FDA Briefing Package

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DIVISION MEMORANDUM

Date: January 9, 2009

From: Sally Seymour, MD
Deputy Director for Safety, Division of Pulmonary and Allergy Products

To: Members, Pulmonary-Allergy Drugs Advisory Committee

Subject: Overview of the FDA background materials for BLA# 125277, Kalbitor (ecallantide) Injection 30mg, for the treatment of acute attacks of hereditary angioedema (HAE) in patients 10 years of age and older

Introduction

Thank you for your participation in the Pulmonary-Allergy Drugs Advisory Committee (PADAC) meeting to be held on February 4, 2009. As members of the PADAC you provide important expert scientific advice and recommendation to the US Food and Drug Administration (the Agency) on the regulatory decision making process related to the approval of a drug or biologic product for marketing in the United States. The upcoming meeting is to discuss the Biologic Licensing Application (BLA) from Dyax Corp., seeking approval for ecallantide 30mg for the treatment of acute attacks of hereditary angioedema in patients 10 years of age and older. The proposed trade name is Kalbitor.

Hereditary angioedema (HAE) is a rare, autosomal dominant disorder estimated to affect 1 in 10,000 to 50,000 individuals, without known differences among ethnic groups. HAE is characterized by intermittent, unpredictable attacks of subcutaneous or submucosal edema of the face, larynx, gastrointestinal tract, limbs, and/or genitalia. Attacks can vary in severity and location and can be life-threatening, particularly those attacks involving the airway. In addition to potentially life threatening laryngeal edema, HAE can also cause significant morbidity.

The treatment options for HAE are usually divided into three categories – chronic long-term therapy, short-term prophylaxis to prevent attacks, and treatment of acute attacks[1]. Recently, recombinant C1 inhibitor (Cinryze™) administered intravenously was approved for routine prophylaxis of HAE attacks in adults and adolescents in the United States (US). Androgenic steroids are also approved for use in patients with HAE in the US. Danazol is approved and marketed in the US with the label indication "prevention of attacks of angioedema." The drug is also used for chronic long-term therapy[1,2]. Stanazol and oxymetholone are also approved with similar indication, but are no longer marketed in the US. Elsewhere in the world epsilon aminocaproic acid (EACA) and tranexamic acid (TA) are approved for use in HAE patients. EACA and TA are used as chronic long-term therapy in HAE, but these are not thought to be effective in acute attacks [1,2]. Fresh frozen

[1] MM Frank. Hereditary angioedema: The clinical syndrome and its management in the United States. Immunol Allergy Clin N Am 2006; 26:653-668.
[2] MM Frank, Jiang H. New therapies for hereditary angioedema: Disease outlook changes dramatically. J Allergy Clin Immunol 2008; 121:272-280.

plasma is often used for short-term prophylaxis to prevent acute attacks and for treatment of acute attacks, but the use of fresh frozen plasma in HAE is controversial as it may worsen an attack by providing more substrate that can be acted on to release additional mediators such as high molecular weight kininogens[1].

Currently, no products are approved for the treatment of acute attacks of HAE in the United States. Ecallantide is a new molecular entity proposed for the treatment of acute attacks of hereditary angioedema in patients 10 years of age and older. Ecallantide is a plasma kallikrein inhibitor, which reversibly binds human kallikrein. Ordinarily, kallikrein activity is regulated by C1-esterase inhibitor (C1 INH). In HAE patients with low or absent levels of functional C1-INH, kallikrein activity goes unchecked and is thought to lead to widespread release of bradykinin. In turn, bradykinin increases vascular permeability which leads to the swelling characteristic of acute HAE attacks.

The materials to be discussed in this meeting and the opinions we are seeking are primarily related to the clinical issues of ecallantide and statistical issues related to the study results. Keep in mind that in the regulatory decision making process to determine approvability of a product, the Agency takes into consideration various factors in addition to clinical issues, including manufacturing and controls of a product and preclinical considerations. These will not be the focus of this Advisory Committee meeting.

Attached are the background materials for this meeting. The background materials include the following: a clinical briefing document, a statistical briefing document, a brief summary of the clinical pharmacology program, a brief summary of the immunoassays utilized in the ecallantide program, the proposed product label for Kalbitor, and reference articles.

This memorandum summarizes the contents of the Agency background material and the key issues and questions for discussion at the meeting. The materials prepared by the Agency contain findings and opinions based on reviews of information submitted by Dyax. These background materials represent preliminary findings, and do no represent the final position of the Agency. An important piece in our decision on this application will be the opinions and input that we receive from you at this meeting.

Background

Ecallantide is a recombinant human plasma kallikrein inhibitor, which reversibly binds human kallikrein. Ecallantide is a 60 amino acid protein containing 3 intra-molecular disulfide bonds, with a molecular weight of 7054 Daltons. It was identified through iterative selection and screening of phage display libraries of the first Kunitz domain of human tissue factor pathway inhibitor. Ecallantide is produced by expression in the yeast, *Pichia pastoris*, then recovered and purified by chromatography. Biologic activity is determined by an *in vitro* activity assay. Glycosylation, oxidation, and N-terminal truncation can occur forming ecallantide related variants. The product related variants have been characterized and are biologically active.

The drug product is a sterile solution for injection containing ecallantide in phosphate buffered saline. There are no preservatives and the pH of the solution is 7.0. The solution is contained in a clear glass vial, in which each vial contains 1mL of ecallantide solution

10mg/mL. The proposed dose of ecallantide is 30mg (3mL), which is to be administered subcutaneously (SC) in three (1mL) divided doses away from the angioedema location. No dilution is necessary.

Pharmacology/Toxicology

Dyax submitted a complete pharmacology/toxicology program to support the chronic intermittent use of ecallantide. The program included 6 month, repeat dose, subcutaneous toxicology studies in rats and monkeys as well as other short term toxicology studies. Reproductive toxicology assessment included a fertility study in rats, teratology studies in rats and rabbits, and a perinatal/postnatal study in rats.

In the 6-month rat and monkey toxicology studies, the primary finding was local injection site reaction. In the rat study, deaths were noted in the high dose group and control group. Brain necrosis was observed in one of these high dose females. The causes of death were not determined except one of the male rat deaths was considered procedure-related. In rats, an increase in transaminases was also noted, but no associated histology changes in the liver. The No Observed Adverse Effects Level (NOAEL) in the rat study was determined to be the mid-dose group, which provides a safety margin of approximately 4-fold or 10-fold for the proposed human dose. There were no deaths or other significant systemic toxicities observed in monkeys.

In terms of immunogenicity, ecallantide antibodies were noted in both rats and monkeys and at a higher frequency in the high dose groups. Based upon the pharmacokinetic data, clearance of ecallantide was reduced and systemic exposure was increased following the development of ecallantide antibodies. However, there was no increase in toxicity noted with the higher exposure.

In animal studies, ecallantide caused a dose-dependent, reversible prolongation of aPTT, which is thought to be due to ecallantide inhibition of activation of factor XII to factor XIIa in the clotting cascade. However, there was no evidence of gross bleeding in the animals with the increase in aPTT.

Clinical Pharmacology

The bioavailability of ecallantide following SC administration is approximately 90% and maximum plasma concentrations are observed approximately 2 to 3 hours after dosing. The elimination half-life is approximately 2.0 hours. As a small polypeptide, ecallantide is expected to be eliminated by metabolic catabolism and renal elimination. But no clinical or preclinical studies were conducted to assess mass balance, route of excretion, or metabolism of the drug. Details regarding the pharmacokinetic data are summarized in the clinical pharmacology memorandum. It should be noted that there is a question of assay validation, so the pharmacokinetic data should be considered preliminary until assay validation is confirmed.

Clinical Program

To support the safety and efficacy of ecallantide for the proposed indication, Dyax submitted a full clinical program including 5 completed studies in HAE patients

(EDEMA0, EDEMA1, EDEMA2, EDEMA3, and EDEMA4) and an ongoing open label study. EDEMA0 and EDEMA1 were early phase 2 studies using IV doses of ecallantide and provide some safety information, but limited efficacy data. EDEMA2 is a dose ranging study that includes subcutaneous (SC) administration of ecallantide. EDEMA3 and EDEMA4 are the two phase 3 controlled clinical trials. EDEMA2, EDEMA3, and EDEMA 4 are the main sources of efficacy and safety data and will be the primary focus of this memo.

Dose Selection

Dose ranging in HAE patients for the proposed indication can be challenging due to the limited patient population and intermittent nature of HAE attacks. Dyax performed three phase 2 studies (EDEMA0, EDEMA1, EDEMA2) that provide some information regarding dose selection; however, each study has its limitations. EDEMA0 was not controlled and only included 9 HAE patients; thus, the results provide little information regarding dose selection. EDEMA1 was a randomized, placebo-controlled, double blind study that evaluated 4 doses of IV ecallantide in patients with HAE, but did not include a SC dose of ecallantide.

EDEMA2 was a phase 2, open-label dose ranging study designed to assess the safety and efficacy of repeated doses of ecallantide in patients 10 years of age and older with acute HAE attacks. Three IV dosage groups (5 mg/m^2, 10 mg/m^2, and 20 mg/m^2) as well as a more convenient dosage, ecallantide 30mg SC, were included in EDEMA2. The 30mg SC dose of ecallantide was expected to provide exposure similar to a 10-20mg/m^2 IV dose group. Although EDEMA2 was not controlled, the results provide some information regarding dose response.

Seventy-seven patients were enrolled and treated with ecallantide for a total of 240 acute HAE attacks in EDEMA2. Of the 240 attacks, there was a range in the number of attacks treated with each dose of ecallantide as shown in the table below. The primary efficacy assessment was the proportion of patients with a successful outcome defined as onset of resolution of symptoms within 4 hours of dosing and continuing for 24 hours. With regards to the primary efficacy assessment, the ecallantide 30mg SC group had the highest proportion of successful outcomes (82%) and lowest proportion of partial response compared to the other treatment groups as shown in the table below.

Table 1 Key Results EDEMA2

	Ecallantide 5 mg/m^2	**Ecallantide 10 mg/m^2**	**Ecallantide 20 mg/m^2**	**Ecallantide 30 mg SC**
Number of patients*	18	55	9	31
Number of attacks treated	24	141	15	60
Proportion of patients with successful outcome**	46%	68%	60%	82%
Proportion of patients with partial response***	33%	16%	27%	12%

* the number of patients exceeds 77 because patients could receive different doses of ecallantide
** successful outcome defined as onset of resolution within 4 hours of dosing and continuing for 24 hours following a single dose
*** partial response defined as response to dosing followed by a relapse within 24 hours

While the results should be interpreted with caution due to the design limitations of EDEMA2, the results suggest that the selection of ecallantide 30mg SC for the phase 3 program is reasonable.

Patient Reported Outcomes – TOS and MSCS

Assessment of efficacy for HAE attacks, which are highly variable in terms of symptoms and location, ideally should be based upon patient symptoms. However, there are no patient reported outcome (PRO) instruments that are the gold standard for assessing symptoms in this population. Therefore, the Applicant developed two PRO scores to assess patient symptoms and response to intervention.

The PROs developed by Dyax are the Mean Symptom Complex Severity (MSCS) and the Treatment Outcome Score (TOS). The MSCS is a global measure of symptom severity at a point in time, while the TOS is a composite measure of response to therapy. The conceptual frameworks for both PROs are shown in the figure below.

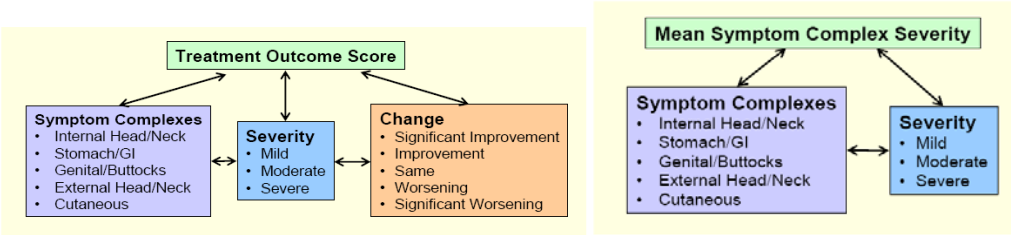


Figure 1 Conceptual Framework for TOS and MSCS

Upon presentation, patients identified HAE symptoms grouped by a symptom complex, i.e. Internal Head/Neck, Stomach/GI, Genital/Buttocks, External Head/Neck, or Cutaneous. The patient ranked each symptom complex severity as normal (0), Mild (1), Moderate (2), or Severe (3). Following study medication, patients assessed response as follows: Significant Improvement (a lot better), Improvement (a little better), Same (unchanged), Worsening (a little worse), or Significant Worsening (a lot worse), scored as 100, 50, 0, -50, -100, respectively.

Using the information recorded in the patient diaries, the TOS at 4 hours was calculated to weight the response for each complex based upon the severity at baseline. In the determination of the TOS, the symptom complex score is the response to treatment (score of -100 to 100) and the complex weight is the severity (0 to 3).

$$TOS = \frac{\sum symptom\ complex\ score \times symptom\ complex\ weight}{\sum symptom\ complex\ weight}$$

As illustrated in the above paragraphs, the TOS is quite complicated to explain and because of this, the results of the TOS are difficult to interpret. The inclusion of a response score ranging from -100 to 100 is not necessarily intuitive and can possibly amplify small effects. The MSCS is the arithmetic mean of the severity of the individual symptom complexes.

The MSCS is measured as a change from baseline and is more straightforward to understand and thus, may be easier to interpret.

Phase 3 Study Design

EDEMA3 and EDEMA4 were the phase 3 studies and were similar in design with a few key differences. EDEMA3 and EDEMA4 were randomized, double-blind, placebo-controlled clinical trial to assess the efficacy and safety of ecallantide for the treatment of acute attacks of HAE. In EDEMA3, the single-dose, randomized, blinded, placebo-controlled period was followed by an open label extension in which all patients could receive ecallantide.

Patients with a documented diagnosis of Type I or Type II HAE who were 10 years of age and older were enrolled. Eligible patients had to present within 8 hours of a moderate to severe acute HAE attack. Patients were randomized to ecallantide 30mg or placebo SC (3 separate 1mL SC injections to upper arm, thigh, or abdomen). Patients were stratified by anatomic location of the attack (laryngeal vs. abdominal vs. peripheral in EDEMA3; laryngeal vs. other in EDEMA4) and based upon prior enrollment in other ecallantide studies.

Efficacy was measured by patient assessment of symptom severity and response to treatment, utilizing the PROs discussed above, the MSCS and the TOS. For EDEMA3, the primary efficacy variable was the TOS at 4 hours post-dose. Based upon discussions with the Agency, the primary efficacy endpoint in EDEMA4 was specified as the change from baseline in MSCS at 4 hours instead of the TOS at 4 hours. This decision was based upon the complexity of the TOS and concerns with interpretation. The MSCS was thought to be more straightforward. Key secondary efficacy variables included time to significant improvement in overall response, the durability of response at 24 hours, proportion of patients receiving medical intervention, time to onset of sustained improvement, and open-label experience due to severe upper airway compromise.

In EDEMA3, data imputations were included in the primary and secondary analysis for medical intervention and emerging symptoms. For example, if medical intervention was given for a specific symptom complex, the data was imputed so that the severity of that symptom complex was severe (3). In order to have a more transparent picture of the effects of ecallantide, the Agency requested that data imputations for medical intervention and emerging symptoms not be performed in EDEMA4 for the primary analyses. Data imputations could be performed as sensitivity analyses.

For EDEMA4, Dyax submitted a protocol amendment to modify the sample size from 52 to 96 patients. This modification of sample size was based upon the results from EDEMA3 and the fact that the MSCS was the primary endpoint in EDEMA4. The Agency agreed to the sample size modification provided it was not based upon an unblinded assessment of EDEMA4. In addition, the Agency noted that patient selection or study conduct should not change to ensure that the sample size modification did not affect other aspects of the study, e.g. patient demographics or baseline disease characteristics. The protocol amendment for this change in sample size was dated [redacted]. The other modification in this

protocol amendment was allowance for use of paper diaries if the electronic diary could not be used.

The single-dose, randomized, blinded, placebo-controlled portion of EDEMA3 was followed by an open label extension (OLE) in which all patients could receive ecallantide. In addition, patients who had not participated in the controlled phase could also enroll in the open-label extension. The purpose of this extension was to assess efficacy and safety with repeat dosing of ecallantide. The results of the EDEMA3 OLE are addressed in the FDA briefing package. Limited safety results regarding hypersensitivity reactions from the ongoing OLE study were submitted in the original submission and are included in the FDA briefing package.

Efficacy Results

A total of 168 patients were included in the randomized, placebo-controlled portion of the phase 3 studies (72 in EDEMA3 and 96 in EDEMA4). Because patients could participate in more than one study, it is important to note that there were 143 unique patients in the controlled portion of the phase 3 studies. In general, patients enrolled in the EDEMA3 and EDEMA4 were primarily females (65-67%) and Caucasian (85-90%) with a mean age of 35 years. The demographic profile and HAE attack history were fairly balanced between treatment groups although the ecallantide arm in EDEMA4 had more females compared to the placebo group. The most common symptom complexes were stomach/GI and cutaneous and overall the distribution of symptom complexes and severity was similar between treatment groups with the exception that there were more stomach/GI symptoms in the placebo group and more cutaneous symptoms in the ecallantide group in EDEMA4. EDEMA3 was conducted in the United States, Europe, Israel, and Canada, while EDEMA4 was conducted in the United States and Canada.

As the double-blind, placebo controlled portion of the studies was only single dose, all but two patients completed this portion of the phase 3 studies: one patient was lost to follow up after Visit 1 in EDEMA3 and one patient left against medical advice in EDEMA4. One important point to note is that two patients in EDEMA3 were incorrectly administered study medication (one in each treatment group) and thus, the analyses were based upon the ITT-randomized and ITT-as treated populations.

The primary efficacy variables were the TOS and MSCS described in detail above. The results for EDEMA3 and EDEMA4 as reported by Dyax are presented in Table 2. In EDEMA3, the TOS at 4 hours was the primary endpoint, and although the results numerically favored ecallantide, the results for the ITT-randomized population were not statistically significant. When the results for the ITT–as treated population were analyzed, the results were statistically significant compared to placebo as shown in the table below. A similar pattern was noted with the MSCS, which was a key secondary efficacy variable in EDEMA3. Because the statistical significance of the results in EDEMA3 was affected by two patients, the results are not robust.

