

Protocol

This trial protocol has been provided by the authors to give readers additional information about their work.

Protocol for: Zuraw BL, Busse PJ, White M, et al. Nanofiltered C1 inhibitor concentrate for treatment of hereditary angioedema. *N Engl J Med* 2010;363:513-22.

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LEVP2005-1: CHANGE Trial (C1-Inhibitor in Hereditary Angioedema Nanofiltration Generation evaluating Efficacy): A Double-blind, Placebo Controlled, Clinical Study to Investigate the Efficacy and Safety of Purified C1 Esterase Inhibitor (Human) for the Treatment of Hereditary Angioedema (HAE) in Acute Attacks and as Prophylactic Treatment to Prevent HAE Attacks

1. SYNOPSIS

Name of Sponsor Lev Pharmaceuticals, Inc		
Name of Finished Product C1INH-nf Currently no trade name		
Name of Active Ingredient C1 esterase inhibitor		
Investigators 12-20 investigators.		
Study centers 12-20 study sites.		
Study period Part A Proposed start date: February 1, 2005 Proposed end date: December 31, 2006 Part B To begin after Part A	Phase of development Phase III	
Objectives This study consists of two parts to investigate the efficacy and safety of C1INH-nf for the treatment of HAE in acute attacks and as prophylactic treatment to prevent HAE attacks.		
Methodology Part A The results of the study will be evaluated according to the statistical methods after 70 randomizations occur. Part B The results of the study will be evaluated according to the statistical methods after 24 subjects complete the study.		

Number of subjects

Part A

A total of 124 subjects will be enrolled in part A of the study and 70 subjects will be randomized in order to achieve 68 evaluable subjects.

Part B

A total of 24 subjects screened in Part A will be enrolled into Part B in order to achieve 20 evaluable subjects. These 24 subjects will be selected based on having relatively frequent angioedema attacks (at least 2/month on average).

Design and main criteria for inclusion

Part A

Part A will be a Phase III multi-center, randomized, placebo-controlled, double-blind trial to confirm the efficacy and safety of C1INH-nf as a therapeutic agent for acute attacks of HAE.

Part B

Part B will be a Phase III multi-center, randomized, placebo-controlled, double-blind cross-over trial to confirm the efficacy of prophylactic C1INH-nf in preventing acute attacks of angioedema in HAE patients.

Inclusion Criteria:

- Age \geq 6 years
- Documented HAE based on 1) evidence of a low C4 level plus either a low C1INH antigenic level or a normal C1INH antigenic level and a low C1INH functional level; or 2) evidence of a low C4 level plus a known HAE-causing C1INH mutation
- Normal C1 level
- Signed informed consent

Exclusion Criteria:

- Age $<$ 6 years
- Low C1 level
- B-cell malignancy
- Presence of an anti-C1INH autoantibody
- History of allergic reaction to C1INH or other blood products
- Narcotic addiction
- Current participation in any other investigational drug study or within in the past 30 days

- Participation in a C1 esterase inhibitor trial, received blood or received a blood product in the past 90 days
- Pregnancy or lactation
- Any clinically significant medical condition, such as renal failure, that in the opinion of the investigator would interfere with the subject's ability to participate in the study

Test product dose and mode of administration, batch number

Dosage Form for Active Drug: Powder for solution for intravenous injection, 500 U/vial to be dissolved in 5 ml sterile water for injection.

Dosage Form for Placebo: Matching saline (5 ml) for injection.

Route of Administration: Intravenous.

Dosing Regimen:

Part A: 1000 U per episode; a second dose of 1000 U may be given if the subject has not responded to the initial treatment within 60 minutes (active drug or placebo).

Part B: 1000 U two times per week (active drug or placebo).

Open Label: 1000 U per episode; a second dose of 1000 U may be given if the subject has not responded to the initial treatment within 60 minutes (active drug).

Batch Number: To be determined.

Duration of treatment

Part A

Part A will consist of the treatment for a single acute attack.

Part B

Part B will last for a total of 24 weeks. Each subject will be randomized either to receive 12 weeks of C1INH-nf followed by 12 weeks of placebo, or 12 weeks of placebo followed by 12 weeks of C1INH-nf.

Open Label

One open label treatment will consist of the treatment for a single acute attack.

Criteria for evaluation

Efficacy

Part A

The primary efficacy endpoint of Part A will be the time to the beginning of unequivocal relief of the defining symptom following initial treatment (Time 0).

Part B

The primary efficacy endpoint for Part B will be the number of attacks of angioedema during each treatment phase, using each subject as his/her own control.

Safety

Safety will be assessed (in Part A, Part B and after open label treatment) by the number and severity of adverse experiences and drop outs (Part B), and by examining treatment failures, changes in clinical laboratory safety parameters, physical findings and vital signs from pre to post infusion.

Statistical methods

Part A

Primary treatment comparison of the primary endpoint will be done by a log-rank test. Part A will have more than 80% power to detect a reduction by 50% in the median time to relief of the defining symptom between the treatment and placebo groups.

Part B

The study design provides more than 90% power to detect the treatment effect assuming angioedema attack rates will be 1 every 2 weeks in the placebo phase and 1 per 12 weeks in the prophylactic C1INH-nf phase. The analysis will be based on a Poisson assumption; a test for extra-Poisson variation will make use of generalized linear interactive modeling (GLIM). Should there be evidence of such variation, a GLIM model with a term that incorporates such variation will be used to make treatment comparisons.

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2. OVERALL STUDY AIMS

2.1 General

This study consists of two parts, both of which will investigate the efficacy and safety of a purified C1 esterase inhibitor (C1INH-nf) for the management of hereditary angioedema (HAE). Investigators and subjects may choose to participate in both parts or Part A alone. Subjects must have a history of angioedema attacks ≥ 2 /month in order to participate in Part B.

Throughout this protocol, "C1INH-nf" will be used to refer to the product under investigation and "C1INH" will be used to refer to the protein itself.

Part A will be a Phase III multi-center, randomized, placebo-controlled, double-blind trial to confirm the efficacy and safety of C1INH-nf as a therapeutic agent for acute attacks of HAE. The primary efficacy endpoint of Part A will be the time to the beginning of unequivocal relief of the defining symptom following treatment. Secondary efficacy endpoints will be the presence or absence of unequivocal beginning of relief of the defining symptom within 4 hours following treatment as well as the time to complete resolution of the attack, and the ability of C1INH-nf to raise C1INH and C4 levels.

Part B will be a Phase III multi-center, randomized, placebo-controlled, double-blind cross-over trial to confirm the efficacy of prophylactic C1INH-nf in preventing acute attacks of angioedema in HAE patients. The primary efficacy endpoint of Part B will be the number of attacks of angioedema during each treatment phase, using each subject as his/her own control. Secondary efficacy endpoints will be number of subjects dropping out at each stage, quality of life, average severity of attacks, the average duration of attacks, number of open label C1INH-nf infusions, and C1INH and C4 levels.

Safety will be assessed (in Part A, Part B and after open label treatment) by the number and severity of adverse experiences and drop outs (Part B), and by examining treatment failures, changes in clinical laboratory safety parameters, physical findings and vital signs from pre to post infusion.

Reserve laboratory samples will be collected in this study and will be used in the event that there is insufficient material to rerun a test, and may also be used for future scientific study of HAE pathogenesis or treatment where no patient identifying information is associated with the sample.

2.2 Rationale

Heterozygous deficiency of C1INH leads to the clinical disease known as HAE. This disease is characterized by attacks of non-itching swellings of the skin or mucosa. These swellings in general last for 2 to 3 days after which they resolve. Acute attacks of angioedema may be life-threatening if sites like the larynx are affected, and are often associated with significant morbidity during gastrointestinal attacks. Hence, HAE attacks require prompt treatment, often in an emergency room. Administration of C1INH-nf might also be warranted as prophylactic treatment. Administration of C1INH products purified from pooled plasma from normal donors has become routine clinical practice to treat attacks of HAE in countries where the therapy is available.

2.3 Objective

This study is divided into two parts to investigate the efficacy and safety of purified C1INH-nf: 1) for the treatment of HAE in acute attacks and 2) as prophylactic treatment to prevent HAE attacks.

2.4 Part A Hypothesis

This is a superiority trial to demonstrate that C1INH-nf treated subjects have shorter median time to relief of the defining symptoms compared to placebo. The null hypothesis for this study is that the difference in the time to beginning of relief for the defining symptom of an eligible attack of angioedema in HAE subjects treated with either C1INH-nf or placebo will be zero. The alternative hypothesis will be that the time to relief is different in subjects treated with C1INH-nf compared to placebo.

2.5 Part B Hypothesis

This is a superiority trial to demonstrate that subjects treated with prophylactic C1INH-nf have fewer attacks of angioedema compared to placebo. The null hypothesis for this study is that the difference in the total number of attacks of angioedema an HAE subject experiences during the period when he/she is receiving prophylactic C1INH-nf treatments compared to the period when he/she is receiving placebo treatments will be zero. The alternative hypothesis will be that the subject will experience a different number of angioedema attacks during the placebo treatment period compared to the C1INH-nf treatment period.

3. BACKGROUND

The syndrome of HAE is characterized by recurrent, acute, local circumscribed edema of the skin or mucosa (1). Acute attacks of HAE are frequently preceded by trauma or emotional stress but also occur in the absence of any recognized precipitating event (1). The frequency of attacks during pregnancy is variable, but often increases during the first trimester and decreases during the third trimester (2, 3). The therapeutic management of HAE is primarily based on long-term prevention with attenuated androgens (4). Thus testosterone has been shown to effectively prevent acute attacks of HAE (5). Although androgen derivatives effectively prevent acute attacks, they cannot be used to treat them because they require at least 1-2 days before they begin to be effective (6). Furthermore, use of danazol or stanozolol is associated with numerous side effects including: secondary hypoestrogenism in women, weight gain, acne, gastrointestinal disturbances, decrease in libido, depression, sleep disorders, tremor, dizziness, muscle spasm, and occasionally biochemical or other evidence of liver injury (7-9). These side effects are dose-related. The treatment of pregnant women and children presents particular difficulties at the current time. Androgens may interfere with normal sexual maturation, and drug effects on the fetus are unknown. Therefore pregnant women are normally not treated with androgens, despite the fact that they often experience a significant increase in angioedema attacks during the first one or even two trimesters of pregnancy. This situation represents an extremely difficult issue in the care of these patients. Because of similar concerns, the use of androgen derivatives in children is controversial.

Patients who are refractory or intolerant of androgen derivatives may be prophylactically treated with the antifibrinolytic drugs, epsilon aminocaproic acid (EACA) or tranexamic acid (10-12). These drugs, however, are not always effective and are associated with an increased risk of thrombosis. Therefore in a significant number of cases, HAE patients are either untreated, under-treated, or suffer from undesirable side effects from therapy. Yet, acute attacks of angioedema may be life-threatening if sites like the larynx are affected (13), and are often associated with significant morbidity during gastrointestinal attacks.

Therapy based on substitution with C1INH, the plasma protein which is deficient in patients suffering from HAE, was developed for the control of acute attacks (14-16). C1INH concentrates are prepared from pooled human plasma. The efficacy of purified C1INH concentrates in the treatment of acute angioedema in HAE has been described (17-20), and the concentrate is approved for treatment of HAE in Europe. The great advantage of purified C1INH concentrates is that it becomes effective virtually immediately following intravenous infusion. To the extent that efficacy data are available, a difference in outcome between treatment and control group is strikingly apparent (14, 21).

