Phase III Multicenter, Randomized Controlled Inhibitor Prevention Trial, Comparing Eloctate (rFVIIIFc) and Emicizumab (Hemlibra) to Prevent Inhibitor Formation in Severe Hemophilia A:

The Inhibitor Prevention Trial

Short Title: The INHIBIT Prevention Trial

Protocol Number: PRO19040140

National Clinical Trial (NCT) Identified Number: NCT04303559

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PROTOCOL SUMMARY

Title: **The Inhibitor Prevention Trial (PREVENT):** A Phase III Multicenter, Randomized, Controlled Trial, comparing *Eloctate* (rFVIIIFc) vs. Emicizumab (Hemlibra) to Prevent Inhibitor Formation in Severe Hemophilia A.

Study Description: This is a prospective, multicenter randomized phase III 48-week clinical trial comparing rFVIIIFc (Eloctate (Eloctate®) vs. Emicizumab (Hemlibra®) in severe hemophilia A patients. Subjects will include previously untreated male children with severe hemophilia A (FVIII < 0.01 U/ml). We *hypothesize* that preemptive weekly prophylaxis with *Emicizumab* is non-inferior to *Eloctate* begun before the first bleed (excluding circumcision), in reducing or preventing inhibitor formation in children 4 months to 4 years of age with severe hemophilia A.

Objective: The **Primary Objective** is to determine if Emicizumab is no worse than Eloctate in preventing inhibitor formation in previously untreated patients (PUPs) with severe hemophilia A, with no more than 3 exposures to FVIII (FVIII, cryo, FFP) including circumcision, and no detectable inhibitor, anti-FVIII < 0.6 B.U.
The **Secondary Objectives** are to evaluate the safety and mechanism of Eloctate vs. Emicizumab prophylaxis in preventing inhibitor eradication.

Endpoints: The **Primary Endpoint** is inhibitor development, defined as the development of anti-FVIII $\geq$ 0.6 B.U. The anti-FVIII will be repeated will be repeated on the sample immediately, and a second sample obtained within 4 weeks to confirm the result. The **Secondary Endpoints** include a) *safety* by bleeding, measured by the number and frequency of bleeding episodes (i.e. hematoma, joint, CNS bleed), and time to port infections; and b) *mechanism* of *inhibitor suppression (prevention)* by FVIII-specific T cell ELISPOT, 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 microbiome stool sample, each compared with anti-FVIII.

Study Population: Study subjects will be male children age $\geq$ 4 months to 4 years of age with severe hemophilia A, defined as factor VIII < 0.01 IU/ml, with no more than 3 FVIII exposures (FVIII concentrate, cryoprecipitate, or FFP) including for circumcision. A total of 66 eligible subjects with severe hemophilia A will be enrolled in this 6-year trial.

Site Enrolling Subjects: Up to 41 Participating Hemophilia Treatment Center (HTCs).

Description of Study Intervention: There are two study interventions: Eloctate vs. Emicizumab.
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.

Trial Arms	
Arm A	Eloctate 65 IU/kg IV weekly for 48 weeks
Arm B	Emicizumab 1.5 mg/kg SQ weekly (after 3.0 mg/kg/wk x 4 wk induction) 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 Prevention Trial,** in which *Eloctate (rFVIII-Fc)* will be compared with *Emicizumab (Hemlibra)* using Bayesian platform design to prevent 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 Prevention Trial** is a 48-week randomized phase III trial, in which 66 previously untreated patients (PUPs) with severe hemophilia A will be enrolled. Subjects will include children from 4 months of age up to 4 years of age who have not been previously treated with clotting factor. Once enrolled, subjects who meet all the inclusion and none of the exclusion criteria will be randomized to *preemptive* weekly *Eloctate* (rFVIIIFc) vs. weekly *Emicizumab* (Hemlibra) to prevent inhibitor formation, defined as anti-FVIII $\geq$ 0.6 BU. 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 Prevention 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 Prevention Trial (PRO19040140) is linked to the Inhibitor Eradication Trial (PRO19070080), as part of the INHIBIT Clinical Trials Platform, with both trials will be conducted efficiently in the same hemophilia treatment centers (HTCs), with the same MDs, coordinators, visit frequency, blood sampling, and assays.

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Principal Investigator: Margaret V. Ragni, MD, MPH

Protocol Title: The Phase III Multicenter, Randomized, Controlled Inhibitor Prevention Trial, comparing *Eloctate* (rFVIIIFc) vs. *Emicizumab* (Hemlibra) to Prevent Inhibitor Formation in Severe Hemophilia A.

