# Phase III Multicenter, Randomized Trial, Controlled Inhibitor Eradication Trial, Comparing Eloctate Immune Tolerance Induction (ITI) plus Emicizumab vs. Eloctate ITI Alone to Eradicate Inhibitor Formation in Severe Hemophilia A:

# The Inhibitor Eradication Trial

*Short Title:* **The INHIBIT Eradication Trial**

**Protocol Number:** PRO19070080

**National Clinical Trial (NCT) Identified Number:** NCT04303572

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**PROTOCOL SUMMARY**

**Title:** **The Inhibitor Eradication Trial (ERADICATE):** A Phase III Multicenter, Randomized, Controlled Trial, Comparing *Eloctate* Immune Tolerance Induction (ITI) plus Emicizumab vs. Eloctate ITI Alone to Eradicate Inhibitor Formation in Severe Hemophilia A.

**Study Description:** This is a prospective, multicenter, randomized phase III 48-week clinical trial, comparing rFVIIIFc (Eloctate®) Immune Tolerance Induction (ITI) plus Emicizumab (Hemlibra®) vs. Eloctate ITI alone in severe hemophilia A patients with current or past high-responding inhibitors (anti-FVIII $\geq$ 5.0 B.U.) to eradicate inhibitor formation. Subjects will include male adults or children who are ITI-refractory or ITI-naïve, or who developed an inhibitor on the linked Inhibitor Prevention Trial. We *hypothesize* that tolerance induction with Eloctate ITI plus Emicizumab is superior to Eloctate ITI alone in eradicating inhibitors in children and adults with severe hemophilia A and inhibitors.

**Objective:** The **Primary Objective** is to determine if Eloctate ITI plus Emicizumab is different from Eloctate ITI alone in eradicating inhibitors in severe hemophilia A patients with high-responding inhibitors who are ITI-refractory or ITI-naïve, or those arising during the Inhibitor Prevention Trial.
The **Secondary Objectives** are to evaluate the safety and mechanism of Eloctate ITI plus Emicizumab vs. Eloctate ITI alone in eradicating inhibitor eradication.

**Endpoints:** The **Primary Endpoint** is the probability of becoming inhibitor-free by 48 weeks, where the eradication of inhibitor formation is defined as anti-FVIII < 0.6 B.U. by chromogenic Nijmegen Bethesda assay (CBA). The **Secondary Endpoints** include a) *safety* by number and frequency of bleeding episodes (i.e. hematoma, joint, CNS bleed) and frequency and time to port infections; and b) *mechanism of inhibitor suppression (eradication)* by FVIII-specific T cell ELISPOT assay, B cell, Ig, and RNA assays and cytokine assays to determine the role of T regs in controlling immune response to FVIII; and by FVIII genotype; HLA type; and chromogenic FVIII trough levels; and by micriobiome stool sample, each compared with anti-FVIII.

**Study Population:** Study subjects will be male adults and children age $\geq$ 4 months of age with severe hemophilia A, defined as factor VIII $\leq$ 0.01 IU/ml and a current or past high-responding inhibitor (anti-FVIII $\geq$5.0 B.U.), either ITI-refractory, ITI-naïve, or developing on the linked Inhibitor Prevention Trial. A total of 90 eligible severe hemophilia A patients with inhibitors will be enrolled in this 6-year trial.

**Site Enrolling Subjects:** There are up to 41 participating sites: Hemophilia Treatment Center (HTCs).

**Description of Study Intervention:** There are two study interventions: Eloctate ITI plus Emicizumab vs. Eloctate ITI alone.
Recombinant factor VIII Fc (Eloctate®) is an FDA-approved clotting factor approved for treatment and/or prevention of bleeds in hemophilia A. It is an intravenous agent supplied as single-use vials containing approximately 250, 500, 750 IU per vial. The vials are reconstituted with 5-10 ml vial of sterile water for injection, USP, which is transferred by two-way needle into the lyophilized powder for reconstitution, and the reconstituted vial infused over 5-10 minutes, at 65 IU/kg once weekly for 48 weeks.
Emicizumab (Hemlibra®) is an FDA-approved agent for the prevention of bleeding in hemophilia A. It is a subcutaneous agent supplied in vial sizes of approximately 30 mg per ml, 60 mg per 0.4 ml, and 105 mg per 0.7 ml. A tuberculin syringe allows transfer of the drug from the vial for subcutaneous injection over 1-2 minutes, at 1.5 mg/kg weekly (following a 4-week induction with 3.0 mg/kg/week) for up to 48 weeks.

| **Trial Arms** | |
|---|---|
| **Arm A** | Eloctate 100 IU/kg IV qod plus Emicizumab 1.5 mg/kg SQ weekly (after 3.0 mg/kg/wk x 4 wk) for 48 weeks |
| **Arm B** | Eloctate 100 IU/kg IV qod alone for 48 weeks |

**Study Duration:** This is a 48-week outpatient trial in which all subjects will be randomized to one of two treatment arms and followed for 48 weeks.

**Abstract**

This is a multi-center randomized phase III clinical trial, the **Inhibitor Eradication Trial,** in which *Eloctate (rFVIIIFc)* ITI plus *Emicizumab (hemlibra)* will be compared with *Eloctate ITI* alone, using Bayesian platform design to eradicate inhibitors in patients with severe hemophilia A. This design is necessary as randomized trials in rare diseases are often not possible. The INHIBIT Clinical Trials Platform includes two linked trials, the Inhibitor Prevention Trial (Prevention Trial) and the Inhibitor Eradication Trial (Eradication Trial) that will be conducted at up to 41 U.S. hemophilia treatment centers (HTCs) affiliated with universities. The **Inhibitor Eradication Trial** is a 48-week randomized phase III trial, in which 90 previously treated patients (PTPs) with severe hemophilia A and current or past high-responding inhibitors (anti-FVIII $\geq$ 5.0 B.U.) will be enrolled. Subjects will include individuals with severe hemophilia A who develop inhibitors during the linked Inhibitor Prevention Trial *and* adults or children at the same HTCs who are immune tolerance induction (ITI)-regractory or ITI-naive. Once enrolled, subjects who meet all the inclusion and none of the exclusion criteria, will be randomized to weekly *Eloctate* ITI plus weekly *Emicizumab* vs. weekly *Eloctate* ITI alone to eradicate inhibitor formation, defined as anti-FVIII<0.6 B.U. Blood draws will be minimized to 6 timepoints, baseline, 4, 12, 24, 36, and 48 weeks post-randomization, and validated for small volumes, 3.8 cc (¾ tsp) each. The Inhibitor Eradication Trial is considered greater than minimal risk as study drug is given *before* the first bleed and special inhibitor studies are obtained. (NB: The Inhibitor Eradication Trial (PRO19070080) is linked to the Inhibitor Prevention Trial (PRO19040140), as part of the INHIBIT Clinical Trials Platform, and both trials will be conducted efficiently in the same hemophilia treatment centers (HTCs), with the same MDs, coordinators, visit frequency, blood sampling, and assays.

**Principal Investigator:** Margaret V. Ragni, MD, MPH

**Protocol Title: The Phase III Multicenter, Randomized Controlled Inhibitor Eradication Trial,** comparing *Eloctate* Immune Tolerance Induction (ITI) plus *Emicizumab* vs. *Eloctate* ITI alone to Eradicate Inhibitor Formation in Individuals with Severe Hemophilia A.

## 1.0 Objective and Specific Aims

The purpose of **the Inhibitor Eradication Trial** is to eradicate inhibitor formation in children and adults with severe hemophilia A. This 48-week phase III open-label, randomized, controlled trial, comparing *Eloctate* immune tolerance induction (ITI) plus the bispecific monoclonal antibody FVIII mimic, *Emicizumab,* vs. *Eloctate* ITI alone, to eradicate inhibitor formation in children and adults with severe hemophilia A, including those developing inhibitors in the Prevention Trial or those ITI-refractory or ITI-naïve never-tolerized inhibitors at the same HTCs. In this trial, time to inhibitor eradication with *Eloctate* ITI plus *Emicizumab* vs. *Eloctate* ITI will be assessed in the eradication of inhibitor formation in children and adults with severe hemophilia A and current or past high-responding inhibitors, defined as anti-FVIII $\geq$ 5.0 B.U. The research in this trial is considered greater than minimal risk as it involves combining agents to eradicate inhibitors and obtaining special inhibitor studies. The primary endpoint will be the probability of inhibitor eradication by 48 weeks, defined as anti-FVIII < 0.6 B.U. by chromogenic Nijmegen Bethesda assay (CBA), repeated immediately and confirmed within 4 weeks. Secondary endpoints will include a) safety, measured by frequency of bleeds (hematomas, joint, CNS bleeds) and b) mechanism of inhibitor eradication, measured by FVIII-specific T cell ELISPOT assay, performed on peripheral blood mononuclear cells (PBMCs) at baseline and at 4, 12, 24, 36, and 48 weeks. The B cell, Ig, and RNA assays and cytokine assays will be performed on an aliquot of PBMCs to determine the role of T regs in eradication of immune response to FVIII. Studies will be performed on T cell populations depleted of regulatory T cells (T reg) (CD4+CD25+FoxP3+) to determine the role of T regs in controlling immune response to FVIII. Hemophilia genotype and HLA assays will be determined at baseline on the buffy coat of plasma. A microbiome (stool sample) will be obtained at baseline. The trial will assess inhibitor eradication after each subject completes the 48-week trial. The Inhibitor Eradication Trial is designed to test whether *Eloctate ITI* plus *Emicizumab* is superior to *Eloctate ITI* alone in inhibitor eradication by 48 weeks. At the interim, once 75% of the subjects are randomized, the Bayesian posterior probability that either arm, *Eloctate ITI plus Emicizumab* or *Eloctate ITI* alone, is more likely to be inhibitor-free must be at least 99.5% to stop the trial. Assuming that the trial does not stop at the interim to declare superiority at the end of the trial, the Bayesian posterior probability that either arm, *Eloctate ITI plus Emicizumab* or *Eloctate ITI* alone, is more likely to be inhibitor-free must be at least 97.9%. This maintains the type 1 error at 5% with at least 80% power for superiority if we assume that the true condition is that *Eloctate ITI plus Emicizumab* is 15% superior to *Eloctate ITI* alone (95% vs 80% inhibitor-free).

## 2.0 Background and Significance

### 2.1 Background

Hemophilia A is an X-linked inherited bleeding disorder caused by deficient or defective coagulation factor VIII (FVIII) that affects approximately 20,000 in the U.S. and 80,000 worldwide. It is characterized by spontaneous and traumatic bleeding into joints, soft tissue, and muscles. Among the most severe complications of hemophilia treatment is the formation of inhibitor alloantibodies directed against exogenous factor VIII which occurs in up to 30% or more of hemophilia patients. Inhibitor formation is a T cell-dependent B-cell immune response, which was first recognized in HIV(+) hemophilia inhibitor patients whose inhibitors disappeared when CD4+ T cells fell below 200/µl and re-appeared after HIV antiviral therapy restoration of CD4 cells to >200/ µl. Inhibitor formation typically occurs early in life, after a median of 10-20 exposure days. Inhibitors bind to exogenous FVIII, neutralize its activity, interfering with clinical hemostasis and, despite bypass therapy (e.g. rFVIIa or factor IX concentrates) often leads to uncontrolled bleeding and increased morbidity, cost, hospitalization. Thus, inhibitors are a major cause of morbidity and high healthcare cost. CDC studies have determined inhibitor patients are twice as likely to require hospitalization for bleeding complications and cost on average ten times more than hemophilia patients without inhibitors.