The use of C1INH concentrates has thus far proven to be safe. The only major drawback of plasma-derived concentrates has been the risk of transmission of viral agents. Although of uncertain clinical significance, hepatitis G virus has been reported to be transmissible by C1INH concentrates (22). During the past years a variety of viral inactivation methods have been developed to eliminate this risk. The C1INH concentrate to be used in this study, C1INH-nf, is pasteurized and nanofiltered in the manufacturing process, and these methods have been validated as effective measures for removing viruses that may be present in plasma.

Dosing Rationale

A standard dose of 1000 U C1INH-nf will be used in this study. This dosing regimen is based on both extensive clinical experience in Europe as well as published information predicting the level of C1INH required to abort attacks of angioedema. As is the practice in Europe, a repeat dose of 1000 U C1INH-nf will be administered if a subject fails to respond to the initial dose within 60 minutes.

A retrospective study was performed to identify the time to complete relief of symptoms in 35 HAE subjects who received Cetor (C1INH-Sanquin) in the Netherlands. Three patients received 500 U, 29 patients received 1000 U, 2 patients received 1500 U and 1 patient received 2000 U. The mean time to complete relief was 145 minutes among the entire group of 35 patients; among the 29 patients treated with 1000 U the mean and median times to complete relief were 140 and 120 minutes respectively. Although time to beginning of relief was not collected for these subjects, it is undoubtedly much shorter.

Data are also available on the time to beginning of relief from 206 attacks of HAE treated with 1000 U of Tim3 (C1INH-Baxter/Immuno) in Italy. Patients reported beginning of relief within 60 minutes of treatment in 199 of the 206 attacks treated with 1000 units of Tim 3. Among the 7 patients with attacks that did not report beginning of relief within 60 minutes, 1 responded in 120 minutes, 5 responded in 180 minutes, and 1 failed to respond. Taken together, the data demonstrate that the overwhelming majority of HAE patients treated with 1000 U of C1INH show rapid clinical improvement. Furthermore, no evidence of a more rapid response was seen with higher doses of C1INH. These studies, therefore, provide a strong rationale for the dosing schedule used in this study.

Because the studies summarized above were open label studies, it is important to ascertain how these results might compare to a placebo controlled blinded study. Kunschak *et al* (21) performed a randomized, double-blind study of C1INH (Tim 3-Baxter/Immuno) versus placebo. They showed that the mean and median times to beginning of improvement in 11 patients treated with placebo were 921 and 1,020 minutes, respectively. Thus, it is clear that the rapid improvement reported in the open label studies is not likely due to a placebo effect. The treated subjects in this study reported mean and median times to beginning of improvement of 162 and 50 minutes respectively. One difference that should be noted is that the dosing scheme used by

Kunschak *et al* was 25 units/kg, which is different than the dosage to be used in this study.

Finally, Spath *et al* (23) demonstrated that a functional C1INH level >40% of normal is sufficient to prevent attacks of angioedema. Pharmacokinetic analysis of Cetor demonstrates that a dose of 1000 U will increase C1INH levels by 21.3% in a 75 kg subject. Since baseline functional C1INH levels are typically around 20% in HAE patients, the dose of 1000 U should increase the C1INH level sufficiently to end the attack, and a second dose (if necessary) will certainly accomplish this.

4. STUDY DESIGN

This is a multi-center, phase III study conducted in two parts with an open label treatment option:

- Part A includes a randomized, placebo-controlled, double-blind treatment to confirm the efficacy and safety of C1INH-nf as a therapeutic agent for acute attacks of HAE
- Part B includes a randomized, placebo-controlled, double-blind cross-over treatment to confirm the efficacy and safety of prophylactic C1INH-nf in preventing acute attacks of angioedema in HAE subjects
- Part A and Part B include an open label treatment option

4.1 Study Population

The study will take place at approximately 12-20 study sites in the United States and 1 study site in Israel. The number of subjects anticipated to be enrolled at the site in Israel is 3, and the total number of subjects expected to take part in Part A of the study is 124. Seventy subjects will be randomized to achieve 68 evaluable randomized treatments. Only subjects with HAE will be eligible; subjects with acquired C1INH deficiency may not participate.

A total of 24 subjects screened in Part A will be enrolled into Part B of the study to achieve 20 evaluable randomized treatments. These subjects will be selected based on their having relatively frequent angioedema attacks (at least 2/month on average).

4.2 Selection Criteria for Enrollment

4.2.1 Inclusion Criteria

Inclusion criteria are:

- Age \geq 6 years
- Documented HAE based on 1) evidence of a low C4 level (C4 < 14 mg/dL; normal range 14 to 40 mg/dL, lab code 8171 from Mayo Clinical Trial Services) plus:
 - a) A low C1INH antigenic level (C1INH < 19 mg/dL normal range 19 to 37 mg/dL, lab code 8198 from Mayo Clinical Trial Services)

OR

- b) A normal C1INH antigenic level (C1INH \geq 19 mg/dL) and a low C1INH functional level (functional C1INH < 41% abnormal, 41-67% equivocal abnormal, > 67% normal, lab code 81493 from Mayo Clinical Trial Services)

OR

- c) A known HAE-causing C1INH mutation
- Normal C1 level
 - Signed informed consent

4.2.2 Exclusion Criteria

Exclusion criteria are:

- Age < 6 years
- Low C1q level (C1q < 12 mg/dL; normal range 12 to 22 mg/dL, lab code 8851 from Mayo Clinical Trial Services)
- B-cell malignancy
- Presence of an anti-C1INH autoantibody (C1INH free autoantibody > 15.7% of standard; normal = 2.3 to 15.7 % of standard, Test Name "CEIAP Free" from National Jewish Medical and Research Center or C1INH bound autoantibody >35% of standard, normal = 0 to 35% of standard, Test Name "CEIAP Bound" from National Jewish Medical and Research Center)

- History of allergic reaction to C1INH or other blood products
- Narcotic addiction
- Current participation in any other investigational drug study or within the past 30 days
- Participation in a C1 esterase inhibitor trial, received blood or received a blood product in the past 90 days
- Pregnancy or lactation
- Any clinically significant medical condition, such as renal failure, that in the opinion of the investigator would interfere with the subject's ability to participate in the study

4.3 Part A

Part A is a Phase III multi-center, randomized, placebo-controlled, double-blind trial comparing the efficacy and safety of C1INH-nf concentrate with placebo, in subjects with HAE, during acute episodes of angioedema involving the genitourinary tract, abdomen, or face. No participating subject may be randomized more than once during Part A. Following the randomized C1INH-nf treatment, the subject may be eligible (but not required) to enter Part B.

A Time and Events Table of required study assessments and safety observations for Part A is contained in Section 11.

4.3.1 Part A Eligibility and Screening Visit

Screening procedures

Prior to screening evaluations, each subject eligible for the study will be given an orientation session in which the nature and purpose of the study and the risks and benefits of C1INH-nf will be described. If an eligible subject agrees to participate in the study, he/she or the subject's parent or legal guardian will be required to sign an informed consent form.

Screening evaluations include:

- Review selection criteria
- Review medical history
- Review medication history, including any past participation in a clinical trial investigating a medical treatment for HAE

- Subject symptom rating
- Record history of HAE attack frequency
- Physical examination including vital signs (blood pressure, pulse, and respirations)
- Quality of life assessment (SF-36 or SF-10 for children under 14 years old)
- Screening blood samples and reserve samples (see schedule of laboratory tests)

Each subject will be given a symptom diary and instructed to record all angioedema episodes, whether treated or not. Subjects must be able to evaluate symptoms of HAE, even if a minor or a ward.

4.3.2 Randomization Criteria

The following are the criteria for randomization:

- The subject must arrive at the study site within 4 hours of the onset of the swelling episode
 - The time of onset will be considered the point at which a subject first recognizes with certainty that he/she is having an angioedema attack with the defining symptom or symptoms; peripheral angioedema or a rash will not define the beginning of an angioedema attack.
- Subjects must meet or continue to meet Selection Criteria for Enrollment (see section 4.2)
- An abdominal attack with moderate or greater abdominal pain (with or without symptoms of nausea, vomiting, or diarrhea), a genitourinary attack with moderate or greater swelling of scrotum or vulva, or a facial attack with moderate or greater swelling that does not involve the airway
 - The subject will be asked to rate the severity of the attack. If subjects present with moderate or greater symptoms involving more than one eligible area, the subject will be asked to rate which site is the most severe and this will be used as the defining symptom.
 - Subjects having swelling of the extremities alone or airway will not be eligible for randomized treatment.
 - Although swelling of the extremities will not be sufficient to qualify for treatment as a randomized attack, the presence of swelling in the extremities will not by itself exclude a subject who also has abdominal, genitourinary, or facial angioedema.

- Subjects presenting with laryngeal angioedema will be not be eligible to be randomized for that attack. Laryngeal angioedema will be defined as swelling perceived by the subject as being in the area of the throat with any of the following accompanying signs or symptoms: change in voice, trouble or discomfort swallowing, or difficulty breathing. Subjects with airway swelling will be eligible to receive open label C1INH-nf.
- No narcotic pain medications used within the prior 7 days
- The swelling episode must be new, and not the continuation of a previous HAE attack
 - This means that the attack at the site to be included in the randomization must be new. An attack that begins in the extremities and progresses to the face, abdomen, or genitals will not preclude randomizing the subject as long as the subject can identify a distinct time when the includable attack began, and that the timing of the symptoms is compatible with the normal course of a single HAE attack.
- No increased anabolic androgen medications within 5 days of the time of presentation
- Negative urine pregnancy test (females of child bearing potential)

4.3.3 Randomization Visit

Subjects enrolled in Part A of the Study will be randomized when they present with an eligible acute HAE attack.

Procedures prior to treatment

Pre-treatment evaluations include:

- Review selection and randomization criteria
- Review medical history
- Review medication history
- Subject symptom rating
- Adverse event assessment
- Collect and review diary
- Baseline physical examination including vital signs (blood pressure, pulse, and respirations)

Subjects will rate the severity of their symptoms (gastrointestinal, genitourinary, facial, extremities and respiratory) individually at each potential swelling site using the following scale:

- no swelling or pain
- mild swelling or pain
- moderate swelling or pain
- severe swelling or pain

Only subjects who rate their swelling or pain as moderate or severe at gastrointestinal, genitourinary, or facial sites will be eligible for randomization. Prior to randomization, the subject will be asked if he/she believes he/she will be able to refrain from rescue with narcotics until at least 60 minutes after the second infusion (for a total of 2 hours from the initial infusion). Only subjects who respond in the affirmative will be randomized. Subjects who respond no should be treated with standard medical care and should not be randomized at this time.

As described above, subjects with symptoms of laryngeal angioedema will be excluded from randomization. Additionally, for subjects presenting with more than one moderate or greater attack of the same severity, the following priority list will be used to select the defining attack for randomization:

- 1) gastrointestinal
- 2) facial
- 3) genitourinary

A visual analogue scale will also be used (0-100mm) to rate:

- Abdominal attacks: No pain(0) to Most severe pain (100)
- Genitourinary attacks: No swelling/discomfort (0) to Most severe possible swelling/discomfort (100)
- Facial attacks: No swelling (0) to Most severe possible swelling (100)
- Respiratory attacks: No swelling (0) to Most severe possible swelling (100)
- Swelling of an extremity: No swelling (0) to Most severe possible swelling (100)

Randomization and treatment procedures

Randomization will be done at the beginning of the trial for each site. The site randomization codes will be held by the study pharmacist. The study drug (C1INH-nf or

placebo) will be reconstituted (see section 6.1.3) at the study site pharmacy according to the randomization procedures, and will be provided to the investigator to maintain blinding.

