

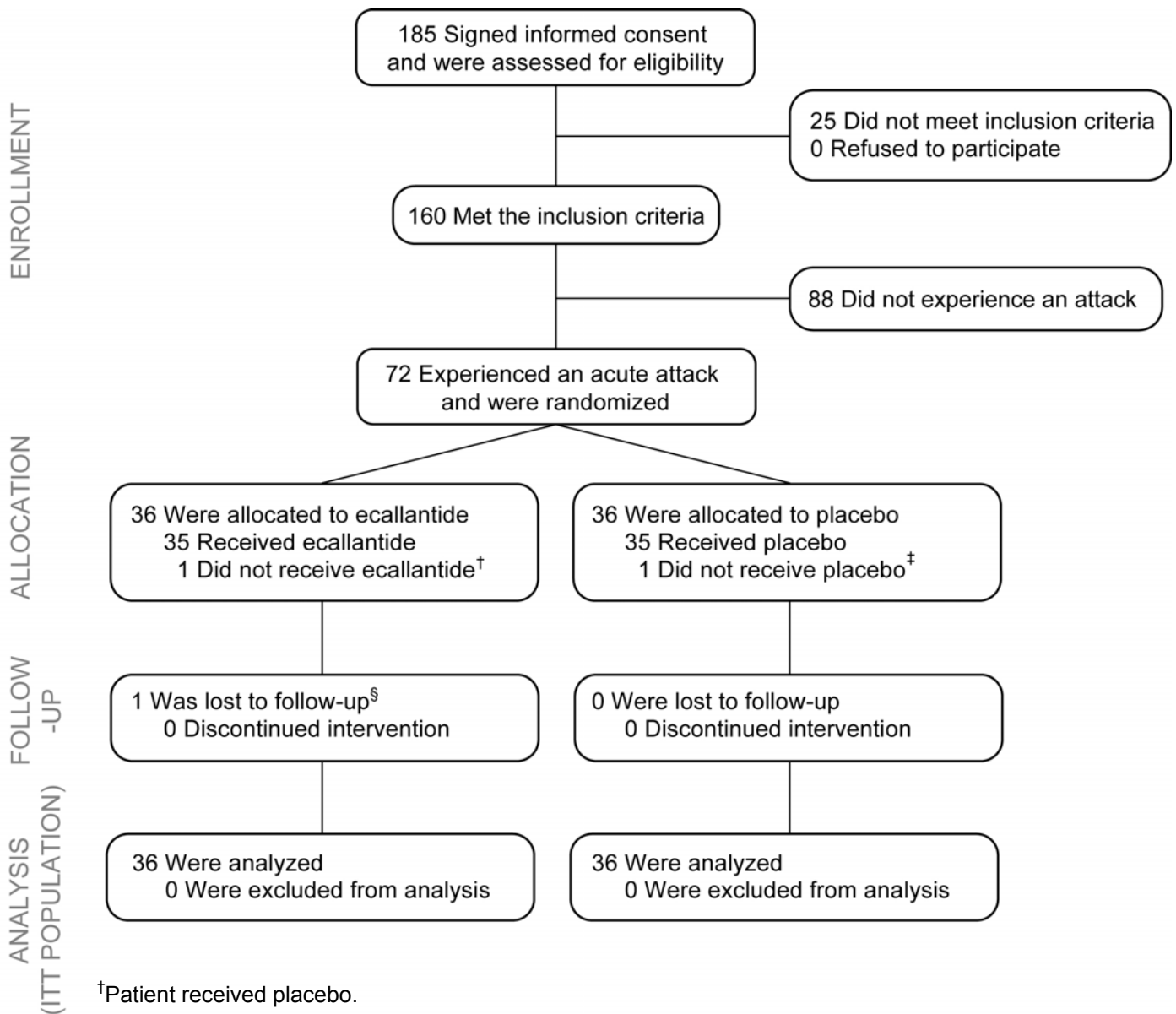
Supplementary Appendix

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

Supplement to: Cicardi M, Levy RJ, McNeil DL, et al. Ecallantide for the treatment of acute attacks in hereditary angioedema. *N Engl J Med* 2010;363:523-31.

Supplementary Appendix Figure 1.