Medical management of individuals with inhibitors is difficult. The use of bypass agents, including the response of inhibitor patients to alternative or bypass agents, such as factor rVIIa or activated IX, which is somewhat unpredictable and suboptimal, and morbidity is high as are healthcare costs. For over 30 years, the only approach to eradication of inhibitors has been immune tolerance induction therapy (ITI), a program of regular FVIII infusions over a prolonged period

of time, which is inconvenient, expensive, and effective in only 75-80% of those treated. Thus, a major goal of comprehensive hemophilia care is to *eradicate* them once they occur, incorporating potentially more effective therapies, such as the novel therapies in development and FDA-approved, e.g. *Eloctate* and *Emicizumab*, to promote better outcomes in those with hemophilia.

**Equipoise**: We are in a state of equipoise as the optimal approach to eradicate inhibitors in hemophilia is not known. While a randomized trial established that 200 IU/kg daily has been adopted as the standard of care for immune tolerance induction (ITI). However, only 70-80% respond, and ITI is time intensive, burdensome, taking up to 9-12 months with some requiring ports and lines, and costly. Further, those not tolerized within the first year of inhibitor onset are often refractory to ITI. Thus, simpler, more effective approaches to eradicate inhibitors is critical to improve outcomes. With the availability of Eloctate, small studies of ITI using 100 IU/kg every other day has reduced time to inhibitor eradication by nearly 50%, but these studies were not randomized. Among the reasons time to ITI eradication is long is the problem of breakthrough bleeds, which may activate the immune system "danger", and thus, use of Emicizumab by promoting hemostasis and avoiding factor VIII, could potentially reduce bleeds during ITI and reduce time to ITI. However, no data exist on whether Emicizumab reduces time to ITI. However, if breakthrough bleeds occur on Emicizumab, the requirement for FVIII to treat these bleeds could negate the potential effect of Emicizumab on ITI could be lost. In small nonrandomized studies, Emicizuab have indicated it is safe in infants but there are no data and no randomized trials to determine who might benefit or the optimal dose and/or combination of these agents to shorten ITI.

*Eloctate (*rFVIIIFc) is an FDA-approved FVIII protein that fuses recombinant a FVIII-Fc fusion protein that prolongs FVIII half-life in the circulation and induces regulatory T cells (Tregs) to promotes tolerance to the hapten to which it is attached, i.e FVIII. Eloctate prevents and treats bleeds in hemophilia A patients of all ages. It remains an important question, whether promoting Tregs reduces time to inhibitor eradication (ITI). In patients who develop an inhibitor, the inhibitor neutralizes FVIII activity, and bypass therapy (rFVIIa, FEIBA or factor VIII inhibitor bypass activity) is used to treat bleeds, but at least 50% are unresponsive to bypass agents or have a persistent shortened FVIII half-life. Thus, while it is standard of care to attempt immune tolerance induction (ITI) in patients with inhibitors, the burden of daily infusions and intercurrent bleeds, persistent shortened FVIII half-life even if successful ITI is achieved is significant, and it is not possible to predict the 20-30% who will not respond to ITI. In small, non-randomized studies, *Eloctate* ITI, when given at 100-200 IU/kg/day to hemophilia A patients with existing inhibitors, never tolerized, or previously refractory to ITI, has been shown to shorten the median time to inhibitor eradication, defined as anti-FVIII < 0.6 BU, was 33.8 months in 4 first-time ITI patients and 67.7 months in 4 rescue ITI patients (Carcao). These data suggest there may be potential utility of *Eloctate* in inhibitor eradication.

*Emicizumab (Hemlibra)* is an FDA-approved bispecific monoclonal antibody that mimics FVIII and has been shown to be safe and effective in phase III trials in hemophilia A with (Oldenburg) or without inhibitors (Mahlangu), as well as in children (Young) and is increasingly being used because of its simpler subcutaneous route. Whether *Emicizumab* may promote inhibitor eradication by avoiding bleeds during ITI and may reducing "danger" is not known. Further, whether *Emicizumab* potentiates *Eloctate* ITI in shortening the time to inhibitor eradication in severe hemophilia A patients with inhibitors is not known.

For these reasons, we will study if *Eloctate* ITI plus *Emicizumab* is superior to *Eloctate* ITI alone in <u>eradicating</u> inhibitor formation in patients with severe hemophilia A and inhibitors to FVIII of all ages, and anti-FVIII > 5.0 B.U. when continued weekly for 48 weeks to eradicate inhibitors. We *hypothesize* that *Eloctate* ITI upregulation of T regs, and *Emicizumab* prevention of bleeds ("danger") will be superior to *Eloctate* ITI alone in eradicating inhibitor formation. The concept is innovative, as it combines the "avoidance of danger" concept with the "promotion of tolerizing T regulatory cells" to improve inhibitor eradication. The concept of avoiding FVIII exposure by preventing bleeds with *Emicizumab* during ITI may be important in eradicating inhibitor formation. It's effectiveness in preventing bleeds in hemophilia inhibitor patients makes it a potentially powerful agent in the management of hemophilia, but there are no data on its use in eradicating inhibitors. Further, if it is not successful in preventing breakthrough bleeds during ITI may negate its potential beneficial effect and simpler subcutaneous administration.

**Specific Aim 1:** To compare *Eloctate* immune tolerance induction (ITI) every-other-day (q.o.d.) plus *Emicizumab* weekly prophylaxis vs. *Eloctate* ITI q.o.d. alone for 48 weeks in eradicating inhibitors in children and adults with severe hemophilia A in the Eradication Trial. We will conduct this multi-center, randomized phase III clinical trials at up to 41 HTC sites, with the same study visit frequency, blood sampling, and lab assays as in the companion Prevention Trial (PRO 19040140). The primary endpoint in the Eradication Trial is the eradication of anti-FVIII antibody < 0.6 BU by chromogenic Nijmegen-modified Bethesda assay (CBA) by 48 weeks.

*Hypothesis 1:* We *hypothesize* that *Eloctate ITI q.o.d. plus weekly Emicizumab is superior to Eloctate ITI q.o.d. alone* in eradicating inhibitors, anti-FVIII < 0.6 B.U. (CBA), when given for 48 weeks in children and adults with severe hemophilia A and high-responding inhibitors (historic anti-FVIII > 5.0 B.U.). Yet there is equipoise, as while Eloctate ITI may reduce the time to tolerance it is burdensome with qod dosing and data are available from small, nonrandomized studies. While both drugs may shorten ITI and even be synergistic, there is equipoise in the absence of randomized trials. While Emicizumab has been tried in hemophilia A inhibitor patients in small nonrandomized studies, there are no data on whether it reduces time to tolerance. As *Emicizumab* is given subcutaneously weekly, if, when given with *Eloctate ITI*, it was *superior* to *Eloctate* ITI alone in reducing time to ITI, it would be the "winner" over *Eloctate ITI alone.*

**Specific Aim 2:** To evaluate the safety and mechanism of inhibitor suppression *eradication* in patients with severe hemophilia A and inhibitors. Secondary endpoints include a) *safety,* measured by number and frequency of bleeding episodes (i.e. hematomas, hemarthroses) and associated factor use and port infections; and b) *mechanism of inhibitor suppression eradication*, measured by FVIII-specific T cell ELISPOT assay; FVIII genotype; HLA type; and chromogenic FVIII trough levels; and by micriobiome stool sample, each compared with anti-FVIII.

*Hypothesis 2:* We *hypothesize* that safety, measured by bleeds requiring rescue factor or prophylaxis escalation, and port infections, will not differ between treatment arms; and the mechanism by which *Eloctate* suppresses (prevents, eradicates) inhibitor formation is via Fc induction of regulatory T cells (Tregs) promoting FVIII tolerance, measured by ELISPOT assay. This novel approach to eradicate inhibitors is compelling, and, if successful, will reduce the burden of inhibitor formation and the associated morbidity, hospitalization, cost, and early mortality, and be adopted immediately into clinical practice.

The Inhibitor Eradication trial is a 48-week outpatient trial conducted in 90 inhibitor patients from up to 41+ Hemophilia Treatment Centers (HTCs) (2-4 subjects locally). Case report forms and laboratory assessment forms will be completed and uploaded to a web-based database. While small, nonrandomized studies have suggested that *Eloctate* ITI may shorten inhibitor eradication, and that Emicizumab may eradicate inhibitors in some patients who are ITI refractory or ITI naive, inhibitor formation remains a major complication of hemophilia for which the optimal approach is not known. Further, whether *Eloctate* can potentiate the effect of *Emicizumab* or prevent bleeds during ITI and improves the proportion who are tolerized remains unknown. A single non-randomized trial of Emicizumab in inhibitor patients has been initiated, but data are not available on its impact on inhibitor eradication or time to eradication. Despite these small studies, effective inhibitor eradication remains a compelling problem, for which an effective approach would be practice changing.

**2.2 Significance:** Inhibitor formation is the most serious complication of hemophilia, affecting 25-30% of those with severe hemophilia A. Inhibitor formation is a T cell-dependent B-cell response to exogenous FVIII that renders lifesaving factor treatment ineffective and leads to poorly controlled bleeding, severe morbidity, frequent hospitalizations, and high healthcare costs. Thus, *eradicating* inhibitors *once* they occur would promote better health outcomes for children and adults with hemophilia, an approach that would be adopted immediately into clinical practice. The drugs we will study are FDA-approved and safe and effective in preventing bleeds in adults and children with hemophilia A, and preliminary data from small studies support the need for clinical trials to determine optimal use of these agents to eradicate inhibitor formation in severe hemophilia A. The Inhibitor Eradication Trial is innovative as it uses a Bayesian Platform design to compare novel agents in a randomized phase III trial. Subjects who enroll in the Eradication Trial will include those with pre-existing refractory inhibitor patients, those who failed, or those who never tolerized at the same HTCs, along with those who develop inhibitors on the companion Prevention Trial (PRO19040140), to compare time tolerance with single or combination novel agents. The two trials are integrated for efficiency and conducted at the same HTCs, with the same visit frequency, blood draws, and lab assays. Moreover, as promising new agents emerge, they could potentially be incorporated into the trial platform design. Finally, the findings of this trial will potentially provide new information on treatment of a major complication of hemophilia and promote blood product safety, per the goals of Healthy People 2020.