The following is the description of the procedures to be performed at the randomization visit after it is determined that the subject qualifies for treatment:

- 1) Insert IV and obtain pre-infusion blood samples and reserve samples (see schedule of laboratory tests).
- 2) Perform vital signs (blood pressure, pulse, and respirations) immediately before the study drug is given.
- 3) Administer 1000 U C1INH-nf or placebo by IV at a rate of ≤ 1 ml per minute. Time 0 will be the time when this initial infusion begins. A calibrated timing device will be used and times will be recorded. The infusion syringe should be immediately disposed of after the infusion is completed.
- 4) Perform post infusion subject symptom assessments (using the scales described below) beginning at 15 minutes following time 0:

Subjects will rate symptom relief (gastrointestinal, genitourinary, facial, extremities and respiratory) individually at each potential swelling site using the following scale:

- absent now and before
- absent now but present before
- present, symptoms new
- present, symptoms worse or the same
- present, symptoms better

Visual analog scale (0-100mm):

- Abdominal attacks: No pain(0) to Most severe pain (100)
- Genitourinary attacks: No swelling/discomfort (0) to Most severe possible swelling/discomfort (100)
- Facial attacks: No swelling (0) to Most severe possible swelling (100)
- Respiratory attacks: No swelling (0) to Most severe possible swelling (100)
- Swelling of an extremity: No swelling (0) to Most severe possible swelling (100)

Symptom assessments must be done every 15 minutes after time 0 for 4 hours or until substantial relief of symptoms occurs. Substantial relief is

defined as at least 3 consecutive reports that symptoms are “absent now but present before” or “present, symptoms better.”

In the case where the subject first reports improvement at 4 hours, the subject will also be asked to assess his/her symptoms at 4 ¼ and 4 ½ hours. Subjects will also be asked to inform study personnel whenever they believe the attack has begun to remit. The beginning of unequivocal relief of the defining symptom will be considered to be the first of the three consecutive reports of either “absent now but present before” or “present, symptoms better” verifying substantial relief.

- 5) Second infusion: If at 60 minutes following the initial infusion the subject has not responded to the treatment, a second infusion of the study drug (1000 U C1INH-nf or placebo) will be administered. While the decision to administer the second infusion will be made at the 60 minute mark, the second infusion should be given as soon as possible thereafter. Evaluation times will still be based on the time that the first infusion was given.
- 6) Vital signs (blood pressure, pulse, and respirations) will be obtained at 30 minutes after the initial infusion, and again at 30 minutes after a second infusion, if administered.
- 7) Blood samples (study and reserve) will be obtained from the opposite arm than the one used to give the infusion and should be taken at the following intervals:
 - 1, 4, and 12-24 hours following the initial infusion (see schedule of laboratory tests)
 - 1 hour after the second infusion if administered (the remaining blood samples will remain on the schedule of 4 and 12-24 hours after the initial infusion)
- 8) Local tolerance testing will be accomplished as follows:
 - The observer will ask the subject to rate any discomfort at the injection site at both 30 minutes and 4 hours after the initial infusion. The scale will be from 1 to 10, with 1 being no discomfort, and 10 being extreme discomfort.
 - The observer will also rate the extent, if any, of the irritation (defined as local erythema or swelling) at the injection site at both 30 minutes and 4 hours after the initial infusion. The scale will be from 1 to 10, with 1 being no noticeable irritation, and 10 being extreme irritation.
 - Both sets of observations will be recorded on the case report form
- 9) Study discharge:

- Subjects may be discharged after the 4 hour blood draw if they have reported symptom relief and the investigator believes it is safe to do so.
- Subjects who continue to have symptoms after open label treatment will be monitored and/or hospitalized and provided with standard HAE therapy. The costs of the hospitalization will be paid for by the Sponsor.
- Subjects who are discharged before the 12 hour time point should come back to the site within 24 hours of the initial infusion for the final blood draw (12-24 hours post initial infusion).

Additional therapy

Fluid replacement can be given, however it should be used at the lowest possible rate of infusion based on the discretion of the investigator. Rescue with narcotics will be considered a treatment failure and the last observation will be carried forward for the remaining time of observation. Criteria for rescue with narcotic treatment will be pain or discomfort that the subject indicates is intolerable. Prior to randomization, the subject will be asked if he/she believes he/she will be able to refrain from rescue with narcotics until 60 minutes after the second infusion, however, no subject will be forced to go without symptomatic treatment if he/she indicates that he/she requires relief.

Randomized subjects who are rescued with narcotic treatment are not eligible for the 4-hour open label treatment. They should continue to be observed as described above and all blood draws (60 minutes, 4 hours, and 12-24 hours post infusion 1) should still be obtained.

Failure to respond to treatment

Subjects treated with study medication or open-label C1INH-nf will be observed for clinical improvement as described above. Failure to respond within 4 hours or evidence suggesting another potential cause of the symptoms (total WBC > 20,000 with left shift; fever > 38° C, or onset of atypical symptoms) should prompt the investigator to consider alternative diagnoses. Serum amylase and a urinalysis should be obtained, and consideration of other diagnostic testing (abdominal ultrasound, CT or KUB) given. Any subject with abdominal pain who has not begun to improve within 9 hours of treatment start should have had an abdominal imaging study performed to exclude other causes.

Telephone follow-up

Subjects will be contacted by telephone 72-96 hours following discharge. The time of complete resolution of the attack will be recorded, and adverse events will be assessed.

4.3.4 Open Label Rescue (Part A)

Subjects treated for a randomized attack may receive open label C1INH-nf during the same visit under the following circumstances: 1) failure to achieve significant symptom relief within 4 hours after the initial treatment at the randomization visit; or 2) development of airway compromise following the initial treatment. In the case where the subject first reports improvement at 4 hours, the investigator should delay open label treatment and ask the subject to assess his/her symptoms at 4 ¼ and 4 ½ hours, and only proceed with the open label treatment if the subject fails to report substantial symptom relief as described above.

Subjects enrolled into Part A may also be treated with C1INH-nf under an open label indication when presenting with laryngeal angioedema (defined under section 4.3.2 above) or when emergency or non-cosmetic surgical procedures are required. Following open label treatment, the subject will be monitored as described for the randomized attack. Subjects not previously randomized who receive open label treatment may subsequently be randomized for an acute HAE attack; however a minimum of 7 days from the open label treatment must have elapsed.

Additional information about open label treatment is contained in Section 4.5, which describes open label infusions on days in which the randomized treatment is not given. If open label treatment is given on a day when randomized treatment is given (e.g. because of failure of the randomized treatment) then:

- the randomized treatment will be considered to have failed
- one or two open label infusions can be given
- other procedures, defined above, should be followed for the randomization visit

4.3.5 3 Month Post-Treatment Safety Follow-up Visit

AE assessment will be performed and blood will be drawn for viral serology studies at 3 months after treatment (see Section 4.6).

4.4 Part B Protocol

Part B will be a Phase III multi-center, randomized, placebo-controlled, double-blind cross-over trial to confirm the efficacy of prophylactic C1INH-nf. Each subject will be randomized either to receive 12 weeks of C1INH-nf followed by 12 weeks of placebo, or 12 weeks of placebo followed by 12 weeks of C1INH-nf. Subjects may not enter Part B until either they have received their randomized treatment in Part A or Part A has ended (once the 70th subject has been randomized).

A Time and Events Table of required study assessments and safety observations for Part B is contained in Section 11.

4.4.1 Part B Eligibility and Initiation Visit

Subjects must meet or continue to meet Selection Criteria for Enrollment (see section 4.2). In addition, subjects must have relatively frequent angioedema attacks (at least 2/month on average) while on their current therapeutic regimen.

Subjects who qualify for entry into Part B and wish to decrease their dose of 17-alpha-alkylated androgens or Amicar (aminocaproic acid, EACA) may do so prior to beginning Part B. If they choose to do so, the dosage will be decreased and entry into Part B will be delayed for 1 month. Once a subject is enrolled in Part B, changes in the dose of 17-alpha-alkylated androgens or Amicar will not be allowed; and any further change will lead to the subject being dropped from this part of the study.

For Part B, subjects will be randomized into one of two groups: 1) placebo treatment for 12 weeks followed by prophylactic C1INH-nf treatment for 12 weeks; or 2) prophylactic C1INH-nf treatment for 12 weeks followed by placebo treatment for 12 weeks. At the beginning of each 12 week treatment period the subject will have an initiation visit.

Initiation visit procedures

Initiation evaluations include:

- Review selection criteria
- Review medical history
- Review medication history
- Assess adverse events
- Physical examination including vital signs (blood pressure, pulse, and respirations)
- Quality of life assessment (SF-36 or SF-10 for children under 14 years old)
- Blood samples (study and reserves) (see schedule of laboratory tests)

Following the initiation visit evaluations, the first prophylactic infusion will be given.

The subject will be dispensed a diary card or provided with an electronic process to record his/her angioedema symptoms throughout the prophylactic treatment period (see section 4.4.3).

4.4.2 Prophylactic Treatments and Visit Procedures

Subjects will be given prophylactic infusions (C1INH-nf or placebo) every 3-4 days (approximately 2 times a week) for the entire 24 week period, beginning at the first initiation visit. If a subject does not receive a scheduled treatment, it will be given as close to the scheduled time as possible. All infusions will be given at the study site. A minor protocol deviation will be: 1) any interval between treatments that is shorter than 2 days or more than 5 days after the last infusion; or 2) any 14 day period in which there are fewer than 3 infusions that have been given. A major protocol deviation will be: 1) any interval between treatments that is longer than 12 days; or 2) any 28 day period in which there are fewer than 3 infusions that have been given. Subjects with this major protocol deviation will be discharged from Part B.

Prophylactic treatment visit procedures

The following is the description of the procedures to be performed at each prophylactic treatment visit:

- Collect and review subject's diary
- Review medication history
- Assess adverse events
- Assess vital signs (blood pressure, pulse, and respirations) immediately prior to prophylactic treatment and again at 30 minutes post-infusion for all treatments
- Collect pre-infusion and post infusion blood samples (study and reserves) monthly beginning at the first initiation visit (see schedule of laboratory tests)
 - Samples should be obtained immediately prior to infusion, and again at 60 minutes post-infusion
- Dispense the subject diary at each visit

4.4.3 Frequency of Angioedema Attacks

At the initiation visit, subjects will be given diary cards or data will be collected electronically and subjects will be instructed to document all attacks on a daily basis in the mornings, evaluating symptoms over the past 24 hours, indicating:

- the location of the swelling
 - abdominal
 - genitourinary
 - facial

- respiratory including laryngeal
- extremity
- the severity of the attack at each location that is listed
 - mild
 - moderate
 - severe
- the duration of the swelling at each location that is listed
 - time in minutes

Subjects will be evaluated at least weekly by study personnel to determine whether they had any attacks during the prior week.