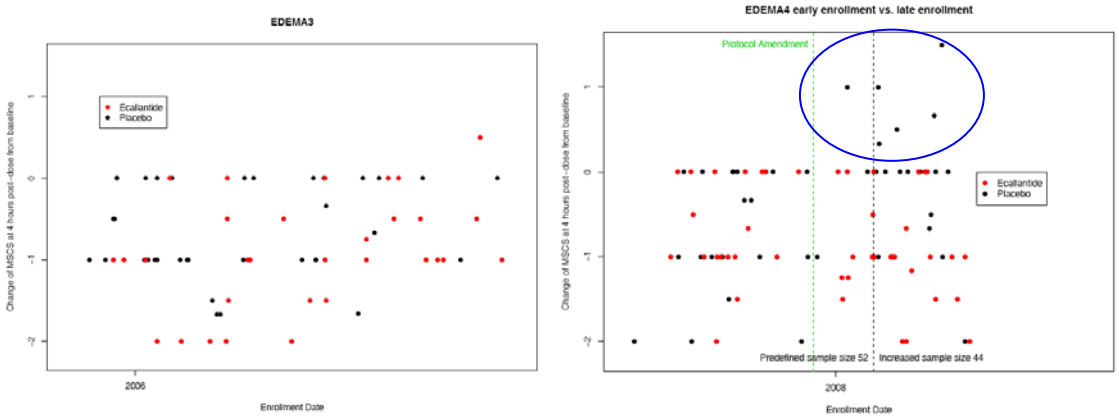
Table 2 Efficacy Results from EDEMA3 and EDEMA4						
	EDEMA3			**EDEMA4**		
	Ecallantide 30 mg N=36	**Placebo N=36**	**Diff from Pbo *(p value)***	**Ecallantide 30 mg N=48**	**Placebo N=48**	**Diff from Pbo *(p value)***
Mean Symptom Complex Score (MSCS)						
MSCS – mean Δ from baseline 4 hrs *ITT as randomized* [baseline]	-0.88 [2.15]	-0.51 [2.26]	-0.37 ***(0.094)***	-0.81 [2.18]	-0.37 [2.02]	-0.44 ***(0.01)***
MSCS – mean Δ from baseline 4 hrs *ITT as treated* [baseline]	-0.91 [2.17]	-0.48 [2.24]	-0.43 ***(0.044)***			
Treatment Outcome Score (TOS)						
TOS at 4 hrs (mean) *ITT as randomized*	46.8	21.3	25.5 ***(0.100)***	53.4	8.1	45.3 ***(0.003)***
TOS at 4 hrs (mean) *ITT as treated*	49.5	18.5	31.0 ***(0.037)***			

In EDEMA4, the results for the MSCS and TOS are statistically significant for ecallantide compared to placebo. However, additional analyses call into question the robustness of the data in this study also. Analysis of the results pre and post protocol amendment (for adjustment in sample size) shows very different results. This analysis was performed after finalization of the clinical briefing document and is not captured in that review, but is addressed in the statistical briefing document. In the table below, the Agency's statistical reviewer provided the results for the original sample size of 52 patients and for the additional 44 patients included after the sample size adjustment in EDEMA4. The results for the original 52 patients planned for EDEMA4 are not significant, while the results for the additional 44 patients are statistically significant and drive the overall results for EDEMA4.

Table 3 Efficacy Results from EDEMA4 Pre and Post Sample Size Adjustment						
	EDEMA4 Pre sample size adjustment (52 patients)			**EDEMA4 Post sample size adjustment (44 patients)**		
	Ecallantide 30 mg N=28	**Placebo N=24**	**Diff from Pbo *(p value)***	**Ecallantide 30 mg N=20**	**Placebo N=23**	**Diff from Pbo *(p value)***
Mean Symptom Complex Score (MSCS)						
MSCS – mean Δ from baseline 4 hrs [baseline]	-0.71 [2.27]	-0.62 [2.12]	-0.09 ***(0.826)***	-0.94 [2.06]	-0.06 [1.92]	-0.88 ***(<0.001)***
Treatment Outcome Score (TOS)						
TOS at 4 hrs (mean)	43.3	19.2	24.1 ***(0.24)***	67.1	-5.3	72.4 ***(0.006)***

It is unclear why there is a discrepancy in the results for the patients pre and post sample size adjustment. A look at the individual patient data may provide some insight. In the following figure the MSCS change from baseline is shown for each patient in EDEMA4. The green line represents the date for the protocol amendment and the black line separates the original sample size of 52 from the additional patients enrolled.

Figure 2 Individual Patient Data for Change from Baseline MSCS



EDEMA 3 **EDEMA 4**

As shown in the figure, there is a group of placebo patients in EDEMA4 that were outliers and performed poorly (increase in the MSCS, i.e. symptoms worsened). These patients performed differently than patients earlier in the study and performed differently than patients in EDEMA3. The statistical reviewer performed a test for interaction and there was significant interaction, meaning that the chance to observe such a difference pre and post sample size adjustment is small provided there was no bias in patient recruitment and the study was conducted in the same way before and after sample size change. This issue calls into question the robustness of the data in EDEMA4.

In addition, a discussion of the clinical significance of the effect size is warranted. Because the TOS and MSCS are novel PROs, the clinical significance of the treatment group difference is unclear. Based upon the PRO validation study (DX88-103) performed by Dyax, the minimum clinical important difference is 30 for the TOS and 0.3 for the MSCS; however, it must be recognized that these are novel PROs with limited experience. Interpretation of the MSCS is more straightforward. Using a baseline severity of moderate (2), a treatment group difference of -0.4 corresponds to symptoms improving from moderate severity towards mild severity. The clinical meaning of this treatment group difference remains open for discussion.

In EDEMA3 and EDEMA4, the results for many of the secondary variables, including time to significant improvement, and change from baseline MSCS at 24 hours were supportive of efficacy as the results numerically favored ecallantide. Two secondary efficacy variables are worth noting as they do no depend on the TOS and MSCS. The proportion of patients with significant improvement in overall response (near complete symptom resolution) favored the ecallantide group in both studies – approximately 50% of ecallantide patients vs. approximately 36% of placebo patients. The proportion of patients receiving medical intervention favored the ecallantide group in both studies - EDEMA3 (14% of the ecallantide patients and 36% of placebo patients) and EDEMA4 (33% of the ecallantide patients and 50% of the placebo patients).

In accord with our regulations, the Agency usually requires more than one adequate and well-controlled study to provide independent substantiation of an efficacy claim. We ask that you consider whether the results of EDEMA3 and EDEMA4 provide substantial evidence of efficacy.

Repeat dosing

Data regarding repeat dosing of ecallantide primarily comes from the OLE of EDEMA3. In the EDEMA3 OLE, 67 patients were treated, including 19 new patients. The majority of patients were treated for one or two attacks. The repeat dose data is somewhat limited as the data is uncontrolled and there were few patients treated for more than 5 or 6 HAE attacks. Without a comparator group, it is difficult to draw definitive conclusions, but following treatment for 1 to 6 HAE attacks, the mean change in MSCS at 4 hours was -0.9 to -1.4, which was similar to the change from baseline in the ecallantide group from the double blind portions of EDEMA3 and EDEMA4.

Pediatrics

Dyax proposes an indication for ecallantide in HAE patients 10 years of age and older. In the ecallantide program, there were 8 patients 16 to 17 years of age and 18 patients <16 years of age. Of the pediatric patients (<18 years of age), only 4 received ecallantide as part of the double-blind, controlled portion of the phase 3 studies. The youngest patient that received ecallantide in the controlled portion of a phase 3 study was a 15-year-old patient. Younger patients were studied during the open-label dosing, but the numbers were small. Extrapolation of efficacy can be considered if the disease is sufficiently similar in adults and pediatric patients, but ideally there should be sufficient representation of patients less than 18 years of age, which is not the case in this program. We ask you to consider whether the pediatric database is adequate to evaluate the efficacy of ecallantide.

Safety

The safety database for ecallantide is based primarily on the 5 HAE studies: EDEMA0, EDEMA1, EDEMA2, EDEMA3, and EDEMA4 (Analysis Population I). There were 219 unique HAE patients, including 18 pediatric patients < 16 years of age in the ecallantide program. In these 219 patients, 609 doses of ecallantide were administered - approximately half received one dose; 40% received 2 to 4 doses of ecallantide; and less than 15% of patients received 5 or more doses. In the controlled portion of the phase 3 studies, 100 patients received 125 doses of ecallantide (Analysis Population II). The safety information below is based upon the phase 3 studies, unless noted otherwise.

Safety assessments in the phase 3 clinical trials included adverse events (AEs), physical examinations, vital signs, electrocardiograms, laboratories, and testing for antibodies. Intensive ECG monitoring was performed in EDEMA4. Antibody testing was performed for IgE and non-IgE antibodies to ecallantide and IgE antibodies to *P pastoris*.

There were no deaths in EDEMA3 and EDEMA4. There was one death in EDEMA1 in a patient with a history of kidney transplant 1 year prior to enrollment. The patient was reported to have chronic rejection of the transplant and died of chronic renal failure 29 days after administration of ecallantide. Serious adverse event (SAE) data were significant for hypersensitivity/anaphylaxis SAEs (discussed below). Other than anaphylaxis, HAE was

the only SAE reported in more than one patient and this occurred at a similar frequency between treatment groups. There were no discontinuations due to AEs during the controlled period.

In the phase 3 studies, AEs were reported in a similar percentage of patients in the ecallantide and placebo treatment groups. AEs more common in the ecallantide treatment group included: upper abdominal pain, nausea, headache, fatigue, injection site pain, pyrexia, nasopharyngitis, pharyngolaryngeal pain, and erythematous rash. Injection site reactions were noted in both treatment groups and tended to be mild and transient.

In terms of the laboratory, physical exam, vital sign, and ECG data, there were generally no safety signals suggested. Because of the potential effect of ecallantide on the coagulation cascade, coagulation parameters are of interest. There were no significant changes in the mean values of coagulation parameters. Outliers and shift table results were reviewed for the coagulation parameters and shifts were noted in both treatment groups, but there were no significant differences noted other than 3 patients with an elevated thrombin time in the ecallantide group and none in the placebo group. There were no hemorrhagic or thrombotic AEs noted with the exception of a contusion in a patient treated with placebo in EDEMA4.

Hypersensitivity and Immunogenicity

Since ecallantide is a therapeutic protein, a discussion of hypersensitivity and immunogenicity is warranted. Because of the nature of HAE attacks, identifying a hypersensitivity reaction can be difficult; however, administration associated reactions were more common in the ecallantide group (13%) compared to the placebo group (10%) in the phase 3 studies. Using diagnostic criteria for anaphylaxis as outlined by the 2006 Joint NIAID/FAAN Second Symposium on Anaphylaxis[3], the clinical reviewer identified 8 potential cases of anaphylaxis in HAE patients treated with ecallantide. See Section 7.3.4 in the Clinical Briefing Document and brief summaries of these patients in the Appendix to this memo. As stated above, 219 HAE patients received 609 doses of ecallantide in the ecallantide HAE studies (Analysis Population I). Using this population, an anaphylaxis rate of 3.7% patients (8 cases of 219 HAE patients) or 1.3% doses (8 of 609 doses) is observed. Most of these reactions occurred following repeat dosing of ecallantide. In addition to these events, other various hypersensitivity reactions, including: urticaria, flushing, pruritis, itchy throat, erythematous rash, shortness of breath, conjunctival erythema, and eye swelling were noted. We ask you to discuss the hypersensitivity reactions, including anaphylaxis and consider in the safety assessment for ecallantide.

In terms of antibody seroconversion, 13% of patients treated with ecallantide in the HAE program seroconverted to testing positive for anti-ecallantide antibodies. There appears to be an increase in the probability of seroconversion with each treated HAE attack through the 5th attack. The number of patients treated beyond 5 attacks limits drawing further conclusions. Antibody status did not appear to increase the frequency of AEs. Of note, the antibody data should be interpreted with caution because preliminary review of Dyax's immunogenicity assays raises concern that the IgE and neutralizing antibody assays may be

[3] Sampson HA et al. J Allergy Clin Immunol 2006; 117:391-7.

limited in sensitivity, resulting in an underestimation of seroconversion. Refer to the Immunoassay Summary in the Agency briefing package for more details.

Pediatrics

As noted above, the pediatric data in the ecallantide program is limited. The safety data from the pediatric patients do not suggest a unique safety signal in this population, but the limitations of the data are noted. We ask you to consider whether the pediatric database is adequate to evaluate the safety of ecallantide.

Self-Administration

In the clinical program, ecallantide was administered by a healthcare professional. There is concern about the potential for self-administration of ecallantide for patient convenience. After interactions with the Agency regarding this issue, Dyax does not propose self-administration for ecallantide at this time and plans to evaluate self-administration further in a future clinical study. However, off-label self-administration remains a possibility and should be considered in the benefit-risk assessment, especially given the safety signal of hypersensitivity reactions/anaphylaxis.

Summary

The purpose of the PADAC meeting is to discuss the adequacy of the efficacy and safety data submitted by Dyax to support the approval of ecallantide for the treatment of acute attacks of HAE in the United States. This is an important discussion as ecallantide is a new molecular entity and there currently are no drug products approved for the proposed indication.

At the PADAC meeting, Dyax will present an overview of the clinical program, which will be followed by the Agency's presentation of the efficacy and safety data. Please keep in mind the following questions that will be discussed and deliberated upon following the presentations and discussion.

Draft Questions

1. Discuss the hypersensitivity/anaphylaxis data and provide recommendations for further evaluation, if necessary

2. Does the data provide substantial and convincing evidence that ecallantide provides a clinically meaningful beneficial effect on acute attacks of hereditary angioedema? **(Voting Question)**
   a) In patients 18 years of age and older
      If not, what further efficacy data should be obtained?
   b) In patients 10 to 17 years of age.
      If not, what further efficacy data should be obtained?

3. Has the safety of ecallantide been adequately assessed for the treatment of acute attacks of hereditary angioedema? **(Voting Question)**
   a) In patients 18 years of age and older
      If not, what further safety data should be obtained?

b) In patients 10 to 17 years of age.

If not, what further safety data should be obtained?

4. Does the committee have recommendations regarding the following:
    a) Labeling
    b) Risk mitigation strategies for hypersensitivity/anaphylaxis reactions
    c) Potential for self-administration
    d) Other

Appendix
Potential Anaphylaxis Cases

- Patient 8805051099 (EDEMA3) experienced anaphylaxis twice – the first time after her 17th dose of ecallantide and the second during a rechallenge procedure. Her first event was characterized by generalized erythema, pruritus, and decreased blood pressure (82/50 mmHg) with an oxygen saturation of 90% on room air. She received epinephrine, diphenhydramine, and supplement oxygen and her blood pressure increased to 110.80 mmHg. Serum tryptase taken 4 hours after the event was 10.4 mcg/L (normal range: 1.9-13.5 mcg/L). The second event was characterized by dyspnea, generalized rash, anxiety, pharyngeal edema, vomiting, diarrhea, urinary incontinence, hypotension and hypoxia following rechallenge with a partial dose. The patient was noted to have tested intermittently positive to IgE against *P. pastoris* up to 2 years before the first event as well as non-IgE to ecallantide.
- Patient 8820401009 (EDEMA4 OLE, DX-88/19) developed anaphylaxis after her 4th dose of ecallantide, consisting of erythema, generalized pruritus, tingling of the tongue, lethargy, change in mental state, and vomiting. She was treated with 2 doses of 0.3 mg epinephrine, hydroxyzine, solumedrol, and IV fluids. A serum tryptase taken 6 hours after the event was 30 ng/ml (normal range 2-10 ng/ml). The patient had intermittently tested positive for non-IgE and IgE antibodies to ecallantide since her 2nd dose and 3rd doses, respectively, although she tested negative for IgE to ecallantide immediately prior to the event.
- Patient 8805024097 (EDEMA2) developed anaphylaxis 10 minutes after her 6th dose. She experienced nausea, diaphoresis, dizziness, and a feeling of faintness before receiving treatment with epinephrine, hydrocortisone, cetirizine and ranitidine. Serum tryptase taken 4 hours and 12 minutes after the event was within normal range (2.7 ng/ml). The patient tested positive for non-IgE antibodies to ecallantide after the 5th dose and positive for IgE 7 days after the anaphylaxis. The patient went on to complete a successful rechallenge procedure and received 11 additional doses of ecallantide.
- Patient 8802003005 (EDEMA0) was identified as having an "anaphylactoid" (per study report) reaction consisting of dysphagia, pruritus, urticaria, edema, dyspnea, abdominal pain, and enteritis 5 minutes after her first dose of ecallantide (40 mg/m^2 IV). She was treated with epinephrine, polaramine, and hydrocortisone. She tested positive for ecallantide antibodies per the investigator's own immunoblot, but subsequently negative on the Applicant's ELISA assays. No rechallenge procedure was attempted.
  Reviewer comment: Although reported as an anaphylactoid reaction in the study report, this patient meets the NIAID/FAAN criteria for anaphylaxis.
- Patient 8804013011 (EDEMA1) reported 3 separate episodes of sneezing, throat itchiness, congestion, rhinorrhea, and shortness of breath following the 1st, 2nd , and 4th doses of 20 mg/m2 ecallantide IV. The time to onset is not recorded and patient's medical history is confounded by a history of asthma and allergic rhinitis. The patient has not tested positive for antibody formation to ecallantide or *P. pastoris*.

