitor formation is reduced, that is, mutations in mild and moderate patients resulting in endogenous production of FVIII or gene therapy-induced sustained FVIII levels resulting from *in vivo* protein expression from tolerogenic organs, for example, liver, or via immunosuppressive conditioning agents to achieve high level FVIII expression.

There are a few caveats to consider. While in practice, a FVIII level of 1% correlates poorly with bleeding, *trough* FVIII 1% is an *important* determinant of bleeding risk: the longer FVIII remains <0.01 U/ml, the greater the bleeding risk. This is the basis for prophylaxis, that is, to prevent bleeds by maintaining a FVIII level above 1%, and, we argue, it may also be the basis for inhibitor reduction, that is, to reduce bleeds and factor exposure, ‘danger signals,’ associated with inhibitor formation. Finally, it is recognized that FVIII levels may be maintained above 1% with standard FVIII prophylaxis. However, the potential advantage of *Eloctate* is that it not only prolongs half-life and area under the curve, that is, the time FVIII is above 0.01 U/ml, but it may also be tolerogenic like other Fc fusion proteins, suggesting the potential utility of an approach to reduction of inhibitors by reducing ‘danger’ and prolonging half-life with a potentially tolerogenic protein, *Eloctate*.

FVIII-specific T-cell immune response & tolerance

There is also ample evidence that tolerance plays an important role in inhibitor reduction. The initial recognition that inhibitor formation to infused FVIII is a T-cell-dependent immune response [1,23,54] originated with the clinical observation that anti-FVIII inhibitor immune response waned in HIV(+) hemophilia inhibitor patients as their CD4+ T cells fell with progression to AIDS [15,16]. The pathogenesis of FVIII inhibitor formation is poorly understood but involves FVIII peptide binding to human leukocyte antigen class II molecules, antigen presentation of FVIII by CD4+ T cells following recognition by the T-cell receptor and CD4+ T-cell differentiation through interaction with regulatory Th1 and Th2 cytokines [54–56]. Inhibitor formation is accompanied by T-cell proliferation, localized to the CD4+-enriched T-cell fraction [57], and in the hemophilia A mouse model (exon 16 knockout), FVIII injection is associated with FVIII-specific splenic T-cell proliferative responses even before an inhibitor develops [57], suggesting that T-cell activation underlies inhibitor formation. Furthermore, several groups have shown that blocking T-cell response in mouse models before injection of FVIII via FVIII-primed immature dendritic cells, which are CD4+CD25+ professional antigen-presenting cells, significantly reduces inhibitor response [23,24], and is accompanied by an increase IL-10 production by splenic T lymphocytes [23,30], consistent with the association of inhibitor formation and IL-10 gene polymorphisms [58,59]. Long-term FVIII tolerance induced by lentiviral FVIII gene transfer in neonatal mice [30] is accompanied by induction of CD4+CD25+ Foxp3+ Tregs [23–25]. These findings confirm the central role of T-cell activation in inhibitor formation and the potential utility of suppressing FVIII-specific T effector response to induce FVIII tolerance, while avoiding ‘danger’ and prolonging FVIII half-life.

INHIBIT trial concept

We, therefore, propose that by combining the two concepts, avoiding ‘danger’ at first FVIII exposure and prolonging FVIII half-life, it may be possible to reduce inhibitor formation in children with hemophilia A. We propose a multicenter, Phase II, single-arm, 48-week trial, the INHIBIT trial, to determine if *Eloctate* reduces inhibitor formation in previously untreated children when begun *before* a bleed or surgery or trauma (*preemptive*) and continued once weekly to prevent bleeds (*prophylaxis*). We *hypothesize* that by avoiding danger *and* prolonging FVIII half-life and area under the curve, inhibitor formation will be reduced. Adopting once-weekly dosing addresses the greatest drawback to current three- to

four-times weekly prophylaxis, namely, access by a central line. This approach is novel and, if successful, will eliminate the greatest complication of hemophilia. Given the public health burden of hemophilia inhibitor formation, it is critical and timely to prevent inhibitor formation.