1.0 Objective and Specific Aims

The purpose of **the Inhibitor Prevention Trial** is to prevent inhibitor formation in children with severe hemophilia A. This 48-week phase III open-label, randomized, controlled trial, compares the recombinant factor VIII Fc fusion protein *Eloctate* (rFVIIIFc) vs. the bispecific monoclonal antibody FVIII mimic *Emicizumab* (Hemlibra), each given as weekly *preemptive* prophylaxis, to prevent inhibitor formation in children with severe hemophilia A. *Eloctate* is approved by the FDA to prevent and treat bleeds in children and adults with hemophilia A; while *Emicizumab* is approved by the FDA to prevent bleeds in children and adults with hemophilia A with and without inhibitors. In this trial, *Eloctate* and *Emicizumab* will be assessed in the prevention of inhibitor formation in children with severe hemophilia A who are previously untreated patients (PUPs). The research in this trial is considered greater than minimal risk as it involves initiating weekly dosing *before* the first bleed and obtaining special inhibitor studies. The primary efficacy endpoint will probability of inhibitor development by 48 weeks, defined as anti-FVIII $\geq$ 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 prevention, measured by FVIII-specific T cell ELISPOT assay, performed on peripheral blood mononuclear cells (PBMC) at baseline and at 4, 12, 24, 36, and 48 weeks. The B cell, Ig, and RNA assays will be performed on an aliquot of PBMCs. 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 formation after each subject completes the 48-week trial. The Inhibitor Prevention Trial is designed to test whether Emicizumab is non-inferior or superior to Eloctate in preventing inhibitor formation by 48 weeks. At the interim, once 75% of subjects are randomized, the Bayesian posterior probability that the *Emicizumab* arm is more likely to prevent inhibitor formation than the *Eloctate* arm must be at least 99% to stop the trial. Assuming the trial does not stop at the interim to declare non-inferiority at the end of the trial, the Bayesian posterior probability that *Emicizumab* is non-inferior to *Eloctate* in preventing inhibitor formation must be at least 95.7%. If non-inferiority is met, then to declare *Emicizumab* superior to *Eloctate* at the end of the trial, the Bayesian posterior probability that the Emicizumab arm is more likely to prevent inhibitor formation than the *Eloctate* arm must be at least 95.1%. This maintains type 1 error at 5%, one-sided, with 85% power for non-inferiority and 28% power for superiority if we assume that the true condition is that *Emicizumab* is 10% superior to *Eloctate* (85% vs. 75% 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 *prevent* inhibitors before 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.

Among those who develop inhibitors, 50% do so within the first 20 factor VIII exposures, and over 95% within the first 50 factor VIII exposures, suggesting there may be a potential window of opportunity to intervene to prevent inhibitor formation. To date, however, it has been difficult to identify those at risk who might benefit from inhibitor prevention efforts. Risk factors for inhibitor formation are well established, including patient-related (genetic) factors such as race (African American), family history, and genotype (large deletion mutations); and treatment-related (environmental) factors, especially high-intensity factor exposure at an early age. Despite this information and recently developed scoring systems, it has been difficult to identify those at risk early enough to target prevention efforts.

Equipoise: We are in a state of equipoise as the optimal approach to prevent inhibitor formation in hemophilia is not known. Studies of children with hemophilia have determined that when the first treatment with factor VIII (first "exposure") is for a severe bleed, surgery, or trauma, an inhibitor is more likely to form than when the first treatment is for prevention of a bleed. This is thought to be because severe bleeds, surgery, or trauma may trigger the immune system to make an antibody to any foreign protein, and the first factor VIII exposure occurs during a severe bleed, surgery, or trauma, the immune system may identify it as a "foreign" protein or threat ("danger") and make an antibody ("inhibitor") against it. This is called the "danger" theory (Matzinger). Yet, avoiding danger is not sufficient to prevent inhibitor formation as shown in previous studies of previously untreated children (PUPs) receiving rFVIII.