- Patient 8804013003 (EDEMA1) developed rhinitis, itchy throat, and shortness of breath following receipt of her 1st dose of ecallantide 20 mg/m2 IV. The patient was treated with epinephrine, antihistamines, and corticosteroids. The patient underwent a rechallenge procedure and developed rhinitis symptoms 42 minutes after the start of the test dose infusion. The patient has not tested positive for antibody formation to ecallantide or *P. pastoris*.
- Patient 8805019001 (EDEMA2) experienced symptoms suggestive of anaphylaxis during a rechallenge procedure. Her initial reaction consisted of worsening allergic rhinitis symptoms, conjunctival erythema, eye swelling, and urticaria 2 minutes after the start of the 1st ecallantide dose (10 mg/m2 IV). The patient tested positive for IgE antibodies to *P. pastoris* 1 year prior to the reaction but had tested negative in subsequent assays. On rechallenge 18 months later, she developed sneezing, nasal congestion, throat itchiness, and cough.
- Patient 8805050097 (EDEMA2) developed abdominal pain, nausea, vomiting, throat itchiness, and nasal congestion following receipt of the 1st dose of ecallantide for treatment of an external head/neck HAE attack. Study drug infusion was stopped. No antibodies were detected and the patient did not undergo a rechallenge procedure.

PULMONARY-ALLERGY DRUGS ADVISORY COMMITTEE MEETING

February 4, 2009

CLINICAL BRIEFING DOCUMENT

BLA 125277

Kalbitor (ecallantide) 30mg for the treatment of acute attacks of hereditary angioedema

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1 Executive Summary

1.1 Brief Overview of Clinical Program

Dyax submitted a Biologic Licensing Application (BLA) for ecallantide solution for injection for the treatment of acute attacks of hereditary angioedema (HAE) in patients 10 years of age and older. HAE is a rare, inherited condition characterized by intermittent, unpredictable attacks of angioedema and is categorized as an orphan disease. HAE attacks are potentially life-threatening, particularly in cases of airway compromise. Currently, there are no drug products approved for the treatment of acute attacks of HAE and the standard of care remains supportive therapy. Several drug products are available for prophylaxis, but their effectiveness in preventing acute attacks is limited.

Ecallantide is a new molecular entity and a novel recombinant inhibitor of human plasma kallikrein. It is a 60-amino-acid protein produced in *Pichia pastoris* yeast cells by recombinant DNA technology. The proposed trade name is Kalbitor to be marketed as a 10mg/mL solution in 1mL single use vials. Kalbitor is a sterile solution that contains ecallantide in a phosphate buffered solution. The proposed dosing regimen is 30 mg ecallantide SC, administered as three separate 1mL injections. In cases of insufficient relief or recurrence of symptoms, an additional 30 mg dose may be administered within a 24-hour period.

The dose ranging data in this clinical program is limited and primarily comes from early phase 2 studies in patients with HAE. In one phase 2 dose ranging study (EDEMA2), the data suggested that 10mg mg/m^2 to 20 mg/m^2 IV ecallantide and 30mg SC ecallantide were efficacious based on patient-reported symptomatology; however, the efficacy measures used in this study were not validated. Based upon pharmacokinetic data, the 30 mg SC dose of ecallantide corresponds approximately to a 15 mg/m^2 IV dose of ecallantide. The challenge of performing dose ranging studies for the proposed indication is noted. Although the dose ranging data is limited, based upon the submitted data, the selection of 30mcg SC dose of ecallantide was reasonable to carry forward into the phase 3 program.

The clinical development program to establish the safety and efficacy of ecallantide 30mg SC for the proposed indication included two small, randomized, placebo-controlled Phase 3 studies, EDEMA3 and EDEMA4. The design and conduct of the studies were similar. Both studies consisted of a single-dose double-blind phase followed by an optional, open-label, uncontrolled extension (OLE) study of repeat doses of ecallantide for new acute HAE attacks. Patients with a documented diagnosis of Type I or Type II HAE who were 10 years of age and older were enrolled. Eligible patients had to present within 8 hours of a moderate to severe acute HAE attacks. Patients were randomized to ecallantide 30mg or placebo SC (3 separate 1mL SC injections to upper arm, thigh, or abdomen). Patients were stratified by anatomic location of the attack (laryngeal vs. other) or if patients had prior enrollment in other ecallantide studies.

During the OLE phase of both studies, patients presented with new acute HAE attacks and received ecallantide 30 mg SC.

Efficacy was measured by patient assessment of symptoms, severity and response to treatment, utilizing the novel patient reported outcome instruments, the Mean Symptom Complex Severity (MSCS) and the Treatment Outcome Score (TOS), which are discussed in detail in this review. Because there is no established regulatory pathway for the proposed indication, Dyax developed the MSCS and TOS as novel patient reported outcome efficacy variables.

Although EDEMA3 and EDEMA4 were similar in design, two major differences between the studies are worth noting: 1) different primary efficacy endpoints and 2) differing pre-specified statistical analyses with imputation for missing data (EDEMA3) in contrast to no imputation (EDEMA4). EDEMA3 used the TOS at 4 hours as the primary efficacy endpoint; change in MSCS from baseline at 4 hours was a secondary endpoint. During discussion regarding design of EDEMA4, the Agency raised concerns about the transparency of the TOS and recommended using the MSCS as the primary efficacy variable in EDEMA4. As a result, the MSCS was the pre-specified primary efficacy variable and the TOS was a key secondary efficacy variable in EDEMA4. In terms of data imputation, EDEMA3 employed imputations for emerging symptom complexes and medical interventions. In both studies, sensitivity analyses were performed using imputations for emerging symptoms and medical interventions to test the robustness of the study conclusions.

Safety assessments included adverse events (AEs), physical examinations, vital signs, electrocardiograms, laboratories, and testing for antibodies. Intensive ECG monitoring was performed in EDEMA4. Because of the concern regarding immunogenicity with ecallantide, there is an expanded discussion of immunogenicity and hypersensitivity AEs.

The clinical program did not include a placebo-controlled evaluation of repeat exposures. The OLE results from EDEMA3 provide data regarding chronic, repeat use of ecallantide. Additional data is provided by open-label data obtained from the Phase 2 study, EDEMA2. OLE efficacy data from EDEMA4 were not included in the original submission and were not submitted in time for inclusion in this briefing document. Limited safety data from EDEMA4 was submitted and is included in this review.

1.2 Efficacy

Primary Efficacy Variables

As discussed above, Dyax developed patient reported outcome variables, the Mean Symptom Complex Severity (MSCS) and the Treatment Outcome Score (TOS) to assess efficacy of ecallantide for the proposed indication. Because these are novel efficacy variables, a brief discussion of the MSCS and TOS is warranted prior to discussing the results of the phase 3 program. The TOS is based upon severity of symptoms and response/change to therapy whereas the MSCS is based solely upon symptom severity.

Upon presentation, patients identified HAE symptoms grouped by a symptom complex, i.e. Internal Head/Neck, Stomach/GI, Genital/Buttocks, External Head/Neck, or Cutaneous. The patient and physician ranked each symptom complex severity as normal (0), Mild (1), Moderate (2), or Severe (3). Patients were then administered study medication and assessed response as follows: Significant Improvement (a lot better), Improvement (a little better), Same (unchanged), Worsening (a little worse), or Significant Worsening (a lot worse). Response was scored as 100, 50, 0, -50, -100, respectively.

The Treatment Outcome Score (TOS) is a composite, weighted symptom complex score intended to assess global symptom response to treatment. Each individual symptom complex score is based on a severity rating for that particular group of symptoms multiplied by a "response-to-treatment" factor, so that the outcome is incorporated into the final TOS value.

$$TOS = \frac{\sum symptom\ complex\ score \times symptom\ complex\ weight}{\sum symptom\ complex\ weight}$$

In this equation, "symptom complex score" = response-to-treatment and symptom complex weight = baseline severity assessment. Severity is scored on a scale of 0 to 3, with 3 being the most severe. Response assessment is scored as -100, -50, 0, 50, or 100, with -100 representing significant worsening and a score of 100 representing significant improvement. The maximum and minimum possible TOS was 100 and -100, respectively, with a higher value corresponding to greater improvement; a TOS value of 0 signified no change.

The Mean Symptom Complex Score (MSCS) is an arithmetic mean of the severity of the individual symptom complexes. Unlike the TOS, there is no inherent time/outcome element in the MSCS; hence, response to treatment is assessed as "the change from baseline MSCS." The maximum possible calculated MSCS value is 3.0 and the minimum possible value is 0; accordingly, the greatest possible change from baseline is ±3.0.

The TOS is a complicated score that is difficult to interpret, due in part to the response and severity multipliers used. Overall, a higher number corresponds to a better response to study drug, although the magnitude of response for a given TOS value is not intuitively clear. The response multiplier appears to exaggerate small differences, which may or may not be clinically meaningful. For this reason, in the EDEMA4 study the Agency recommended that the applicant use the change from baseline MSCS as the primary endpoint with the TOS as a supportive secondary endpoint. The MSCS was felt to be more transparent and more similar to symptom scoring used for other conditions.

Phase 3 Efficacy Results

There were 72 and 96 patients enrolled in the placebo controlled portion of EDEMA3 and EDEM4, respectively. The majority of patients were female and Caucasian, with a mean age of 35. Demographics were generally balanced between treatment groups, with the exception that there was an imbalance in females in the treatment groups in EDEMA4.

The results for the key efficacy variables, TOS and MSCS as reported by Dyax are shown in the table below. Two patients in EDEMA3 received the wrong medication; therefore, the results for EDEMA3 include the ITT-as randomized and ITT-as treated datasets.

Table 1 Efficacy Results from EDEMA3 and EDEMA4

	EDEMA3			EDEMA4		
	Ecallantide 30 mg N=36	Placebo N=36	Diff from Pbo **(p value)**	Ecallantide 30 mg N=48	Placebo N=48	Diff from Pbo **(p value)**
TOS at 4 hrs (mean) *ITT as randomized*	46.8	21.3	25.5 **(0.100)**	53.4	8.1	45.3 **(0.003)**
TOS at 4 hrs (mean) *ITT as treated*	49.5	18.5	31.0 **(0.037)**			
MSCS – mean Δ from baseline 4 hrs *ITT as randomized* [baseline]	-0.88 [2.15]	-0.51 [2.26]	-0.37 **(0.094)**	-0.81 [2.18]	-0.37 [2.02]	-0.44 **(0.01)**
MSCS – mean Δ from baseline 4 hrs *ITT as treated* [baseline]	-0.91 [2.17]	-0.48 [2.24]	-0.43 **(0.044)**			

EDEMA4 had robust results for a change from baseline MSCS at 4 hours. The treatment difference of 0.4 is greater than the MCID estimated in the PRO validation studies. Looking at additional sensitivity analyses that include imputation for emerging symptoms and medical interventions, the difference between ecallantide and placebo is further supported. Similar statistically significant findings for the TOS at 4 hours were also reported in EDEMA4. EDEMA3, in contrast, did not have robust results. The efficacy results based upon the ITT-as randomized dataset was not statistically significant. When the efficacy endpoints were recalculated using a dataset based on the ITT as treated population, the differences between the ecallantide and placebo arms were found to be statistically significant. These results support ecallantide's efficacy, although the results do not appear to be robust and the limitations of a small sample size are apparent. The MSCS scores suggest that the placebo groups performed similarly across studies and indicate that the sample size of EDEMA3 may have contributed to the non-significant findings. The MSCS scores also highlight the difficulty in TOS interpretation, since the TOS does not permit a comparison of baseline status and the subsequent change from baseline.

Other secondary endpoints to consider included the TOS and MSCS at 24 hours as a measure of durability of response, responder analysis, and medical interventions as a different measure of efficacy. Overall, the secondary efficacy endpoints provide support of ecallantide's efficacy.

Repeat Dosing

With regards to repeat dosing, the clinical program did not include a placebo-controlled evaluation of chronic, intermittent dosing. The support for repeat dosing is based primarily on information obtained from the open-label experience in EDEMA3, EDEMA4, and EDEMA2 in conjunction with extrapolation from the controlled single-dose experience. Given the underlying pathophysiology and the fact that HAE attacks are generally unique events, it is reasonable to assume that ecallantide would be equally efficacious for future attacks. In general, the number of treatment episodes was not associated with any decrease in efficacy. Although there are limitations with the repeat dose data – lack of placebo control and potential for selection bias –

the uncontrolled, repeat dose data combined with extrapolation of the single-dose, placebo controlled data supports the efficacy of ecallantide with repeat dosing.

Pediatrics
Dyax proposes an indication for ecallantide in patients 10 years of age and older. A limited number of pediatric patients were evaluated in the clinical program. There were 18 total pediatric patients (< 16 years of age) in the development program, but of the pediatric patients, only 3 received ecallantide as part of a double-blind study, the youngest being a 15-year-old patient in EDEMA3. Younger patients were studied during the open-label dosing, but the numbers were small. Although it is expected that ecallantide would behave similarly in a pediatric patient, there should be sufficient representation of patients less than 18 years of age to support an indication in this age group. This clinical reviewer does not believe there is adequate controlled data with ecallantide in adolescents/children < 18 years of age to support the use of ecallantide in this age group.

1.3 Safety

The safety of ecallantide at the proposed 30 mg SC dose is supported by the submitted clinical study data. Safety data showed that ecallantide is most commonly associated with headache, nausea, diarrhea, pyrexia, and injection site reactions. The most concerning adverse events are anaphylaxis and other hypersensitivity reactions. The size of the safety database is somewhat limited due to the rarity of HAE and the difficulty of conducting controlled trials to evaluate unpredictable, acute HAE attacks. However, given the potential severity of HAE and the lack of effective treatment alternatives, the safety profile for the proposed dose is acceptable with appropriate risk management strategies for hypersensitivity reactions.

Anaphylaxis and Hypersensitivity
Anaphylaxis and hypersensitivity reactions appear to be the most serious potential adverse events associated with use of ecallantide. Based on a safety population including all HAE patients in the formal development program (excluding compassionate use and rechallenge patients), an anaphylaxis rate of 3.7% patients (8 cases of 219 HAE patients) or 1.3% doses (8 of 609 doses) is observed. Anaphylaxis reactions are unpredictable and life-threatening events. However, HAE is also unpredictable and life-threatening and there are currently no approved therapies for use in acute attacks. Medical care facilities equipped to treat manifestations of acute HAE attacks such as laryngeal edema are an appropriate setting for administering ecallantide and monitoring for anaphylaxis. In addition, HAE patients, given the nature of their disease and the rarity of the condition, tend to be a relatively sophisticated patient population that would be receptive to patient education about anaphylaxis and drug hypersensitivity. Therefore, the clinical review concludes that the risks of ecallantide use in a controlled setting with healthcare provider supervision are balanced by the potential benefits.

The clinical review also recommends further study of these reactions to elucidate risk factors and promote development of effective screening tools. The Applicant has proposed a pharmacovigilance system to monitor and follow-up on AE of special interest, namely anaphylaxis and other drug hypersensitivity reactions associated with chronic, intermittent use of

ecallantide. The database will include information on patient antibody status where available, as well as any other information on rechallenge or desensitization procedures if utilized.

Pediatrics
Patients below the age of 18 years were included in the phase 3 studies, but only 3 patients below age 18 actually received ecallantide during the double-blind phase of the studies. While there is no scientific rationale to expect that pediatric patients would respond differently to ecallantide, the application lacks sufficient controlled safety data to make an assessment in patients under the age of 18 years for the proposed indication.

Self-Administration
The potential for self-administration of ecallantide remains a safety concern. Although self-administration may offer certain benefits in terms of patient convenience and potentially greater efficacy, the safety and feasibility of self-administration have not been evaluated in the clinical development program to date. Dyax does not propose self-administration; however, off-label self-administration remains a possibility and should be considered in the benefit risk assessment. If ecallantide is approved, Dyax should have post-marketing risk mitigation strategies including extensive education materials for both patients and healthcare providers regarding the risk of hypersensitivity events.

2 Introduction and Regulatory Background

2.1 Product Information

The established name for the proposed product is ecallantide and the proposed tradename is Kalbitor™. The established name will be used in this review to refer to the product. Ecallantide is supplied as a colorless, sterile, preservative-free isotonic solution with an ecallantide concentration of 10 mg/ml in a 2 ml glass vial. Each vial contains 10 mg ecallantide, 8.0 mg sodium chloride, 0.76 mg disodium hydrogen orthophosphase (dihydrate), 0.2 mg monopotassium phosphate, and 0.2 mg potassium chloride in water for injection, USP. The active ingredient, ecallantide, is a new molecular entity and a novel recombinant inhibitor of human plasma kallikrein. It is a 60-amino-acid protein produced in *Pichia pastoris* yeast cells by recombinant DNA technology. Ecallantide was identified through iterative selection and screening of phage display libraries of the first Kunitz domain of human tissue factor pathway inhibitor (TFPI) and shares 88% homology with endogenous TFPI.

The proposed indication for ecallantide is the treatment of acute attacks of HAE in patients 10 years of age and older. The proposed dosing regimen is 30 mg SC, administered as 3 separate injections. In cases of insufficient relief or recurrence of symptoms, an additional 30 mg dose may be administered within a 24-hour period.

2.2 Currently Available Treatments for Proposed Indications

Currently, there are no drug products approved for the treatment of acute attacks of HAE in the US. The standard of care for acute attacks remains supportive therapies, e.g. opiates for pain management, anti-emetics for nausea, and intubation for airway obstruction. Several drug products are available for prophylaxis, although their effectiveness in preventing acute attacks is limited or not established. Danazol (NDA 74-582) is approved for the prevention of attacks of hereditary angioedema of all types (cutaneous, abdominal, and laryngeal). Oxymetholone (NDA 22-965) and stanazolol (NDA 12-885) had similar indications but are no longer marketed in the US. Another androgen, oxandrolone, is used off-label in the US as an alternative to danazol. Most recently, recombinant C1 inhibitor (Cinryze™) administered intravenously was approved for routine prophylaxis of HAE attacks in adults and adolescents.

2.3 Availability of Proposed Active Ingredient in the United States

Ecallantide is currently not marketed in the US.

2.4 Important Safety Issues With Consideration to Related Drugs

No other members of the pharmacologic class are currently marketed.