The number of attacks will consist of all angioedema attacks that occur during the treatment irrespective of whether the subject obtained open label C1INH-nf or not. An angioedema attack will be defined as a discrete episode during which the subject progresses from no angioedema to symptoms of angioedema. Attacks that progress from one site to another will be considered as a single attack. Attacks that begin to regress and then become worse before complete resolution will be considered to be one attack.

4.4.4 Part B at Cross-Over and at Final Prophylactic Treatment Visit

The following is the description of the procedures to be performed at the end of the first 12 weeks of treatment, at the time that subjects cross-over and at the final prophylactic treatment visit:

- Collect and review diary
- Dispense diary (end of first 12 week treatment period)
- Review medical history
- Review medication history
- Assess adverse events
- Evaluate quality of life questionnaire (SF-36 or SF-10 for children under 14 years old)
- Perform physical examination including vital signs (blood pressure, pulse, and respirations)
- Collect blood samples (study and reserves) (see schedule of laboratory tests)

4.4.5 Open Label Treatment of Angioedema Attacks

Subjects enrolled in Part B may receive open label treatment if in the opinion of the principal investigator this treatment is required. This treatment is described in section 4.5. The regularly scheduled prophylactic infusions shall continue on the normal schedule after an open label treatment. In the event that the next prophylactic infusion is scheduled to occur within 24 hours of the open label treatment, the next prophylactic infusion should be rescheduled to occur at least 24 hours after the last open label infusion.

4.4.6 3 Month Follow-up Visit for Laboratory Safety Evaluation

AE assessment will be performed and blood will be drawn for viral serology studies at 3 months after the last treatment in Part B.

4.5 Open Label Treatment of Angioedema Attacks

Subjects enrolled in Part A or Part B will be eligible to receive open label C1INH-nf (see sections 4.3.4 and 4.4.5).

If the open label treatment is given on the day of a randomization visit then the site should make sure that they follow the procedures defined for the randomization visit. The following is the description of the procedures to be performed at the beginning of an open label treatment visit:

- Review medical history
- Review medication history
- Assess adverse events
- Perform physical examination including vital signs (blood pressure, pulse, and respirations)

Subjects will rate their symptoms using the following scale:

- the location of the swelling
 - abdominal
 - genitourinary
 - facial
 - respiratory including laryngeal
 - extremity

- the severity of the attack at each location that is listed
 - mild
 - moderate
 - severe

Subjects will also rate their symptoms using this visual analog scale (0-100mm):

- Abdominal attacks: No pain(0) to Most severe pain (100)
- Genitourinary attacks: No swelling/discomfort (0) to Most severe possible swelling/discomfort (100)
- Facial attacks: No swelling (0) to Most severe possible swelling (100)
- Laryngeal attacks: No swelling (0) to Most severe possible swelling (100)

After the evaluation is performed the following will be done:

- Obtain vital signs (blood pressure, pulse, and respirations)
- Obtain pre-infusion blood samples and reserves (see schedule of laboratory tests) immediately prior to prophylactic treatment
- Administer open label C1INH-nf
- Obtain vital signs 30 minutes after infusion
- Obtain post-infusion blood samples and reserves one hour after infusion (see schedule of laboratory tests)
- If the attack has abated by the 1 hour time point, the subject will be allowed to leave
- If the attack has not abated by the 1 hour time point, a second dose of 1000 U open label C1INH-nf may be administered, and the subject will be observed and provided medical treatment as required until the attack subsides.

4.6 3 Month Follow-up Visits for Laboratory Safety Evaluation

AE assessment will be performed and blood will be drawn for viral serology studies at 3 months after treatment in Part A, at 3 months after each open label treatment, and after the last treatment in Part B. However, testing for viral serology studies shall not take place more frequently than every three months, except testing related to the final infusion received by the subject, which shall take place 3 months from the last infusion regardless of any previous tests.

4.7 Efficacy and Outcomes

This will be an intent-to-treat study. A detailed statistical analysis plan will be developed based on the statistical discussion presented below (in sections 4.7.1, 4.7.2 and 4.7.3).

4.7.1 Discussion of Assessment of Efficacy for Part A

The primary endpoint of Part A will be the time to the beginning of unequivocal relief of the defining symptom following initial treatment (Time 0). Selection of this endpoint is based on pathophysiologic, clinical and statistical considerations. Each of these considerations is discussed below.

The natural history of an HAE attack is characterized by an extended period of slowly but progressively increasing angioedema, typically lasting 24 hours; followed by an even longer period of gradual resolution of the angioedema, typically over 48-72 hours. Considerable progress has been made in understanding the mechanisms that underlie these two phases of an angioedema attack. The angioedema occurs as a consequence of a permeability defect allowing plasma fluid to move into the interstitial space. This permeability defect is mediated via generation of vasoactive mediators due to insufficient inhibition of proteases normally inhibited by C1INH. Once the generation of vasoactive mediators ceases, the angioedema attack begins to resolve. The slow process of resolution involves movement of fluid out of the interstitial fluid through the combined effects of tissue hydrostatic pressure and lymphatic drainage. C1INH deficiency thus plays a direct role in the development of angioedema but has no bearing on the rate of resolution. Therefore, if an HAE patient is given effective treatment to stop an acute attack of angioedema, generation of additional vasoactive mediators would cease and the swelling would quickly stop progressing. In this situation, patients rapidly note the beginning of relief as the fluid pressure within the affected tissue begins to decrease. Full resolution of the angioedema, however, will continue to depend upon hydrostatic pressure and lymphatic drainage – processes that proceed relatively slowly and are not directly affected by the treatment that stopped the acute attack.

Beginning of relief is also the most important clinical parameter used to assess the status of HAE patients during an acute attack. Clinical experience has shown that the beginning of symptom relief correlates with the onset of the resolution phase. This has important prognostic and practical implications. In the management of airway attacks, for instance, patients are no longer considered at risk of asphyxiation once beginning of relief has occurred. Patients are generally discharged home to await full resolution of the angioedema once it is established that the attack has begun to resolve. Patients are also typically able to resume normal daily activities well before the angioedema has fully resolved; thus, beginning of symptoms relief is also a better indicator of functional severity.

Finally, a previous double-blind randomized study showed that the beginning of symptom relief is a much more sensitive outcome parameter than full resolution of angioedema following C1INH replacement therapy. The study by Kunschak *et al* (21) examined the time to beginning of relief and resolution following the first treatment with either C1INH or placebo. In the 11 subjects treated with C1INH, the median and mean times to beginning of relief were 0.83 and 2.70 hours respectively, while these times were 17.00 and 15.35 hours respectively in the 12 subjects treated with placebo. By contrast, the median and mean time to resolution of symptoms were 14.08 and 17.23 hours respectively in the C1INH treated subjects, but 26.00 and 34.58 hours respectively in the placebo treated subjects. It is clear that time to beginning of relief was a much more sensitive measure of the effect of C1INH. Both this study as well as an earlier one by Waytes *et al* (14), demonstrated that beginning of relief provided a statistically significant outcome measure to detect the effect of C1INH replacement therapy for acute attacks in HAE patients.

Secondary endpoints will be the presence or absence of unequivocal beginning of relief of the defining symptom within 4 hours following initial treatment (time 0) as well as the time to complete resolution of the attack, and the ability of C1INH-nf concentrate to raise C1INH and C4 levels.

4.7.2 Statistical Analysis of Outcomes for Part A

The primary analysis will be time to beginning of relief of the defining symptom from the first dose and will be analyzed by a log rank test. Patients who do not achieve symptom relief within 4 hours of the first dose will be censored at 4 hours. However, if a patient receives narcotic rescue medication prior to 4 hours s/he will be censored at that time.

Part A will have more than 80% power to detect a reduction by 50% in the median time to relief of the defining symptom between the treatment and placebo groups. The assumptions used in the calculation of sample size for this study is based on M. Kunschak *et al* (21). In this study each patient was monitored for adverse reactions during 24 hour hospitalization and so, it will be reasonable to assume that the study duration is 24 hours and the value of $t=24$ was used in working with the N-Query Advisor software for the calculation of sample size using the log-rank test procedure. In the Kunschak study, 7 subjects in the C1INH group and 6 subjects in the placebo group never received treatment. In order to perform a valid intent-to-treat analysis, Kunschak *et al* assigned the time of beginning of relief observed in the 12 placebo treated subjects to all 13 untreated subjects. Despite this conservative procedure, analysis of the data showed that the median time-to-relief was 6.17 hours in the C1INH group as compared to 15.35 hours in the placebo group, thereby showing a 50% reduction in the median time to relief. The difference for time-to-relief was highly significant ($p=0.007$, Mann-Whitney U test.) In order to achieve a power greater than 80% to show a 50% reduction in the median time to relief in this study, 34 evaluable subjects are needed in each of the treatment groups. A

total of 124 subjects will be enrolled in Part A of the study and 70 subjects will be randomized in order to achieve 68 evaluable subjects.

C4 levels have been shown to fall consistently during an acute attack of HAE (1). To control for the possibility that some episodes might not represent actual HAE attacks, we will measure the C4 antigenic level at the screening visit (the baseline) and again prior to study drug administration for an acute attack. Subjects who fail to exhibit a C4 level that is lower during the randomized attack than their baseline level will be considered to not have had a true HAE attack, and therefore will be excluded from the intent to treat analysis for efficacy but will remain in the safety analysis. However, subjects will not be excluded if their C4 levels were below the detection limit both at screening and at the time of the randomized attack.

Effects of the study drug on other secondary endpoints (C1INH antigenic and functional levels and C4 levels) will be analyzed by Wilcoxon rank sum test. The subjects rating their symptoms using a VAS will also be analyzed using Wilcoxon rank sum test.

Safety will be assessed by the number and severity of adverse experiences, and changes in clinical laboratory safety parameters and vital signs from pre to post infusion. Two post infusions records, one at the end of the first dose and the other at the end of second dose (if there is one) will be used to study safety parameters (laboratory, vital signs and adverse events). Viral serology studies will be performed immediately prior to the first infusion, then at 3 months after treatment.

Local tolerance testing will be assessed by descriptive statistics.

4.7.3 Statistical Measures of Outcome for Part B

The primary endpoint for Part B will be the number of attacks of angioedema during each treatment phase, using each subject as his/her own control, thereby controlling the variability. This will enable us to attribute the difference in the number of attacks of angioedema between the treatment groups entirely to the treatment. Assuming angioedema attack rates will be 1 every two weeks in the placebo phase and 1 per 12 weeks in the prophylactic C1INH-nf phase, 10 subjects per arm provides more than 90% power to detect the treatment effect. The analysis will be based on a Poisson assumption; a test for extra-Poisson variation will make use of generalized linear interactive modeling (GLIM). Should there be evidence of such variation, a GLIM model with a term that incorporates such variation will be used to make treatment comparisons.

Secondary endpoints will be number of subjects dropping out at each stage, quality of life, average severity of attacks, average duration of attacks, number of open label C1INH-nf infusions, and C1INH and C4 levels. Wilcoxon rank-sum tests will be used for treatment comparisons of these endpoints. The subjects rating their symptoms will also be analyzed using Wilcoxon rank sum tests.

Safety will be assessed by the number and severity of adverse experiences, and changes in clinical laboratory safety parameters.

5. SAFETY

Safety will be assessed by the number and severity of adverse experiences, and changes in clinical laboratory safety parameters and vital signs from pre- to post-infusion. Viral serology studies will be performed immediately prior to the first infusion, and at 3 months after the first treatment (Part A) and after the last treatment (Part B).