## 3.0 Research Design and Methods

### 3.1 Drug/Device Information

*Eloctate* (rFVIIIFc) and *Emicizumab* (hemlibra) are FDA-approved for the prevention and/or treatment of bleeds in hemophilia A, and are safe and effective in adults, adolescents, and children. For this trial, subjects will use their own supply of study drug to which they are randomized. Clinical trials of *Eloctate* and *Emicizumab* in adults and children with severe hemophilia A have demonstrated safety and efficacy when used to prevent bleeds or promote hemostasis. In this trial, subjects will use *Eloctate* ITI (immune tolerance induction) with or without *Emicizumab* to eradicate inhibitors: this approach is supported by preliminary data from anecdotal small, non-randomized studies. Subjects will maintain a home inventory of the study drug(s) to which they are randomized. *Eloctate* in vial sizes of approximately 250, 500, and 750 is available for children, and 1000, 1500, 2000, 3000 IU for adults: a two-way needle will allow transfer of the diluent into the lyophilized powder for reconstitution and slow infusion over 5-10 minutes. *Emicizumab* in vial sizes of approximately 30 mg per ml, 60 mg per 0.4 ml, 105 mg per 0.7 ml, and 150 mg per ml is available for children and adults: a tuberculin syringe will allow transfer of the drug from the vial for subcutaneous injection over 1-2 minutes. Study drug(s) will be available by physician prescription which can be filled at the HTC pharmacy or at another pharmacy of the patient's or parent/caretaker's choice.

Once a subject is consented and identified as eligible for the trial, a unique subject number will be assigned to each subject by the Data Coordinating Center (DCC), Graduate School of Public Health (GSPH), University of Pittsburgh, Pittsburgh, PA. Study drug dosing will be given by the hemophilia center nurse, clinic or hospital infusion center, or, if on home treatment, by patient or parent/ care provider, or visiting nurse, in the usual manner: this is either by intravenous infusion (*Eloctate*) over 5-10 minutes through a winged infusion needle (butterfly) heplock placed in the forearm, or, if required, a central line; or by subcutaneous injection (*Emiczumab*) over 1-2 minutes with a tuberculin syringe in the abdomen or thigh. This study site will maintain accurate records, demonstrating dates, times, and doses administered, and patients or parent/caretakers will maintain a diary to record this information.

### 3.2 Research Design and Methods

This is a 48-week outpatient, randomized phase III clinical trial that employs Bayesian Platform design to assess inhibitor eradication in patients with severe hemophilia A, FVIII <0.01 IU/ml, and a current or past high-responding inhibitor (anti-FVIII $\geq$5.0 B.U.), ITI-refractory or ITI-naive. Because hemophilia is a rare disease, we incorporated Bayesian Platform design and will use historical and prospective ongoing clinical trial data on inhibitor formation (Bayesian "priors") to efficiently use rare patients and increase power. The Inhibitor Eradication Trial is linked to the Inhibitor Prevention Trial, and the trials are integrated so there is efficiency and economy, using the same HTCs, staff, frequency of blood sampling, visits, data collection, and lab assays. The **Eradication Trial** is a randomized phase III open-label trial designed to evaluate the efficacy of *Eloctate* immune tolerance induction (ITI) (100 IU/kg every other day) plus weekly *Emicizumab* 1.5 mg/kg weekly (after a 4-week induction) vs. *Eloctate* ITI alone (100 IU/kg every other day), to eradicate inhibitor formation in adults or children with severe hemophilia A and current or past high-responding inhibitors to FVIII, anti-FVIII $\geq$ 5.0 B.U. Approximately 2-4 children or adults with severe hemophilia A and inhibitors will be recruited at each HTC. The study drugs will be administered to each subject for up to 48 weeks. *Eloctate* will be given either by weekly intravenous infusion over 5-10 minutes, and (*Eloctate*) will be given by weekly subcutaneous injection over 1-2 minutes. For treatment of breakthrough bleeds, for safety reasons, rFVIIa will be given by intravenous infusion over 5-10 minutes at 90 mcg/kg per standard of care, at MD discretion, or if the inhibitor titer has fallen, additional Eloctate will be given, per MD discretion. The treating physician may be the study physician. *FEIBA will not be allowed on the trial as it may result in thrombosis or thrombotic microangiopathy in patients taking Emicizumab*. All bleeds and study drug(s) will be recorded by patient or parent/ caretaker for each subject in a patient diary. All randomized subjects will be followed 48 weeks or until they reach the study outcome of inhibitor eradication. Follow-up visits will be monthly until end-of-study, week 48.

The Data Safety Monitoring Board (DSMB) will be responsible for reviewing all data generated for the study interventions, from the time of dosing through the end of each 48-week trial. Each study will consist of three phases; a Screening Period, a Treatment Period, and a Post-Treatment Period.

Screening Period: Subject eligibility for the study will be determined prior to study drug dosing. A history of past bleeds, factor treatment, and circumcision bleeding and treatment will be obtained. A baseline blood sample will be collected for anti-FVIII inhibitor titer by chromogenic Nijmegen Bethesda (CBA) assay, FVIII-specific T cell (ELISPOT), trough

FVIII (chromogenic assay), HLA, hemophilia genotype, and sample storage for future testing. A microbiome stool sample will also be obtained. A baseline height, weight and brief physical exam will be obtained.

Treatment Period: After random assignment to one of the two treatment regimens, the treatment period will extend for up to 48 weeks, beginning with every other day *Eloctate* ITI plus weekly *Emicizumab or* with *Eloctate* ITI alone. If a breakthrough bleed occurs during the trial, in the presence of an inhibitor, rFVIIa may be used as needed up to 4 times daily until resolution, per physician discretion. *FEIBA will not be allowed on the trial as it may result in thrombosis or thrombotic microangiopathy in patients taking Emcizumab*. If a breakthrough bleed occurs in the absence of an inhibitor (e.g. if it has resolved), either *Eloctate* (65 IU/kg) or rFVIIa (90 µg/kg) may be used to treat bleeds, per physician discretion.

The frequency and number of inhibitors that are tolerized (eradicated) will be analyzed in subjects by assigned treatment during the study; if a subject receives another clotting protein for any reason, he will be monitored and followed to 48 weeks and included in the primary intention-to-treat analysis. All bleeds and factor/drug use will be recorded in a patient diary. Height, weight, and a brief physical exam will be performed every month at routine study visits, and factor supply reviewed for each study subject, and, if necessary, reordered at that visit. Laboratory assays including anti-FVIII inhibitor by chromogenic Nijmegen modified Bethesda (CBA) assay, antigen-specific T cell assays (ELISPOT), hemophilia genotype, trough FVIII (chromogenic assay), and sample storage for future studies will be obtained at baseline and at week 4, 12, 24, 36, and 48. Hemophilia genotype and HLA will be performed at baseline only. All samples must be drawn before the study drug dose.

Post-Treatment Period: The post-treatment period begins after the last day of study follow-up at week 48 or when the inhibitor is eradicated. All clinical and lab data will be reviewed and entered on the INHIBIT Trial DCC Data Management System website through the end of the entire study period.

This is a randomized Phase III Clinical Trials Platform that includes 2 clinical trials, the **Prevention Trial** (PRO19040140) and the **Eradication Trial** (PRO19070800) in a rare population which utilizes the same HTCs, sampling time, volume, and frequency, visits, and lab assays to enhance trials efficiency. In the **Eradication Trial**, study drug will be initiated as early as the day of enrollment and randomization. Subjects will receive *Eloctate* ITI 100 IU/kg every other day plus weekly *Emicizumab* 1.5 mg/kg (after standard 3.0 mg/kg/wk x4 wk induction) or *Eloctate* ITI 100 IU/kg every other day alone. The doses of these drugs are consistent with standard of care guidelines for bleed eradication (immune tolerance). Subjects will receive the drug(s) to which they are randomized, preferably between 7 am and 9 am on days they are given and continued through study week 48. All bleeds and factor use will be recorded in the Subject Diary. This is an open-label trial, so subjects enrolled on the study will receive study drug by physician prescription. Baseline variables, all potential risk factors, including age, family history of inhibitors, race, and circumcision, will be collected prior to randomization and prior to initiating study drug. (Circumcision is commonly performed in over 50% of children with hemophilia in the U.S., but not an established risk for inhibitor formation).

*Bleeding Episodes Occurring during Study*

For breakthrough bleeds occurring during the study, *Eloctate* 65 IU/kg will be the treatment of choice for non-inhibitor or tolerized inhibitor patients (BU< 0.6 BU), while rFVIIa 90 mcg/kg will be the treatment of choice for inhibitor patients unresponsive to *Eloctate* or FVIII, per discretion of the treating physician (may be the study physician). The HTC coordinator will review study drug(s) supply with the patient/ parent/ caretaker to assure they are re-ordered when three doses are left. There will be no change in study visits.

*Subjects Developing the Primary Endpoint*

The primary endpoint will be eradication of an inhibitor, defined as anti-FVIII < 0.6 B.U. by chromogenic Nijmegen Bethesda assay (CBA). A local lab anti-FVIII < 0.6 B.U. will be repeated on this sample or, if insufficient, a repeat sample within 7 days, and a second sample obtained within 4 weeks for confirmation. A central lab anti-FVIII < 0.6 B.U. will be repeated within 4 weeks for confirmation. All subjects whose inhibitor is eradicated will remain on study follow-up to week 48. Additional tests will be done, per standard of care, including peak and trough to advise when ITI can be stopped and regular prophylaxis can begin. Subjects whose inhibitor is eradicated, i.e. anti-FVIII < 0.6 B will receive *Eloctate* with/without *Emicizumab*, per physician discretion, to treat or prevent bleeds: if bleeding occurs that is unresponsive to *Eloctate* with/without *Emicizumab*, rFVIIa will be used. FEIBA will not be allowed on this trial for safety reasons.

It is anticipated that 2-4 subjects will be enrolled locally, contributing to the overall study enrollment of 90 subjects at one of up to 41+ participating HTCs. The duration of the study is approximately 48 weeks per subject, with an expectation the study will last up to 7 or more years, subject to enrollment and completion of all procedures. For this study, all study visits will occur at the HTC.

**SCREENING VISIT, RANDOMIZATION, AND INITIATION OF ASSIGNED TREATMENT**
**Study Visit 1, Screening/ Baseline Visit, Wk 0**

This visit will take place prior to administration of *Eloctate* with/without *Emicizumab* in order to confirm eligibility requirements. Randomization and initiation of treatment may occur at this visit or within 4 weeks of the visit. The following tests or assessment will be performed at the Screening/ Baseline Visit.