2.5 Summary of Presubmission Regulatory Activity Related to Submission

BBIND 10426 was originally opened in [redacted] in CBER prior to transfer to the Division of Pulmonary and Allergy Products (DPAP) in CDER in [redacted]. The following is a timeline of pertinent regulatory proceedings:

- [redacted] – Orphan Drug designation granted.
- [redacted] – Meeting with sponsor. Following deficiencies in the clinical development program were identified:
    - Inadequate support for 30 mg SQ dose selection; lower doses may be efficacious. Advised to conduct additional dose-ranging studies with SQ doses of 10, 40, and 80 mg doses with clinically meaningful endpoints.
    - Need for validation of PRO instrument used in primary efficacy endpoint for Phase 3 study
    - Long-term safety data needed
- [redacted] – End-of-Phase-2 meeting with sponsor. The following issues were addressed:
    - Agreement that the TOS and MSCS are appropriate efficacy variables for use in pivotal studies if validated. The Division advised the sponsor to submit a cognitive debriefing protocol for review.
    - The Division advised the sponsor to add a placebo arm to confirmatory study for comparison to 30 mg dose.
    - The Division advised that the unit of observation should be at patient level, not number of individual attacks, which may introduce bias into the efficacy analysis.

  - The Division advised a long-term, open-label safety study with a sample size larger than the proposed 30 patients and a pre-specified study duration. Antibody testing should be performed throughout treatment.
- [redacted] – request for Special Protocol Assessment for EDEMA4. Comments were communicated to the Sponsor, including a discussion of the proposed efficacy endpoints. The Division recommended that the MSCS be designated as the primary efficacy variable and the TOS be a secondary efficacy variable, in contrast to the EDEMA3 study design, due to difficulties with the interpretation of the TOS. Other issues were the management of severe upper airway compromise in the study and the need for validation of the PRO instruments. The Sponsor agreed to the Division's recommendations.
- [redacted] – The Applicant proposed BLA submission without EDEMA4. The Division informed the Sponsor that preliminary review of the EDEMA3 results indicated that EDEMA3 would not be sufficient support for drug approval.
- [redacted] – Fast Track designation on the basis that ecallantide was proposed for an unmet medical need and life-threatening disease condition.
- [redacted] – Proposed assessment of QT prolongation request. Given the largely negative results from the preclinical studies, the lack of effect observed in the clinical studies, and the expected manner of use and indication for the proposed drug product, a thorough QT study for ecallantide did not appear warranted. More intensive ECG monitoring in the Phase 3 program beyond the proposed ECG monitoring for EDEMA4 was unlikely to provide much additional information given the small numbers of patients enrolled, the intermittent dosing, and in consideration of the life-threatening potential of HAE attacks.

3 Ethics and Good Clinical Practices

The Applicant states that no debarred investigators participated in the study, and all studies were conducted under Good Clinical Practices. The Applicant certifies that no financial arrangements were made with the clinical investigators requiring disclosure.

The Division requested an audit by the Division of Scientific Investigations (DSI) for this NDA since ecallantide is a new molecular entity proposed for a novel indication and the data for efficacy and safety is based on small sample sizes due to the rarity of HAE. A single investigator, Dr. Robyn Levy, MD (Atlanta, GA), was responsible for a relatively large number of patients enrolled in both pivotal studies (n=8 in EDEMA8 and n=15 in EDEMA4), so her site was recommended for audit.

Reviewer's comment: At the time of this review, results of the DSI audit are pending.

4 Issues Related to Other Review Disciplines

4.1 Chemistry Manufacturing and Controls

Dyax submitted a complete CMC package for this BLA. The CMC/Office of Therapeutic Proteins review of this application is pending at the time of finalization of this briefing document. In addition, a CMC site inspection is planned for pending at the time of finalization of this briefing document. Based on preliminary review, the CMC review has noted that glycosylation, oxidation, and N-terminal truncation can occur and lead to formation of ecallantide-related variants. The product-related variants have been characterized and are biologically active.

In addition, the CMC reviewers have stated that both the assays for neutralizing antibodies and IgE antibodies lack sensitivity, which may lead to an underestimation of patients who have seroconverted upon exposure to ecallantide. The assays for non-IgE antibody to ecallantide appear adequate. The CMC reviewers have also noted that the Applicant has not made an assessment of potential cross-reactivity with endogenous tissue factor pathway inhibitor (TFPI). Ecallantide shares 88% homology with TFPI. In knock-out mouse models, TFPI deficiency is an embryonic lethal due to hypercoagulability. Based on this literature, TFPI cross-reactivity may theoretically predispose to thrombotic events in humans.

4.2 Preclinical Pharmacology/Toxicology

Dyax submitted a complete pharmacology/toxicology package for this BLA. The Preclinical Pharmacology/Toxicology review of this application is pending at the time of finalization of this briefing document. The program included 6 month, repeat dose, subcutaneous toxicology studies in rats and monkeys and other short term toxicology studies. Reproductive toxicology assessment included a rat fertility study and teratology studies in rats and rabbits. The most prominent toxicity observed in both species was severe injection site reactions. Similar reactions have not been observed in clinical studies to date; only mild, self-limited injection site reactions have been reported in humans. In rats, an increase in transaminases was also noted. In the rat study, deaths were noted in female rats in the high dose groups, but the causes of death were not determined although histologic changes in the heart of a couple of animals suggested a possible cardiac etiology. No deaths occurred in male rats nor in any of the monkeys. Ecallantide also caused a dose-dependent, reversible prolongation of aPTT, presumably due to inhibition of the kallikrein-mediated activation of Factor XII to XIIa in the intrinsic coagulation cascade. The aPTT elevations were not associated with any bleeding.

In terms of immunogenicity, ecallantide antibodies were noted in both rats and monkeys. Clearance of ecallantide was reduced and systemic exposure was increased following the development of ecallantide antibodies. No increase in toxicity was noted with the higher exposure.

A carcinogenicity study was not submitted with this BLA; however, this is acceptable given the proposed indication and patient population. The animal data indicates that a carcinogenicity study in 1 species would be feasible. If the BLA is approved, a carcinogenicity study may be performed post-marketing.

4.3 Clinical Pharmacology

The Applicant submitted a complete clinical pharmacology package for this BLA. The clinical pharmacology review of this application is pending at the time of finalization of this briefing document, but a brief summary of the submitted information is included below.

4.3.1 Mechanism of Action

Ecallantide binds plasma kallikrein with high affinity and high specificity, blocking the action of plasma kallikrein. Ordinarily, kallikrein activity is regulated by C1-esterase inhibitor (C1 INH). In HAE patients with low or absent levels of functional C1-INH, kallikrein activity goes unchecked and is thought to lead to widespread release of bradykinin. In turn, bradykinin increases vascular permeability which leads to the swelling characteristic of acute HAE attacks.

4.3.2 Pharmacodynamics

Limited dose-ranging was performed in the clinical program. Briefly, EDEMA2 evaluated efficacy based on patient-reported symptomatology between doses of 5 mg/m^2 to 20 mg/m^2 IV. These data demonstrated the most efficacy for the 30 mg SC dose followed by the 10 and 20 mg/m2 IV doses; a clear dose response was not demonstrated. Based upon pharmacokinetic data, the 30 mg SC dose corresponds approximately to a 15 mg/m^2 IV dose. Exposure was dose-proportional in this dose range. No exposure-response relationships for ecallantide to components of the complement pathway or kallikrein-kinin pathway have been established. In vitro, ecallantide causes a dose-dependent, reversible prolongation of activated partial thromboplastin time (aPTT). The transient prolongation in aPTT is due to inhibition of the kallikrein-mediated activation of Factor XII to XIIa in the intrinsic coagulation cascade.

Reviewer's comment: A rigorous comparison of different dose levels for efficacy was not performed and only EDEMA2 included the 30 mg SC dose used for the phase 3 program. The primary efficacy endpoints used in EDEMA2 were the following: 1) proportion of successful outcomes (i.e. attack resolution begun by 4 hours after a single dose and maintained for greater than 24 hours after a single dose) and 2) the proportion of patients who have a partial response (i.e. an initial response to dosing followed by a relapse 4 to 24 hours after the dosing). These endpoints were gross patient-reported measures and were not validated endpoints.

4.3.3 Pharmacokinetics

Following administration of a single 30 mg ecallantide dose in healthy subjects, the mean maximum plasma concentration of 586±106 ng/ml was observed 2 to 3 hours after dosing. Plasma levels declined rapidly with a mean elimination half-life of 2.0±0.5 hours. Plasma

clearance was 153±20 ml/min and the V_d was 26.4±7.8L. The maximum ecallantide concentration expected in HAE patients receiving the 30 mg SC dose is 0.6 mcg/ml or 85 nM. Ecallantide is a small protein (7054 Da) and it is presumed that it undergoes renal elimination. According to the application, population PK analysis demonstrated that no dose adjustment is needed for age, gender, or race, assuming normal renal and hepatic function. Studies in renal and hepatic impairment have not been conducted. The plasma concentrations at 1, 2, and 4 hours post dosing for various doses of ecallantide administered intravenously (5, 10, and 20 mg/m^2) and subcutaneously (30 mg) are shown in the table below.

Table 2 EDEMA2: Plasma ecallantide concentrations (ng/ml) at 1, 2, and 4 hours post-dose by dosage level (PP population)

Dosage level	1 hour	2 hours	4 hours
5 mg/m^2 IV			
N	23	23	24
Mean (SD)	192.5 (109.6)	135.1 (234.0)	23.0 (22.4)
Median	191.4	84.3	19.1
Range	30.0-402.1	12.1-1165.7	0-66.9
10 mg/m^2 IV			
N	138	138	139
Mean (SD)	602.8 (778.1)	265.2 (217.8)	86.1 (65.8)
Median	415.4	222.0	71.2
Range	0-5438.2	0-1768.5	0-447.8
20 mg/m^2 IV			
N	11	14	14
Mean (SD)	1235.1 (1205.6)	276.2 (121.3)	170.4 (186.1)
Median	729.0	265.7	104.4
Range	594.7-4613.3	104.3-609.3	24.2-672.8
30 mg SC			
N	70	68	70
Mean (SD)	509.7 (281.2)	627.5 (326.7)	473.8 (208.5)
Median	488.2	586.7	477.0
Range	66.1-1323.9	78.5-1623.6	0-1016.5

Source: dx-88-5-csr-body.pdf, Section 11.4.2, Table 26

5 Sources of Clinical Data

5.1 Tables of Clinical Studies

The Applicant conducted 10 clinical studies with ecallantide, two of which are ongoing. These studies include 4 trials in healthy volunteers, 5 studies in HAE, and 1 study in cardiothoracic surgery (CTS). At the time of BLA submission, two studies remained ongoing: 1 open-label HAE study (DX-88/19, EDEMA4 OLE) and the CTS study. To support the efficacy and safety of ecallantide for the proposed indication, the Applicant relied primarily on the completed HAE studies. Safety data from rechallenges, compassionate use, and SAEs from the two ongoing studies (as of July 31, 2008) were also provided. Comprehensive efficacy and safety data from the EDEMA4 OLE were not provided in the original submission; only limited report of hypersensitivity reactions was provided.

To date, a total of 222 HAE patients have received 638 ecallantide doses. Of these 222 patients, 108 patients received a single dose, 80 patients received 2 to 4 doses, 19 patients received 5 to 9 doses, and 12 patients received >9 doses. The HAE development program is summarized in the table below.

Table 3 Ecallantide clinical development program for HAE

Study	Patients	Patients treated*	#Doses	Design	Duration/ Dosing interval	Dose	Endpoints
Phase 1							
DX-88/1	Healthy	12	12	DB, SD	SD	10 mg IV 20 40 80 placebo	tolerability
DX-88/6	Healthy	8	29	OL, MD	4 weeks (weekly dose)	20 mg/m^2 IV	Safety and PK
DX-88/13	Healthy	18	51	OL, MD, X-over	(weekly dose)	30 mg IV 10mg SC 30 mg SC	Safety, PK
DX-88/15	Healthy	24	47	DB, R, X-over	SD	30 mg liquid SC 30 mg lyophil SC Placebo	PK
Phase 2							
DX-88/2 EDEMA0	HAE/ AAE (≥18yo)	9	9	OL, SD	SD	10 mg IV 40 80	• Proportion with resolution of attack by 4h post-dose • Safety
DX-88/4 EDEMA1	HAE (≥10yo)	41	41	DB, SD	SD	5 mg/m^2 IV 10 20 40 Placebo	• Proportion with significant improvement by 4hr • Safety
DX-88/5 EDEMA2	HAE	77	273	OL, MD	≥7 days between attacks	5 mg/m2 IV 10 20 30 mg SC	• Safety • Proportion of successful outcomes
Phase 3							
DX-88/14 EDEMA3-DB	HAE	37	39	DB, R, PC, with OLE	SD	30 mg SC Placebo	• Treatment outcome score (TOS) • Safety
EDEMA3-RD (open-label extension)	HAE	67	161	OL, repeat-dose	≥72h between attacks	30 mg SC	• TOS at 4h • Safety
DX-88/20 EDEMA4	HAE	70	86	DB, R, PC with OLE	SD, extra OL dose for airway compromise or incomplete response/ relapse	30 mg SC Placebo	• Change in Mean Symptom Complex Score (MSCS) at 4h • Safety
DX-88/19 (OLE) (ongoing)	HAE	77 as of 31-Jul-08	?	OL, RD	≥72h between attacks	30 mg SC	• Change in Mean Symptom Complex Score (MSCS) at 4h • Safety

*Patients randomized to receive ecallantide. Patients could enroll in sequential studies.

5.2 Review Strategy

The two Phase 3 studies (EDEMA3 and EDEMA4), the open-label dose-ranging repeat dose study (EDEMA2), and the two other Phase 2 studies (EDEMA0 and EDEMA1) in Table 3 were reviewed, with the greatest emphasis placed on the pivotal Phase 3 efficacy and safety studies. Data from the OLE phases of both pivotal studies were also included in the review, but the results of EDEMA4 OLE were not available to include in this briefing document. EDEMA3 and EDEMA4 are presented and discussed in Section 5.3 below; more detailed review of these two studies and the other studies are located in the Individual Study Reviews found in Section 10. EDEMA2 was reviewed to assess the extent of dose-ranging performed in the clinical development program and for additional safety and information on repeat doses, given the small number of patients exposed in the overall clinical development program. A detailed review of EDEMA2 is located in Section 10 (Individual Study Reviews). Additional studies not shown in Table 3 that were also reviewed include the PRO validation studies intended to support the primary and secondary efficacy variables used in the Phase 3 studies and a rechallenge study in patients with hypersensitivity reactions to ecallantide. Data from the Phase 1 program and compassionate use were also evaluated for additional safety information.

Reviews of the studies are based primarily on the Dyax study reports, original protocols, and statistical analysis plans. The Applicant's summary data tables were reviewed in details. Appendix tables were also reviewed in varying amounts of detail, depending upon the endpoint and review issue. Case report forms (CRFs) were also reviewed.

The Applicant provided bibliographies within the study reports and expert opinion reports in the application. These references in addition to the results of a literature search conducted by the reviewer were reviewed to the extent of their relevance to the review.

5.3 Discussion of Individual Studies

This section presents an overview of efficacy data from the two pivotal studies; more detailed discussion of these studies and the other clinical studies can be found in Section 6 and in the Individual Study Summaries located in Section 10, . A detailed discussion of safety data is presented separately in Section 7.

The clinical development program included two randomized, placebo-controlled Phase 3 studies, EDEMA3 and EDEMA4. The design and conduct of the studies were similar. Each study consisted of a double-blind phase and an optional, open-label phase. During the double-blind phase, patients presenting within 8 hours of onset of symptoms of a moderate to severe, acute HAE attack were randomized to receive a single 30 mg dose of ecallantide or placebo. In EDEMA3, patients were eligible to receive an additional unblinded 30 mg ecallantide dose (Dose B) for severe upper airway compromise (SUAC); in EDEMA4, patients were eligible for Dose B for SUAC *or* recurrent, persistent symptoms. During the OLE phase of both studies, patients presented with new acute HAE attacks and received ecallantide 30 mg SC. In the EDEMA3 OLE, the initial dose could be followed by a second, blinded dose (Dose B;

randomized 1:1 ecallantide:placebo) for persistent or worsening symptoms. In EDEMA4, Dose B was open-label ecallantide.

Although EDEMA3 and EDEMA4 were similar in design, two major differences between the studies make an individual presentation of each study useful: 1) different primary efficacy endpoints and 2) differing pre-specified statistical analyses with imputation for missing data (EDEMA3) in contrast to no imputation (EDEMA4). EDEMA3 used the TOS at 4 hours as the primary efficacy endpoint; change in MSCS from baseline at 4 hours was a secondary endpoint. During the SPA discussion of EDEMA4, the Division raised concerns about the transparency of the TOS and recommended switching the two endpoints. As a result, EDEMA4 was conducted under SPA using the MSCS as the pre-specified primary efficacy variable and the TOS as a key secondary efficacy variable. A more detailed description of these endpoints and the validation studies conducted to support these PRO instruments is included below and in Section 6 of this review. In terms of data imputation, EDEMA3 employed imputations for emerging symptom complexes and medical interventions. In both studies, sensitivity analyses were performed using imputations for emerging symptoms and medical interventions to test the robustness of the study conclusions.

The clinical program did not include a placebo-controlled evaluation of repeat exposures. The OLE efficacy results from EDEMA3 and EDEMA4 are described in this section, as clinical data to support chronic, repeat use of ecallantide is derived primarily from the OLE phase of EDEMA3. Additional support is provided by open-label data obtained from the Phase 2 study, EDEMA2. The inclusion/exclusion criteria and efficacy assessments performed in EDEMA2 were not as rigorous as those performed in the Phase 3 program, so the EDEMA2 results are considered as secondary support. The design and results of EDEMA2 are presented here and in further detail in the Individual Study Summaries located in Section 10. OLE efficacy and safety data from EDEMA4 were not included in the original submission and were not submitted in time for inclusion in this briefing document.

5.3.1 EDEMA3

Study design and conduct

EDEMA3 was a 2-part Phase 3 study conducted in the US, Canada, and Europe. The first phase was a randomized, double-blind, placebo-controlled, single-dose phase (97 days duration for DB phase) followed by an open-label extension phase where patients could receive treatment for additional acute HAE attacks. Patients with symptoms of a moderate to severe HAE attack presenting within 8 hours of symptom onset were eligible for treatment with a single dose of 30 mg ecallantide SC or placebo.