Trial feasibility

To design this trial, we conducted a U34 HL-114674 feasibility study in which we distributed surveys to HTC MDs to determine prophylaxis practice and age at first bleed; interviewed parents and HTC MDs to determine acceptance and barriers to a *preemptive* rFVIII Fc prophylaxis trial to prevent inhibitors; validated T-cell assays for pediatric volumes and optimized trial design.

Surveys—Despite the established standard of three-times weekly prophylaxis to prevent bleeds [30], little is known regarding prophylaxis practice. Yet, this is critical to trial design. We surveyed HTCs using the CDC website [60] and the Hemostasis & Thrombosis Research Society website [25,61] (TABLE 2). Of 62 responding HTCs, representing 44.0% of the 141 National Hemophilia Foundation-listed HTCs, only 28 (45.2%) initiate prophylaxis three-times weekly beginning *after* the first bleed [24,30] and 41 (66.1%) avoid central lines in over 50% of their patients. Barriers to participation in an inhibitor prevention trial include nursing costs and staffing to prepare institutional review board submissions in 35.5% (22 HTCs). Of 53 HTCs reporting new births in 2008–2009, there were 2.13 births/HTC/year, which we confirmed in the 2014 survey indicating 2.01 births/HTC/year in 41 HTCs. The median age at first non-circumcision bleed requiring factor was 7 months, with 72% bleed-free at 4 months, 50% at 7 months and 22% at 12 months. Among 113 (53.5%) children undergoing circumcision, 62 (54.9%) bled, but none was associated with inhibitor formation, consistent with US, European, Israeli and African experience [KENET G, PERS. COMM., POTGIETER JC, PERS. COMM., MAHLANGU JN, PERS. COMM.] [26,27,29,38]. Our survey suggests that initiating factor *before* the first bleed, with the exception of circumcision, is an uncommon practice, occurring in fewer than 10% of US HTCs [25]; thus, it is possible that children recruited to the trial *before* a first bleed or ‘danger’ may represent a ‘low danger signal’ cohort, for example, the RODIN study [14], not representative of all inhibitor patients. By contrast, children recruited to the trial with a family history of inhibitors may represent a ‘high risk signal’ cohort. Recognizing these possibilities, we will perform a *post-hoc* analysis to determine whether our cohort differs from other PUP study cohorts [5–14].

Structured interviews—We conducted 30-min structured interviews on 20 HTC physicians and 20 parents of children with hemophilia to determine acceptability of the trial approach and barriers to participation. Despite the burden of weekly factor infusion *before* a child’s first bleed, parents and MDs found this approach acceptable, given the ‘pain of,’ ‘expense of’ and ‘lack of treatment to prevent’ inhibitors. All were willing to participate even if it did not benefit their children directly, but several stated ‘not if a port was required’ and some had concerns about ‘five blood draws during the study.’ The main *parental* concern was time and travel required for weekly visits. The main *physician* concern in 10 interviews, 44 surveys and two investigator meetings (n = 19) was the ability to recruit 1–2

subjects in 5 years due to past factor and bleeds, difficulty of weekly infusions, nursing costs and competition with Baxter HIPS and Biogen PUP studies.