*Eloctate (*rFVIIIFc) is an FDA-approved FVIII protein that "fuses" recombinant FVIII (rFVIII) to IgG (globulin protein), prolongs FVIII half-life in the circulation. In human clinical trials, *Eloctate* provides longer protection against bleeds than standard rFVIII via a longer half-life than rFVIII. Further, Fc has been shown to induce tolerance to the hapten to which it is fused, in this case rFVIII: specifically, *Eloctate* induces regulatory T cells (T regs) which promotes tolerance to rFVIII. In hemophilia A mice receiving weekly *Eloctate*, the rate of inhibitor formation was significantly lower than in mice given standard factor VIII, i.e. full length rFVIII (Advate) or B-domain deleted rFVIII (Xyntha) (Liu). In clinical trials, while *Eloctate* is safe and effective in adults, adolescents, and children (Powell), inhibitors still develop in ~25% of 89 previously untreated patients (PUPs) receiving *Eloctate* prophylaxis (A-LONG trial), similar to prospective studies in rFVIII-treated PUPs, 28-30% (Gouw). To date there has been no randomized comparison between these agents, in part because this is a rare population and there is waning interest in use of rFVIII in young children because it requires three infusions per week. Taken together, these findings suggest the potential utility of an inhibitor prevention approach that employs a once weekly infusion that avoids initial factor exposure at the time of a bleed ("danger") by avoiding bleeds/ surgery/ trauma, *and* potentially promotes FVIII tolerance.

Emicizumab (Hemlibra) is an FDA-approved bispecific monoclonal antibody that has been shown to be safe and effective in phase III trials in hemophilia A with (Oldenburg) or without inhibitors (Mahlangu) and is increasingly being used because of its simpler subcutaneous route. Whether *Emicizumab* may reduce inhibitor formation by avoiding FVIII exposure in the vulnerable first 50 exposure days in PUPs is not known; however, breakthrough bleeds (hematomas, hemarthroses) during *Emicizumab* prophylaxis that requiring FVIII may increase "danger" and potential inhibitor formation, and potentially negating its potential benefit.

For these reasons, we will study if *Emicizumab* is noninferior to *Eloctate* in <u>preventing</u> inhibitor formation in PUPs when begun *before* FVIII given for a bleed or surgery or trauma *(preemptive)* and continued weekly to prevent bleeds (*prophylaxis).* We *hypothesize* that avoiding danger, prolonging FVIII half-life, and promoting T reg tolerance will reduce or prevent inhibitor formation. The concept of initiating *Eloctate* in children *before* they have a bleed is innovative, as it advances the "avoidance of danger" concept. Dosing once weekly addresses the greatest drawback to current prophylaxis regimens requiring 3-4 times weekly infusion for prophylaxis in children, namely difficult access and need for central lines or ports. The concept of avoiding FVIII exposure during the vulnerable first 50 exposure days by the non-factor hemostatic agent, *Emicizumab*, may be important in reducing inhibitor formation, and its effectiveness in preventing bleeds in hemophilia with or without inhibitors makes it a potentially powerful agent in the management of hemophilia: yet, the potential for breakthrough bleeds may complicate its potential use.

Specific Aim 1: To compare weekly *Emicizumab* and *Eloctate* in preventing anti-FVIII inhibitor formation when each is initiated before the first bleed in previously untreated children (PUPs) with severe hemophilia A in the Prevention Trial. We will conduct this multi-center, randomized phase III clinical trial at 31 (up to 40) HTC sites, with the same study visit frequency, blood sampling, and lab assays as in the companion Eradication Trial (PRO19070080). The primary endpoint is the development of anti-FVIII antibody ≥ 0.6 BU by chromogenic Nijmegen-modified Bethesda assay (CBA) by 48 weeks.

Hypothesis 1: We *hypothesize* that *Emicizumab is non-inferior to Eloctate* in preventing inhibitors, anti-FVIII ≥ 0.6 B.U. (CBA), in children with severe hemophilia A, when each is given weekly for 48 weeks, beginning before the first bleed, in the absence of "danger." Yet, there is equipoise, as *Eloctate* induces Tregs and promotes FVIII tolerance and may be *superior* to *Emicizumab* in inhibitor prevention; but as *Emicizumab* avoids FVIII exposure, it may be *superior* to *Eloctate*, *unless* breakthrough bleeds occur that require FVIII, i.e. "danger," and trigger an immune activation and inhibitor formation, making *Emicizumab inferior* to *Eloctate.* However, as *Emicizumab* is given subcutaneously, if it were *non-inferior* to *Eloctate* in reducing inhibitors, it would be the "winner" over *Eloctate.*

Specific Aim 2: To evaluate the safety and mechanism of inhibitor *prevention* in patients with severe hemophilia A. Secondary endpoints include a) *safety,* measured by number and frequency of bleeding episodes (i.e. hematomas, hemarthroses) and port infections; and b) *mechanism of inhibitor prevention*, measured by FVIII-specific T cell ELISPOT assay; FVIII genotype; HLA type; and chromogenic FVIII trough levels; and by microbiome 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 prevent inhibitor formation is compelling, and, if successful, will eliminate a major complication of hemophilia treatment and the associated morbidity, hospitalization, cost, and early mortality, and be adopted immediately into clinical practice.