Enrollment, Allocation, Follow-up, and Analysis of Study Participants.



†Patient received placebo.

‡Patient received ecallantide.

§Patient was lost to follow-up after Visit 1.

ITT denotes intent-to-treat.

Patient-Reported Outcomes Instruments

To capture the relevant symptoms and severity of an attack of angioedema, as well as to quantify the improvement of symptoms after treatment, two novel patient-reported outcomes instruments were developed: the treatment outcome score and the mean symptom complex severity score. The treatment outcome score and the mean symptom complex severity score were developed with input from external psychometric experts, practicing clinicians, patients with hereditary angioedema, and the US Food and Drug Administration (FDA).

The treatment outcome score is a composite patient-reported outcome measure composed of 3 components: 1) the anatomic location of attack (“symptom complex”); 2) the severity of each symptom complex at baseline; and 3) response of each symptom complex after study drug administration. Symptom complexes are classified as laryngeal, gastrointestinal, genital/buttocks, external head/neck, and cutaneous. The last three of these symptom complexes are collectively referred to as “peripheral”. Baseline symptom severity is graded on a scale of 1 to 3 (1 = mild, 2 = moderate, 3 = severe). After study drug administration, the patient’s symptoms are reassessed at 1, 2, 3, 4, and 24 hours. The change in severity for each symptom complex at follow-up (outcome score) is graded as follows: significant improvement = 100; improvement = 50; same = 0; worsening = -50; and significant worsening = -100.

The treatment outcome score is calculated by taking the sum of individual symptom complex outcome scores multiplied by their individual severity score, divided by the sum of individual severity scores as presented in the following formula:

$$\text{Treatment outcome score} = \frac{\sum(\text{symptom complex outcome score} \times \text{symptom complex severity})}{\sum \text{symptom complex severity}}$$

Example 1: Patient with “severe” laryngeal symptoms (score = 3) and “mild” gastrointestinal symptoms (score = 1) at baseline. At 4 hours the laryngeal symptom complex is scored as “improvement” (score = 50) and the gastrointestinal symptom complex is scored as “same” (score = 0). The resulting 4 hour treatment outcome score is $[(3 \times 50) + (1 \times 0)] / 4 = [(150) + (0)] / 4 = 37.5$.

Example 2: Patient with “severe” laryngeal symptoms (score = 3) and “severe” gastrointestinal symptoms (score = 3) at baseline. At 4 hours the laryngeal symptom complex is scored as “significant improvement” (score = 100) and the gastrointestinal symptom complex is scored as “same” (score = 0). The resulting 4 hour treatment outcome score is $[(3 \times 100) + (3 \times 0)] / 6 = [(300) + (0)] / 6 = 50$.

The primary trial end point was treatment outcome score at 4 hours after study drug administration. The 4-hour time point was chosen based on previous evidence that drug efficacy can be discriminated from placebo effect within this time frame [Waytes AT et al. N Engl J Med 1996;334:1630-4].

The mean symptom complex severity score reflects a comprehensive assessment of symptom burden at a single point in time. The mean symptom complex severity score is an arithmetic mean based on symptom complex identification and severity assessment of each symptom complex. The mean symptom complex severity score uses the 3-point severity scale described above for the baseline evaluation. At 4 and 24 hours after study drug administration, patients rate severity of all symptom complexes identified at baseline as well as any symptoms that emerged since presentation (0=no symptoms; 1 = mild; 2 = moderate; 3 = severe). The ratings from all presenting and emerging symptom complexes are averaged to generate the mean symptom complex severity score. A decrease in mean symptom complex severity score from baseline reflects an improvement in symptoms.

Example: Patient with “severe” laryngeal symptoms (score = 3), “moderate” head and neck symptoms (score = 2) and “mild” gastrointestinal symptoms (score = 1) at baseline. At 4 hours the laryngeal symptoms are “mild” (score = 1), the head and neck symptoms are “mild” (score = 1) and the gastrointestinal symptoms have resolved (score = 0). At 24 hours the laryngeal

symptoms have resolved (score = 0), the head and neck symptoms are “mild” (score = 1) and the gastrointestinal symptoms have resolved (score = 0).