- Study physicians will discuss the study with the patient or parent/caretaker of potential study subjects, answer questions and assure there is adequate time for the patient or parent/caretaker to decide about participation in the study. The patient or parent/caretaker may sign the consent or take the consent with them to discuss with family members and/or other members of their healthcare team. If the patient or the parent/ caretaker is interested in study participation, they may **sign the consent and they or the child considered enrolled in the trial**. No study procedures will occur until after the patient or parent/care giver has signed the consent document.
- Subjects currently receiving *Emicizumab* will be asked to discontinue the medication prior to randomization. If the subject or parent/caretaker is not willing to discontinue treatment, they will be deemed ineligible. If a subject discontinues *Emicizumab* prior to randomization, there is no need for a washout period and there are no known risks of stopping *Emicizumab.* It is possible that bleed frequency could increase if prophylaxis (eg. factor VIIa) is not given or infrequently given due to the more difficult intravenous route factor VIIa than *Emicizumab*. If a bleed occurs, factor VIIa will be given per physician discretion until it resolves and monitored by home treatment records, per standard of care.
- Demographics of the subject will include age, date of birth, race, and hemophilia genotype, if known.
- Other medical and surgical history of the subject will include height, weight, past bleeds, circumcision, and past factor treatment, dose and frequency.
- Hemophilia diagnosis by history, with historical laboratory test result for confirmation.
- Medication history including all concomitant medications within 4 weeks of the first dose of study drug.
- Brief physical exam including vital signs, height (cm) and weight (kg).
- Blood draw: A 3.8 ml (¾ tsp) blood sample will be collected for anti-FVIII chromogenic Nijmegen Bethesda (CBA) assay (plasma), antigen-specific (FVIII) T cell ELISPOT assay (PBMC), HLA and hemophilia genotype (buffy coat of the plasma sample), trough FVIII chromogenic activity (aliquot of plasma), and a sample (plasma) stored for future studies. All samples must be drawn prior to the study drug dose. Amounts of blood drawn will be less for children weighing less than 14 pounds. The Appendix detailing timing of visits and blood draws is attached at the end of this protocol.
- The samples (all de-identified) and labeled with subject ID number will be processed, frozen, and shipped to a repository in the Coagulation Laboratory at Vitalant, Pittsburgh PA, under the direction of Mr. Michael Meyer. The inhibitor titer will be run by chromogenic Nijmegen Bethesda (CBA) assay real time, with confirmation on stored aliquots for those testing negative (< 0.6 B.U.). An aliquot of plasma sample will be assessed for trough FVIII chromogenic assay (aliquot of plasma sample). The micriobiome (stool) sample will be shipped to the Vitalant Lab and transported to the microbiome, Vascular Medicine Institute (VMI), Pittsburgh, under the direction of Dr. Alison Morris. The PBMC sample will be snap frozen in dimethyl sulfoxide (DMSO) and stored in liquid nitrogen until batch testing for antigen-specific (FVIII) T cell responses by ELISPOT assay in the research laboratory of Dr. Kathleen Pratt, Uniformed Services of the Health Sciences (USUHS), Bethesda, MD. Samples for future testing (for hemophilia genotype or immunologic studies) will be stored indefinitely.

- Stool sample: A small stool sample will be collected at home to assess genes and bacteria in the gut (micriobiome) and placed in a small tube and returned in a container mailer to Vitalant, and couriered to VMI, Pittsburgh PA.
- After the study physician determines eligibility, the subject will be **randomly assigned to one of the two treatment strategies,** *Eloctate ITI* plus *Emicizumab* or *Eloctate ITI alone*.
- The patient or parent/caretaker will obtain *Eloctate* with/without *Emicizumab* by physician prescription through a clotting factor pharmacy of their choice. *Eloctate* will be administered by venipuncture, using a winged infusion needle (butterfly needle), heplock, or, if required, by central line per physician discretion, over 5-10 minutes. *Emicizumab* will be administered by subcutaneous injection, using a small tuberculin needle over 1-2 minutes. It is anticipated in children and some adults that all study drug doses will be administered in the Hemophilia Center, or, for those living a distance away from the HTC, at a nearby infusion center, emergency room, by patient or parent/ caretaker if trained, or by a visiting nurse.
- All doses of study drug(s), including day, date, time, and dose will be recorded in subject diaries. All bleeds, including day, date, site, and resolution will be recorded by the patient or parent/caretaker. All diaries will be returned to the HTC nurse coordinator at the monthly visits.
- Subjects will begin *Eloctate* with/without *Emicizumab*, with the first dose as early as the day of consent and randomization, should the subject already have received a prescription and supply of study drug as part of standard care; otherwise, study drug(s) may be started as soon as the prescription is received.
- The weekly dose of study drug, 100 IU/kg *Eloctate ITI* with/without 1.5 mg/kg *Emicizumab* (following the 3.0 mg/kg weekly x 4 weeks induction), will be begun following enrollment and screening. The HTC nurse will discuss study visit scheduling with the patient or parent /caretaker. The Screening/ Baseline visit, which may include randomization ant treatment initiation, will last approximately 1 hour.

**FOLLOW-UP VISITS:**

**Study Visit 2: Week 4 (Month 1) Follow-up Visit**

- Subject diaries will be reviewed for accuracy and completeness. Data will be extracted for frequency, type, and site of bleeds, including site, and for factor usage by day, date, time, and dose.
- Study drug(s) use will be reviewed and more *Eloctate* with/without *Emicizumab* ordered as needed.
- Concomitant medications will be recorded.
- Assessment for adverse events will be completed.
- Vital signs [blood pressure (BP), pulse (P), respiratory rate (RR), and oral temperature (ºC)] will be taken, and weight (kg) will be obtained.
- A 3.8 ml (¾ tsp) blood sample will be collected for anti-FVIII chromogenic Nijmegen Bethesda assay (plasma), antigen-specific (FVIII) T cell ELISPOT assay (PBMC), trough FVIII chromogenic activity (aliquot of plasma), and a sample (aliquot of plasma) stored for future studies (for hemophilia genetic or immunologic studies). All samples must be drawn prior to the *Eloctate* with/without *Emicizumab* dose.

**Study Visits 3, 5, 6, 8, 9, 11, 12: Monthly Follow-up Visits (Can be done remotely or in person)**

- Subject diaries will be reviewed for accuracy and completeness. Data will be extracted for frequency and type of bleeds including site, and for factor usage by day, date, time, and dose.
- Study drug(s) use will be reviewed and more *Eloctate* with/without *Emicizumab* ordered as needed.
- Concomitant medications will be recorded.
- Assessment for adverse events will be completed.

**Study Visits 4, 7, 10: Quarterly Follow-up Visits**

- Subject diaries will be reviewed for accuracy and completeness. Data will be extracted for frequency, type, and site of bleeds, including site, and for factor usage by day, date, time, and dose.
- Study drug(s) use will be reviewed and more *Eloctate* with/without *Emicizumab* ordered as needed.
- Concomitant medications will be recorded.
- Assessment for adverse events will be completed.
- Vital signs [blood pressure (BP), pulse (P), respiratory rate (RR), and oral temperature (ºC)] will be taken, and weight (kg) will be obtained.

  A 3.8 ml (¾ tsp) blood sample will be collected for anti-FVIII chromogenic Nijmegen Bethesda assay (plasma), antigen-specific (FVIII) T cell ELISPOT assay (PBMC), trough FVIII chromogenic activity (aliquot of plasma), and a sample (aliquot of plasma) stored for future studies (for hemophilia genetic or immunologic studies). All samples must be drawn prior to the *Eloctate* with/without *Emicizumab* dose.

**END-OF-STUDY VISIT**

**Study Visit 13: Week 48, End-of-Study Visit**

- Subject diaries will be reviewed for accuracy and completeness. Data will be extracted for frequency, type, and site of bleeds, including site, and for study drug(s) usage by day, date, time, and dose.
- Study drug(s) use will be reviewed.
- Concomitant medications will be recorded.
- Assessment for adverse events will be completed.
- Vital signs [blood pressure (BP), pulse (P), respiratory rate (RR), and oral temperature (ºC)] will be taken, and weight (kg) will be obtained.
- A 3.8 ml (¾ tsp) blood sample will be collected for anti-FVIII chromogenic Nijmegen Bethesda inhibitor assay (CBA) (plasma), antigen-specific (FVIII) T cell ELISPOT assay (PBMC), trough FVIII chromogenic activity (aliquot of plasma), and a sample (aliquot of plasma) stored for future studies (for hemophilia genetic or immunologic studies). All samples must be drawn prior to the *Eloctate* with/without *Emicizumab* doses.

The total blood volume for all tests is 22.8 ml (4½ tsp) during the 48-week study. Monthly blood sampling will be 3.8 ml (¾ tsp). Amounts of blood drawn will be less for children weighing less than 14 pounds. The Appendix detailing timing of visits and blood draws is attached on the last page of this protocol.

### 3.3 Data and Specimen Collection

Data Collection. Data will be collected by the study physician(s) and the HTC research nursing staff. All data required by the study protocol will be adequately documented in the source documents (e.g. hospital records, clinical and office charts, laboratory notes, memoranda, subjects' dairies, evaluation checklists, pharmacy dispensing records, lab assay results, copies or transcriptions certified after verification as being accurate copies, microfilm, magnetic media, subject files, pharmacy and laboratory records). Subject information will be captured and managed by electronic Case Report Forms via web-based electronic data capture by the INHIBIT DCC Data Management System. All information will be stored on their server in a secure database (housed in a locked room). No personal identifiers will be associated with the data. Subjects will be children or adult males of all ages with severe hemophilia A and a current or past high-responding inhibitor, anti-FVIII $\geq$5.0 B.U., who fulfill the inclusion and exclusion criteria, and are cared for at one of the up to 41+ participating HTCs. Prognostic factors will be collected at baseline, include age, race, family history of inhibitors, current or past emicizumab use, and past circumcision. Follow-up data will be collected through week 48, including adherence

(diary), bleeds (no., type, location treatment), factor use (dose, date), and if used, central line use or infection (time to, number). Data collected from subjects at baseline and follow-up visits will include demographic, medical, and health information, including site and frequency of bleeds, procedures, hospitalizations, study drug(s) usage, and other medications. Source data will be stored at the HTC in a locked file cabinet in a locked room.

Laboratory Specimens. A 3.8 ml (¾ tsp) blood sample, including a citrate (plasma) tube and a heparin (PBMC) tube will be drawn on all subjects at baseline, week 4, 12, 24, 36, and 48 of the trial. All samples will be drawn before administration of the study drug(s). All assays in the study are validated for small pediatric volumes, so total blood draw is 22.8 ml (4½ tsp) over the 48-week study for the anti-FVIII by chromogenic Nijmegen-modified Bethesda (CBA) assay, FVIII-specific T cell ELISPOT assay, trough FVIII (chromogenic assay), HLA, hemophilia genotype, and sample for storage (See Appendix, Table). De-identified plasma (citrate) blood samples will be processed, frozen, and shipped to a repository under the direction of Mr. Michael Meyer, Coagulation Laboratory, Vitalant, Pittsburgh PA. The inhibitor (anti-FVIII) by chromogenic Nijmegen Bethesda assay (CBA) will be run real time on citrate plasma samples, with confirmation on stored aliquots for those testing positive (anti-FVIII $\geq$ 0.6 B.U.). The hemophilia genotype and HLA type will be performed on buffy coat from the citrate sample at baseline only, and the FVIII trough by chromogenic assay will be run on an aliquot of the citrate sample. Samples for future testing will be stored up to 5 years after the end of the study. De-identified heparinized samples (PBMC) will be sent to the laboratory of Dr. Kathleen Pratt, Uniformed Services University of the Health Sciences (USUHS), Bethesda, MD. The PBMC sample will be snap frozen in dimethyl sulfoxide (DMSO) and stored in liquid nitrogen until batch testing for FVIII-specific T cell responses by ELISPOT assay. Samples for future testing (hemophilia genetic and immunology studies) will be stored indefinitely after the end of the study. Any part of subject blood samples that remains after the planned blood tests are done will either be destroyed or all identifiers and means of linking it back to the subject will be permanently removed prior to sending to repository for storage. The remaining sample will be used only for genetic and immunologic studies related to inhibitor development and hemophilia. The stool sample will be collected by patients at home, placed in a tube, and returned in a container to Vitalant, and transported locally to VMI, Pittsburgh PA.