5.1 Adverse Events (AEs)

5.1.1 Definition

An AE is any unfavorable or unintended sign, symptom, or disease temporally associated with the use of study treatment, whether or not considered related to the study treatment. All AEs must be fully recorded in the source documents and on the AE case report form (CRF), regardless of whether or not the event is considered related to study medication.

For all AEs, the investigator must pursue and obtain information adequate both to determine the outcome of the AE and to assess whether it meets the criteria for classification as a serious adverse event (SAE) requiring immediate notification of the CRO. Follow-up of the AE, even after the date of therapy discontinuation, is required if the AE or its sequelae persist. Follow-up is required until the event or its sequelae resolve or stabilize at a level acceptable to the investigator and the sponsor.

5.1.2 Intensity

The intensity or severity of an AE will be graded as follows:

Mild	Events that are usually transient, requiring no special treatment, and do not interfere with the subject's daily activities.
Moderate	Events that introduce some level of inconvenience or concern to the subject, and may somewhat interfere with daily activities, but are usually ameliorated by simple therapeutic measures (may include drug therapy).
Severe	Events that are unacceptable or intolerable, significantly interrupt the subject's usual daily activity, and require systemic drug therapy or other treatment.

5.1.3 Relationship to Study Treatment

The relationship or association of the AE to a study treatment will be characterized as follows:

- Unlikely** It is improbable that the study treatment caused the AE and other factors are more likely the cause, such as concurrent illness, progression or expression of a disease, or a reaction to a concomitant medication.
- Possible** The AE cannot be fully explained by other causes, and it is possible that the study treatment caused the event.
- Probable** A reasonable temporal association exists between the AE and treatment administration, and, based on the investigator's clinical experience, the association of the AE with the study treatment seems probable.

5.1.4 Serious Adverse Events

An SAE is defined as any event that:

- Results in death
- Is life-threatening
- Results in persistent or significant disability/incapacity
- Results in or prolongs an existing inpatient hospitalization
- Is a congenital anomaly/birth defect

Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered an SAE when, based upon appropriate medical judgment, they jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. Examples of such events are allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in an in-patient hospitalization, or the development of drug dependency or drug abuse.

5.1.5 Serious Adverse Event Reporting

Events that are life-threatening, result in a subject's death, are considered related to the use of the study drug, and are unexpected according to the current Investigator's Brochure for the investigational product must be reported to the FDA by fax or telephone within 7 days, and a written report must follow within 8 days after the initial notification to the FDA. All other SAEs that are considered related to the use of study drug and are

unexpected (per the current Investigator's Brochure) require a written report to the FDA within 15 days of site notification that an event has occurred.

The principal investigator is responsible for notifying the Institutional Review Board (IRB) of all SAEs that occur at his or her site and those reportable events occurring at other sites.

5.1.6 Investigator Reporting Requirements

All SAEs, regardless of treatment group or suspected relationship to study drug, must be reported immediately by telephone to the contract research organization (CRO). In addition, if the investigator learns of an SAE occurring after the last follow-up visit, but within 30 days after the last administration of the study drug, he/she should immediately report the event to the CRO. All SAEs will be reported to Sanquin's Drug Safety Officer as well.

The investigator will complete and submit a SAE Form within 24 hours for all SAEs. The investigator is obligated to provide information requested by the clinical monitor in addition to that on the SAE Form. In general, this will include a description of the AE in sufficient detail to allow for a complete medical assessment of the case and independent determination of possible causality. In the case of a subject's death, a summary of available autopsy findings must be submitted as soon as possible to the CRO.

5.1.7 Clinical Laboratory Assessments

Blood will be collected for serum chemistry and hematology. Any authorized and qualified person can collect biological samples from the subject. The central laboratory will process all of the clinical laboratory samples. The investigator will be provided with laboratory kits, shipping containers, and specific instructions for the collection and shipment of samples at the study initiation visit. The schedules for laboratory testing are in Tables 1 through 5; these tables include the safety laboratory variables (HBV, HCV, HIV, Parvo B19, CBC, BUN, and creatinine), as well as laboratory variables used for efficacy.

5.1.8 Vital Signs

Vital signs will be measured before and after the initial infusion and again after a second infusion if one is administered. An authorized and qualified person will perform the measurements. Blood pressure will be measured in the same arm by using a manual or automated sphygmomanometer, and the results will be recorded in mmHg. Pulse rate will be measured in the radial artery in the dominant arm for 30 seconds and will be

recorded as beats/minute. Respiratory rate will be measured and recorded in breaths/minute.

5.1.9 Unblinding Instructions

In the event of an extreme emergency, where knowledge of the investigational product is essential for the welfare and clinical management of the subject, the investigator may obtain the treatment assignment from the unblinded pharmacist. The investigator must record the date and reason for unblinding in the subject's source document and in the CRF. In the event of any unblinding of the treatment assignment, the CRO must be notified immediately.

6. DESCRIPTION AND ACCOUNTABILITY OF TREATMENTS ADMINISTERED

6.1 Description

The C1INH-nf is supplied as a powder for injection. After being dissolved in the prescribed volume of water for injection the preparation contains 100 U of C1INH per ml. 1 U of C1INH corresponds to the quantity of C1INH present in 1 ml of normal fresh plasma.

Different batches of the study drug will be used. The batch numbers will be documented in the Certificates of Analyses, which are filed in the study master file. On all the bottles the actual batch number will be printed. This number will be documented by the pharmacist in the Drug Accountability Log as well as in the Drug Distribution Chart. A detailed listing of all batches used will be included in the final study report.

Matching placebo will also be provided (saline).

6.1.1 Conditions for Transport and Storage

The investigational product should be stored in a locked refrigerator in a safe area of limited access at 2 °C to 8 °C and protected from light. The product must not be frozen. A temperature log should be kept by the site to ensure that the proper temperatures are maintained.

6.1.2 Packaging and Labeling

The investigational product will be labelled, packed and delivered by Sanquin Blood Supply Foundation, Amsterdam, the Netherlands. The investigational product will be

shipped either to the study site directly or to the site's hospital pharmacy, depending on local regulations.

Each bottle will be labelled and packed in a labelled box with water for injection. The following label will be used:

Study No.

500 U C1 Esterase Inhibitor (Human)

FOR CLINICAL TRIAL USE ONLY

Batch number: _____ EXPIRATION DATE: _____

DO NOT FREEZE

STORE AT 2°C to 8°C, PROTECTED FROM LIGHT

KEEP OUT OF REACH OF CHILDREN

SPONSOR: Lev Pharmaceuticals, Inc. (LevPharma)

MANUFACTURER: Sanquin Blood Supply Foundation, Amsterdam, the Netherlands

6.1.3 Reconstitution

Drug kits will be provided to the participating sites. Instructions will be provided to sites to describe the process of reconstitution of study drug.

6.2 Subject Compliance

Because the study drug will be administered intravenously and only under medical supervision, compliance is not an issue.

7. REGULATORY COMPLIANCE

7.1 Compliance

This Study will be conducted in compliance with the protocol, Good Clinical Practice regulations, and all applicable regulatory requirements.

7.2 Consent

Informed consent will be obtained for each subject entering the Study. Institutional Review Boards may require additional written consent using their own locally approved consent form. The Study will be conducted in accordance with FDA 21CFR 50, Subpart B-Informed Consent of Human Subjects and the applicable regulatory requirements.

The investigator will obtain written informed consent from each subject participating in this Study, after adequate explanation of the aims, methods, anticipated benefits, and potential hazards of the Study, prior to applying any Study-related procedure to a subject.

For subjects not qualified to give legal consent, written consent must be obtained from the subject's legal guardian. If children are old enough to understand the risks and benefits of the Study (generally 6 years to 18 years), they should also be informed and provide their written assent or consent.

The investigator will explain that the subjects are completely free to refuse to enter the Study or to withdraw from it at any time, without any consequences for their further care and without need for justification. The investigator will complete and sign the informed consent section of the CRF for each subject enrolled.

If a subject is unable to read or if their legal guardian is unable to read, an impartial witness should be present during the entire informed consent discussion. After the informed consent form and any other written information is provided, read, and explained to the study participant or their legal guardian, and after oral consent has been obtained, if capable of doing so, the study participant or their guardian should sign and date the informed consent form. The witness should also sign and date the consent form. In addition, the study participant or guardian should, if possible, sign an additional form confirming that the materials provided have been read and explained to them. The witness should also sign and date this additional form.

Each subject will be informed that his or her medical records may be reviewed during the course of the Study or afterwards, including review by government agencies. Each subject will be informed that his or her medical data will be included in a database and may be reviewed during the course of the Study or afterwards, including review by government agencies of US and of other countries. However, only authorised personnel will review these data. Each subject should be informed that the investigator will protect any personal information not related to the Study, and that individuals associated with the Study are bound by the same confidentiality obligations as other health care professionals with regard to subject confidentiality.

7.3 Data Safety Monitoring Board

A Data and Safety Monitoring Board (DSMB) will be established to monitor this trial. The responsibilities of the DSMB are to:

- 1) Familiarize themselves with the research protocol and plans for data and safety monitoring.
- 2) Review interim analyses of outcome data and cumulative toxicity data summaries to determine whether the trial should continue as originally designed, should be changed, or should be terminated based on these data. The DSMB will review trial performance information such as accrual information. The DSMB will also determine whether and to whom outcome results should be released prior to the reporting of study results.
- 3) Review reports of related studies to determine whether the monitored study needs to be changed or terminated.
- 4) Review major proposed modifications to the study prior to their implementation (e.g., termination, dropping an arm based on toxicity results or other reported trial outcomes, increasing target sample size).
- 5) Following each DSMB meeting, provide the study Principal Investigator (PI) with written information concerning findings for the trial as a whole related to cumulative toxicities observed and any relevant recommendations related to continuing, changing, or terminating the trial. The study PI will provide information on cumulative toxicities and relevant recommendations to the site PIs to be shared with their IRBs.

7.4 Emergency Reporting

All emergencies and serious adverse events should be reported by telephone immediately to:

INC Research SAE Hotline
Phone: 877-462-0134
Fax: 434-817-2298

8. USE OF DATA AND PUBLICATION

Results may be published in peer-reviewed journals with acknowledgment given to LevPharma for this product. LevPharma may disclose data derived from the Study to other investigators and domestic or foreign drug regulatory authorities.

To the extent permitted by law, the Investigator will hold all matters relating to the Study in confidence between itself and LevPharma provided, however, that should any of this

confidential information be published or otherwise made available to the public through sources entitled to disclose the information, the Investigator shall be free to disclose such publicly available information. The Investigator will maintain Study documents until at least 2-years after the last approval of a marketing application.

The Investigator will be free to disclose at scientific meetings and to publish in the scientific literature data resulting from this Study. The Investigators agree to transmit in writing to LevPharma the nature of such disclosures within a reasonable period of time.