The baseline mean symptom complex severity score is $[3 + 2 + 1] / 3 = 6 / 3 = 2$.

The 4 hour mean symptom complex severity score is $[1 + 1 + 0] / 3 = 2 / 3 = 0.67$.

The 24 hour mean symptom complex severity score is $[0 + 1 + 0] / 3 = 1 / 3 = 0.33$.

The change in mean symptom complex severity score from baseline to 4 hours is $0.67 - 2 = -1.33$.

The change in mean symptoms complex severity score from baseline to 24 hours is $0.33 - 2 = -1.67$.

To establish the minimally important difference for both the treatment outcome score and the mean symptom complex severity score, a secondary analysis of pooled ecallantide and placebo data from EDEMA3 was undertaken [Vernon MK et al. Qual Life Res 2009;18:929-939]. Anchor- and distribution-based approaches provided estimates of the minimally important difference for the treatment outcome score of 30 points at 4 hours and for the change in mean symptom complex severity score from baseline at 4 hours of -0.30 points. The anchor-based approach was assessed through an analysis of covariance (ANCOVA) model comparing treatment outcome scores at 4 hours after study drug administration between five categories of overall symptom change (significant improvement, improvement, same, worsening, and significant worsening), controlling for baseline MSCS score. A similar analysis of variance (ANOVA) model comparing change in mean symptom complex severity score from baseline at 4 hours after study drug administration between the five overall symptom change categories was also conducted. The minimally important difference was determined by examining the differences between the stable group (same) and the group defined as reporting improvements (improvement). Distribution-based methods were also calculated. As suggested by some researchers, standard error of the mean, which was computed after the reliability assessment, may approximate the minimally important difference. The second distribution-based approach utilized was an assessment of one half of a standard deviation of the treatment outcome score at 4 hours after study drug administration and the mean symptom complex score at baseline.

Emerging Symptoms and Medical Interventions

Emerging symptoms within 24 hours after study drug administration were observed in 1 ecallantide-treated patient and 3 placebo-treated patients. The ecallantide-treated patient presented with cutaneous symptoms and developed gastrointestinal symptoms. In the placebo group, 1 patient who presented with cutaneous symptoms developed genital/buttocks symptoms, 1 patient who presented with a laryngeal attack developed cutaneous symptoms, and 1 patient who presented with a gastrointestinal attack developed laryngeal symptoms.

Fewer ecallantide-treated patients (n = 5) required medical interventions within 24 hours after study drug administration than placebo-treated patients (n = 13; P=0.012, logistic regression model). In most cases (2 ecallantide-treated and 12 placebo-treated patients), medical intervention consisted of emergency medications including pain medications, anti-nausea medications, or C1-INH. One patient in each group received fresh frozen plasma. Open-label ecallantide was administered to 2 ecallantide-treated patients and 1 placebo-treated patient with severe upper airway compromise. All 3 patients experienced symptom resolution; only the placebo-treated patient received additional medical intervention (diphenhydramine and epinephrine).

Adverse Events

Serious adverse events were experienced by 3 (4.2%) ecallantide-treated patients and 2 (2.7%) placebo-treated patients. All 5 events were acute attacks of angioedema unrelated to study treatment; all patients recovered without sequelae.

The most common adverse events occurring more often in ecallantide-treated patients than in placebo-treated patients were headache (4 patients), diarrhea (3 patients), pyrexia (3 patients), and nasal congestion (2 patients).

Four patients treated with ecallantide reported headache. Patient 1 experienced a headache (CTC Grade 1) with onset within hours of study drug administration. The headache was intermittent for 5 days and was treated with intermittent acetaminophen and ibuprofen. Concurrent adverse events were loose stools and upset stomach (both CTC grade 1). All 3 adverse events were considered “not related” by the investigator.

Patient 2 experienced a headache (CTC Grade 1) with onset the day following study drug administration and resolution on the same day. Treatment consisted of 2 doses of acetaminophen. Concurrent adverse events were stomach spasms, fever, and rigors (all CTC Grade 1). All of the adverse events were considered by the investigator to be possibly related to study drug.

Patient 3 experienced a headache (CTC grade 1) with onset the day of study drug administration and resolution on the same day. There were no concurrent adverse events and no treatments were administered. The investigator considered this adverse event to be unrelated to study drug.