### 3.4 Intervention

The intervention for subjects randomized to the Eradicate Trial is *Eloctate* ITI 100 IU/kg IV every other day plus *Emicizumab* 1.5 mg/kg SQ weekly (after 4-week induction at 3 mg/kg/week) vs. *Eloctate* ITI at 100 IU/kg every other day alone. *Eloctate* is an FDA-approved drug that was shown in clinical trials to be safe and effective in prevention and treatment of bleeds, with improved half-life and safety comparable to standard rFVIII with no differences in inhibitor frequency, thrombosis or allergic reaction. *Emicizumab* is an FDA-approved bispecific monoclonal antibody FVIII mimic that was shown in clinical trials to be safe and effective in the prevention of bleeds and is increasingly being used because of its simpler subcutaneous once weekly dosing. Parents, caretakers, or patients will review and provide written consent, after which subjects will be randomized by computer-generated randomization codes by the INHIBIT DCC Data Management System. Data will be stored in a secure database, housed in a locked room, which will track each drug prescription and infusion, including the initial dose within 24 hours of enrollment, and continued every other day for 48 weeks. *Eloctate* will be reconstituted by two-way needle to allow transfer of the diluent into the lyophilized powder and given by slow intravenous infusion over 5-10 minutes by heplock in an arm or hand vein; and Emicizumab will be given by subcutaneous injection by tuberculin syringe, which will allow transfer of the drug from the vial for injection over 1-2 minutes, in the abdomen, by HTC study nurse, visiting nurse, or trained parent/caretaker. *Elocate* and *Emicizumab* are covered by insurance, and prescriptions will be provided to patients/ parents/ caretaker by HTC MDs. Both drugs are FDA approved, *Eloctate, exempt under IND 15996, and/*or *Emicizumab* prophylaxis, as used in this trial, and thus, "exempt" from IND requirement**.**

### 3.5 Study Population

The Inhibitor Eradication Trial will enroll 90 adults and children $\geq$4 months of age with severe hemophilia A with high-responding inhibitors (anti-FVIII $\geq$ 5.0 BU) who are ITI-refractory or ITI-naïve, or developed on the Prevention Trial.

The Inclusion Criteria for the Eradicate Trial include children and adults $\leq$ 4 months of age; severe hemophilia A (FVIII < 0.01 U/ml); current or past high-responding inhibitor to FVIII (anti-FVIII $\geq$ 5.0 B.U.), or ITI-refractory or ITI-naive. Exclusion Criteria include acquired hemophilia or any bleeding disorder other than hemophilia A; use of an experimental drug(s); surgery anticipated in the next 48 weeks (except port placement); life expectancy less than 5 years; patient/parent/caretaker unable or unwilling to keep a personal diary of bleeding frequency and study drug treatment,

make monthly visits and blood draws; or other illness, condition, or reason in the opinion of the investigator that would make the patient unsuitable for the trial.

Our 2019 survey indicates there are 274 refractory inhibitor patients at 31 HTCs. When combined with 12 inhibitor patients from the Prevention Trial (8x25% plus 52x15% = 12), that is 25% of N=8 Eloctate-treated and 15% of N=52 Emicizumab-treated, for a total 4+8=12, we will achieve 90 inhibitor subjects (78+12) over the 6-year enrollment. With *only* 29% participating, we will enroll 78 refractory inhibitor patients plus 12 inhibitor patients from the Prevention Trial, we will enroll 90 severe hemophilia A inhibitor subjects over the 6-year enrollment period (Table 1).

**Table 1: 2019 Survey of 31 HTCs: Potential Subjects to Enroll on the Eradication Trial**

| Inhibitors in 6 yr Prevention Trial | Non-Trial Inhibitors at HTC | % of 6 yr Total for 6 yr Trial | Sample Size, Power for 6 Yr Trial |
|---|---|---|---|
| N=12 (8 x 25% = 4) + (52 x 15% = 8) | N = 274 refractory/untreated | 29% of 274 = ~78 in 6 yr | 90 (78 + 12) Inhib: β=0.80, α=0.05 |

### 3.6 Statistical Considerations

The purpose of the **Inhibitor Eradication Trial** is to compare novel agents, *Eloctate ITI* with or without *Emicizumab,* in the eradication of inhibitor formation. The results will be compared with hemophilia genotype, trough FVIII chromogenic activity, HLA, and FVIII-specific T cell response by ELISPOT assay from baseline, in order to determine correlation of each assay with inhibitor development over time by descriptive statistics.

Demographic and other baseline characteristics will be summarized using descriptive statistics or counts and percentages, as appropriate. Data to be tabulated will include, but not be limited to, age, race, height, weight, frequency and site of bleeds (hematoma, joint, CNS), emicizumab use, circumcision bleeding and/or treatment, surgery or procedures. All subjects enrolled in the study and who receive *Eloctate ITI* plus *Emicizumab* or *Eloctate ITI alone* will be included in the evaluation for safety. Data on treatment administration and adherence to assigned treatment will be presented. All subjects enrolled in the study and who receive *Eloctate ITI* with/without *Emicizumab* will be included in the evaluation for safety.

Safety measures will include the number and frequency of bleeding events (hematomas, joint, CNS bleeds) during the 48-week study, and the frequency and time to central line infections. Patient diaries will be reviewed monthly for bleeding frequency and site, and for dose and frequency of treatment. If breakthrough bleeds occur, additional treatment will be given at the discretion of the subject's physician, after which treatment may be continued at least weekly or escalated per physician discretion. Subjects will be closely monitored for adverse events, including allergic reactions, thrombosis, bleeding: these events will be summarized over time on study drug. Adverse events will be evaluated for frequency, relatedness, and severity.

Adverse events will be classified using CTCAE System, Vol 4.03, 2010. Tabulations of adverse events by frequency, relatedness, and severity will be presented. Serious adverse events, laboratory parameters, and vital signs will be presented using descriptive statistics for changes over time. Concomitant medications and physical exam data will be summarized over time.

The **Primary Endpoint** is the probability of becoming inhibitor-free by 48 weeks, where the eradication of inhibitor formation is defined as anti-FVIII < 0.6 B.U. by chromogenic Nijmegen Bethesda assay (CBA). We *hypothesize* that tolerance induction with *Eloctate ITI* plus *Emicizumab* is superior to *Eloctate ITI* alone in eradicating inhibitors in children and adults with severe hemophilia A and inhibitors. While both drugs may shorten ITI and even be synergistic, there is equipoise.

Analysis of the Primary Endpoint: We propose to take advantage of prior historical information on inhibitor formation to improve power on the Prevention Trial, and efficient use of inhibitors developing on the Prevention Trial (PRO19040140) to enroll in the Eradication Trial. For the **Eradication Trial,** we propose a Bayesian design and analysis plan to test if *Eloctate* ITI plus *Emicizumab* is superior to *Eloctate ITI* alone. Given the lack of data on either arm, we used two non-informative priors and 1:1 randomization allocation. Non-informative Bayesian priors were used for both arms as there are no published data regarding the time to inhibitor eradication with *Emicizumab* or Eloctate. The time to inhibitor eradication will be compared between the two assigned treatment arms by piecewise exponential survival models to determine the probability of being inhibitor-free at 48 weeks. Monte-Carol Markov-Chains (MCMC) were utilized to determine the posterior probability of the outcome when determining the operating characteristics. At the interim, once 75% of the subjects are randomized, the Bayesian posterior probability that either *Eloctate ITI plus Emicizumab* or *Eloctate ITI alone* is more likely to be inhibitor-free must be at least 99.5% to stop the trial. Assuming the trial did not stop at the interim, then to declare significance at the end of the trial, the Bayesian posterior probability that either arm, *Eloctate ITI*

*plus Emicizumab* or *Eloctate ITI alone* is more likely to be inhibitor-free at must be 97.9%. This maintains type 1 error at 5%, two-sided, with at least 80% power for superiority if we assume the true condition is that *Eloctate ITI plus Emicizumab* has an inhibitor-free rate of 95% and Eloctate ITI has an inhibitor-free rate of 80%.

Subjects reaching the primary endpoint: The primary endpoint is inhibitor eradication, defined as anti-FVIII < 0.6 B.U., by chromogenic Nijmegen Bethesda assay (CBA). A local lab anti-FVIII < 0.6 B.U. will be repeated on this, or if insufficient, a repeat sample within 7 days, and a second sample obtained within 4 weeks for confirmation. A central lab anti-FVIII < 0.6 B.U. will be repeated within 4 weeks for confirmation. If confirmed, subjects will either continue *Eloctate or rFVIIa* for treatment of bleeds, depending on the inhibitor titer per discretion of the subject' treating physician, but remain on study follow-up through week 48. For clarification, time to peak FVIII >60% will be determined at subsequent testing after confirmation of anti-FVIII < 0.6 B.U. Prognostic factors collected at baseline, including age, race, family history of inhibitors, current or past emicizumab use, and past circumcision, will be compared by treatment arm.

The **Secondary Endpoints** include a) safety measured by the number and frequency of bleeding events (hematoma, joint, CNS bleeds), the frequency and time to central line infections; and the number and frequency of allergic reaction, and thrombosis; and b) mechanism of inhibitor prevention by FVIII-specific T cell ELISPOT (Ig, RNA), performed on an aliquot of PBMCs; and genotype, HLA, and trough chromogenic FVIII drawn on the buffy coat of anti-FVIII plasma samples. ELISPOT assays will be performed on T cell populations depleted of regulatory T cells (T reg) (CD4+CD25+FoxP3+) to determine the role of T regs in controlling immune response to FVIII.

Analysis of Secondary Endpoints: The trial is not powered to analyze secondary endpoints statistically, but will collect these endpoints and perform descriptive statistics and correlations of safety and T cell mechanism by study arm, and with hemophilia genotype, HLA, and antigen-specific (FVIII) T cell responses (ELISPOT) from baseline, in order to determine correlation of each assay with inhibitor development over time by descriptive statistics.

Subjects reaching secondary clinical endpoints: The clinical secondary endpoint will include safety measures, including the number and frequency of bleeding events (hematomas, joint, CNS) during the 48-week study, and the frequency and time to central line infections. Patient diaries will be reviewed monthly for bleeding frequency and site, and for dose and frequency of treatment. If breakthrough bleeds occur, additional treatment will be given at the discretion of the subject's physician, after which treatment may be continued at least weekly or escalated per physician discretion. Subjects will be closely monitored for adverse events, including allergic reactions, thrombosis, bleeding: these events will be summarized over time on study drug. Adverse events will be evaluated for frequency, relatedness, and severity.