The Inhibitor Prevention trial is a 48-week outpatient trial conducted in 66 PUPs from up to 41+ Hemophilia Treatment Centers (HTCs) (1-4 subjects locally). Case report forms and laboratory assessment forms will be completed and uploaded to a web-based database. While a landmark study established three times weekly prophylaxis as *standard* prophylaxis dosing, based on reduction in joint bleeds and improved joint range of motion, only 46% of surveyed HTC physicians initiate prophylaxis at this dose and frequency. In fact, 32% prescribe once-weekly prophylaxis, with escalation for break-through bleeds, similar to the published Canadian experience (Feldman), thereby avoiding invasive central lines and attendant infection risk. Although inhibitor frequency was lower, 8%, with the latter approach, the frequency of joint bleeds was higher, 19.6% vs. 12.5% at 6 months. Further, most centers wait to initiate prophylaxis until *after* the first bleed. A survey of HTCs physicians indicates that 54% of physicians use a once- or twice-weekly prophylaxis regimen in young children, beginning *after* the first bleed (Ragni). Despite these small studies, effective inhibitor prevention 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, *preventing* inhibitors *before* 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 prevent inhibitor formation in severe hemophilia A. The Inhibitor Prevention Trial is innovative as it uses a Bayesian platform design to compare novel agents in a randomized phase III trial. The trial will incorporate historical data on inhibitor formation (*Bayesian priors)*, which allows for more efficient use of rare subjects and improved power. Subjects from the Prevention Trial who develop inhibitors, along with pre-existing refractory inhibitor patients at the same HTCs, can be enrolled in the Eradication Trial (PRO 19070080) to compare immune tolerance induction 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 prevention 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 surgery. In this trial, subjects will use *Eloctate* or *Emicizumab* to prevent bleeds, which is within the current standard of care guidelines in which clotting factor is begun before or after 1-2 bleeds, per physician discretion, to prevent bleeds (prophylaxis). 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: 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 and 105 mg per 0.7 ml is available for children: a tuberculin syringe will allow transfer of the drug from the vial for injection over 1-2 minutes. The study drugs 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 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, including dates, times, and doses administered, and families will maintain a diary to record the same information.

3.2 Research Design

This is a 48-week outpatient, randomized phase III clinical trial, that employs a Bayesian platform design to conduct the Prevention Trial in patients with severe hemophilia A, FVIII < 0.01 IU/ml. 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 Prevention Trial is linked to the Inhibitor Eradication 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 **Prevention Trial** is a randomized phase III open-label trial designed to evaluate the efficacy of *preemptive* weekly prophylaxis with *Eloctate* vs. *Emicizumab* to prevent inhibitor formation in children $\geq$ 4 months of age up to 4 years with severe hemophilia A. Approximately 1-4 previously untreated children with severe hemophilia will be recruited at each HTC. The study drugs will be initiated prior to the first bleed and administered to each subject for up to 48 weeks. *Eloctate* will be given by weekly intravenous infusion of 65 IU/kg by weekly intravenous infusion over 5-10 minutes. *Emicizumab* will be given weekly subcutaneous injection of 1.5 mg/kg SQ weekly (after a 4-week induction at 3.0 mg/kg/wk x 4 weeks) over 1-2 minutes. For treatment of breakthrough bleeds, for safety reasons, additional *Eloctate* doses will be given, after which study drug dosing will be continued at least weekly and escalated per physician discretion, as is standard of care per a recent HTC survey. The treating physician may be the study physician. 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 formation. 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 assay (CBA), 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 two treatment regimens, the treatment period will extend for up to 48 weeks, beginning with weekly *Eloctate or Emicizumab* prophylaxis begun *before* the first bleed. If a break-through bleed occurs during the trial, *Eloctate* will be given per standard of care to all subjects: in those on the *Eloctate* arm, this may lead to escalation of the dose from once to twice weekly as in typical practice, per physician discretion; whereas in subjects on the *Emicizumab* arm, Emicizumab dosing remains unchanged as it is not a treatment but rather a prophylaxis to prevent bleeds. If a subject develops an inhibitor, either *Eloctate* (65 IU/kg), in the case of a low-responding inhibitor, or *rFVIIa* (90 µg/kg), in the case of a high-responding inhibitor, will be used to treat bleeds, at physician discretion. *NOTE: FEIBA (factor VIII inhibitor bypass activity) will not be allowed on these trials as it may result in thrombosis or thrombotic microangiopathy in patients taking Emicizumab.*