9. REFERENCES

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10. SCHEDULE OF TESTING***Table 1: Schedule of Laboratory Testing for Part A Randomized Attacks***

	Screen	Pre-infusion	60 min ¹	240 min	12-24 hrs	3 months
C1INH Antigenic	X	X	X	X	X	X
C1INH Functional	X	X	X	X	X	X
C4	X	X	X	X	X	X
C1q	X	X	X	X	X	X
C1INH autoAb	X	X				X
Anti Parvo B19		X ²				X ³
Parvo B19 NAT					X ³	
HBsAg	X	X				X
Anti HCV	X	X				X
Anti HIV	X	X				X
HIV NAT	X	X				X
HCV NAT	X	X				X
CBC		X			X	
BUN		X			X	
Creatinine		X			X	
Reserves	X	X	X	X	X	X

¹If a second 1000 U infusion is given, these labs will be repeated at 1 hour after that infusion. The remaining schedule will continue to be based on the initial infusion (Time 0).

² If positive, Parvo testing complete, if negative run NAT at 12-24 hr post infusion.

³ If Parvo B19 NAT is positive post infusion, repeat Anti Parvo B19 at 3 months.

Table 2: Schedule of Laboratory Testing for Open-Label Treatment in Part A

	Pre-infusion	60 min	3 Months*
C1INH Antigenic	X	X	
C1INH Functional	X	X	
C4	X	X	
C1q	X	X	
C1INH autoAb	X		
Anti Parvo B19	X		X
HBsAg	X		X
Anti HCV	X		X
Anti HIV	X		X
HIV NAT	X		X
HCV NAT	X		X
Reserves	X	X	X

*Testing shall take place 3 months after each open label treatment (but not more frequently than every three months, except testing for the final infusion, which shall take place 3 months from the last infusion regardless of previous tests.)

Table 3: Schedule of Laboratory Testing for Prophylactic Treatment in Part B

	Screen Visit (see Part A)	Visit 1a		Visit 8a		Visit 16a		Visit 24a		Visit 1b		Visit 8b		Visit 16b		Visit 24b		270 Days
		Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	
C1INH Antigenic		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	
C1INH Functional		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	
C1INH autoAb										X						X		X
Anti Parvo B19 ¹		X						X								X		X
Parvo B19 NAT ¹			X						X								X	
HBsAg		X						X								X		X
Anti HCV		X						X								X		X
Anti HIV		X						X								X		X
HIV NAT		X						X								X		X
HCV NAT		X						X								X		X
CBC		X																X
BUN		X																X
Reserves		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	

¹ If the Anti-Parvo B19 testing is positive at any visit, Parvo B19 testing is complete.

Table 4: Schedule of Additional Laboratory Testing for Prophylactic Treatment in Part B

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Table 5: Schedule of Laboratory Testing for Open Label Treatment in Part B

	Pre-infusion	60 min	3 Months*
C1INH Antigenic	X	X	
C1INH Functional	X	X	
C4	X	X	
C1q	X	X	
C1INH autoAb	X		
Anti Parvo B19	X		X
HBsAg	X		X
Anti HCV	X		X
Anti HIV	X		X
HIV NAT	X		X
HCV NAT	X		X
Reserves	X	X	X

*Testing shall take place 3 months after each open label treatment (but not more frequently than every three months, except testing for the final infusion, which shall take place 3 months from the last infusion regardless of previous tests.)

11. TIME AND EVENTS TABLES**Table 1: Part A-Randomization**

	Screening Visit	Randomization Visit	3 Day Telephone Follow-up	3 Month Follow-up
Informed Consent	X			
Inclusion/Exclusion Criteria	X	X		
General Medical History	X			
Medical History Update		X		
History of HAE Attacks	X			
Medication History	X	X		
Adverse Event Evaluation		X	X	X
Physical Examination	X	X		
Laboratory Testing	X	X		X
Diary Card Dispensed	X			
Diary Card Collected		X		
Evaluation of Eligibility for Treatment		X		
SF-36 or SF-10 Evaluation	X			
Randomization		X		
Administer Single Dose Blinded Treatment		X		
Evaluation of Symptoms		X		
Urine Pregnancy Test (Women)		X		
Vital Signs	X	X		
Assess HAE Attack Resolution		X	X	
Emergency/Open Label Treatment Option		X		

Table 2: Part B-Prophylactic Treatment

	Part B1 Initiation	Infusion Visits	Part B2: Initiation	Infusion Visits	Part B: Final Visit	Follow-up
	Day 0	Days 2-88	Day 90	Days 92 - 188	Day 190	Day 270
Inclusion/Exclusion Criteria	X					
Medical History Update	X		X		X	
Medication History	X	X	X	X	X	
Adverse Event Evaluation	X	X	X	X	X	X
Physical Examination	X				X	
Laboratory Testing	X					X
Pre/Post Infusion Labs (monthly at infusion visits)	X	X	X	X		
Diary Card Dispensed	X	X	X	X		
Diary Card Collected		X	X	X	X	
Evaluation of Eligibility for Treatment	X					
SF-36 or SF-10 Evaluation	X		X		X	
Randomization (Part B)	X					
Vital Signs	X	X	X	X	X	
Telephone Contact (optional)		X		X		
Prophylactic Treatment	X	X	X	X		
Emergency/Open Label Treatment Option	X	X	X	X	X	

Table 3: Open Label Treatment

Medical History Update	X
Medication History	X
Evaluation of Symptoms (VAS)	X
Physical Examination	X
Open Label Treatment	X
Vital Signs (Pre/30 minutes post)	X
Laboratory Assessment (Pre/60 minutes post)	X
3-month follow-up (Laboratory testing)	X

Summary of Important Changes to LEVP2005-1

Amendment 1

The number of randomized subjects in Part A was increased from 62 in the original protocol to 70 (to achieve 68 evaluable events).

The number of randomized subjects in Part B was increased from 20 in the original protocol to 24 (to achieve 20 evaluable).

Viral serology studies were changed to be three months after every open label treatment, but no more often than every three months in the event of multiple open label visits.

Viral serology studies for Part B were added during the study at every three month time point.

Amendment 2

The number of sites was increased to 20

The inclusion and exclusion labs were changed from Specialty to Mayo Clinical Trial Services (with resulting reference range changes)

The lab visits for Part B were changed from every 30 days in the original protocol to coincide with specific visits during Part B.

Amendment 3

The time point for rescue with open label C1INH-nf was changed from the 8 hour time point in the original protocol to 4 hours.

Symptom assessment was changed from up to 8 hours in the original protocol to up to 4 hours.

Subjects were allowed to be released from the site anytime after 4 hours (once the patient was well enough to leave in the opinion of the investigator). This was changed from 12 hours in the original protocol.

Subjects were instructed to return to the site for the last blood draw anytime between 12 and 24 hours after the first infusion



Statistical Analysis Plan

**CHANGE Trial (C1-Inhibitor in Hereditary Angioedema
Nanofiltration Generation evaluating Efficacy): A Double-blind,
Placebo Controlled, Clinical Study to Investigate the Efficacy and
Safety of Purified C1 Esterase Inhibitor (Human) for the Treatment
of Hereditary Angioedema (HAE) in Acute Attacks and as
Prophylactic Treatment to Prevent HAE Attacks
(Part A)**

Protocol No: LEVP2005-1

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**Final
July 18, 2006**

List of Abbreviations

AE	Adverse Event
CI	Confidence Interval
CRF	Case Report Form
h	Hour
HAE	Hereditary Angioedema
ITT	Intent-to-Treat
MedDRA	Medical Dictionary for Regulatory Activities
PID	Pain Intensity Difference
SAP	Statistical Analysis Plan
SAS [®]	Statistical Analysis Software
SPID	Sum of the Pain/Swelling Intensity Difference
TEAE	Treatment-emergent Adverse Events
VAS	Visual Analog Scale
WHO	World Health Organization

1. Study Design

This is a multi-center, 2-part Phase III study.

Part A is a randomized, placebo-controlled, double-blind study designed to evaluate the efficacy and safety of C1INH-nf as a therapeutic agent for acute attacks of Hereditary Angioedema (HAE). In Part A, the use of C1INH-nf will be assessed during the treatment of a single acute attack of HAE. Open-label rescue is permitted under the following circumstances: 1) failure to achieve significant symptom relief after the initial treatment at the randomization visit; or 2) development of airway compromise following the initial treatment. In addition, subjects who present with laryngeal angioedema (and who are therefore ineligible for randomization at that attack) may be treated with open-label C1INH-nf. Subjects completing Part A are eligible (but not obligated) to participate in Part B.

Subjects may enter Part B after they have completed their randomized treatment in Part A or Part A has ended.

Part B is a randomized, placebo-controlled, double-blind cross-over study to confirm the efficacy and safety of prophylactic C1INH-nf in preventing acute attacks of angioedema in HAE subjects. Subjects enrolled in Part B may receive open label treatment, if in the opinion of the principal investigator, treatment is required.

The following Statistical Analysis Plan (SAP) describes the summary and analysis of Part A only.

2. Objectives

The objective of Part A is to investigate the efficacy and safety of purified C1INH-nf for the treatment of acute attacks of HAE.

2.1 Primary Objective

The primary objective of Part A is to compare the median time to the beginning of unequivocal relief of the defining symptom in subjects treated with C1INH-nf and subjects treated with placebo.

2.2 Secondary Objective(s)

Secondary efficacy assessments are:

1. To determine and compare the percentage of subjects in the C1INF-nf and placebo treatment groups with presence or absence of unequivocal beginning of relief of the defining symptom within 4 hours following treatment.
2. To determine and compare the time to complete resolution of the attack in the C1INF-nf and placebo treatment groups.
3. To evaluate and compare the ability of C1INH-nf concentrate to raise C1INH and C4 levels in the C1INF-nf and placebo treatment groups.
4. To compare the subjects' Visual Analog Scale (VAS) rating of severity of the defining symptom between the C1INF-nf and placebo treatment groups.

3. Sample Size Determination

Based on an estimate of 0.83h for the median time to start of relief for subjects treated with C1 inhibitor, 34 subjects per treatment group are required, to give a total of 65 observed events. This would provide 80% power, at the 5% level of significance (2-sided test), to detect a 50% reduction in median time to unequivocal beginning of relief of defining symptom. A total of 124 subjects will be enrolled in Part A of the study and 70 subjects will be randomized in order to achieve 68 evaluable subjects.

Estimates for time to start of unequivocal relief of defining symptom are based on the study described by Kunschak *et al.*¹

4. Analysis Datasets

All efficacy analyses will be performed on the set of patients who received study drug and had a C4 level (based on blood drawn prior to study drug administration) that confirmed a true HAE attack. Details on this determination are described in [appendix 1, "SOP For Determining Randomization Evaluability for LEVP2005-1"](#). Subjects who fail to exhibit a C4 level that is lower during the randomized attack (prior to study drug administration) than their baseline level (screening visit) will be considered as not having had a true HAE attack, and therefore will be excluded from the efficacy dataset. Subjects will not be excluded if their C4 levels were below the detection limit both at screening and at the time of the randomized attack.

The Safety dataset will consist of all subjects who received a complete or partial infusion of randomized therapeutic treatment, together with subjects who received open-label C1INH-nf for treatment of laryngeal HAE. For the safety summary tables, non-randomized subjects who receive open-label medication will be presented as a third treatment group.

5. Interim Analysis

There is no interim analysis planned for this study.

6. Subject Disposition

A summary table of subject disposition will be provided, to show the number of subjects enrolled, and the number of subjects either randomized to C1INH-nf or placebo, or presenting with symptoms of a laryngeal HAE attack. From the former group, the number of treated subjects, the number of subjects who did not have a true HAE attack, and the number of subjects in the efficacy dataset will be tabulated by treatment group. Of the subjects with laryngeal HAE, the number of subjects who were treated with open-label C1INH-nf will be presented. A separate row will clarify the number of subjects in the safety dataset.