## 4.0 Human Subjects

### 4.1 General Characteristics - Minority Inclusion and Non-Discriminatory Statements

For the Inhibitor Eradication Trial, adults or children ≥ 4 months of age with severe hemophilia A and a current or past high-responding inhibitor to FVIII, anti-FVIII ≥ 5.0 B.U., are eligible to participate. Subjects will include those who develop inhibitors in the Prevention Trial or those who have been refractory or never tolerized at the same HTCs. As hemophilia is an X-linked congenital disorder, >98% of whom are male, it is anticipated that the study population will be male, and as almost 90% are Caucasian, it is anticipated that the majority of subjects will be Caucasian males. For the trial, subjects will be children or adults ≥4 months of age with severe hemophilia A, FVIII < 0.01 IU/ml), with current or past high-responding inhibitor to FVIII, anti-FVIII ≥ 5.0 B.U.

Currently there is no known effective approach to eradicate inhibitors, and they remain major complications of hemophilia. The trial will assess *Eloctate* ITI (tolerance induction) with or without *Emicizumab* to eradicate inhibitors, and rFVIIa will be used to treat bleeds. There will be no discrimination based on race or ethnicity (see Targeted/Planned Enrollment Table). The racial, gender and ethnic characteristics of the proposed subject population reflects the demographics of Pittsburgh, surrounding area, and the subject population of the University of Pittsburgh Medical Center. We shall attempt to recruit subjects in proportion to these demographics. All subjects will be screened prior to enrollment to confirm the diagnosis of severe hemophilia A, FVIII < 0.01 U/ml. Written, informed consent will be obtained from all subjects at the screening visit. Once eligibility is confirmed, visit 1 will be the start of enrollment. For the Eradicate Trial, 90 subjects (2-4 per site) will be enrolled.

### 4.2 Inclusion of Children:

The optimal approach to prevention and eradication of inhibitor formation in hemophilia is unknown. Among those

who develop inhibitors, 95% occur within 50 treatment exposures, usually in the first two years of life, but unless inhibitors are tolerized, they may last a lifetime, and thus affect children and adults of all age. Thus, the **Inhibitor Eradication Trial** is limited to male children or adults with severe hemophilia A and an inhibitor to factor VIII, anti-FVIII $\geq$ 5.0 B.U.

### 4.3 Inclusion/Exclusion Criteria

***Inclusion criteria:***

1. Children or adults $\geq$ 4 months of age.
2. Severe hemophilia A, FVIII < 0.01 U/ml.
3. Current or past high-responding inhibitor, anti-FVIII $\geq$ 5.0 B.U., ITI-refractory or ITI-naive.

***Exclusion criteria:***

Subjects will be excluded from study entry if any of the following exclusion criteria exist:

1. Acquired hemophilia or any bleeding disorder other than hemophilia A.
2. Unwilling to discontinue pre-trial *Emicizumab* before randomization.
3. Use of an experimental drug(s).
4. Surgery anticipated in the next 48 weeks (except port placement).
5. Life expectancy less than 5 years.
6. Patient/parent/caretaker unable or unwilling to keep a personal diary of bleeding frequency and study drug treatment, make monthly visits and blood draws.
7. Other illness, condition, or reason in the opinion of the investigator that would make the patient unsuitable for the trial.

### 4.4 Recruitment Procedures

It is anticipated that 2-4 subjects will be enrolled in the Eradication Trial at this study site. Patients or parents/caretakers of children with a diagnosis of severe hemophilia A and inhibitors will be approached for participation in the trial. Patients or parents will specifically be contacted during routine clinic visits to determine if they or their child are interested in participating in the study. There will be no cold-calling. The study physicians, as their clinical care doctors, see FVIII-deficient children at routine clinic visits and will determine the patient or parent/caretaker interest in study participation. If they are interested, the study physicians will discuss the study in further detail and review the informed consent. Each and every potential patient or parent/caretaker will discuss the study with their study physicians and will be encouraged to take time to decide on participation and ask questions. If a patient or parent/caretaker decides to take a consent form home for further viewing, they may contact a study nurse if they wish to participate, at which time; they will be scheduled again to visit/discuss/review consent with study physicians for enrollment. Discussion will include the purpose, safety issues, and risks and benefits of the study. The study physicians will answer any questions and will obtain the consent. No experimental procedures or interventions will occur until after informed consent is obtained. The investigator's certification statement will be signed at the time consent is obtained. If any new information occurs during the conduct of the study, the patient or the parent/caretaker who has provided consent will be informed and will be re-consented with this information at the next visit. A de-identified prescreening/screening log will be kept, and all reasons for exclusion documented in study source documents and screening log. Patients or parent/caretakers who read the consent form are free to refuse enrollment, and they will be free to withdraw their or their child's consent at any time. If patients or parents/caretakers wish to withdraw consent, they may do so by addressing a letter to the principal investigator.

Any data collected or blood samples drawn prior to the time of withdrawal will continue to be used, but no additional information or blood samples will be collected. Processed blood sample results will continue to be used for the research study; however, remaining samples will be destroyed or used as indicated by the patient or parent/caretaker's letter. The reason (e.g. AE, lost to follow-up, etc.) and date of withdrawal for all subjects withdrawn from this study will be recorded. Subject information will be captured and managed by study sites on electronic CRFs via the INHIBIT Trial DCC Data Management System. The sIRB may inspect the records of this study. Every effort will be made to keep the subject's personal medical data confidential.

Subject eligibility for the study will be determined after screening. Subjects are to use their own supply of *Eloctate or Emicizumab* during the study. The patients or parents/caretakers will sign informed consent and baseline labs will be

drawn, including anti-FVIII level, FVIII-specific T cell assays (ELISPOT), FVIII trough level, hemophilia genotype and HLA type. Subject enrollment and screening will be conducted by the local Investigator, in communication with the INHIBIT Trial DCC Data Management System. Subjects will be considered enrolled in the study after all assessments have been completed during the Screening period and just prior to *Eloctate or Emicizumab* administration. No subject may begin treatment prior to enrollment and assignment of a unique subject identification number. Any subject identification numbers that are assigned will not be reused even if the subject does not receive treatment.

**Demographic Chart: Targeted Enrollment for Study**

| **TARGETED/PLANNED ENROLLMENT: Number of Subjects** | | | |
|---|---|---|---|
| **Ethnic Category** | **Sex/Gender** | | |
| | **Females** | **Males** | **Total** |
| Hispanic or Latino | 0 | 3 | 3 |
| Not Hispanic or Latino | 0 | 87 | 87 |
| **Ethnic Category: Total of All Subjects *** | 0 | 90 | 90 |
| **Racial Categories** | | | |
| American Indian/Alaska Native | 0 | 0 | 0 |
| Asian | 0 | 4 | 4 |
| Native Hawaiian or Other Pacific Islander | 0 | 0 | 0 |
| Black or African American | 0 | 8 | 8 |
| White | 0 | 78 | 78 |
| **Racial Categories: Total of All Subjects *** | 0 | 90 | 90 |

* The "Ethnic Category: Total of All Subjects" must be equal to the "Racial Categories: Total of All Subjects."

Subjects are only enrolled after the Investigator has verified that they are eligible. Subjects withdrawn from the study after enrollment and prior to receiving *Eloctate with/without Emicizumab* will be replaced. A subject eligibility form will be provided for the Investigator to verify that the Inclusion and Exclusion Criteria have been satisfied for each subject. The site (HTC) coordinator will upload the screening information to the INHIBIT Trial DCC Data Management System. Following confirmation of the subject's eligibility along with the subject identification number, an accession number will be generated in the database. Once the subject identification number has been assigned, the subject may be randomized and then initiate the assigned treatment, *Eloctate with/without Emicizumab*, preferably in the morning.

### 4.5 Risk/Benefit Ratio

Risk to Subjects

There are risks associated with the study drug and having blood drawn. *Eloctate and Emicizumab* will be administered at the HTC, an outpatient infusion center, emergency room or at home by parent/caretakers/ or visiting nurses. Patients or parents/ caretakers will be asked to report any safety problems or side effects associated with *Eloctate or Emicizumab* administration.

*Risk of Discontinuing Emicizumab Prior to Randomization*

There are no known risks of discontinuing *Emicizumab*. If a bleed occurs, factor VIIa will be given per physician discretion until it resolves and monitored by home treatment records, per standard of care.

*Risk of Blood Drawing*

There may be discomfort with drawing blood, which is common, occurring in up to 25%, or 25 in 100 individuals: this may include pain, lightheadedness, fainting, bruising, or bleeding or infection in the tissue around the vein. This may be alleviated or reduced by applying pressure to the blood draw site for 5 minutes, and assuming a recumbent position (lying on the back). The risk of repeated blood draws is anemia, but this will be carefully monitored. Injections may rarely, in less than 1%, or less than 1 in 100 people, cause pain, soreness, redness, warmth, itching, numbness, tenderness, swelling, skin changes (discoloration, breakdown, or thickening), or swelling or lymph nodes near the injection site.

*Risk of Stool Collection*

There are no known risks of stool sample collection.

*Risk of Study Drug(s) Administration*

Treatment with *Eloctate or Emicizumab* may include minor pain or bruising at the infusion or injection site in up to 25%, or 25 in 100 individuals. A local anesthetic, Emla cream, may be used to minimize this discomfort. In young children, difficulty obtaining venous access due to small veins may require the placement of a central line. The insertion of a central line may reduce the discomfort of needle sticks and will be suggested at the discretion of the treating physician. Placement of central lines may cause bleeding, and thus additional factor treatment may be necessary. There is also the risk of anesthesia required for the line placement procedure. In addition, central lines may be complicated by infections that require hospitalization, antibiotics, and/or removal and replacement of the line. The insertion of a central line is part of clinical care and not required for this study.

*Risk of Inhibitor Development*

Inhibitor development occurs in 25-30% or more of individuals with hemophilia A (Factor VIII deficiency). Of those who develop inhibitors, 95% do so within 50 exposure days, usually in childhood after 10-20 exposure days, by the age of 2. Currently there is no known way to prevent or eradicate inhibitor formation. Study subjects will be followed closely to monitor this risk, and samples for inhibitor assessment will be taken at several time points in the study to monitor for this possibility during the study. Should an inhibitor occur, subjects may use *Eloctate or Emicizumab* for immune tolerance, and rVIIa for bleeding at the discretion of the patient's physician. Children and adults with current or past high-responding inhibitors, anti-FVIII $\geq$ 5.0 B.U., will be enrolled in this trial.

*Risk of Allergic or Anaphylactic Reaction*

Allergic-type reactions including anaphylaxis are rarely, if ever, reported for factor VIII products, including *Eloctate or Emicizumab*, and therefore, is expected to be rare, occurring in <0.001%, or less than 1 in 100,000 people. Symptoms may include chills, fever, nausea and vomiting, or rarely may include, in decreasing order of severity, death, anaphylaxis (life-threatening difficulty breathing), low blood pressure, heart beat irregularity, increase in body fluids, paresthesia (numbness or prickling sensation), urticaria (hives), chest tightness, rash, pruritus (itching), edema (swelling), fever, and/or chills. Should these symptoms occur, benadryl, a medication which reduces inflammation, or an epi-pen, which causes vasoconstriction and bronchial smooth muscle relaxation, may be given, with close monitoring of these symptoms. Benadryl may cause drowsiness, dizziness or low blood pressure. An epi-pen may cause tachycardia, palpitations, sweating, nausea, and anxiety. Subjects will be monitored for these symptoms. Subjects will be monitored closely for early symptoms and signs of hypersensitivity reactions, including hives, generalized urticaria, angioedema, chest tightness, dyspnea, wheezing, faintness, hypotension, tachycardia, and anaphylaxis. If any subject develops signs or symptoms of an allergic type reaction or anaphylaxis during the administration of *Eloctate or Emicizumab*, the infusion will be immediately be stopped and appropriate medical care initiated.