Validated T-cell assay—Our ELISPOT assays and experiments with fluorescent MHC tetramers, which label FVIII-specific T cells after expansion in culture, have indicated that the fraction of CD4 cells specific for FVIII rarely exceeds 1–5%, even in high-titer inhibitor patients [62–65]. The ELISPOT assay, which detects as few as one in 10^6 antigen-specific T cells, is the preferred method to characterize antigen-specific immune responses in pediatric samples; it detects T cells secreting proinflammatory cytokines, for example, IFN- γ , IL-4 and IL-5; anti-inflammatory cytokines, for example, IL-10 [58,59] and antigen-specific antibodies secreted by plasma cells. We validated the FVIII-specific ELISPOT assay for pediatric (2 cc) volumes, stimulating peripheral blood mononuclear cells (serially diluted from 2×10^5 peripheral blood mononuclear cells/well) with 5 nM FVIII to allow detection and titration of anti-FVIII responses, consuming $<1.5 \times 10^6$ peripheral blood mononuclear cells per cytokine assayed. Inhibitor patients PT-001 had varied responses, with one exhibiting Th2 (IL-5) and IL-10 T-cell responses to FVIII, while two others showed Th1 (IFN- γ) responses to FVIII, but none secreted IL-4. Thus, these lab assays are validated for pediatric volumes, and cytokine responses to IL-17 and IL-10 appear to be key cytokines to assess in children who will be enrolled in the INHIBIT trial.

Study design—As hemophilia is a rare disease for which a randomized trial is not possible, we worked with statisticians who are experts in rare disease trial design to identify a better approach. After running statistical simulations, we chose a Bayesian Phase II single-arm trial design to test the hypothesis that early treatment will reduce the probability of developing inhibitors from 28% to 14% (FIGURE 1). With a sample size of 45 subjects, a Bayesian design provides a high probability (>0.90) of rejecting the current rate of inhibitors (0.28) in favor of the lower rate when the lower rate is correct and a very low probability (<0.05) of rejecting (0.28) when the new treatment offers no improvement in the incidence of inhibitors. A stopping rule was set up such that after 20 subjects have completed follow-up, if more than 10 subjects have developed inhibitors, the trial will be halted as an observed rate of 50% would be highly unlikely if the true rate was 28%.

We identified in our 2014 survey 41 HTC's that agree with this approach and are willing to participate in the trial, and we have a strong working collaboration with most of these HTC's on past clinical trials and cohort studies. Based on current births at these HTC's, 329 children are projected to be born with severe hemophilia A over the next 4 years, of whom we estimate 50% (~164) will be disqualified because of past bleeds or factor use; 10% (~32) for travel, insurance or family barriers and 10% (~32) for participation in competing trials, leaving 101 potentially eligible subjects. If only 50% of these remaining 101 agree to participate, there will be approximately 50 eligible subjects, sufficient to enroll 45 subjects in 4 years, with 1-year follow-up per subject, to complete the study in 5 years (FIGURE 1).

Thus, the preliminary U34 study findings indicate that the trial design is acceptable to parents and MDs, lab assays are validated for pediatric volumes, and sufficient sites and potential subjects are available to conduct the trial. To promote participation, infusions will be weekly to avoid ports; enrollment will be at >4 months of age, factor use for circumcision

will not be an exclusion; blood draws will be pre-infusion to avoid extra sticks; parent travel and nurse costs will be compensated and central IRB submission will be offered.

Innovation

The concept we propose is *innovative* as it investigates a preventive rather than reactive approach to induce tolerance to FVIII, and thereby reduce inhibitor formation in PUPs with severe hemophilia A, arguably the most *important challenge* of hemophilia treatment. To test this hypothesis, the trial will evaluate the recently US FDA-approved novel long-acting FVIII fusion protein, *Eloctate* (rFVIII-Fc), which prolongs FVIII half-life and is safe and effective in adults and children with hemophilia A. Our approach combines the ‘avoid danger’ and ‘prolong half-life’ concepts to promote FVIII tolerance to prevent inhibitor formation. Evaluating the tolerizing effect of rFVIII-Fc as *preemptive prophylaxis* to prevent inhibitor formation is innovative. Elucidating the mechanism of FVIII-specific tolerance by Tregs (CD24+CD25+) and FVIII-specific T-cell ELISPOT assays and comparing these findings with FVIII genotype, HLA type and serial pretreatment trough FVIII levels will enhance our understanding of inhibitor formation (TABLE 3). The proposed trial challenges the current paradigm of treating inhibitors *after* they occur, which does not avoid morbidity, and if successful, will be *practice-changing*, as once-weekly infusion is less invasive than currently recommended prophylaxis, avoids central lines and limits ‘danger’ during the vulnerable first 50 exposure days. Despite competing studies, surveys and structured interviews of physicians and parents indicate acceptance of the concept and sufficient subjects to conduct the proposed trial. Finally, these goals are consistent with Healthy People 2010 objectives to promote safety and prevent complications of existing products [66].