The frequency and number of new (developing) inhibitors 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 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 assay CBA), 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 subject develops an inhibitor. 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 phase III randomized controlled clinical trials, the **Prevention Trial** (PRO1904140) and the **Eradication Trial** (PRO19070800) in severe hemophilia A which utilize the same HTCs, sampling time, volume, and frequency, visits, and lab assays to enhance trials efficiency. In the **Prevention Trial**, all subjects will be enrolled *before* no more than 3 FVIII exposures (FVIII in concentrate, cryoprecipitate, or FFP) including circumcision. Subjects will be randomized to receive weekly *Eloctate* 65 IU/kg or weekly *Emicizumab* 1.5 mg/kg (after standard 3.0 mg/kg/wk x4 wk induction) beginning *before* the first bleed. The doses of these drugs are consistent with standard of care guidelines for bleed prevention (prophylaxis) per physician discretion. Subjects will receive the drug to which they are randomized weekly, preferably between 7 am and 9 am, beginning *before* a bleed and continued weekly 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. (NB: Circumcision is performed in over 50% of hemophilic children in the U.S. and is 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, and escalated, per physician discretion, from once weekly to twice weekly or three times weekly prophylaxis for bleeds. In subjects who develop an inhibitor (anti-FVIII $\geq$ 0.6 BU), once confirmed, treatment will be based on the type inhibitor: in those developing a low-responding inhibitor (defined as no inhibitor increase after FVIII dosing), *Eloctate* is the treatment of choice for breakthrough bleeds; however, for those unresponsive to *Eloctate,* or for those who develop a high-responding inhibitor (defined as inhibitor increase, i.e. anamnestic response, after FVIII dosing), rFVIIa 90 mcg/kg will be the treatment of choice for breakthrough bleeds, per physician discretion. The HTC coordinator will review study drug(s) supply with the parent/ caretaker to assure it is 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 development of an inhibitor, defined as anti-FVIII ≥ 0.6 B.U. (chromogenic Nijmegen Bethesda assay, CNA). 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 developing inhibitors will remain on study follow-up to week 48. At the time an inhibitor is detected, the subject's treating physician (who may be the study physician) is encouraged to obtain an inhibitor test locally, if possible. The treatment approach will be at physician discretion: once the anti-FVIII is confirmed positive, subjects will be able to enroll on the Eradicate Trial, or receive, at physician discretion, immune tolerance induction (ITI) with *Eloctate* or other FVIII product, to suppress the inhibitor; and rFVIIa for bleeds. FEIBA will not be allowed on this trial for safety reasons.

It is anticipated that 1-4 subjects will be enrolled locally, contributing to the overall study enrollment of 66 subjects at one of up to 41+ participating HTCs (hemophilia treatment centers). 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* or *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 parent/caretaker to decide if their child will participate 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 parent/ caretaker is interested in the child participating in the study, they may **sign the consent and the child will be enrolled in the trial**. No study procedures will occur until after the 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*. If a bleed occurs, factor VIII (FVIII) 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 on the last page of this protocol.
- The samples (all de-identified) and labeled with study 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 positive (≥ 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 Vascular Medicine Institute (VMI) in 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 (microbiome) 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* or *Emicizumab*.
- The parent/caretaker will obtain *Eloctate* or *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 of 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 that all study drug doses may be administered in the Hemophilia Center, or, for those living a distance away from the HTC, at a nearby infusion center, emergency room, by 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 parent/caretaker. All diaries will be returned to the HTC nurse coordinator at the monthly visits.
- Subjects will begin *Eloctate* or *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, 65 IU/kg Eloctate; and 1.5 mg/kg *Emicizumab* (following the 3 mg/kg weekly x 4 week-induction), will be begun following enrollment and screening, *before* the first bleed. The HTC nurse will discuss study visit scheduling with the parent/caretaker. The Screening/Baseline/Randomization and Treatment Initiation visit 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* or *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 (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* or *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* or *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* or *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 (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* or *Emicizumab* dose. Preferably, the ELISPOT assay will be drawn after 5 exposure days, rather than week 12 or week 24, whichever is closer in time.