- ***Treatment Outcome Score (TOS)***

The primary efficacy endpoint was the Treatment Outcome Score (TOS) at 4 hours. The TOS is a composite, weighted symptom complex score intended to assess global symptom response to treatment. Each individual symptom complex score is based on a severity rating for that particular group of symptoms multiplied by a “response-to-treatment” factor, so that the outcome is incorporated into the final TOS value.

$$TOS = \frac{\sum symptom\ complex\ score \times symptom\ complex\ weight}{\sum symptom\ complex\ weight}$$

In this equation, "symptom complex score" = response-to-treatment and symptom complex weight = baseline severity assessment. Severity is scored on a scale of 0 to 3, with 3 being the most severe (see definitions of severity ratings in Table 4). Response assessment is scored as -100, -50, 0, 50, or 100, with -100 representing significant worsening and a score of 100 representing significant improvement. The following symptom complexes were assessed: 1) internal head/neck, 2) stomach/GI, 3) genital/buttocks, 4) external head/neck, and 5) cutaneous. The maximum and minimum possible TOS was 100 and -100, respectively, with a higher value corresponding to greater improvement; a TOS value of 0 signified no change.

- ***Mean Symptom Complex Score (MSCS)***

The secondary efficacy endpoint was the change from baseline Mean Symptom Complex Score (MSCS) at 4 hours. The MSCS is an arithmetic mean of individual symptom complexes. Unlike the TOS, there is no inherent time/outcome element in the MSCS; hence, response to treatment is assessed as "the change from baseline MSCS." The maximum possible calculated MSCS value is 3.0 and the minimum possible value is 0; accordingly, the greatest possible change from baseline is ±3.0. The table below shows the scoring for severity assessment used in the MSCS calculation.

Table 4 Severity assessment for MSCS calculation

Severity Assessment	Score	Definition
Severe	3	treatment or intervention required due to inability to perform activities of daily living (e.g. throat swollen/difficulty breathing, lips swollen/cannot eat, feet swollen/cannot walk)
Moderate	2	treatment or intervention highly desirable and symptoms impact activities of daily living (e.g. hands swollen/cannot button shirt, feet swollen/discomfort wearing shoes)
Mild	1	noticeable symptoms but do not impact activities of daily living
Normal	0	patient's state absent of an acute HAE attack

Reviewer's comment: The primary efficacy variable, TOS, is a complicated score that is difficult to interpret, due in part to the response and severity multipliers used. Overall, a higher number corresponds to a better response to study drug, although the magnitude of response for a given TOS value is not intuitively clear. The response multiplier appears to exaggerate small differences, which may or may not be clinically meaningful. For this reason, in the EDEMA4 SPA, the Division recommended that the applicant use the change from baseline MSCS as the primary endpoint with the TOS as a supportive secondary endpoint. The MSCS was felt to be more transparent and more similar to symptom scoring used for other conditions.

<u>Study results</u>

A total of 36 patients received one 30 mg dose of ecallantide. Two of these 36 received a second 30 mg dose for SUAC. One placebo patient also received an open-label 30 mg dose for SUAC. The disposition of the patients and the demographic information are summarized Table 5 and Table 6.

Table 5 EDEMA 3: Patient disposition

	Ecallantide N=36 N (%)	Placebo N=36 N (%)	Total N=72 N (%)
Intent to treat population[a]	36 (100.0)	36 (100.0)	72 (100.0)
Per protocol population[b]	35 (97.2)	36 (100.0)	71 (98.6)
Safety population[c]	36 (100.0)	36 (100.0)	72 (100.0)
Patients completing double-blind phase	35 (97.2)	36 (100.0)	71 (98.6)
Patients rolling over to continuation study[d]	21 (58.3)	27 (75.0)	48 (66.7)
Patients withdrawing from study	1 (2.8)	0	1 (1.4)
Adverse event	0	0	0
Noncompliance or protocol violation	0	0	0
Withdrawal of consent	0	0	0
Lost to follow-up	1 (2.8)	0	1 (1.4)
Investigator discretion	0	0	0
Left study site against medical advice	0	0	0

[a] Patients who received any amount of study drug and completed the 4-hour follow-up
[b] Patients who received a complete dose of study drug with no major protocol violations and completed the 4-hour follow-up
[c] Patients who received any amount of study drug
[d] All patients were eligible to enroll in the open-label extension study.
Source: dx-88-14b-csr-body.pdf, Section 10.1, Table 3

Table 6 EDEMA3: Patient demographics

	Ecallantide N=36	Placebo N=36	Total N=72
Age			
Mean (SD)	38.5 (14.6)	32.2 (13.8)	35.4 (14.5)
Range	18-77	11-57	13-77
Sex (N,%)			
Male	12 (33.3)	13 (36.1)	25 (34.7)
Female	24 (66.7)	23 (63.9)	47 (65.3)
Race (N,%)			
White	33 (91.7)	32 (88.9)	65 (90.3)
Black	1 (2.8)	4 (11.1)	5 (6.9)
Hispanic	2 (5.6)	0	2 (2.8)
Prior use of ecallantide	8 (22.2)	11 (30.6)	19 (26.4)

Source: dx-88-14db-csr-body.pdf, Section 11.2.1, Table 4

Details regarding the patients' HAE history and concomitant mediations can be found in the individual study review located in Section 10. In EDEMA3, the most commonly reported symptom complexes of at least moderate to severe severity in the ecallantide group were cutaneous (n=21) and stomach/GI (n=20). In the placebo group, 14 patients reported cutaneous symptoms and 21 reported stomach/GI symptoms. Laryngeal attacks were reported in 9 ecallantide patients and 4 placebo patients. Results of the main efficacy analyses are presented below. Recall that EDEMA3 includes data imputations in the primary analyses.

Table 7 EDEMA3 Efficacy analyses

Endpoint	ITT			ITT as treated*		
	Ecallantide N=36	Placebo N=36	P	Ecallantide N=36	Placebo N=36	P
Mean TOS at 4h (SD)	46.8 (59.34)	21.3 (69.04)	0.100	49.5 (59.43)	18.5 (67.78)	0.037
Change from baseline MSCS at 4h (SD)	-0.88 (1.11)	-0.51 (0.68)	0.094	-0.91 (1.10)	-0.48 (0.68)	0.044

* Population based on treatments as received

Reviewer's comment: Two patients mistakenly received the wrong study drug: 1 placebo patient received ecallantide and 1 ecallantide patient received placebo. When the efficacy endpoints are recalculated using a dataset corrected for these protocol violations, the differences between the ecallantide and placebo arms are statistically significant. These results suggest that ecallantide has some efficacy, although the results do not appear to be robust and the limitations of a small sample size are apparent.

Although a formal subgroup analysis for the individual study was not provided by the Applicant, post hoc analyses performed by the Division's statistical reviewer did not show any clear differences in efficacy based on anatomical attack site, gender, or history of prior exposure to ecallantide. Subgroup analysis by age or race is limited by the small sample sizes.

Other secondary efficacy endpoints assessed were numerically supportive if not statistically significant when based on the ITT population. In terms of time to significant improvement, a median time of 165.0 minutes was reported for the ecallantide group, in comparison to 240 minutes for the placebo group (p=0.136). Using a cutoff value of 70 for TOS at 4 hours, 15 patients (42%) in the ecallantide group qualified as having a successful response assessment in comparison to 12 (33.3%) patients in the placebo group (p=0.47). No statistically significant differences were observed when adjusted for attack location or prior use of ecallantide. At the 24-hour timepoint, the median TOS was 75.0 for the ecallantide group compared to 0 in the placebo group (p=0.044). Rescue medication usage patterns also favored the ecallantide arm over placebo; 5 (14%) in the ecallantide arm required medical intervention in comparison to 13 (36%) in the placebo arm. The most commonly administered interventions were emergency medications such as opioids for pain control and anti-emetics. No patients required intubation or urgent surgical decompression. In both treatment groups, fewer patients with peripheral attacks required intervention than patients with a laryngeal attack (p=0.014).

Reviewer's comment: The secondary efficacy endpoints are generally supportive of ecallantide's effectiveness for the proposed indication. Although not statistically significant, the findings suggest durability of response and a reasonable response rate for the drug. Rescue medication use also supports the efficacy of ecallantide over placebo.

Extension, repeat-dose phase

Following the double-blind, placebo-controlled phase of EDEMA3, patients were eligible to continue in the repeat-dose, open-label extension for up to 20 separate HAE attacks. New

patients who did not participate in the double-blind phase were also eligible to enroll in the repeat-dose phase. A new attack was defined as an HAE attack that presented after a return to normal state following a previous acute attack. Patients were treated with a single, 30 mg dose of ecallantide. If symptoms did not resolve completely, patients could be given a second blinded dose of 30 mg ecallantide or placebo within 4 to 24 hours of the initial single dose.

From the double-blind phase, 22 ecallantide and 26 placebo patients received at least 1 dose of ecallantide in the OLE phase. Another 19 new patients also joined the study, for a total of 67 patients in the safety population. A total of 160 attacks were treated during the OLE. The majority of patients were treated for 1 attack during the OLE; 1 patient was treated for 13 attacks. Sixty-five of 153 treated attacks in the ITT population involved multiple symptom complexes. Thirty-three attacks had laryngeal involvement. The Applicant reported heterogeneity in individual patients, both in attack site and in severity, from one attack to the next, which is consistent with the natural history of HAE described in the literature.

The TOS at 4 hours and the change from baseline in MSCS at 4 hours varied by treatment episode. The first treatment episode only includes new patients who did not participate in the double-blind phase. The following tables summarize these results.

Table 8 EDEMA3 OLE: TOS at 4 hours by treatment episode

Treatment episode	N	Median (IQR)	Mean (SD)
1	18	68.8 (50, 100)	71.3 (28.9)
2	51	100 (50, 100)	73.3 (44.9)
3	30	100 (70, 100)	81.9 (28.5)
4	21	100 (38, 100)	81.2 (24.5)
5	11	100 (0, 100)	48.5 (68.5)
6	9	60 (50, 100)	60.4 (49.3)

Source: dx-88-14rd-csr-body.pdf, Section 11.4.1.1, Table 15

Change in MSCS at 4 hours

Table 9 EDEMA3 OLE: Mean change in MSCS at 4 hours by treatment episode

Treatment episode	N	Median (IQR)	Mean (SD)
1	17	-1.0 (-1.5, -1.0)	-1.2 (0.9)
2	51	-1.0 (-1.8, -0.5)	-1.1 (0.9)
3	30	-1.0 (-2.0, -1.0)	-1.3 (0.9)
4	21	-2.0 (-2.0, -1.0)	-1.4 (0.8)
5	11	-1.0 (-1.3, 0)	-0.9 (0.7)
6	9	-1.0 (-1.0, -0.3)	-0.9 (0.8)

Source: dx-88-14rd-csr-body.pdf, Section 11.4.1.1, Table 16

Based on subgroup analysis provided by DPAP's statistical reviewer, there were no major efficacy differences between ecallantide-naïve patients and patients with a history of prior exposure. Only 3 patients received Dose B, limiting analysis. Of the 2 patients who received placebo as Dose B, both patients reported symptoms to be "a lot better or resolved" at the 4- and 24-hour assessments. The third patient who received ecallantide as Dose B reported symptoms to be the "same" and did not receive further treatment in the study.

Reviewer's comment: The TOS values suggest efficacy over repeated doses, although the number of patients upon which the TOS is based decreases with each episode. This may be a function of the underlying rate of attacks; alternatively, these results could be due to self-selection of responders vs. non-responders, meaning that patients with incomplete or unsatisfactory responses may have chosen not to present for treatment of future attacks. The MSCS scores appear consistent with the TOS, which is expected as the MSCS is a component of the TOS calculation. In the absence of a control, these results are difficult to interpret as the natural course of an HAE attack is gradual improvement. Numerically, the magnitude of the MSCS results appears comparable to those observed for the ecallantide arm in the double-blind phase.

Conclusions

EDEMA3 is generally supportive of ecallantide's efficacy in the treatment of acute HAE attacks but the study did not demonstrate a statistically significant difference between ecallantide and placebo for the ITT population as randomized. The Applicant attributes the non-significant results to the accidental administration of placebo to 1 patient assigned to ecallantide and ecallantide to 1 patient assigned to placebo. When the data was reanalyzed using an as-treated dataset to correct for this error, the results were found to be statistically significant. While this post hoc analysis along with secondary and tertiary endpoints suggest efficacy, these results are not robust and confirmatory results from the second placebo-controlled trial, EDEMA4, are needed.

5.3.2 EDEMA4

Study design and conduct

EDEMA4 was the second pivotal Phase 3 study conducted in the US and Canada and similar in design to EDEMA3. Patients presenting within 8 hours of onset of moderate to severe HAE symptoms were randomized to treatment with 30 mg ecallantide SC or placebo. Patients were stratified by location of attack (laryngeal vs. other sites). Patients with evidence of upper airway compromise within 4 hours of dosing were eligible for an open-label dose of ecallantide. Similarly, patients with symptom relapse/recurrence at least 4 hours after dosing and within 24 hours of dosing were also eligible for open-label treatment with a single dose. Unlike EDEMA3, change from baseline MSCS at 4 hours post-dose was the designated primary efficacy endpoint for EDEMA4; the TOS was a key secondary efficacy endpoint. As noted above, the MSCS is the arithmetic mean of the severity grade of the individual symptom complexes, where each symptom complex is assessed a severity grade of severe to normal. A decrease from baseline MSCS corresponds to a reduction in severity. The same anatomic symptom complexes as in EDEMA3 were assessed.

No imputations were made for the primary analysis. Sensitivity analyses performed to assess the effects of emerging symptom complexes and medical interventions were performed using the following imputations: Emerging symptom complexes were included in the MSCS calculation if present at the 4-hour and 24-hour MSCS assessment timepoints. If medical interventions were performed during an attack, the affected symptom complex(es) were assigned a severity of "severe" at 4 and/or 24 hours.

Efficacy results

Ninety-six patients were enrolled; 48 in the ecallantide arm and 48 in the placebo arm. The disposition of the patients and baseline demographics are shown in Table 10 and Table 11.

Table 10 EDEMA 4: Patient disposition

	Ecallantide N=48 N (%)	Placebo N=48 N (%)	Total N=96 N (%)
Intent to treat population[a]	48 (100.0)	48 (100.0)	96 (100.0)
Per protocol population[b]	47 (97.9)	48 (100.0)	95 (99.0)
Safety population[c]	48 (100.0)	48 (100.0)	96 (100.0)
Patients rolling over to continuation study[d]	47 (97.9)	46 (95.8)	93 (96.9)
Patients withdrawing from study		1 (2.1)	1 (1.0)
Adverse event	0	0	0
Noncompliance or protocol violation	0	0	0
Withdrawal of consent	0	0	0
Lost to follow-up	0	0	0
Investigator discretion	0	0	0
Left study site against medical advice	0	1 (2.1)	1 (1.0)

[a] Patients who received any amount of study drug
[b] Patients who received a complete dose of study drug with no major protocol violations
[c] Patients who received any amount of study drug
[d] All patients were intended to roll over to the open-label extension study (DX-88/19) for follow-up safety assessments. A total of 2 patients (1 in the ecallantide arm and 1 in the placebo arm) declined further participation. An additional patient in the placebo arm left the study site against medical advice and was not enrolled in the follow up study.
Source: dx-88-20-csr-body.pdf, Section 10.1, Table 2

Table 11 EDEMA4: Patient demographics

	Ecallantide N=48	Placebo N=48	Total N=96
Age			
Mean (SD)	37.0 (13.1)	38.0 (12.2)	37.5 (12.6)
Range	15-72	13-72	13-72
Sex (N,%)			
Male	11 (22.9)	20 (41.7)	31 (32.3)
Female	37 (77.1)	28 (58.3)	65 (67.7)
Race (N,%)			
White	39 (81.3)	43 (89.6)	82 (85.4)
Black	3 (6.3)	3 (6.3)	6 (6.3)
Asian	1 (2.1)	1 (2.1)	2 (2.1)
Hispanic	4 (8.3)	1 (2.1)	5 (5.2)
Other	1 (2.1)	0	1 (1.0)
Prior use of ecallantide	17 (53.4%)	19 (39.6%)	36 (37.5)

Source: dx-88-csr-body.pdf, Section 11.2.1, Table 4

In the ITT population, a total of 36 patients (17 in the ecallantide arm and 19 in the placebo arm) had previously participated in another ecallantide study. The groups appeared mostly comparable, although the ecallantide group had a higher proportion of female participants than the placebo arm. The potential impact of this discrepancy on efficacy findings is unclear. Details regarding the patients' HAE history and concomitant mediations can be found in the individual study review located in Section 10. In EDEMA4, the most commonly reported moderate-severe symptom complex in the ecallantide group was cutaneous, with 22 patients

reporting cutaneous symptoms of moderate-severe severity compared to 17 patients in the placebo arm. The placebo arm had a larger number of patients reporting moderate-severe GI symptoms in comparison (n=26 compared to n=13 in the ecallantide arm). Laryngeal symptoms of moderate-severe severity were reported with similar frequency in the treatment groups (8 patients in the ecallantide group and 7 patients in the placebo group).

Reviewer comment: The distribution of attack sites is not equal, with cutaneous attacks predominating in the ecallantide group versus stomach/GI attacks in the placebo group. This uneven distribution could impact efficacy findings, if ecallantide works better on cutaneous symptoms, for example, or if the PRO instruments do not assess different attack site symptoms similarly. However, the literature and the PRO validation studies actually suggest the opposite, that GI symptoms, primarily pain, tend to be considered more significant in HAE attacks and perhaps more easily assessed by PRO measures.

Results from the primary efficacy analysis are shown below. The treatment arms had comparable baseline MSCS scores. A statistically significant greater decrease in MSCS from baseline was observed in the ecallantide group compared to the placebo arm (Table 12). Similar results were observed for the per-protocol population analysis as well (p=0.011). A statistically significant difference between the ecallantide group (mean TOS 53.4, SD 49.7) and the placebo group (mean TOS 8.1, SD 63.2) was observed (p=0.003). Similar TOS results were also reported for the PP population.

Table 12 EDEMA4: Primary efficacy endpoint, Change from baseline MSCS at 4 hours post-dose

	Baseline MSCS	Change from baseline at 4h	P
Ecallantide	2.2 (0.5)	-0.8 (0.6)	0.01
Placebo	2.0 (0.4)	-0.4 (0.8)	

Source: dx-88-20-csr.pdf, Section 11.4.1.1, Table 14

Imputations for emerging symptom complexes and medical interventions were also performed. These results are displayed in Table 13 EDEMA4: Primary efficacy endpoint sensitivity analyses.