Conclusion

In summary, these data provide preliminary evidence that an approach which avoids ‘danger’ with initial and early FVIII exposure and prolongs FVIII half-life might successfully prevent inhibitor response, and this trial represents a unique opportunity to collect clinical, immunological and genetic markers to enhance our understanding of FVIII-specific immune response and FVIII tolerance.

Expert commentary

To date, no clinical trial of inhibitor prevention has been conducted with a long-lasting FVIII protein in PUPs with severe hemophilia A. Despite the difficulties in conducting clinical trials in rare populations, preventing inhibitor formation has the potential to be among the most important clinical improvements in hemophilia management. Inhibitor formation is associated with considerable morbidity, hospitalization and high cost. Treatment of inhibitor patients with bypass agents is less effective than FVIII in those without inhibitors, and, for those with inhibitors for more than 1 year, immune tolerance induction may fail. Not only would prevention of inhibitors lead to clinical improvement, it would also lead to economic gains.

Five-year view

The next 5 years will undoubtedly see the licensure and availability of a number of novel long-lasting therapeutics for hemophilia A. If inhibitor formation is lower with the Fc fusion protein *Eloctate*, we predict that other long-lasting FVIII proteins, whether pegylated or albumin fusion proteins, will potentially also prevent inhibitor formation, likely based on their capacity to prolong half-life and potential tolerogenic properties. Whether this is so will require clinical trials that are challenging in rare diseases.

Acknowledgments

MV Ragni designed the research and collected the data. MV Ragni and LM Malec reviewed the findings, formulated the conclusions and wrote the manuscript. We acknowledge the statistical expertise of DJ Brambilla, Research Triangle Institute, Rockville, MD and the clinical expertise of the members of the NHLBI State of the Science Hemophilia von Willebrand Disease Subcommittee, including CM Kessler (Georgetown, Washington, DC), PF Fogarty (University of Pennsylvania, Philadelphia, PA), NC Josephson (University of Washington, Seattle, WA), AT Neff (Vanderbilt, Nashville, TN) and L Raffini (Children's Hospital of Philadelphia, Philadelphia, PA). The study was supported by NHLBI U34 114674 Feasibility of INHIBIT Trial and CTSC/CTSI NIH NCRR/CTSA UL-1 RR024153.

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Key issues

- Inhibitor formation is among the most serious complications of hemophilia, for which response to treatment is poorer than factor VIII (FVIII) in non-inhibitor patients, and prevention is unknown.
- Anti-FVIII antibody formation is a T-cell response to foreign infused FVIII.
- Increasing evidence suggests that *reducing ‘danger,’* that is, avoiding immune system activation by bleeds, trauma, infection or vaccination at the time of first FVIII exposure and *prolonging FVIII half-life* may be critical to achieving FVIII tolerance.
- CONCEPT 1: Initiating FVIII before a first bleed or trauma may avoid ‘danger’ signals that activate the immune system and prevent inhibitor formation.
- CONCEPT 2: Initiating weekly prophylaxis with the novel, US FDA-approved long-lasting factor, rFVIII-Fc or *Eloctate*, which improves FVIII half-life may reduce inhibitor formation.
- We propose a multicenter, Phase II, single-arm, 48-week trial, the INHIBIT trial, to evaluate whether *Eloctate* reduces inhibitor formation in previously untreated children when begun *before* a bleed or surgery or trauma (*preemptive*) and continued once weekly to prevent bleeds (*prophylaxis*).
- If successful, this approach will suggest a new paradigm in the management of individuals with hemophilia.