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* or *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 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 male children, ≥ age 4 months up to 4 years, with severe hemophilia A, 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, 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). 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 ≥ 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 Prevent Trial is *Eloctate* 65 IU/kg IV weekly or *Emicizumab* 1.5 mg/kg sq (after 4-week induction at 3 mg/kg/week) beginning before the first bleed. *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, to begin within 24 hours of enrollment, and continued weekly 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/ caretakers 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 Prevent Trial will enroll 66 PUPs with severe hemophilia A, FVIII <0.01 IU/ml, ≥ 4 months of age up to 4 years and no anti-FVIII antibody (anti-FVIII < 0.6 B.U.). The age chosen was based on our 2010 survey found that the median age at first bleed is 5.5 months, and 72% remain bleed-free at 4 months.

Inclusion Criteria for the Prevent Trial include male children ≥ 4 months and up to 4 years of age, severe hemophilia A (FVIII<0.01 U/ml), no evidence of an inhibitor (anti-FVIII < 0.6 B.U.), and no more than 3 exposures to FVIII, including FVIII for circumcision (factor VIII concentrate, cryoprecipitate or fresh frozen plasma). 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; 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 420 infants with severe hemophilia A are expected to be born in the next 6 years at 31 HTCs. With *only* 16% participating, we will enroll 66 severe hemophilia A PUPs over the 6-year enrollment (Table 1).

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

Sev Hemophilia A Births (2016-18)	No. Births In 6 yr	% of 6 yr Total to Enroll in 6 yr Trial	Sample Size, Power for 6 yr Trial
N = 210/ 3 yr	N = 420 pt/ 6yr	16% of 420 = ~ 66 in 6 yr	66 PUPs: β=0.80, α=0.05

3.6 Statistical Considerations

The purpose of the **Inhibitor Prevention Trial** is to compare novel agents *Eloctate* and *Emicizumab* in the prevention 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. Data on treatment administration and adherence to assigned treatment will be presented. All subjects enrolled in the study and who receive *Eloctate* or *Emicizumab* will be included in the evaluation for safety. 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 time to the development of an inhibitor, defined as antiFVIII ≥0.6 B.U. (CBA). We *hypothesize* that preemptive weekly prophylaxis with *Emicizumab* is non-inferior to *Eloctate* begun before the first bleed (excluding circumcision), in reducing or preventing inhibitor formation in children ≥ 4 months up to 4 years of age with severe hemophilia A. The goal is to test the hypothesis that early treatment with *Emicizumab* is noninferior to *Eloctate* in reducing inhibitor formation. The primary analysis will be conducted according to the intention-to-treat principle. Sensitivity analyses will include a per protocol analysis of patients who initiated the assigned treatment regimen.

Analysis of the Primary Endpoint: We propose to take advantage of prior historical information on inhibitor formation to improve power on the Prevention Trial. We propose a Bayesian design and analysis plan to test if *Emicizumab* is non-inferior to *Eloctate* by a 10% non-inferiority margin. Interim data presented by the PUPs A-Long trial (Königs, EAHAD, 2019) was used to inform a prior distribution for the *Eloctate* arm which incorporates the previously observed *Eloctate* data along with appropriate uncertainty to acknowledge these "borrowed" data were not observed concurrently in our study. This prior conceptually borrows the equivalent of 10 events (new inhibitors) occurring in our proposed study, allowing us to adjust our randomization ratios to assign more subjects to the lesser-studied *Emicizumab* arm, using 1:3 *Eloctate* to *Emicizumab* randomization. A non-informative Bayesian prior will be used for the *Emicizumab* arm as there are no data published regarding inhibitor formation with this drug. The time to inhibitor formation will be compared between the two assigned treatment arms analyzed using 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 by operator characteristics. One interim analysis is planned to occur after 75% of the subjects are randomized. The trial will declare early success if *Emicizumab* demonstrates superiority to *Eloctate* at the

interim. Otherwise, the trial will continue to the final analysis for non-inferiority. Extensive simulations were performed to set thresholds such that the type 1 error rate is maintained and power is maximized while recruiting 66 subjects. At the interim, once 75% of the subjects are randomized, the Bayesian posterior probability that *Emicizumab* is more likely to prevent inhibitor formation than *Eloctate* must be at least 99% to stop the trial. Assuming that the trial does not stop at the interim, in order to declare non-inferiority at the end of the study, the Bayesian posterior probability that *Emicizumab* is non-inferior to *Eloctate* in preventing inhibitor formation at the end of the study, must be 95.7%. If non-inferiority is met, then to declare superiority at the end of the trial, the Bayesian posterior probability that *Emicizumab* is more likely to prevent inhibitor formation than *Eloctate* must be at least 95.1%. This maintains the type 1 error rate at one-sided 5%, while having 85% power for non-inferiority and 28% power for superiority if we assume the true condition is that *Emicizumab* is 10% superior to *Eloctate* in prevention of inhibitor formation (85% vs. 75% inhibitor-free rates).