Table 13 EDEMA4: Primary efficacy endpoint sensitivity analyses

	Mean change from baseline MSCS at 4 hours		
	Ecallantide (N=47)	Placebo (N=48)	P
Imputation for emerging symptoms	-0.8 (0.6)	-0.2 (0.9)	<0.001
Imputation for emerging symptoms and medical intervention	-0.8 (0.7)	-0.1 (0.9)	<0.001

Source: dx-88-20-csr.pdf, Summary tables 14.2.3.2.1 and 14.2.3.2.2

Secondary efficacy endpoints were also generally supportive of ecallantide's efficacy in terms of numerical trends, if not statistically significant. The response appeared to be durable, statistically significant differences in terms of MSCS scores and TOS being observed at the 24-

hour mark post-dose. Again, attack site location and prior exposure to ecallantide were not determinants of response. Using the same TOS cutoff value of 70 that was used in EDEMA3 to distinguish responders from non-responders, more ecallantide patients (22 of 48, 45.8%) qualified as responders compared to the placebo arm (9 of 47, 19.1%) [p=0.011]. Also, fewer patients in the ecallantide group (16 of 48, 33.3%) received medical intervention than in the placebo group (24 of 48, 50.0%).

Conclusions

EDEMA4 provides efficacy and safety support for ecallantide as a treatment of acute HAE attacks. The study used a related but different endpoint for the primary efficacy analysis and was also greater in sample size compared to EDEMA3, which may explain in part the different statistical outcomes in the two studies. In terms of effect sizes and treatment differences, the MSCS results from EDEMA4 and EDEMA3 were similar, which suggests that EDEMA3's non-significant findings may be due in part to the smaller sample size.

5.3.3 EDEMA2 (Study DX-88/5)

Study design and conduct

EDEMA2 was an open-label, dose-ranging repeat dose study of ecallantide for the treatment of acute HAE attacks. Qualified patients presenting within 4 hours of the onset of an acute attack of at least moderate severity were treated with a single dose of ecallantide (Dose A). If no improvement was noted within 4 hours, a second dose (Dose B) could be administered. Patients could receive a maximum of 20 doses for separate attacks. Escalating IV doses (5 mg/m^2, 10 mg/m^2, or 20 mg/m^2) were administered by sequential dose cohorts. The transition from each dosage cohort to the next was based on the review of safety and efficacy in the EDEMA1 study by the DSMB. For example, once the DSMB had determined the 10 mg/m^2 dose level safe in EDEMA1, patients enrolled in EDEMA2 were then given 10 mg/m^2. Patients were not restricted to a particular dose cohort and could receive repeated doses of ecallantide at a different dose level from the one received previously. From July 2005 to study conclusion, IV infusions were changed to ecallantide 30 mg SC fixed dose. Patients who had an incomplete response were eligible for Dose B.

Efficacy results

A total of 77 patients from 26 study sites were enrolled and treated for 240 HAE attacks. This population constitutes the ITT population. Twenty of the 77 (25.9%) had had prior exposure to ecallantide. Peripheral HAE attacks were reported as the first study-treated attacks for 35 (45.5%) patients. Abdominal attacks were reported for 32 (41.6%) patients. Ten (13.0%) patients presented with laryngeal attacks for their first study-treated attack. The baseline demographics of the patients are described in Table 14.

Table 14 EDEMA2: Patient demographics

	5 mg/m^2 N=14	10 mg/m^2 N=40	20 mg/m^2 N=9	30 mg N=14	Overall N=77
Age					
Mean (SD)	34.6 (13.6)	31.7 (15.2)	28.7 (12.4)	38.0 (11.8)	33.0 (14.1)
Range	11-53	13-78	12-52	10-55	10-78
Sex (N,%)					
Male	6 (42.9%)	11 (27.5%)	4 (44.4%)	8 (57.1%)	50 (64.9%)
Female	8 (57.1%)	29 (72.5%)	5 (55.6%)	6 (42.9%)	27 (35.1%)
Race (N,%)					
White	10 (71.4%)	38 (95.0%)	8 (88.9%)	11 (78.6%)	67 (87.0%)
Black	3 (21.4%)	2 (5.0%)	0	0	5 (6.5%)
Hispanic	1 (7.1%)	0	1 (11.1%)	2 (14.3%)	4 (5.2%)
Asian	0	0	0	1 (7.1%)	1 (1.3%)

Source: dx-88-5-csr-body.pdf, Section 11.2.1, Table 7

Efficacy was based on patient symptom reports. These symptom reports were largely descriptive and did not include a formal scoring system like the TOS and MSCS. A successful outcome was defined as onset of resolution within 4 hours of dosing and continuing for 24 hours of dosing. Of the 240 treated attacks, 165 attacks (68.9%) were reported to have a successful outcome. Among the 4 dosage levels, the 30 mg SC dose had the highest proportion of successful outcomes (49 of 60 attacks, 81.7%), followed by the 10 mg/m^2 IV and 20 mg/m^2 IV doses (68.1% and 60.0%, respectively). The 5 mg/m^2 IV dose had 11 of 24 attacks (45.8%) with successful outcomes. Another 41 of 240 attacks (17.1%) were reported as having a partial response, meaning a response to dosing for at least 1 symptom at the primary attack site within 4 hours of treatment followed by a relapse within 24 hours or receipt of Dose B.

A number of different instruments were used to assess response to abdominal attacks, including a Visual Analog Scale (VAS) for pain, the McGill Pain Questionnaire, and change in waist girth. According to VAS measurements, pain was reduced by 83.2%, 79.5%, and 66.8% at 4 hours post-dosing for Attacks 1, 2, and 3, respectively. These results corresponded with an average reduction of 2 scale points (total of 0 to 5) on the McGill Pain questionnaire at 4 hours. For Attacks 1 and 2, an average 2 to 4% reduction in waist circumference was measured at 4 hours; for Attack 3, the decrease in average waist circumference was negligible.

Reviewer's comment: These symptom assessments are generally supportive but the clinical benefit cannot truly be assessed in the absence of placebo. HAE attacks gradually resolve on their own, so improvements over time are expected even on placebo. Also, it is worth noting, however, that neither the VAS nor the McGill Pain Questionnaire are PRO instruments validated for use in HAE, nor is waist circumference a routinely utilized clinical measure.

Study conclusions

EDEMA2 is generally supportive of ecallantide's efficacy for acute attacks of HAE and support the dose selection of 30 mg SC. The strength of the efficacy findings for repeat, intermittent dosing are limited by three main factors: 1) the inclusion criteria (specifically, the HAE diagnostic criteria) were not as rigorous as those specified in the Phase 3 program and could have potentially resulted in the inclusion of acquired angioedema (AAE) patients; 2) the efficacy

measurements were based on unvalidated symptom scores that were unrelated to the MSCS and TOS, limiting cross-study comparisons; 3) there was no control arm. As a result, although EDEMA2's results are positive, EDEMA2 remains a secondary study in terms of efficacy support.

6 Review of Efficacy

Efficacy Summary

6.1 Indication: Treatment of acute attacks of HAE

6.1.1 Indication

The proposed indication for ecallantide is "the treatment of acute attacks of HAE" in patients age 10 years and older.

6.1.2 Methods

The review of efficacy relies primarily on the findings of the two pivotal, randomized, placebo-controlled efficacy and safety studies, EDEMA3 and EDEMA4. The design and conduct of these two studies is presented in further detail in Section 5.3 and in the Individual Study Summaries in Section 10. Additional evidence of support for repeat dosing is provided by EDEMA2, a Phase 2 study that involved extended, repeat open-label dosing. Anecdotal support provided by the compassionate use narratives and preliminary efficacy data from EDEMA0 and EDEMA1 were also considered in the assessment of efficacy.

6.1.3 Demographics

Demographic information from the efficacy studies are presented in detail in Table 6, Table 11, and Table 14 in Section 5.3. In general, most patients were female, Caucasian, with a mean age around 35 years. The groups in each efficacy study were generally balanced with the exception of females in the EDEMA4 study. The groups were generally balanced with regards to HAE history and concomitant medication use.

6.1.4 Patient Disposition

Patient disposition is described in detail in Sections 5.3 and 7.2. For the controlled portion of EDEMA3 and EDEMA4, the majority of patients completed the single dose and follow up period. Only two patients did not complete the follow up.

6.1.5 Analysis of Primary Endpoint(s)

Primary endpoint selection and validation: The TOS and MSCS

The Applicant developed two symptom scoring systems with the intent of capturing the full range of signs and symptoms of an HAE attack, the TOS and the MSCS. The TOS includes the MSCS in its calculation along with multipliers for temporal assessment, so the two efficacy variables are related. The Applicant was advised to refer to the draft *Guidance for Industry: Patient Reported Outcomes: Use in Medical Product Development to Support Labeling Claims* (January 2006) during the development of these instruments. In April 2006 at an end-of-phase 2 meeting with the Applicant, the Division confirmed that both the TOS and MSCS would be suitable efficacy variables during the phase 3 studies, presuming adequate validation was available. During discussion of the design of EDEMA4, the Division raised concerns about the complex nature of the TOS. Given the complexity of the scoring system with its severity multipliers and the inclusion of a temporal assessment of response into the score, the Division raised concerns that the TOS was not intuitive and hard to interpret. Due to the response multipliers, small differences of uncertain clinical relevance could be exaggerated. The Division felt that defining a clinically meaningful difference would prove difficult. In addition, the Division was concerned that the TOS would be difficult to represent accurately in a product label and could potentially cause confusion to clinical practitioners. As a result, the Division recommended that the Applicant use the MSCS as the primary efficacy variable for EDEMA4 and include the TOS as a key secondary endpoint to facilitate cross-study comparisons between the two pivotal studies. The MSCS is a more straightforward global symptom score that captures symptom severity at a point in time. To support both PRO instruments, the Applicant has submitted validation reports as well as the results of cognitive debriefing interviews with patients and proxy respondents.

Reviewer's comment: There are no previously validated PRO instruments available for use in HAE. The complex nature of an HAE attack – the various anatomic sites of attack and different symptom manifestations at these locations – makes objective measurement of drug responses in this condition difficult. Usually, an anatomic site will predominate but other sites are frequently involved and an attack may continue to evolve over time. Even for a given individual, attacks can vary from one to the next and affect the intra-individual retest reliability of a PRO instrument.

- ***Cognitive debriefing interviews (Study DX-88/)***

  Cognitive debriefing interviews were conducted in 21 subjects: 15 patients with angioedema (including 2 children) and 6 proxy respondents (1 husband of a patient, 1 mother of a child patient, 3 clinical site coordinators, and 1 physician). On average, the patients reported an attack frequency of 1 attack every 3.5 months that typically lasted in duration from 10 hours to 3 days. When asked about the most recent attack, patients reported symptom complexes consistent with those specified for the MSCS and TOS calculations. Severity was described in terms of effects on daily activities which appeared to be consistent with the severity definitions used in the Phase 3 trials. In addition, patients noted that the most severe symptom within a complex determined their rating of severity. Of note, patients reported a hierarchy in anatomic sites, noting that GI symptoms and laryngeal symptoms were more

severe than cutaneous symptoms due to the pain associated with GI swelling and life-threatening nature of laryngeal swelling. Based on the interview comments, it appeared that a moderate GI attack was considered inherently more severe than a moderate cutaneous attack. Overall, participants appeared to understand the terms used in the MSCS and TOS, with the exception of the term "cutaneous" and the distinction between "internal" versus "external" head and neck symptoms. Based on this feedback, the investigators recommended that patients be presented with all the symptom complexes and their definitions prior to completion of the e-diaries in the study. These recommendations were implemented in EDEMA4 but were made after the completion of EDEMA3.

- ***PRO validation (Study DX88-103)***

Study DX88-103 was intended to assess the psychometric properties of the TOS and MSCS, using data collected from EDEMA3. The study demonstrated moderate test-retest reliability (TOS intra-class correlation coefficient [ICC] = 0.52; MSCS ICC = 0.62) by comparing TOS and MSCS scores to a global improvement measure in a subset of patients who had reported no change or "same" at the 4 hour timepoint on the global improvement measure. The TOS and MSCS correlated with the global improvement score at 4 hours, suggesting construct validity. The TOS and MSCS also discriminated between the global improvement groups at 4 hours, indicating discriminant validity. Using a triangulation approach and comparison to the global improvement measurement scores, a minimum clinically important difference (MCID) for both the TOS and MSCS was estimated: TOS MCID = 30 points and MSCS MCID 0.30 points.

Reviewer's comment: To put the estimated MCID values in context, a difference of 42.1 was found in the mean TOS values for patients reporting no change and those reporting improvement at 4 hours on the global improvement measure. For the MSCS, a difference of 0.5 was found in the change in MSCS values for patients reporting no change versus those reporting improvement at 4 hours.

The Applicant has followed the guidelines set forth in the PRO Guidance for Industry to validate the two instruments, TOS and MSCS. Both symptom scores appear to capture patients' HAE symptoms with some degree of test-retest reliability and differences in the scores appear to correlate statistically with patient-reported clinical changes. In addition to the validation data provided by the Applicant, individual line listings of patients' efficacy TOS, MSCS, and global improvement item scores in both EDEMA3 and EDEMA4 were reviewed and generally appear to corroborate the study's findings. That being said, the TOS remains difficult to interpret and represent and concern remains that the response outcome multipliers may exaggerate differences of questionable clinical relevance. Given these issues with the TOS, this clinical reviewer prefers the MSCS and global response assessments as measurements of efficacy.

Efficacy findings

The two Phase 3 studies, EDEMA3 and EDEMA4, provide the primary efficacy support for the proposed indication, the treatment of acute HAE attacks. EDEMA2 also provided support for the efficacy of repeat dosing, but the strength of these data is limited by the rigor of patient selection criteria and the selection of efficacy measurements, as discussed in Section 5.3. The

study design of the pivotal studies was adequate; both EDEMA3 and EDEMA4 studies were randomized and placebo-controlled and used appropriate inclusion/exclusion criteria and efficacy endpoints. The patients enrolled and their presentations were consistent with typical HAE attacks described in the literature.

EDEMA4 had robust results with a change from baseline MSCS at 4 hours for the ecallantide group of -0.8 versus -0.4 in the placebo group (p=0.01). The treatment difference of 0.4 is greater than the MCID estimated in the PRO validation studies. Looking at additional sensitivity analyses that include imputation for emerging symptoms and medical interventions, the difference between ecallantide and placebo is further accentuated (Section 10, Table 54). Similar statistically significant findings for the TOS at 4 hours were also reported in EDEMA4 (53.4 vs. 8.1; p=0.003).

EDEMA3, in contrast, did not have robust results. As described in Section 5.3, 2 patients mistakenly received the wrong study drug. When the efficacy endpoints were recalculated using a dataset based on the ITT as treated population, the differences between the ecallantide and placebo arms were found to be statistically significant. These results support ecallantide's efficacy, although the results do not appear to be robust and the limitations of a small sample size are apparent. In terms of the TOS, EDEMA3 results (ecallantide vs. placebo, 46.8 vs. 21.3; p=0.100) were generally comparable to the EDEMA4 results, although the placebo group appears to have done relatively worse in EDEMA4 when compared to EDEMA3. However, the baseline values and the magnitude of change in MSCS reported for EDEMA3 were similar to the findings in EDEMA4 (-0.9 vs. -0.5; p=0.09). The MSCS scores suggest that the placebo groups performed similarly across studies and indicate that the sample size of EDEMA3 may have contributed to the non-significant findings. The MSCS scores also highlight the difficulty in TOS interpretation, since the TOS does not permit a comparison of baseline status and the subsequent change from baseline.

Table 15 Efficacy Results from EDEMA3 and EDEMA4

	EDEMA3			EDEMA4		
	Ecallantide 30 mg N=36	Placebo N=36	Diff from Pbo **(p value)**	Ecallantide 30 mg N=48	Placebo N=48	Diff from Pbo **(p value)**
TOS at 4 hrs (mean) *ITT as randomized*	46.8	21.3	25.5 **(0.100)**	53.4	8.1	45.3 **(0.003)**
TOS at 4 hrs (mean) *ITT as treated*	49.5	18.5	31.0 **(0.037)**			
MSCS – mean Δ from baseline 4 hrs *ITT as randomized* [baseline]	-0.88 [2.15]	-0.51 [2.26]	-0.37 **(0.094)**	-0.81 [2.18]	-0.37 [2.02]	-0.44 **(0.01)**
MSCS – mean Δ from baseline 4 hrs *ITT as treated* [baseline]	-0.91 [2.17]	-0.48 [2.24]	-0.43 **(0.044)**			

With regards to repeat dosing, the clinical program did not include a placebo-controlled evaluation of chronic, intermittent dosing. The support for repeat dosing is based primarily on information obtained from the open-label experience in EDEMA3, EDEMA4, and EDEMA2 in conjunction with extrapolation from the single-dose experience. In the whole clinical program, 108 patients (50%) had only a single exposure. Eighty patients (37%) had 2 to 4 doses and 19 patients had 5 to 9 doses. One patient in EDEMA3 had a total of 14 doses. Overall, the MSCS

and TOS in the open label period appeared to be consistent with the single dose data, suggesting that the effects of ecallantide do not diminish with repeat doses. However, these results could be due to self-selection of responders vs. non-responders, meaning that patients with incomplete or unsatisfactory responses may have chosen not to present for treatment of future attacks. Given the underlying pathophysiology and the fact that HAE attacks are generally unique events, it is reasonable to assume that ecallantide would be equally efficacious for future attacks. The exception would be in the case of neutralizing antibodies which could theoretically inhibit drug action at a sufficient titer. Based on the data presented, however, there does not appear to be any negative or positive correlation between the development of non-IgE antibodies to ecallantide and efficacy, with the caveat that the total number of patients represented is quite small. The issue of immunogenicity is addressed in further detail in Section 7. In general, the number of treatment episodes was not associated with any decrease in efficacy, although it cannot be ruled out that patients with less favorable responses may have declined to present for treatment of further episodes, resulting in self-selection of responders for the higher number of doses.