Patient 4 experienced a headache (CTC Grade 1) with onset within hours of study drug administration and a duration of 3 hours. There were no concurrent adverse events. One dose of aspirin was administered. The investigator considered this adverse event to be unrelated to study drug.

Three patients reported diarrhea within 24 hours of receipt of ecallantide. For all three patients, the severity was mild (CTC Grade 1). Two patients reported gastrointestinal symptoms at baseline as part of their initial attack of angioedema, and the third developed gastrointestinal symptoms following receipt of study drug, consisting of mild diarrhea that persisted for 9 days.

Pyrexia was reported for three patients treated with ecallantide. Patient 1 experienced pyrexia approximately 1 day after treatment with ecallantide (no corresponding temperature; patient report only). The event was assessed by the investigator as moderate in severity (CTC Grade 2) and not related to treatment. The patient was administered aspirin (one 325-mg oral dose) and recovered without sequelae.

Patient 2 experienced pyrexia approximately 1 day after treatment with ecallantide. The event was assessed by the investigator as mild (CTC Grade 1) and possibly related to treatment. The patient was administered acetaminophen (one 650-mg oral dose) and acetaminophen with codeine (one 650-mg oral dose) and recovered without sequelae. No specific temperature was reported by the patient. Concurrent adverse events at the time of pyrexia included GI disorder, headache, and rigors, all assessed by the investigator as mild (CTC Grade 1) and possibly related to treatment. All events were nonserious and the patient recovered without sequelae.

Patient 3 experienced pyrexia (38.6°C) approximately 2 hours after treatment with ecallantide. The event was assessed by the investigator as severe (CTC Grade 3) and probably related to treatment. The patient was administered acetaminophen (one oral 1000-mg dose) and recovered without sequelae. Concurrently, the patient experienced the adverse event of influenza-like illness assessed by the investigator as mild (CTC Grade 1) and probably related to treatment. The patient was not treated for this concurrent adverse event. One day after treatment, the patient experienced the adverse event of fatigue that was assessed by the investigator as mild (CTC Grade 1) and

probably related to treatment. All events were assessed by the investigator as nonserious, and the patient recovered without sequelae.

Clinical laboratory results, vital signs, physical examination findings, and ECG results did not indicate any sustained or clinically relevant changes related to ecallantide treatment. A prolonged prothrombin time was observed in no ecallantide-treated and 2 placebo-treated patients; 1 of these cases was attributed to laboratory error. There were no clinically significant changes in cardiac rhythm from baseline to 2 hours after dosing for either the ecallantide or placebo group. The mean change in heart rate was -3.1 beats/min in the ecallantide group and -2.1 beats/min in the placebo group. The mean change in corrected QT interval was 0.9 msec (range, -8.5 to 9.6 msec) in the ecallantide group and -0.1 msec (range, -6.1 to 8.9 msec) in the placebo group.

All serum samples were negative for immunoglobulin E (IgE) antibodies to ecallantide by enzyme-linked immunosorbent assay (ELISA) at all study time points. None of the patients who received ecallantide as first-time exposure developed non-IgE antibodies to ecallantide. Two patients, who had been previously treated with 9 and 11 doses of ecallantide, respectively, tested positive for non-IgE anti-ecallantide antibodies. One patient tested positive for non-IgE antibodies specific to ecallantide at the enrollment visit (prior to study drug administration) and at the first follow-up visit (Day 6-10). The other patient tested positive for non-IgE antibodies specific to ecallantide at separate screening and enrollment visits (prior to study drug administration) and at the first follow-up visit (Day 6-10). Neither patient experienced adverse events related to treatment in this study. Both had improved symptoms, as measured by Treatment Outcome Score, at 4 hours after study drug administration.

Two ecallantide-treated patients and 1 placebo-treated patient seroconverted to anti-*P pastoris* IgE antibodies. Additionally, 5 ecallantide-treated and 3 placebo-treated patients tested positive for anti-*P pastoris* IgE antibodies at baseline. No episodes of potential hypersensitivity (e.g., urticaria, pruritus, rhinitis) or anaphylaxis were noted in either group.