The number of subjects completing the study and withdrawing from the study will be tabulated. Withdrawals will be categorized by reason for withdrawal.

7. Demographics and Baseline Characteristics

Demographics (age, gender and ethnic origin) will be summarized by treatment group for the efficacy and safety datasets.

In addition, the following baseline characteristics will be tabulated for the safety dataset:

- Medical History: abnormalities by body system
- Time since diagnosis of HAE: summary statistics and categorical frequencies
- Historical severity of symptoms: severity grade by area of swelling

For the efficacy dataset only, pre-treatment VAS and severity for the defining symptom will be summarized. This table will then be repeated, subset by each of the possible areas for the defining symptom, in turn.

8. General Analysis Comments

Time-to-event variables will be compared between treatment groups using the Cox-regression proportional hazards model. The estimated median time to event and its 95% confidence interval (CI) will be calculated (if at least 50% of subjects in each treatment group achieve the event) using the Kaplan-Meier estimator. Hazard ratio and its 95% CI will also be estimated. Subjects not experiencing an event will be considered censored. The Kaplan-Meier estimates will be displayed graphically.

Continuous or ordinal variables will be analysed using an ANOVA including treatment and center.

Categorical variables will be compared between treatment groups using the Cochran-Mantel-Haenszel test, stratified by center. For the binary outcome variable, 95% CI for the proportion ratio (estimated from the CMH option of PROC FREQ) between each pair of treatment groups will be provided.

Centers with fewer than one subject in either treatment group will be pooled for all analyses.

All efficacy analyses in Part A will be performed on the efficacy dataset, as defined in Section 4 above.

Summary statistics will consist of frequencies and percentages of responses in each category for discrete measures and of means, medians, standard deviations, minimum and maximum values for continuous measures. For safety summaries, percentages will be based on the number of subjects in the safety dataset.

All analyses and summaries will be produced using SAS[®] version 8.2. All significance tests will be two-sided with statistical significance assessed at the 5% level.

Data will be listed for all randomized and open-label subjects. For listings of subject disposition and inclusion/exclusion criteria, all enrolled subjects will be listed.

9. Efficacy Analyses

9.1 Primary

The primary efficacy variable for Part A is the actual time from the start of the first infusion of study drug to the beginning of unequivocal relief of the defining symptom. Unequivocal relief is defined as having three consecutive reports of either “absent now but present before” or “present, symptoms better”. The beginning of relief is the time after infusion at which the first of these

three reports occurs. Time to relief will be compared between the active drug and placebo arms using the Cox-regression proportional hazards model as implemented in the SAS statistical procedure "PROC PHREG", study center will be included as a factor in the model. P-values for treatment and center will be reported for this primary analysis of the primary efficacy endpoint. Subjects who do not achieve symptom relief within 4 hours of the first dose will be censored at 4 hours. Subjects who receive rescue narcotics or open-label treatment with C1INH-nf due to treatment failure will be censored at the time the rescue medication was administered. Subjects who discontinue the study prior to 4 hours post-infusion and who have not experienced unequivocal relief will be censored at the time of discontinuation if they are withdrawn for a reason(s) unrelated to treatment failure. If the subject discontinues due to disease progression, investigator decision, withdrawn consent, death or adverse event (AE) related to HAE attack, the subject will be considered a treatment failure, and will be censored at 4 hours. Ambiguous reasons described in the "Other" CRF category will be assessed at the Blind Data Review meeting.

As a secondary analysis, treatment, study center, and center-by-treatment interaction will be included in the model to assess the comparability of study centers.

Subgroup analyses for gender and age group will also be conducted as secondary analyses with treatment and center included in the model plus the treatment by subgroup interaction for each of the two subgroup analyses.

9.2 Secondary

The secondary variables for Part A of the protocol are

- Presence or absence of unequivocal relief of the defining symptom within 4 hours following initial treatment. Treatment failures (including subjects who received open-label treatment and subjects with inadequate data) will be counted as failures (i.e., absence of relief within 4 hours). The treatment effect will be analyzed using Cochran-Mantel-Haenszel test, stratified by center.
- Time to complete resolution. The date and time of the complete resolution of the HAE attack are recorded at the 3-day telephone follow-up. Time from first infusion of study drug to the complete resolution of symptoms will be compared between the active drug and placebo arms (efficacy dataset) using the same Cox-regression proportional hazards model described for the primary analysis. Subjects who have not experienced complete resolution of symptoms at the time of the follow-up telephone call will be considered censored at the time of 3-day follow-up. Subjects who were discontinued at or prior to the 12 hour post-infusion time point will be censored at the time of discontinuation. Subjects who cannot be contacted at the 3-day time point will be censored at the time of their discharge from the hospital. Subjects who received rescue medication will be censored at the time rescue drug was administered.
- Change from baseline in other secondary endpoints (C1INH antigenic and functional levels and C4 levels) will each be analyzed by the Wilcoxon rank sum test
- Symptom ratings. The subjects' VAS rating of severity of the defining symptoms will be analyzed by comparing the Sum of the Pain/Swelling Intensity Difference (SPID) from 0-4 hours post-infusion between the two randomized treatment groups. The SPID 0-4h is

calculated as the time-weighted sum of the decrease in pain/swelling VAS from pre-infusion. For the purpose of calculating SPID, subjects who have alternative medication or receive open-label C1INH-nf prior to 4 hours post-infusion will have subsequent VAS scores imputed on a last observation carried forward basis. The SPID 0-4h will be compared between treatment groups in the efficacy dataset, using an ANOVA that includes the effects of treatment group and study center. Below are the details on the calculation of SPID 0-4h:

Calculation of SPID 0-4h

The Pain Intensity Difference is the improvement in VAS score from pre-infusion, and is calculated at each time-point as :

$$PID = \text{pre-infusion VAS} - \text{post-infusion VAS}$$

If $PID_{t(x)}$ is the pain intensity difference at time point x , and $t(x)$ is the relative time post-infusion at scheduled time-point x , then

$$SPID_{0-4h} = \sum_{x=0.25}^{x=4} PID_{t(x)} \cdot [t_{(x)} - t_{(x-0.25)}]$$

If the 4 hour time-point is more than 5 minutes away from the scheduled time point, then the 4-hour value will be imputed by linear interpolation.

9.3 Other Evaluations

There are no other efficacy evaluations planned.

9.4 Missing Data

As a consequence of the intent-to-treat nature of the efficacy analysis for this study, there will be no time-windows established for the regular post-infusion assessments in Part A. Missing symptom relief ratings will not be imputed. Thus, for the time to relief endpoint, the three positive assessments must be made, with no negative assessments intervening. However, intervening missing data will not be considered to have interrupted the three consecutive positive results required for 'unequivocal' relief.

10. Safety Analyses

Safety analyses will be assessed using the following measures: extent of exposure, AEs, vital signs, physical examinations, and laboratory tests. All summary safety analyses will be carried out using subjects included in the safety dataset.

10.1 Extent of Exposure

The number of infusions received by each subject will be summarized in a frequency table. Infusions given in the double-blind phase of the study will be distinguished from open label infusions given as rescue medication.

10.2 Adverse Events

Adverse events will be coded using the MedDRA coding dictionary version 8.0. Events will be classified by system organ class and preferred term. Only treatment emergent adverse events (TEAEs) will be summarized. Treatment-emergent adverse events are defined as those events which start on or after the time of first infusion of trial medication (or whose severity worsened on or after that time) and up to 30 days after the last infusion of study drug.

The incidence of TEAEs will be summarized by intensity ('Mild', 'Moderate' or 'Severe') at preferred term and system organ class level. For each subject and preferred term, only the most severe AE will be counted.

The incidence of AEs related to study drug will be summarized at preferred term and system organ class level. Events with a relationship to study drug classified as 'Definitely', 'Probably', 'Possibly' or 'Unknown' will be categorized as 'Related'. If a subject experiences the same event (at preferred term level) on more than one occasion, then only one event will be counted, and this event will be categorized as 'Related' if any of the events under that preferred term are categorized as 'Related'.

All AEs for each subject, including the same event on several occasions will be listed, giving both preferred term and the original term used by the investigator. Serious AEs will be described separately on an additional listing.

10.3 Laboratory Evaluations

Laboratory values will be obtained for serum chemistry, hematology and immunology at screening (immunology only), pre-treatment and 12 hours post-infusion. The laboratory tests will be performed by a central laboratory.

Descriptive statistics will be provided for serum chemistry and hematology data at pre-treatment and 12 hours post-infusion. Descriptive statistics for change from pre-treatment to 8 hours post-infusion, and shifts with respect to normal ranges for the same 8 hour period will also be presented. Data listings will be provided for all subjects. Laboratory values outside the normal range will be flagged.

Results of immunology tests (Anti Parvo B19, Parvo B19 NAT, HBsAg, Anti HCV, HCV NAT, Anti HIV, HIV NAT) at screening, pre-treatment and 3 months post-infusion will be listed. Urine pregnancy test result, recorded on the CRF at pre-randomization, will also be listed.

10.4 Physical Examination

Physical examination results at screening and pre-treatment will be listed by treatment group, subject, visit and body system. No summary statistics will be presented.

10.5 Vital Signs

Vital signs will be measured before and after the initial infusion and again after a second infusion if one is administered. Pre-infusion, post-infusion and change from pre- to post- infusion values for systolic blood pressure, diastolic blood pressure, heart rate, respiration rate and temperature will be summarized for the first and second infusions, and for any open-label infusions the subject has received.

Height and weight, obtained at the screening visit, will be summarized by treatment group within the demographics table.

10.6 Other Evaluations

Concomitant medications are coded according to the WHO drug dictionary, 2nd quarter, 2004.. Details of medications used are provided in a subject listing.

Discomfort and irritation at the injection site are recorded by the subject and an observer at 30 minutes and 4 hours after the initial infusion. These ratings (from 1 - no discomfort/irritation to 10 - extreme discomfort/irritation) will be presented in a data listing.

11. References

1. Kunschak M, Engl W, Maritsch F, Rosen FS, Eder G, Zerlauth G, Schwarz HP. A randomized, controlled trial to study the efficacy and safety of C1 inhibitor concentrate in treating hereditary angioedema. *Transfusion* 1998; 38:540-549.

12. Attachments

12.1 Attachment 1: Planned Listing Shells

12.2 Attachment 2: Planned Table Shells

12.3 Attachment 3: Planned Figure Shells

13. Appendix

13.1 SOP For Determining Randomization Evaluability for LEVP2005-1

Purpose:

According to the protocol LEVP2005-1, Complement C4 levels are used to determine if a subject is having a true HAE attack for Part A. The baseline C4 result at Screening is compared to the C4 result obtained at Pre-Infusion during an attack. If the C4 level at Pre-Infusion is lower than the C4 level at Screening the subject is found to have had a true attack and this subject's randomization data is determined to be evaluable and is included in the efficacy evaluation. Conversely if the result at Pre-Infusion is not lower than the screening C4, the subject's randomization data is excluded from the efficacy evaluation.

The testing of Complement C4 went thru two major changes during this trial. Originally C4 testing was performed at Specialty Laboratories. On August 16, 2005 Specialty Laboratories changed the C4 testing to a different instrument resulting in a reference range change. In December 2005, the C4 testing was moved to Mayo Laboratories requiring another reference range change.