Although there are limitations with the repeat dose data – lack of placebo control and potential for selection bias – the uncontrolled, repeat dose data combined with extrapolation of the single-dose, placebo controlled data supports the efficacy of ecallantide with repeat dosing.

6.1.6 Analysis of Secondary Endpoints(s)

Both the TOS and MSCS are discussed above, as these were used as primary and key secondary efficacy variables, respectively, in EDEMA3, and vice versa in EDEMA4. Other secondary endpoints to consider include the TOS and MSCS at 24 hours as a measure of durability of response, responder analysis, and medical interventions as a different measure of efficacy. Overall, the secondary efficacy endpoints provide additional confirmatory evidence of ecallantide's efficacy. Several of the secondary efficacy variables are discussed below.

- ***MSCS and TOS at 24 hours***

  Analysis of MSCS and TOS at 24 hours suggests durability in the ecallantide response. In EDEMA3 the median TOS at 24 hours was 75.0 in the ecallantide group versus 0 in the placebo group (p=0.044). The mean change in MSCS at 24 hours was -0.87 (SD 1.0) in the ecallantide group and -0.46 (SD 1.1) in the placebo group (p=0.142). In EDEMA4 the mean TOS at 24 hours was 88.8 in the ecallantide group vs. 55.1 in the placebo group (p=0.029). The mean change in MSCS at 24 hours was -1.5 (SD 0.6) in the ecallantide group and -1.1 (SD 0.8) in the placebo group (p=0.039).

- ***Responder analysis (TOS≥70)***

  Based on the PRO validation studies, a TOS value of 30 was deemed the MCID. The Applicant performed responder analysis using a range of cutoff values for the TOS at intervals approximately based on this MCID: ≥30, ≥50, ≥70, and 100. A similar proportion of patients in each of the phase 3 studies qualified as "responders" based on these cutoff values. For example, in EDEMA3 15 patients (42%) in the ecallantide group compared to 12 (33.3%) in the placebo group had a TOS≥70 at 4 hours (p=0.47). In EDEMA4 more ecallantide patients (22 of 48, 45.8%) qualified as responders compared to the placebo arm (9

of 47, 19.1%) [p=0.011]. No statistically significant differences were observed when adjusted for attack location or prior use of ecallantide.

- ***Medical interventions***

The medical intervention patterns supported ecallantide's efficacy, as more placebo patients required additional intervention during an attack. In EDEMA3, 5 patients (14%) in the ecallantide group compared to 13 (36%) of placebo patients received medical intervention. Similarly, in EDEMA4, fewer patients in the ecallantide group (16 of 48, 33.3%) received medical intervention than in the placebo group (24 of 48, 50.0%). The most commonly administered interventions were emergency medications such as opioids for pain control and anti-emetics. No patients required intubation or urgent surgical decompression.

6.1.7 Other Endpoints

Several patients in both studies received additional dosing for severe upper airway compromise (SUAC) or for incomplete response/relapse (Dose B). The numbers of patients receiving Dose B was limited and formal efficacy measures (MSCS or TOS) were not recorded systematically, limiting the efficacy assessment.

6.1.8 Subpopulations

Pediatrics

A limited number of pediatric patients were evaluated in the clinical program. There were 18 total pediatric patients in the development program, but of the pediatric patients, only 3 received ecallantide as part of a double-blind study, the youngest being a 15-year-old patient in EDEMA3. Although the proposed indication extends down to the age of 10 years, the youngest patient who received ecallantide during the double-blind phase of EDEMA3 or EDEMA4 was a single 15-year-old in EDEMA4. The youngest participants in the double-blind phase of EDEMA3 were one 16-year-old and one 17-year-old. Younger patients were studied during the open-label dosing, but the numbers were small: one 12-year-old, two 13-year-olds, two 16-year-olds, and two 17-year-olds. EDEMA2 included a small number of pediatric patients: one 10-year-old, one 11-year-old, one 12-year-old, two13 year-olds, two 14-year-olds, two 15-year-olds, one 16-year-old, and one 17-year-old. Of these EDEMA2 patients, only one 10-year-old, one 14 year-old, one 16 year-old, and one 17-year-old received the 30mg SC dose. The remainder received IV doses of 5 to 20 mg/m^2 IV. Although it is not expected that ecallantide would behave differently in a pediatric patient, to support an indication in this age group, there should be sufficient representation of patients less than 18 years of age. In addition, for younger patients, consideration of the appropriate dose is recommended. This clinical reviewer does not believe there is adequate experience with ecallantide in adolescents/children < 18 years of age to support the efficacy of ecallantide in this age group.

Subgroup analysis on the basis of anatomic attack site was complicated by the fact that patients frequently presented with multiple symptoms and the symptom scores collected were composite symptom scores. In general, there were no clear differences in efficacy on the basis of predominant attack location. Review of individual case narratives for dosing for SUAC do not

suggest a deleterious effect from drug but conclusions about efficacy in these cases are difficult to make in the absence of a placebo control.

Other subpopulation analyses were limited by the small sample size. Based on the information provided, there does not appear to be any differential efficacy according to gender or race.

6.1.9 Analysis of Clinical Information Relevant to Dosing Recommendations

The total amount of circulating pre-kallikrein is estimated to be 500 nM in HAE patients. With the intent of achieving stoichiometric equivalence, an 18 mg dose of ecallantide was estimated to achieve a plasma concentration of 500nM. The clinical program was intended to assess a range of doses around this projected plasma concentration, and included both IV formulations (5 to 80 mg/m^2 IV) in EDEMA0 and EDEMA1 as well as the 30 mg SC dose in EDEMA2. However, the evaluable dose-ranging data collected in the clinical program was limited. EDEMA0 and EDEMA1 were not designed or powered in such a way as to permit any conclusions to be made about the comparative efficacy among the different dose levels. Details of these two studies are located in the respective Individual Study Reviews in Section 10. On the basis of EDEMA2, the 30 mg SC was the dose selected for study in the Phase 3 program. The SC dose had administration advantages over the intravenous form of the study studied in the earlier dosing cohorts of EDEMA2 and appeared to provide more consistent plasma levels over the initial 4 hour dosing period.

6.1.10 Discussion of Persistence of Efficacy and/or Tolerance Effects

Durability of response over an initial 24 hour period and potential tolerance effects secondary to the development of neutralizing antibodies are discussed above in Sections 6.1.5 and 6.1.6. Given the sporadic, intermittent dosing of the drug and short half-life, more persistent effects or other tolerance issues are not anticipated.

6.1.11 Conclusions

The application supports the efficacy of ecallantide 30mg for the proposed indication of the treatment of acute HAE attacks in patient 18 years of age and older. The robust results of EDEMA4 provide the primary support with additional support from EDEMA3. Repeat dose data is limited given the lack of placebo control and the potential for selection bias, but the submitted data combined with extrapolation of the single-dose, placebo controlled data supports the efficacy of ecallantide for repeat, intermittent dosing.

The data for patients less than 18 years of age is limited. Although it is expected that ecallantide would behave in a similar fashion in adolescent and adult patients, there is insufficient representation of patients less than 18 years of age in the clinical program. The few patients less than 18 years of age included in the clinical program are not sufficient to support the efficacy of ecallantide in this age group.

7 Review of Safety

Safety Summary

7.1 Methods

7.1.1 Clinical Studies Used to Evaluate Safety

The clinical review focused on the studies that used the to-be-marketed SC formulation in HAE patients: EDEMA2, EDEMA3, and EDEMA4. Additional safety information was obtained from the Phase 1 studies, the cardiothoracic study, the rechallenge study, and the compassionate use case narratives. General information on the study design and patient numbers is presented in Table 3, while more detailed information is provided in Section 5.3 and in the individual study reviews located in Section 10.

7.1.2 Adequacy of Data

The data submitted in support for ecallantide for the proposed indication were generally appropriate given the constraints of conducting studies for an orphan disease, as were the safety evaluations performed during the development program with the exception of limited data in patients less than 18 years of age as discussed in Section 6.1.2.8. The Applicant provided patient data listings that were appropriately indexed for review, as well as CRFs for all SAEs. Investigators used NCI CTC criteria for grading AE severity. AE coding was performed using the MedDRA coding dictionary (Version 6.0). In review of SAE case narratives, SAE verbatim terms, and the SAE preferred terms, coding was performed appropriately.

7.1.3 Pooling Data Across Studies to Estimate and Compare Incidence

The Applicant has provided several pooled datasets for the Integrated Summary of Safety:

- Analysis Population I: All HAE patients treated with ecallantide in EDEMA studies, excluding the compassionate use and rechallenge studies.
- Analysis Population II: Patients from controlled phase of EDEMA3 and EDEMA4
- Analysis Population III: EDEMA3 OLE patients
- Analysis Population IV : Healthy volunteers in ecallantide studies

The clinical safety review relies on Analysis Population II to estimate and compare the incidence of various AEs to placebo. This population is representative of the clinical program and appears representative of the general HAE population. Patients were permitted to participate sequentially in multiple ecallantide studies, so 16 patients from EDEMA3 also enrolled in EDEMA4. The Division previously raised concern about the handling of these patients in the safety analysis, so the Applicant has provided longitudinal patient profiles for all patients that include a unique identification number. The Analysis Population II represents 100 unique

patients who have received 125 doses of ecallantide. If a patient received placebo in one study and ecallantide in the next, safety data collected during exposure to placebo was attributed to placebo and the same for ecallantide. Also, any EDEMA4 placebo patient who received a Dose B for airway compromise or incomplete response/relapse was analyzed as a placebo-treated patient up to the time of the open-label dose and as an ecallantide-treated patient from the time of ecallantide to the study conclusion.

As noted in Section 6, the clinical program does not include placebo-controlled data on repeat dosing. The safety data to support repeat dosing is based primarily on the repeat data from the open-label phases of EDEMA3, EDEMA4, and to a lesser extent EDEMA2. In the original BLA submission, the Applicant provided the data from EDEMA3 (Analysis Population III), representing 67 patients treated with 161 doses of ecallantide. Each patient is counted only once regardless of the number of HAE episodes treated in the study. The Applicant also provided a pooled analysis of all HAE patients treated with ecallantide in EDEMA studies (Analysis Population I), excluding the compassionate use and rechallenge studies. Analysis Population I represented 219 patients who received 609 doses of ecallantide. This population included all AEs reported by patients, so that patients who participated in multiple studies may be represented multiple times. While Analysis Population I is of interest due to the greater numbers represented, it includes patients who received the IV formulation of ecallantide in a range of other doses. The generalizability of the Analysis Population I findings to the to-be-marketed SC formulation is uncertain. For example, the IV formulation may not be as immunogenic as the SC formulation, as SC drug administration may be associated with increased sensitization. As a results, Analysis Population I could potentially underestimate the rate of hypersensitivity reactions.

Data from healthy volunteers (Analysis Population IV) and the CTS study patients were reviewed in terms of specific AEs, namely hypersensitivity and anaphylaxis reactions.

7.2 Adequacy of Safety Assessments

7.2.1 Overall Exposure at Appropriate Doses/Durations and Demographics of Target Populations

HAE is an orphan disease with life-threatening potential so the guidelines put forth in the current ICH document (ICH *E1A The Extent of Population Exposure to Assess Clinical Safety: For Drugs Intended for Long-term Treatment of Non-life-threatening Conditions*) and the *Guidance for Industry: Pre-marketing Risk Assessment* (March 2005) on extent and duration of exposure are limited in their applicability. Given the limitations of this rare condition and previous discussions with the Division during the end-of-phase-2 and pre-BLA interactions, including the SPA agreement for EDEMA4, the clinical program includes adequate exposure information at the appropriate dose for an adult HAE population. The limitations of the adolescent/pediatric exposure data was noted in Section 6.1.8. The design of the studies, both open-label and placebo-controlled, was adequate to make a safety assessment.

Total human exposure to ecallantide in the development program (Analysis Population I) is shown below.

Table 16 Total ecallantide exposure for all HAE patients (Analysis Population I)

	Ecallantide (N=219)		
Number of patients with:	**N (%)**	**Min – Max Total cumulative dose (mg)**	**Min – Max duration**
1 dose	108 (49.3)	8.5 – 89.6	1 day
2 to 4 doses	80 (36.5)	27.9 –153.2	1 day – 51 months, 15 days
5 to 9 doses	19 (8.7)	80.2 – 310.8	1 month, 27 days – 59 months, 5 days
>9 doses	12 (5.5)	169.2 – 623.9	13 months, 26 days – 44 months, 13 days

Source: summary-clin-safety.pdf, Table 2.7.4.5

The demographic information for the Phase 1 healthy volunteer studies, the pooled Phase 2-3 studies (Analysis Population I), and the pooled Phase 3 studies (Analysis Population II) are presented in Table 17. The demographics across the clinical program were comparable, with the exception of the healthy volunteer pool being younger on average.

Table 17 Demographics of Phase 1, Phase 2, and Phase 3 ecallantide studies

	Phase I	*Pooled Phase 2-3*	*Phase 3*	
	Analysis Population IV (Healthy subjects)	**Analysis Population I**	**Analysis Population II**	
	Ecallantide N=62	**Ecallantide N=219**	**Ecallantide N=100**	**Placebo N=81**
Age (yrs)				
N	62	219	100	81
Mean (SD)	28.5 (8.9)	34.6 (13.7)	36.5 (12.7)	35.4 (13.4)
Range	18-55	10-78	15-77	10-72
Gender (n, %)				
Female	34 (54.8)	144 (64.8)	66 (66.0)	50 (61.7)
Male	28 (45.2)	75 (34.2)	34 (34.0)	31 (38.3)
Race (n, %)				
Asian	3 (4.8)	3 (1.4)	2 (2.0)	1 (1.2)
Black	6 (9.7)	13 (6.2)	6 (6.0)	6 (7.4)
Caucasian	52 (83.9)	178 (84.8)	4 (84.0)	73 (90.1)
Hispanic	0	13 (6.2)	7 (7.0)	1 (1.2)
Other	1 (1.6)	3 (1.4)	1 (1.0)	-

Source: summary-clin-safety.pdf, Table 2.7.4.8 and iss.pdf, Appendix 4, Table 2.3

Exposure data in pediatric patients is far more limited and the generalizability of the safety findings from the adult population to pediatric patients remains in question. Of 18 total pediatric patients, 10 received 1 dose of ecallantide, 4 received 2 to 4 doses of ecallantide, 2 received 5 to 9 doses, and 2 received >9 doses. Of the pediatric patients, only 3 received ecallantide as part of a double-blind study, the youngest being a 15-year-old patient in EDEMA3. Younger patients were studied during the open-label dosing phases of the Phase 3 program, but the numbers were small: 1 12-year-old, 2 13-year-olds, 2 16-year-olds, and 2 17-year-olds. EDEMA2 included a small number of pediatric patients: 1 10-year-old, 1 11-year-old, 1 12-year-old, 2 13 year-olds, 2 14-year-olds, 2 15-year-olds, 1 16-year-old, and 1 17-year-old. Of the EDEMA2 patients, only 1 10-year-old, 1 14 year-old, 1 16 year-old, and 1 17-year-old received the 30mg SC dose. The remainder received IV doses of 5 to 20 mg/m^2 IV.

Certain other subpopulations, such as patients over 75 years and people with renal or hepatic impairment, were not studied in significant numbers. However, given the rarity of HAE and its life-threatening potential, the pre-marketing safety assessment in these subpopulations is expected to be minimal.

7.2.2 Explorations for Dose Response

Both Phase 3 studies were conducted using a single 30 mg SC dose, which is estimated to provide similar exposure as 15 mg/m^2 IV. Intravenous doses ranging from 5 to 80 mg/m^2 IV were studied in the Phase 1 and 2 programs. The total dose and duration for all HAE patients in the clinical program is summarized in Table 16. In general, there were no evident correlations between AEs and dose, and the types of AEs reported across dose groups were similar. The most serious AE, anaphylaxis, was found to occur at all dose levels, which is consistent with an antibody-mediated hypersensitivity reaction.

7.2.3 Special Animal and/or In Vitro Testing

At the time of this review, the Pharmacology/Toxicology review is ongoing. Upon preliminary review, the preclinical testing was adequate. Two major concerns were raised by the preclinical data: injection site reactions in animals and impaired coagulation in in vitro studies. The clinical correlation regarding these issues are addressed later in this review.

7.2.4 Routine Clinical Testing

Routine clinical testing included the following: CBC with differential, routine serum chemistry, coagulations tests, and urinanalysis. Reference ranges were based on ranges published in the "Laboratory Handbook of Reference Intervals – Massachusetts General Hospital Clinical Laboratories" (February 2007) and "Laboratory Reference Values" as reported in the New England Journal of Medicine (Kratz et al., 2004). Laboratory data was collected at baseline and at appropriate intervals following dosing and at follow-up.

7.2.5 Metabolic, Clearance, and Interaction Workup

The pharmacokinetics of ecallantide are described briefly in Section 4.4. No formal drug-drug interaction studies were included in this program. Ecallantide is a biologic product and not expected to interact with the CYP450 enzymes or p-glycoproteins.

7.2.6 Evaluation for Potential Adverse Events for Similar Drugs in Drug Class

Ecallantide is a biologic, immunogenic product and sensitization with hypersensitivity reactions including anaphylaxis is expected. In addition to screening for adverse events of this nature, the Applicant collected serial antibody samples to evaluate for development of non-IgE antibodies to ecallantide and IgE antibodies to ecallantide and *P. pastoris.* The Applicant also conducted a

rechallenge study to assess the risks and benefits of rechallenge in patients with ecallantide hypersensitivity reactions. These results are presented in more detail in Sections 7.3 and 7.4.

7.3 Major Safety Results

7.3.1 Deaths

Two deaths were reported in the ecallantide program. Patient 8804022001 (EDEMA1) had a history of dual nephrectomy and kidney transplant 1 year prior to enrollment. The patient was reported to have chronic rejection of the transplant and died of chronic renal failure 29 days after administration of ecallantide. Patient 101 (DX88/16, CTS study) died or perioperative myocardial infarction and multi-organ system failure. The treatment assignment for this patient has not yet been unblinded.

Reviewer's comment: Based on the nature and timing of the deaths, neither case appears to be related to the administration of ecallantide.