Figure 1.
Schema for the Hemophilia INHIBIT Trial.

Table 1

Inhibitor formation in PUP clinical trials.

	Year	Patients (n)	n < 0.01	PUP/MTP	rFVIII/pd	Inhibitor overall	High titer inhibitor	ED	Inhibitor Definition	Ref.
Kogenate PUP Trial	1989–1996	101	n = 49	PUP	rFVIII	13/49 (26.5%)	7/49 (14.3%)	Median 9 ED	5 BU	[5]
Recombinate PUP Trial	1990–1998	20	n = 20	PUP	rFVIII	6/20 (30.0%)	2/20 (10.0%)	Up to 8 years	>5 BU	[6]
French PUP Trial	1993–1996	50	n = 50	PUP	rFVIII	10/50 (20.0%)	7/50 (14.0%)	Median 32 months	5 BU	[7]
B-Domain-Deleted rFVIII Trial	1994–1999	101	n = 101	PUP	rFVIII	32/101 (31.6%)	16/101 (15.8%)	Median 143 ED	>5 BU	[8]
Kogenate-FS Trial	1998–1999	212	n = 13	PUP	rFVIII	1/13 (7.7%)	1/13 (7.7%)	Mean 187 ED	5 BU	[9]
EPIC Trial	2001–2007	56	n = 56	PUP/MTP	rFVIII	15/56 (26.8%)	8/56 (14.3%)	>150 ED	>5 BU	[10,11]
Israeli PUP Trial	1984–2008	292	n = 43	PUP	rFVIII	14/43 (32.5%)	9/43 (20.9%)	Up to 7 years	>5 BU	[12]
Polish PUP Trial	2009–2011	39	n = 36	PUP	pdFVIII	4/39 (10.3%)	3/39 (7.7%)	50 ED	5 BU	[13]
RODIN Trial	2000–2010	710	n = 576	PUP	rFVIII	179/576 (31.1%)	118/576 (20.5%)	Median 14.5 ED	5 BU	[14]

ED: Exposure days; MTP: Minimally treated patients; pdFVIII: Plasma-derived FVIII; PUP: Previously untreated patients; rFVIII: Recombinant.

Table 2

HTC survey: patient prophylaxis data.

Year	New patients (n)/ Severe hemophilia A	New patients (n)/HTC	Patients (n) with 1st bleed	Median age at 1st bleed (months)	Median age 1st non- circumcision bleed (months)	No. with circ info	No. undergo circumcision	No. bleed at circumcision
2009	115	2.17/HTC	86 (74.8%)	5	6	108	55 (50.9%)	28 (50.9%)
2008	111	2.09/HTC	100 (90.0%)	4	7	103	58 (56.3%)	34 (58.6%)
Total	226	2.13/HTC	186 (82.3%)	5.5	7	211	113 (53.5%)	62 (54.9%)

Data taken from [25].

Table 3

The INHIBIT trial schedule of events.

Schedule of events	Study weeks 0–48												
	Week 0	Week 4	Week 8	Week 12	Week 16	Week 20	Week 24	Week 28	Week 32	Week 36	Week 40	Week 44	Week 48
Study visit	1	2	3	4	5	6	7	8	9	10	11	12	13
Screening, consent	X												
Initiate study arm	X												
Initiate study diary	X												
Clinical monitoring		X	X	X	X	X	X	X	X	X	X	X	X
End-of-study visit													X
Laboratory tests													
Anti-FVIII Nijmegen	X	X		X			X			X			X
Hemophilia genotype	X												
HLA type	X												
FVIII 1-stage (trough)	X	X		X			X			X			X
T cell (Elispot, Ig, RNA)	X	X		X			X			X			X
Sample for storage	X	X		X			X			X			X