*Risk of Thromboembolism/Thrombogenicity*

Historically hemostatic agents including *Eloctate and Emicizumab* rarely, if ever, are associated with the development of thromboembolic complications. In five adult patients with inhibitors taking FEIBA (factor eight inhibitor bypass activity) with *Emicizumab* in a clinical trial, thrombosis-related events occurred, so for that reason **the use of FEIBA will not be allowed on this trial**, and only factor rFVIIa will be allowed for bypass treatment of bleeds. There is the unlikely possibility, < 0.001%, or less than 1 per 100,000 people, that *Eloctate or Emicizumab*, in the absence of FEIBA (aPCCs), could cause a clot, swelling, or inflammation in a vein. This risk will be very carefully monitored clinically. Should a clot occur, treatment would primarily consist of stopping the study treatment and/or removing the line in which it was given, if that is the source of the clot, as soon as possible. Should a bleeding episode occur during the study period, the subject will be treated with study *Eloctate* or, if an inhibitor is present, rFVIIa, per physician discretion.

*Risk of Bleeding*

Because individuals with hemophilia are enrolling on trials in which study drug(s) will be administered by infusion or injection, there is a risk of bleeding at the infusion or injection site. Contact us if this occurs or seek medical attention. In the event that bleeding at the infusion site cannot be stopped with pressure, it may be necessary to use a stitch or an adhesive material to stop the bleeding. The devices used to administer the stitch or adhesive may cause the following negative side effects: bleeding, a build-up of blood known as a hematoma, infection, allergic reaction, nerve injury, and swelling.

*Other Risks*

Other risks reported in clinical trials of *Eloctate and Emicizumab* but not different than in the general hemophilia population include malaise (feeling unwell) and joint aching in 2% (2 per 100 people); and in 1% (1 per 100 people) feeling hot or cold, blood vessel disease, high blood pressure, slow heart rate, skin rash, and cough.

*Risk of Genetic Testing*

There is the possibility that if the results of the research studies involving a subject's genetic material were to become generally known, this information could affect his ability to be insured, employed, or influence plans for future children, or have a negative impact on family relationships, and/or result in paternity suits or stigmatization. The biological sample or genetic material from subjects enrolled on this study may lead, in the future to new inventions or products. If new products were developed from the use of a subject's biological sample or genetic material, no monetary or other reward that might result from the development of the new product would be provided to any subject. Since the genetic testing for this study is done by a research laboratory, results from this study laboratory cannot be released.

*Risk of Inadvertent Disclosure*

Study participation and related data will be protected to maintain confidentiality. There is a possibility that the subject's personal information could become generally known. This information could impact future insurability, employability, or reproduction plans, or have a negative impact on family relationships, and/or result in paternity suits or stigmatization. In order to reduce risks of disclosure or breach of confidentiality, the research related documents, blood samples and clinical information stored in his/her research files will be assigned an alphanumeric (letters and numbers) identifier (that do not contain personal identifiers). For this study, a linkage key for linking this number and the subject's name will be kept at the HTC under lock and key by HTC physicians and the HTC research staff. Any publication arising from this study will not contain names or other identification unless study subjects grant permission in another signed consent.

*Potential Benefits*

The potential benefits include we will learn more about effective ways to eradicate inhibitor formation in adults and children. The subjects will be under close supervision during the study period. It is possible that administration of the *Eloctate or Emicizumab* weekly beginning before a bleed may shorten the time to tolerance in existing inhibitors in adults and children: however, this is currently not known, and is the purpose of this study. As both drugs prevent bleeds in patients with hemophilia, a potential benefit may be fewer bleeds: however, as bleeds may occur despite the use of these drug(s), treatment for break-through bleeding will be allowed at the discretion of the subject's physician.

Data Safety Monitoring Plan (DSMP)

The **study** will include the following requirements:

1. This study will identify, monitor, and report adverse events (AE) and unanticipated problems (UP).
2. **Expedited reporting to the sIRB** is required for unanticipated problems (UP) or unexpected serious adverse events (SAE) that may be related to the study protocol as follows:

Any event or problem that is **unexpected** AND **possibly, probably, or definitely related** to study participation AND one of the following:

- Is fatal, life-threatening, or serious (SAE + UP)……REPORT within 7 calendar days
- Suggests greater risk of harm to study participant(s) than…… REPORT within 30 calendar days was previously known or recognized

3. **Expedited SAE/UP reporting to the sIRB** should include study and grant number, PI, description of the event or problem, why it merits expedited reporting, dates the event was reported to sIRB, FDA and other governing bodies, and any corrective action planned or taken in response to the event or problem, e.g. study suspension, consent or protocol changes, additional training or security measures.
4. Reporting is required by the investigator to and following the guidance of any other applicable oversight bodies, but not limited to sIRB, DSMB and FDA. All communication from these oversight bodies regarding any applicable SAE/UP must be reported to sIRB according to the Data and Safety Monitoring Board.

The PI, Dr. Ragni, the Co-Investigators, Drs. Seaman, Xavier, Brooks, and Bertolet will be responsible for ongoing monitoring of all recruitment, data collection, and subject confidentiality procedures in the trial. They will meet at least bi-weekly to review all aspects of the study. Trial data, including all subjects enrolled, will be closely monitored by the PI and the clinical investigator team to ensure subject safety and to ensure that procedures are in place to maintain privacy and confidentiality, progress of study, integrity of the data, procedure reviews and for discussion of pertinent scientific literature or events which could affect the benefit to risk ratio. All serious and unexpected adverse events and/or major breaches of confidentiality will be reported to the sIRB according to regulations outlined in the IRB *Reference Manual for the Use of Human Subjects*. All AE's, SAE's generated from the HTC will be sent following reporting guidelines to sIRB. A report summarizing the above local and central DSMP activities will be submitted to the sIRB at the time of annual renewal.

Data will be reviewed on an ongoing basis by the Data Safety Monitoring Board (DSMB). DSMB members will have expertise in hematology, hemophilia, and biostatistics; and all will have voting rights. The DSMB will review data for all subjects enrolled in the study protocol and determine if the risk benefit ratio is sufficiently favorable that it is appropriate to continue the trial.

The events are:

- A subject develops anaphylaxis in association with administration of *Eloctate or Emicizumab*.
- A subject develops a thrombotic event in association with the administration prophylaxis with the exception of intravenous (IV) infusion site thrombophlebitis.
- A subject develops severe or catastrophic bleeding requiring prolonged and/or intense treatment exceeding study-related dosing.
- A subject develops a Grade 2 or greater allergic reaction in association with *Eloctate or Emicizumab*, defined as follows using the CTCAE grading.
    - Grade 2 Transient flushing, rash, or drug fever $\geq$ 38º C.
    - Grade 3 Symptomatic bronchospasm; with or without urticaria; parenteral medication(s) indicated; allergy-related edema/angioedema; hypotension
    - Grade 4 Anaphylaxis

In addition to halting enrollment and further treatment, such an event will be handled as a serious adverse event (SAE) and reported in an expedited time frame to the FDA. The data concerning the event and subject with input from the site HTC Co-Investigator will be reviewed by the DSMB along with all other available data to determine appropriate follow-up, with a decision to continue enrollment and treatment of subjects at that time. If the decision is made to discontinue the

study, the Co-Investigators will be notified and the appropriate final study evaluations (Last Visit) will be performed on all subjects enrolled in the study at that time.

Additionally, the following may also stop further subject enrollment and treatment.

- The DSMB warrants temporary suspension of enrollment for further review of data generated to date.
- The PI determines that a medically important event warrants further evaluation by the DSMB.

In these cases the required follow-up as determined by the PI and/or DSMB will be performed. The DSMB will determine if it is appropriate to reinitiate enrollment in the study. The sIRB will be informed of such decisions.

**Local Adverse Event Reporting**

All adverse events experienced by study subjects from the consent until 30 days after administration is to be recorded on the CRF, regardless of the severity of the event or its relationship to study treatment.

The serious AE reporting procedures are based on the "Common Terminology Criteria for Adverse Events" (CTCAE) v 4.03, June 14, 2010. Subjects will report to HTC co-investigator or to the HTC nursing coordinator, any AE or SAE. AE's will classified as mild (does not interfere with routine activities), moderate (interferes somewhat with routine activities), or severe (impossible to perform routine activities). The following algorithm will be used to assess the causality of all AE's:

- **Not related:** The event can readily be explained by factors not involving *Eloctate or Emicizumab*, and a temporal relationship with *Eloctate or Emicizumab*, does not exist.
- **Possibly related:** A clinical event, including laboratory test abnormality, with a reasonable time sequence to administration of *Eloctate or Emicizumab*, but which could also be explained by concurrent disease or other drugs or chemicals. Information on drug withdrawal may be lacking or unclear.
- **Probably related:** The temporal relationship between the administration *Eloctate or Emicizumab* is compelling, and the event cannot be explained by the subject's medical condition or other therapies.
- **Related:** The event follows a reasonable temporal sequence from administration of *Eloctate or Emicizumab*, follows a known or suspected response pattern to *Eloctate or Emicizumab*, is confirmed by improvement upon stopping the agent (dechallenge) and reappears upon repeated exposure to *Eloctate or Emicizumab*.

All AEs, regardless of severity, will be followed up by HTC Investigator until satisfactory resolution. All subjects experiencing AEs will be monitored until symptoms subside and any abnormal laboratory values have returned to baseline, or until there is a satisfactory explanation for the changes observed, or until death, in which case a full pathologist's report will be supplied, if possible. Withdrawal from the clinical study and therapeutic measures shall be at the discretion of the investigator.

As required by the University of Pittsburgh Institutional Review Board, if there is an unexpected, serious internal adverse event (life threatening or fatal) that is determined to be associated with *Eloctate or Emicizumab*, it will be reported to the sIRB within 24 hours. If the event is not serious, unexpected or related to either agent, it will be reported within 5 days. External adverse events that are unexpected, related to either agent, and determined to place the subject at greater risk than previously recognized will be reported within 10 working days of notification.

**Adverse Event (AE) Reporting at sites**

All events must be assessed to determine the following:

- If the event meets the criteria for an SAE
- The relationship of the event to study treatment
- The severity of the event

An AE is any untoward medical occurrence in a subject in whom a pharmaceutical product is administered and that does not necessarily have a causal relationship with this treatment. An AE can therefore be any unfavorable and unintended

sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a pharmaceutical product, whether or not related to the pharmaceutical product.

A serious adverse event (SAE) is any untoward medical occurrence that at any dose:

- results in death
- in the view of the Investigator, places the subject at immediate risk of death (a life-threatening event), but does not include an event that, had it occurred in a more severe form, might have caused death
- requires inpatient hospitalization or prolongation of existing hospitalization
- results in persistent or significant disability/incapacity, or
- results in a congenital anomaly/birth defect.