Subjects reaching the primary endpoint: The primary endpoint is inhibitor development, defined as anti-FVIII $\geq$ 0.6 B.U., by chromogenic Nijmegen Bethesda assay (CBA). A local lab anti-FVIII $\geq$ 0.6 B.U. will be repeated on this, or if insufficient 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 $\geq$ 0.6 B.U. will be repeated within 4 weeks for confirmation. If confirmed, and a low-responding inhibitor has developed, subjects will use *Eloctate* for breakthrough bleeds; if confirmed and a high-responding inhibitor has developed, *rFVIIa* will be used for breakthrough bleeds, per discretion of the subject's treating physician: but, in either case, subjects will remain on study follow-up through week 48. For clarification, all detectable inhibitor levels will be repeated for confirmation. 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 ad time to central line infections; and the number and frequency of allergic reactions, 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 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.

4.0 Human Subjects

4.1 General Characteristics - Minority Inclusion and Non-Discriminatory Statements

For the Inhibitor Prevention Trial, children $\geq$ 4 months of age up to 4 years with severe hemophilia A, FVIII < 0.01 U/ml, with no previous bleed requiring factor treatment (other than circumcision) and who are cared for at the Hemophilia Center, are eligible to participate. 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 limited to children $\geq$ 4 months up to 4 years of age, with no previous bleeding requiring treatment, before the time of the first treatment. We anticipate 90% of subjects will be 12 months of age or younger and 10% over 12 months up to 4 years or more, and are limited to children, as inhibitor formation occurs in >95% within the first 50 exposure days to factor VIII treatment, typically within the first 2 years of life. Despite the benefits of *standard* prophylaxis three-times weekly to prevent joint bleeds and joint damage, the risk of inhibitor formation remains.

Currently there is no known effective approach in prevention of inhibitors, and this remains a major complication of hemophilia. The Prevention Trial will assess the effectiveness of *preemptive* weekly prophylaxis with *Eloctate or Emicizumab* begun *before* the first bleed (excluding circumcision). These agents will be given as part of clinical care to prevent 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 Prevent Trial, 66 subjects (1-4 locally), 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. Thus, inhibitor formation is primarily a problem in children, while inhibitor eradication is a problem of those who have already developed inhibitors, including children and adults. Thus, the trial is limited to male children with severe hemophilia A.

4.3 Inclusion/Exclusion Criteria

Inclusion criteria:

1. Male children $\geq$ 4 months up to 4 years of age.
2. Severe hemophilia A, FVIII < 0.01 U/ml.
3. No evidence of an inhibitor, i.e. anti-FVIII < 0.6 BU.
4. No more than 3 FVIII exposures (factor VIII concentrate, cryoprecipitate, or fresh frozen plasma), including circumcision.

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. 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 1-4 subjects will be enrolled in the Prevention Trial at this study site. Parents/caretakers of children with a diagnosis of severe hemophilia A, cared for at the HTC will be approached for participation in the trial. Parents/caretakers 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 doctor sees FVIII-deficient children at routine clinic visits, and will determine the parent/caretaker interest in their child's study participation. If they are interested, their study physicians will discuss the study in further detail and review the informed consent. Each and every potential parent/ caretaker will discuss the study with their study physician and will be encouraged to take time to decide on participation and ask questions. If a 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 the consent with their study physicians for enrollment. Discussion will include the purpose, safety issues, and risks and benefits of the study. Their 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 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. Parent/caretakers who read the consent form are free to refuse enrollment, and they will be free to withdraw their child's consent at any time. If 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 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 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.

Demographic Chart: Targeted Enrollment for Study

TARGETED/PLANNED ENROLLMENT: Number of Subjects			
Ethnic Category	**Sex/Gender**		
	Females	**Males**	**Total**
Hispanic or Latino	0	2	2
Not Hispanic or Latino	0	64	64
Ethnic Category: Total of All Subjects *	0	66	66
Racial Categories			
American Indian/Alaska Native	0	0	0
Asian	0	3	3
Native Hawaiian or Other Pacific Islander	0	0	0
Black or African American	0	6	6
White	0	57	57
Racial Categories: Total of All Subjects *	0	66	66

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

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 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 does not receive treatment.