These changes made the original procedure for determining the evaluability or inclusion/exclusion of a particular subject into the efficacy evaluation a challenge. Additionally, small differences in C4 levels between Screening and Pre-Infusion may be the result of normal instrument/method variation and therefore not a good indicator that the subject was/was not having a true HAE attack. Therefore, the decision was made to use the services of a judiciary to determine the evaluability of a certain randomization.

Judiciary Personnel Requirements:

Medical Doctor or PhD with extensive knowledge and background in the treatment of patients with Hereditary Angioedema. The candidate cannot be associated with LEVP2005-1 in any other capacity.

Procedure:

Information will be supplied to the Judiciary to aide in determining whether a subject's randomization is a true HAE attack and therefore is evaluable. Judiciary will be blinded. **Only information prior to infusion will be given.** No outcome results will be given.

Information supplied may include:

1. Screening Laboratory Results.

2. Pre-Infusion Laboratory Results
3. Source Doc Worksheets prior to Infusion.
4. Medical History

Judiciary may call the following people if he has questions:

1. Linda Ubelacker- Clinical Trial Manager
2. Lyle Camblos- Lead CRA
3. Site Coordinator at a given site
4. Principle Investigator at a given site

Based on the above information the Judiciary will decide if the subject had a true HAE attack and therefore should be included or excluded in the Efficacy Evaluation.



Statistical Analysis Plan

**CHANGE Trial (C1-Inhibitor in Hereditary Angioedema
Nanofiltration Generation Evaluating Efficacy): A Double-blind,
Placebo Controlled, Clinical Study to Investigate the Efficacy and
Safety of Purified C1 Esterase Inhibitor (Human) for the Treatment
of Hereditary Angioedema (HAE) in Acute Attacks and as
Prophylactic Treatment to Prevent HAE Attacks**

(Part B)

Protocol No: LEVP2005-1

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Final
April 05, 2007

Amendment 1
July 02, 2007

List of Abbreviations

AE	Adverse Event
CI	Confidence Interval
CRF	Case Report Form
h	Hour
HAE	Hereditary Angioedema
ITT	Intent-to-Treat
MedDRA	Medical Dictionary for Regulatory Activities
SAP	Statistical Analysis Plan
SAS [®]	Statistical Analysis Software
TEAE	Treatment-emergent Adverse Events
VAS	Visual Analog Scale
WHO	World Health Organization

1. Study Design

This is a multi-center, 2-part Phase III study.

Part A is a randomized, placebo-controlled, double-blind study designed to evaluate the efficacy and safety of C1INH-nf as a therapeutic agent for acute attacks of Hereditary Angioedema (HAE). Statistical Analysis Plan (SAP) for Part A is described in a separate document.

Part B is a randomized, placebo-controlled, double-blind cross-over study to confirm the efficacy and safety of prophylactic C1INH-nf in preventing acute attacks of angioedema in HAE subjects. Part B will last for a total of 24 weeks. Each subject will be randomized either to receive 12 weeks of C1INH-nf followed by 12 weeks of placebo, or 12 weeks of placebo followed by 12 weeks of C1INH-nf. Subjects enrolled in Part B may receive open label treatment, if in the opinion of the principal investigator, treatment is required.

This document describes the summary and analysis of Part B only.

2. Objectives

The objective of Part B of the study is to confirm the efficacy and safety of prophylactic C1INH-nf in preventing acute attacks of angioedema in HAE subjects.

3. Sample Size Determination

Assuming angioedema attack rates will be 1 every two weeks in the placebo phase and 1 every 12 weeks in the prophylactic C1INH-nf phase, 10 subjects per sequence provides more than 90% power to detect the treatment effect. A total of 20 subjects is planned to be enrolled into Part B.

4. Analysis Datasets

The Efficacy dataset will consist of all subjects who were randomized into one of 2 treatment sequences and who completed the entire initial treatment phase (B1) and received at least 1 treatment of the crossover phase (B2). The number of attacks will be calculated on a per day basis.

The Safety dataset will consist of all subjects who received a complete or partial infusion of therapeutic treatment.

5. Interim Analysis

There is no interim analysis planned for this study.

6. Subject Disposition

A summary table of subject disposition will be provided, showing the number of subjects enrolled, the number of subjects randomized to either treatment sequence, and the number of subjects who received open-label treatment. For all randomized subjects, the number of subjects who completed the first treatment period and the number of subjects who completed both treatment periods will be tabulated. A separate row will clarify the number of subjects in the safety dataset.

The number of subjects withdrawing from the study will be tabulated. Withdrawals will be categorized by reason for withdrawal.

7. Demographics and Baseline Characteristics

Demographics (age, gender and ethnic origin) will be summarized by treatment sequence for the efficacy and safety datasets.

In addition, the following baseline characteristics will be tabulated for the safety dataset:

- Medical History: abnormalities by body system
- Time since diagnosis of HAE: summary statistics and categorical frequencies
- Historical severity of symptoms: severity grade by area of swelling

8. General Analysis Comments

Descriptive summaries of measurements will be presented by treatment or by treatment sequence. Summary statistics will consist of frequencies and percentages of responses in each category for discrete measures and of means, medians, standard deviations, minimum and maximum values for continuous measures. For safety summaries, percentages will be based on the number of subjects in the safety dataset.

Efficacy analyses in Part B will be performed on the efficacy dataset; except for the analysis on the number of subjects dropping out at each treatment period, which will be performed on the safety dataset. All safety analyses will be performed on the safety dataset, as defined in Section 4 above.

For cross-over analyses, a standard analysis of variance for cross-over study design will be performed with effects for treatment, period, and subject within treatment.

All analyses and summaries will be produced using SAS[®] version 8.2. All significance tests will be two-sided with statistical significance assessed at the 5% level.

By-subject listings will be provided for all data collected and entered into the study database.

9. Efficacy Analyses

9.1 Primary

The primary efficacy endpoint for Part B will be the number of attacks of angioedema during each treatment period, normalized for the number of days the subject participated in that period. This will be done by dividing the total number of attacks in each period by the number of days the patient was in that period. An attack is defined as the subject reported indication of swelling at any location following a report of no swelling on the previous day. The crossover analysis will be based on a Poisson assumption and use the GEE method as implemented in the SAS statistical procedure PROC GENMOD. The goodness-of-fit statistics, deviance and Pearson chi-square, along with the ratios of their values to their degrees of freedom, from the initial model fitting will be used to check for overdispersion. If the Deviance/df and Pearson/df ratios are greater than 1, then there is evidence of overdispersion. In such situation, the GEE approach is able to address the overdispersion issue since it is robust to the misspecification of the covariance structure, and misspecification is occurring in the case of overdispersion.

9.2 Secondary

The secondary efficacy endpoints for Part B are

- Number of subjects dropping out at each treatment period. For the first treatment period, drop-out is defined as not having any Visit 24A records. For the second treatment period, drop-out is defined as not having any Visit 24B records. This is a binary categorical endpoint. At the end of each treatment period, each subject will be assigned a Yes/No drop-out status and a 2x2 table will be produced for treatment by drop-out status. A Fisher's exact test will be carried out to compare between treatments.
- Average severity of attacks. The severity of an attack will be the highest value assigned by the subject to any location at any day during the attack. In order to calculate the average severity of attacks for each period, each mild, moderate, and severe attack will be assigned a score of 1, 2, or 3, respectively. The total severity score will be calculated for each period by multiplying the total number of mild attacks by 1, total number of moderate attacks by 2, and the total number of severe attacks by 3, then adding the results of these three calculations. The average severity of each period is then derived by dividing the total severity score of that period by the total number of attacks in that period. The difference between treatments will be tested by a Wilcoxon Signed Rank Test.
- Average duration of attacks. The duration of an attack will be measured from the first report of swelling at any location until the next report of no swelling at any location. Average duration of attacks for each period will be calculated by first summing the duration of each attack, then dividing that sum by the total number of attacks in that period. The difference between treatments will be tested by a Wilcoxon Signed Rank Test.
- Number of open-label C1INH-nf infusions. The total number of open-label C1INH-nf infusions (counting double infusions as two infusions) while subjects receiving active treatment will be compared with the total number of open-label C1INH-nf infusions (counting double infusions as two infusions) while subjects receiving placebo by using the Wilcoxon Signed Rank Test.
- Change from baseline in C1INH antigenic and functional levels will be compared between study treatments by using the Wilcoxon Signed Rank Test. Baseline value for the first period is defined as the Visit 1a pre-infusion measurement. Baseline value for the second period is defined as the Visit 1b pre-infusion measurement. C1INH antigenic and functional levels will be measured pre and post infusion at Visits 1a, 8a, 16a, 24a, 1b, 8b, 16b, and 24b.

9.3 Other Evaluations

The total number of days of swelling in each study period will be compared between C1INH-nf and placebo. A day of swelling is defined as a day that a subject reported indication of swelling at any location. The difference between treatments will be tested by a Wilcoxon Signed Rank Test.

9.4 Missing Data

Missing data will not be imputed.

10. Safety Analyses

Safety analyses will be assessed using the following measures: extent of exposure, AEs, vital signs, physical examinations, and laboratory tests. All summary safety analyses will be carried out using subjects included in the safety dataset.

10.1 Extent of Exposure

Summary statistics for dose (mL) will be presented for each treatment and open-label drug infusions.

10.2 Adverse Events

Adverse events will be coded using the MedDRA coding dictionary version 8.0. Events will be classified by system organ class and preferred term. Only treatment emergent adverse events (TEAEs) will be summarized. Treatment-emergent adverse events are defined as those events which start on or after the time of first infusion of trial medication (or whose severity worsened on or after that time) and up to 30 days after the last infusion of study drug.

The incidence of TEAEs will be summarized by intensity ('Mild', 'Moderate' or 'Severe') at preferred term and system organ class level. For each subject and preferred term, only the most severe AE will be counted.

The incidence of AEs related to study drug will be summarized at preferred term and system organ class level. Events with a relationship to study drug classified as 'Definitely', 'Probably', 'Possibly' or 'Unknown' will be categorized as 'Related'. If a subject experiences the same event (at preferred term level) on more than one occasion, then only one event will be counted, and this event will be categorized as 'Related' if any of the events under that preferred term are categorized as 'Related'.

All AEs for each subject, including the same event on several occasions will be listed, giving both preferred term and the original term used by the investigator. Serious AEs will be presented separately on an additional listing.

10.3 Laboratory Evaluations

Summary statistics will be presented for each lab assessments. Lab value change and shift from follow-up visit to initial treatment visit will also be presented.

10.4 Physical Examination

Physical examination results at initial and final treatment visits will be listed. No summary statistics will be presented.

10.5 Vital Signs

Summary statistics for vital signs measured at each treatment visit, and the change from post-infusion to pre-infusion at each visit, will be presented by treatment.

10.6 Other Evaluations

Concomitant medications are coded according to the WHO drug dictionary, 2nd quarter, 2004. Details of medications used are provided in a subject listing.

11. Summary of Changes in Amendment 1

The following new text is added to the drop-out analysis description in [Section 9.2](#):

“For the first treatment period, drop-out is defined as not having any Visit 24A records. For the second treatment period, drop-out is defined as not having any Visit 24B records.”

12. Attachments

12.1 Attachment 1: Planned Listing Shells

12.2 Attachment 2: Planned Table Shells