An SAE may also be any other medically important event that, in the opinion of the Investigator, may jeopardize the subject or may require intervention to prevent one of the other outcomes listed in the definition above. (Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or convulsions occurring at home that do not require an inpatient hospitalization.)

In this study, the following events are considered medically important and must be reported as SAEs:

- A subject develops anaphylaxis in association with administration of *Eloctate or Emicizumab*.
- A subject develops a thrombotic event in association with the administration of *Eloctate or Emicizumab*, with the exception of intravenous (IV) infusion site thrombophlebitis.
- A subject develops a Grade 2 or greater allergic reaction in association with administration of *Eloctate or Emicizumab* defined as follows using the CTCAE Evaluation.
    - Grade 2 Transient flushing, rash, or drug fever $\geq$ 38º C.
    - Grade 3 Symptomatic bronchospasm; with or without urticaria; parenteral medication(s) indicated; allergy-related edema/angioedema; hypotension
    - Grade 4 Anaphylaxis

Any SAE experienced by the subject from the time of dosing until 30 days after *Eloctate or Emicizumab* administration is to be recorded on an SAE Form, regardless of the severity of the event or its relationship to study treatment. SAEs must be reported to the PI. Any SAE ongoing when the subject completes the study or discontinues from the study will be followed by the Investigator until the event has resolved, stabilized, or returned to baseline status.

A serious pre-treatment event associated with the conduct of the study experienced by the subject after signing the ICF, but before administration of study treatment is to be recorded on the Serious Adverse Reaction (SAE) CRF and faxed (and electronically uploaded) to the PI within 24 hours of the study site staff becoming aware of the event.

In order to adhere to all applicable laws and regulations for reporting an SAE, the study site will formally notify the PI within 24 hours of the study site staff becoming aware of the SAE. It is the Investigator's responsibility to ensure that the SAE reporting information and procedures are followed. A death must be recorded on the appropriate CRF and electronic CRF (eCRF). All causes of death will be reported as SAEs.

**Reporting Information for SAEs**

Any SAE that occurs after any subject receives *Eloctate or Emicizumab* (Day 1) and any serious pre-treatment events must be reported to the PI within 24 hours of the study site staff becoming aware of the event. This report must be submitted regardless of whether or not the subject has undergone any study-related procedures or received study treatment and *regardless of severity or relationship to study treatment.* To report initial or follow-up SAE information and serious pre-treatment medical event information, enter the information in the CRF and web-based CRF. If the database is not available, fax a completed SAE form to the following or, if fax is not possible, call the number below to report the information. *Emergency Contact Numbers:*
**Fax: 412-209-7281**
**Phone: 412-209-7288 (daytime)**
**Phone: 1-888-990-1100 (evening and weekends)**

**Safety Classifications and Relationship of Events to Study Treatment**
The following definitions should be considered when evaluating the relationship of AEs and SAEs to the study treatment:

**Relationship of Event to Study Treatment**

| | |
|---|---|
| Unrelated | Any event that does not follow a reasonable temporal sequence from administration of study treatment *AND* that is likely to have been produced independently by the subject's clinical state or other modes of therapy administered to the subject. |
| Unlikely | Any event that does not follow a reasonable temporal sequence from administration of study treatment *OR* that is likely to have been produced by the subject's clinical state or other modes of therapy administered to the subject. |
| Possibly | Any reaction that follows a reasonable temporal sequence from administration of study treatment *OR* that follows a known response pattern to the suspected drug *AND* that could not be reasonably explained by the known characteristics of the subject's clinical state or other modes of therapy administered to the subject. |
| Related | Any reaction that follows a reasonable temporal sequence from administration of study treatment *AND* that follows a known response pattern to the suspected drug *AND* that recurs with re-challenge, *AND/OR* is improved by stopping the drug or reducing the dose. |

**Severity of Events**
The following definitions should be considered when evaluating the severity of AEs and SAEs:

**Severity of Event**

| | |
|---|---|
| Mild | Symptom(s) barely noticeable to subject or does not make subject uncomfortable; does not influence performance or functioning; prescription drug not ordinarily needed for relief of symptom(s) but may be given because of personality of subject. |
| Moderate | Symptom(s) of a sufficient severity to make subject uncomfortable; performance of daily activity is influenced; subject is able to continue in study; treatment for symptom(s) may be needed. |
| Severe | Symptom(s) cause severe discomfort; symptoms cause incapacitation or significant impact on subject's daily life; severity may cause cessation of treatment with study treatment; treatment for symptom(s) may be given and/or subject hospitalized. |

A prescheduled or elective procedure or a routinely scheduled treatment will not be allowed during the study period.

## 5.0 Costs and Payments

### 5.1 Research Study Costs

No costs will be incurred by the subjects or their parents/care providers for their participation in this study for research procedures. sIRB preparation costs will be covered, along with research procedures, including screening visits, blood draws for local and central laboratory assays, blood processing, shipping, and HTC charges and services related this study protocol. *Eloctate or Emicizumab* in this study will be the patient's own study drug. The budget is not designed to cover the cost of factor. Should a central line be suggested by the subject's physician, there will be no payment or compensation as this is considered standard clinical care. Research blood draw kits will be provided by the University of Pittsburgh coordinating center for use at local HTC's. Any other expenses for this study not listed above, will be paid by the local HTC.

### 5.2 Research Study Payments

Parents/care providers will receive compensation for their child's participation in this study, to help defray the cost of meals, travel, and time lost from work. There will be no additional costs to parents/caretakers for their child's participation in this study beyond the charges for routine medical care. The study is pending funding by HRSA. Compensation is based on study visits completed. Subjects will receive $40 per visit for up to 13 visits during the 48-week trial. If a subject completes all visits, the total amount will be $480.00. If a subject does not complete any part of the scheduled study days, compensation for missed visits will not be made. If, for whatever reason a subject completes part but not all of the study, the terms of payment will be determined by the number of visits completed.

## 6.0 Appendices

### 6.1 Qualifications of Investigators

Dr. Margaret Ragni is a Professor of Medicine, University of Pittsburgh, and Director of the Hemophilia Center of Western PA, and has conducted numerous clinical research studies at the University, investigator-initiated and in collaboration with the CDC, FDA, and pharmaceuticals. She is an expert in hemophilia and in the management of its complications, including AIDS and hepatitis, and hemophilia inhibitor formation.

Dr. Craig Seaman is an Assistant Professor of Medicine and Associate Director of the Hemophilia Center of Western PA. He has focused his work on von Willebrand disease, bleeding scores, VWF and cardiovascular disease and aging. He has published case studies of patients with VWD

Dr. Frederico Xavier is an Assistant Professor of Pediatrics and Associate Director of the Hemophilia Center of Western PA. He has worked with Dr. Ragni at the Hemophilia Center of Western PA to provide care for children with hemophilia and under her mentorship on numerous research studies in congenital hemostasis and thrombosis. Dr. Xavier has in depth experience as a pediatrician managing children and young adults with hemophilia, von Willebrand disease, and other congenital hemostasis and thrombosis disorders. He serves with as local investigator on this trial.

Debbie Vehec, Clinical Nurse, MSN, RN, will head the nursing and coordinating aspects of this trial. She has past experience in the initiation, monitoring, and coordination of clinical trials, including reporting adverse events, timely communication

Dana Ivanco, Regulatory Coordinator, is a clinical research regulatory coordinator who has had long experience in submission, revision, modification, and all aspects of regulatory protocol work, clinical trials operation, and has served as reviewer for the University of Pittsburgh IRB.

Dr. Maria Mori Brooks, lead DCC, is Professor of Epidemiology and Biostatistics and has over 25 years' experience with clinical trials, study design, coordination and statistical analysis for NIH-sponsored multicenter clinical trials. She is one of the Co-Directors of the Epidemiology Data Center, University of Pittsburgh.

Dr. Marnie Bertolet, lead statistician, is an Assistant Professor of Epidemiology and Biostatistics and has over 10 years' experience in the design and conduct of multicenter trials at the Epidemiology Data Center, University of Pittsburgh. She has expertise in Bayesian statistical methods and design.

Tamara Haller, Data Manager, is an experienced data manager at the Epidemiology Data Center, University of Pittsburgh, who has overseen data collection and data management for numerous federally-funded clinical trials and cohort studies.

Dr. Alison Morris is a Professor of Medicine University of Pittsburgh, Pulmonary, Allergy and Critical Care Disease, who heads the Microbiome Studies at Pitt and will coordinate samples and repository.

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## APPENDIX
## Schedule of Events

| **Table** | **Schedule of Events** | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Study Week** | 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 arms | **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 NBU chromogenic | **X** | **X** | | **X** | | | **X** | | | **X** | | | **X** |
| Hemophilia genotype* | **X** | | | | | | | | | | | | |
| HLA type* | **X** | | | | | | | | | | | | |
| FVIII chromogenic (trough)* | **X** | **X** | | **X** | | | **X** | | | **X** | | | **X** |
| T cell (ELISPOT, Ig, RNA)** | **X** | **X** | | **X**** | | | **X**** | | | **X** | | | **X** |
| Microbiome | **X** | | | | | | | | | | | | |
| Sample for storage | **X** | **X** | | **X** | | | **X** | | | **X** | | | **X** |

*Genotype, HLA are on buffy coat; **FVIII chromogenic is on aliquot of anti-FVIII; **ELISPOT is after 5 exposure days, in lieu of wk 12 or 24, if closer.

**Summary of changes to 08-08-20 protocol (clarification)**
**Protocol v1.0**

1. Page 12. Study population: clarified exclusion: surgery anticipated in the next 48 weeks (except port placement)
2. Page 15. Exclusion criteria: clarified exclusion: surgery anticipated in the next 48 weeks (except port placement)

**Summary of changes to 10-05-20 protocol (clarification)**
**Protocol v1.0**

1. Page 12. Study population: clarified exclusion: if current use of *Emicizumab,* known start date
2. Page 15. Exclusion criteria: if current use of *Emicizumab,* known start date

**Summary of changes to 10-05-20 protocol (clarification)**
**Protocol v2.0**

1. Minor corrections and clarifications throughout protocol
2. Adaptive design updated to Bayesian platform design throughout protocol
3. Page 1. Protocol Version and date updated
4. Page 2. Endpoints: updated to include microbiome stool sample
5. Page 4. Objective and Specific Aims: Addition of microbiome stool sample
6. Page 6. Specific Aim 2: Addition of microbiome stool sample
7. Page 6. Specific Aim 1 and 2: Clerical correction; *Emicizumab* and corrected to Eloctate
8. Page 8. Screening Period: Addition of microbiome stool sample
9. Page 12. Study population: clarified exclusion: if current use of *Emicizumab,* known start date
10. Page 15. Exclusion criteria: if current use of *Emicizumab,* known start date

**Summary of changes to 02-26-21 protocol (clarification)**
**Protocol v3.0**

- Page 10. Study Visits 3, 5, 6, 8, 9, 11, 12: Monthly Follow-up Visits: Added Can be done remotely or in person and removed: Vital signs [blood pressure (BP), pulse (P), respiratory rate (RR), and oral temperature (ºC)] will be taken, and weight (kg) will be obtained.