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 or 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 the assigned treatment initiated, *Eloctate or 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. 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 VIII 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 other FVIII product for immune tolerance, and rVIIa for bleeding at the discretion of the subject's physician. Children with inhibitors will not 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 stopped immediately, 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 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, 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 occurs, rFVIIa, per physician discretion.

Risk of Bleeding

Because individuals with hemophilia are enrolling on a trial in which study drug(s) will be administered by infusion or injection, there is a risk of bleeding at the infusion or injection site. Subjects should contact their physicians if this occurs and/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 the HTC study physicians and the 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 prevent inhibitor formation in 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 decrease inhibitor formation in young 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 local **Data Safety Monitoring Plan** for this trial 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 was previously known or recognized…… REPORT within 30 calendar days
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 IRB according to the Data and Safety Monitoring Policy.

The PI, Dr. Ragni, the Co-Investigators, Drs. Seaman and 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 clinical research 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 statistics, 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 previously untreated subject (PUP) develops a positive Bethesda assay result (anti-FVIII $\geq$ 0.6 B.U.) whether at the local laboratory or the central laboratory.
- 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 "Cancer Therapy Evaluation Program: 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 be 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 a positive (chromogenic Nijmegen Bethesda) inhibitor assay result ($\geq$ 0.6 B.U.) whether at the local laboratory or the central laboratory.
- 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 the end of study, 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 study drugs. 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/caretakers 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 funded 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 long 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 stool microbiome samples.

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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 sample	**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 from 07-01-20 to 08-08-20
Protocol v1.0

1. Page 1. A front page with the INHIBIT Logo, protocol number and NCT number and other identifying information was added to page 1.
2. Page 2. A one-page summary of the protocol was added on page 2.
3. Page 3. Typeset was changed to Calibri Body 11 font, with margin justification throughout, and pagination was switched for ease to the right lower border.
4. Page 3. Abstract, lines 1, 2: Specific drugs to be compared are now included.
5. Page 4-6. Secondary endpoints were revised to specify a precise endpoint, CNS bleed, rather than life-threatening bleed, and use of phrase inhibitor formation rather than term, inhibitor-free, for consistency throughout the protocol, data forms; and redundant phrases were removed.
6. Page 7. The word "caretaker" is now used consistently throughout the protocol.
7. Page 8. Clarifications were made in wording, grammar, terminology for consistency throughout the protocol. Protocol team discussions agreed that up to three bleeds would be allowed, including circumcision, as otherwise impractical to enroll on study.
8. Page 8. The protocol team clarified the primary and secondary endpoints that are now consistent throughout the protocol, including the inclusion of patients if they had no more than 3 FVIII exposures (including FVIII in concentrate, cryoprecipitate, or fresh frozen plasma), including circumcision. This is standardly used in PUP protocols and acknowledges how difficult it is to enroll PUPs on trials with no previous exposure. Up to three exposures is generally viewed as not having impact on inhibitor occurrence.
9. Page 9. Visits were labeled to assure accurate reflection of visit events. Dimethyl sulfoxide is correctly abbreviated "DMSO" for PBMC studies throughout the protocol.
10. Page 13. The table was resized to read more easily. For consistency, patients who will enroll will be age $\geq$ 4 months up to 4 years, and is specified throughout the protocol, including inclusion criteria, Section 3.5 and 4.3 (below). The reasons a subject might be unsuitable are now consistently listed in the exclusion criteria only.
11. Page 13-14. Details of the primary and secondary endpoints are now clarified with input of statisticians on the protocol team.
12. Page 15. Section 4.3 is revised as stated above for Section 3.5 (see 10. above). A sentence was added regarding data capture, electronic data forms, and management.
13. Page 16. The terms site (HTC), randomization, and random assignment, and assigned treatment are clarified.
14. Page 19. Members of the Data Safety Monitoring Board (DSMB) are now more explicitly listed by expertise in hematology, hemophilia, and statistics, all with voting rights.
15. Page 23. Qualifications of investigators: Since Debbie Vehec has joined as the main clinical nurse coordinator, to take the place of Julie Sheline who has left the center.

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 8. Screening Period: Addition of microbiome stool sample
8. Page 12. Study population: clarified exclusion: if current use of *Emicizumab,* known start date
9. 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.