7.3.2 Nonfatal Serious Adverse Events

Of 219 patients in all HAE studies (Analysis Population I), 26 (11.9%) experienced a SAE. Fourteen of the 26 (6.4%) reported an HAE attack as an SAE. Other SAEs reported included a wide range of events: abdominal pain (n=1), colitis (n=1), pancreatitis (n=1), infectious diarrhea and hematochezia (n=1), concussion and contusion due to car accident (n=1), jaw fracture (n=1), skin laceration (n=1), ECG signs of myocardial ischemia (n=1), and chronic renal failure (n=1).

In addition, 3 cases of anaphylaxis and 1 anaphylactoid reaction were reported. These SAEs and other hypersensitivity-related reactions are discussed separately in Section 7.3.4 under Significant Adverse Events.

Reviewer's comment: Although an exacerbating effect cannot be ruled out, most likely the reports of HAE as an SAE reflect the underlying condition. In the Phase 3 studies, the reports of HAE attack as an AE in the placebo group exceeded the number reported in the ecallantide group. Other than HAE, the number and types of SAEs did not suggest a particular safety signal.

7.3.3 Dropouts and/or Discontinuations

Two patients withdrew due to AEs in the OLE of EDEMA3. Patient 8804024001 withdrew 6 weeks after receipt of 10th dose of ecallantide following a new diagnosis of B-cell lymphoproliferative disease and Patient 8805051099 (mentioned in Section 7.3.2) withdrew following anaphylaxis.

Reviewer's comment: On the basis of one case report, a causal relationship between the B-cell disorder and drug cannot be made. In contrast, the anaphylactic event is most likely secondary to drug administration.

7.3.4 Significant Adverse Events

Anaphylaxis

As a protein therapeutic, hypersensitivity reactions to ecallantide are expected. In an attempt to capture these events, the Applicant performed a search using the following MedDRA preferred terms: adverse drug reaction, anaphylactic reaction, anaphylactoid reaction, erythema, flushing, pharyngeal edema, pruritus, pruritus generalized, rash erythematous, rhinitis allergic, throat irritation, urticaria, urticaria localized, and wheezing. For the purposes of the BLA submission, the Applicant defined anaphylaxis as "a severe systemic immunologic reaction, rapid in onset, presumably caused by antibody-mediated release of vasoactive mediators from tissue mast cells and peripheral blood basophils." Anaphylactoid reaction was defined an "immediate, non-immunologic, systemic reaction that mimics anaphylaxis but is caused by non-antibody-mediated release of mediators from mast cells and basophils.

Reviewer's comment: For the purpose of this review, any AEs defined as anaphylaxis or anaphylactoid were accepted as such. In review of other AEs suggestive of anaphylaxis or other hypersensitivity reactions, the clinical review relied on the diagnostic criteria for anaphylaxis as outlined by the 2006 Joint NIAID/FAAN Second Symposium on Anaphylaxis (Sampson HA et al. J Allergy Clin Immunol 2006). The criteria do not make a distinction based on underlying mechanism. These criteria are summarized as follows:

1. *Acute onset of an illness (minutes to several hours) with involvement of the skin, mucosal tissue, or both (e.g., generalized hives, pruritus or flushing, swollen lips-tongue-uvula), and at least one of the following:*
    a. *Respiratory compromise (e.g., dyspnea, wheeze-bronchospasm, stridor, reduced PEF, hypoxemia)*
    b. *Reduced BP or associated symptoms of end-organ dysfunction (e.g., hypotonia [collapse], syncope, incontinence)*
2. *Two or more of the following that occur rapidly after exposure to a likely allergen for that patient (minutes to several hours):*
    a. *Involvement of the skin-mucosal tissue (e.g., generalized hives, itch-flush, swollen lips-tongue-uvula)*
    b. *Respiratory compromise (e.g., dyspnea, wheeze-bronchospasm, stridor, reduced PEF, hypoxemia)*
    c. *Reduced BP or associated symptoms (e.g., hypotonia [collapse], syncope, incontinence)*
    d. *Persistent gastrointestinal symptoms (e.g., crampy abdominal pain, vomiting)*
3. *Reduced BP after exposure to known allergen for that patient (minutes to several hours):*
    a. *Infants and children: low systolic BP (age specific) or greater than 30% decrease in systolic BP*
    b. *Adults: systolic BP of less*

As noted in Section 7.3.2, the Applicant identified 3 cases of anaphylaxis and 1 case of anaphylactoid reaction in the ecallantide program:

- Patient 8805051099 (EDEMA3) experienced anaphylaxis twice – the first time after her 17th dose of ecallantide and the second during a rechallenge procedure. Her first event was characterized by generalized erythema, pruritus, and decreased blood pressure (82/50 mmHg) with an oxygen saturation of 90% on room air. She received epinephrine, diphenhydramine, and supplement oxygen and her blood pressure increased to 110.80 mmHg. Serum tryptase taken 4 hours after the event was 10.4 mcg/L (normal range: 1.9-13.5 mcg/L). The second event was characterized by dyspnea, generalized rash, anxiety, pharyngeal edema, vomiting, diarrhea, urinary incontinence, hypotension and hypoxia following rechallenge with a partial dose. The patient was noted to have tested intermittently positive to IgE against *P. pastoris* up to 2 years before the first event as well as non-IgE to ecallantide.
- Patient 8820401009 (EDEMA4 OLE, DX-88/19) developed anaphylaxis after her 4th dose of ecallantide, consisting of erythema, generalized pruritus, tingling of the tongue, lethargy, change in mental state, and vomiting. She was treated with 2 doses of 0.3 mg epinephrine, hydroxyzine, solumedrol, and IV fluids. A serum tryptase taken 6 hours after the event was 30 ng/ml (normal range 2-10 ng/ml). The patient had intermittently tested positive for non-IgE and IgE antibodies to ecallantide since her 2nd dose and 3rd doses, respectively, although she tested negative for IgE to ecallantide immediately prior to the event.
- Patient 8805024097 (EDEMA2) developed anaphylaxis 10 minutes after her 6th dose. She experienced nausea, diaphoresis, dizziness, and a feeling of faintness before receiving treatment with epinephrine, hydrocortisone, cetirizine and ranitidine. Serum tryptase taken 4 hours and 12 minutes after the event was within normal range (2.7 ng/ml). The patient tested positive for non-IgE antibodies to ecallantide after the 5th dose and positive for IgE 7 days after the anaphylaxis. The patient went on to complete a successful rechallenge procedure and received 11 additional doses of ecallantide.
- Patient 8802003005 (EDEMA0) was identified as having an anaphylactoid reaction consisting of dysphagia, pruritus, urticaria, edema, dyspnea, abdominal pain, and enteritis 5 minutes after her first dose of ecallantide (40 mg/m^2 IV). She was treated with epinephrine, polaramine, and hydrocortisone. She test positive for ecallantide antibodies per the investigator's own immunoblot, but subsequently negative on the Applicant's ELISA assays. No rechallenge procedure was attempted.

Using the diagnostic criteria for anaphylaxis outlined above, the clinical review identified four additional potential case of anaphylaxis:

- Patient 8804013011 (EDEMA1) reported 3 separate episodes of sneezing, throat itchiness, congestion, rhinorrhea, and shortness of breath following the 1st, 2nd , and 4th doses of 20 mg/m2 ecallantide IV. The time to onset is not recorded and patient's medical history is confounded by a history of asthma and allergic rhinitis. The patient has not tested positive for antibody formation to ecallantide or *P. pastoris*.
- Patient 8804013003 (EDEMA1) developed rhinitis, itchy throat, and shortness of breath following receipt of her 1st dose of ecallantide 20 mg/m2 IV. The patient was treated

with epinephrine, antihistamines, and corticosteroids. The patient underwent a rechallenge procedure and developed rhinitis symptoms 42 minutes after the start of the test dose infusion. The patient has not tested positive for antibody formation to ecallantide or *P. pastoris*.

- Patient 8805019001 (EDEMA2) experienced symptoms suggestive of anaphylaxis during a rechallenge procedure. Her initial reaction consisted of worsening allergic rhinitis symptoms, conjunctival erythema, eye swelling, and urticaria 2 minutes after the start of the 1st ecallantide dose (10 mg/m2 IV). The patient tested positive for IgE antibodies to *P. pastoris* 1 year prior to the reaction but had tested negative in subsequent assays. On rechallenge 18 months later, she developed sneezing, nasal congestion, throat itchiness, and cough.
- Patient 8805050097 (EDEMA2) developed abdominal pain, nausea, vomiting, throat itchiness, and nasal congestion following receipt of the 1st dose of ecallantide for treatment of an external head/neck HAE attack. Study drug infusion was stopped. No antibodies were detected and the patient did not undergo a rechallenge procedure.

Anaphylaxis reactions in other patient populations

The Applicant also submitted safety data from studies with ecallantide in cardiothoracic surgery patients. Although the perioperative conditions and surgical/medical comorbities limit comparisons of this patient population to the HAE population, there was one notable case of anaphylaxis (Patient 262). The patient had life-threatening hypotension with bronchoconstriction. No anaphylaxis was reported in the healthy volunteers.

Reviewer's comment: Per the Applicant's submission, 219 HAE patients received 609 doses of ecallantide in the ecallantide HAE studies (Analysis Population I, excludes compassionate use [n=8]and rechallenge protocols [n=9]). Based on Analysis Population I, an anaphylaxis rate of 3.7% patients (8 cases of 219 HAE patients) or 1.3% doses (8 of 609 doses) is observed. Patient 8805051099 had 2 anaphylactic episodes: the first time in EDEMA3 and then again during the rechallenge procedure. Since the rechallenge study is not included in Analysis Population I, only the patient's first event is included in rate calculation.

Other hypersensitivity reactions

In addition to these anaphylactic events, several cases suggestive of a Type I hypersensitivity reaction were also identified.

- Patient 8804013007 (EDEMA1) reported sneezing after the 1st dose of 40 mg/m2 IV ecallantide, relieved by antihistamine. The patient experienced nasal stuffiness during a rechallenge procedure and has not received any further doses of ecallantide. No antibodies to ecallantide or *P. pastoris* were reported for this patient.
- Patient 8805017018 (EDEMA3) developed urticaria 3½ hours following ecallantide 30 mg SC for a laryngeal HAE attack. Non-IgE antibodies to ecallantide were demonstrated at the 28-day follow-up and IgE antibodies to *P. pastoris* at the 57-day follow-up. The patient has not attempted a rechallenge procedure.
- Patient 8805054099 (EDEMA2) reported headache, blurred vision, flushing, urticaria, pruritus, conjunctival injection, increased heart rate (120 → 172 bpm) and increased

blood pressure (122/73 → 152/100 bpm) within 1 minute of completing the 6th dose infusion of 10 mg/m2 IV ecallantide. The patient tested positive for non-IgE antibodies to ecallantide and later neutralizing antibodies in EDEMA3. The patient also tested positive for IgE to *P. pastoris* on two separate occasions. The patient underwent a successful rechallenge and went on to receive 16 additional doses of ecallantide

- Patient 8814326002 (EDEMA3) reported pruritus and nausea 12 minutes after receipt of a 4th dose of ecallantide. The patient tested positive for non-IgE antibodies to ecallantide and IgE to *P. pastoris.* The patient had a positive wheal and flare response during the skin testing phase of rechallenge and has not received additional doses.
- Patient 8814302002 (EDEMA3-RD) experienced increased heart rate and blood pressure and flushing 10 minutes after receipt of a 2nd dose of 30 mg ecallantide SC. The patient tested positive for non-IgE antibodies to ecallantide on ECL bridging assay and negative by ELISA. The patient received 1 additional dose of ecallantide and reported chest tightness and flu-like symptoms following the dose. The time to onset was not reported.
- Patient 8805024099 reported itchy throat after the 2nd and 3rd of 6 ecallantide doses.
- Patient 8804017010 reported an erythematous rash on the buttocks the day following the 11th IV dose and again after the 12th SC dose. The second rash was also accompanied by injection site pain.

Five other patients reported pruritus or generalized pruritus following injection, although the time course in relation to dose administration is not clearly documented in the majority of cases.

Injection site reactions

In Analysis Population II, local injection site reactions were reported in 3 (3.0%) patients in the ecallantide group compared to 1 (1.2%) in the placebo group. All three of the patients were seronegative for antibody to ecallantide and *P. pastoris*. In the total HAE population, injection site reactions were reported in 13 of 219 (5.9%) of patients. The reactions were characterized primarily by pain, pruritus and erythema. One case of local urticaria was reported. The reactions were all transient and resolved without intervention, differing from the severe local reactions observed in preclinical studies.

7.3.5 Submission Specific Primary Safety Concerns

Potential self-administration with ecallantide remains a safety concern, especially given the risk of anaphylaxis. Although self-administration may offer certain benefits in terms of patient convenience and potentially greater efficacy, the safety and feasibility of self-administration have not been evaluated in the clinical development program to date. In the original BLA submission, the Applicant included patient self-administration as an option at the discretion of the healthcare provider and the patient. The Division communicated concern about self-administration given the absence of supportive data in the 60-day filing letter. In response, the Applicant informed the Division in a letter dated December 24, 2008, that the self-administration issue would be deferred. The Applicant stated that post-marketing information on anaphylaxis reactions and a separate clinical study to assess self-administration would be used to inform future decisions on commercial self-administration options. The clinical review agrees with this more conservative approach; however, off-label self-administration remains a possibility and

should be considered in the benefit risk assessment. If ecallantide is approved, Dyax should have post-marketing risk mitigation strategies including extensive education materials for both patients and healthcare providers regarding the risk of hypersensitivity events.

7.4 Supportive Safety Results

7.4.1 Common Adverse Events

The most common AEs associated with ecallantide are headache, nausea, diarrhea, pyrexia, and nasopharyngitis. AEs occurring in >1 patient and at a frequency greater in the ecallantide group than placebo are shown in Table 18. Of note, HAE attack was reported in 3 (3.0%) ecallantide patients versus 4 (4.9%) placebo patients. Prolonged prothrombin time was reported in no ecallantide patients compared to 2 in placebo.

In the total HAE safety database with no placebo control for comparison, the most common AEs reported were headache (n=36, 16.4%), nausea (n=27, 12.3%), fatigue (n=27, 12.3%), diarrhea (n=24, 11.0%), upper respiratory tract infections (n=19, 8.7%), nasopharyngitis (n=13, 5.9%), vomiting (n=12, 5.5%), upper abdominal pain (n=11, 5.0%), and pyrexia (n=11, 5.0%). HAE as an AE was reported in 18 patients (8.2%).

Table 18 Adverse events occurring in >1 patient and at a greater frequency in the ecallantide group vs. placebo (Analysis Population II)

Preferred term	Ecallantide N=100 (n,%)	Placebo N=81 (n,%)
Patients with ≥1 AE	36 (36.0	28 (34.6)
Headache	8 (8.0)	6 (7.4)
Nausea	5 (5.0)	1 (1.2)
Diarrhea	4 (4.0)	3 (3.7)
Pyrexia	4 (4.0)	-
Nasopharyngitis	3 (3.0)	-
Injection site pain or reaction	3 (3.0)	1 (1.2)
Dizziness	2 (2.0)	1 (1.2)
Erythematous rash	2 (2.0)	-
Fatigue	2 (2.0)	-
Pharyngolaryngeal pain	2 (2.0)	-
Upper abdominal pain	2 (2.0)	-

Source: summary-clin-safety.pdf, Table 2.7.4.11

Reviewer's comment: The assessment of common adverse events is limited by the small sample size. The most common AEs identified appear to be consistent in the pooled Phase 3 program (Analysis Population II) when compared to safety data for the total HAE database (Analysis Population I).

7.4.2 Laboratory Findings

Overview of laboratory testing and selection of studies for drug-control comparisons
As presented in Section 7.2.4, routine clinical laboratory testing (CBC with differential, chemistry panel, coagulation parameters, and urinanalysis) were performed at baseline and at appropriate intervals through each study. Serum sampling for antibody formation to ecallantide and *P. pastoris* was also obtained at baseline and at follow-up visits. A detailed schedule of collection timepoints for each study is provided in the Individual Study Reviews located in Section 10.

Measures of central tendency, outliers, and marked outliers were reviewed for each lab parameter. Baseline is defined as the closest observation prior to dosing. Laboratory changes were not performed by study visit because of the variety of time points used for laboratory assessments across studies. Instead, the most abnormal value from all follow-up visits was selected for analysis. For comparison to placebo control, the review focuses on the Analysis Population II, consisting of the pooled Phase III data. The entire HAE population (Analysis Population I) is also reviewed, particularly in terms of repeat dose data and outliers.

Hematology

Mean changes in hematology parameters
No clear differences in hematocrit, total white cell count and differential, or platelet number were observed between baseline and post-baseline ecallantide and placebo-treated groups in the pooled Phase 3 analysis (Analysis Population II) (Table 19). Similar mean values were observed in the pooled Phase 2 and Phase 3 analysis (Analysis Population I).

Table 19 Mean change in hematology parameters (Analysis Population II)

Indices	Ecallantide N=100			Placebo N=81		
	Baseline	Post-baseline		Baseline	Post-baseline	
		Lowest	Highest		Lowest	Highest
Hematocrit (%)						
N	97	97	97	74	74	74
Mean (SD)	43.7 (4.2)	40.7 (3.9)	43.8 (4.0)	43.5 (4.9)	41.1 (5.0)	43.9 (4.7)
Median	43.0	40.0	44.0	43.5	41.0	44.0
(Min, Max)	(33, 51)	(31, 50)	(35, 54)	(34, 54)	(32, 52)	(33, 54)
WBC (x10^3/mcl)						
N	97	97	97	74	74	74
Mean (SD)	8.2 (2.6)	6.7 (1.9)	8.9 (2.6)	8.4 (2.6)	7.2 (2.5)	9.2 (2.2)
Median	7.9	6.7	8.7	8.4	6.8	9.0
(Min, Max)	(3,8, 20.6)	(3.5, !6.2)	(3.9, 20.2)	(2.9, 15)	(3.3, 14.3)	(4.5, 15.8)
Basophils (%)						
N	97	97	97	74	74	74
Mean (SD)	0.7 (0.4)	0.5 (0.3)	0.9 (0.5)	0.8 (0.5)	0.6 (0.4)	1.0 (0.5)
Median	0.7	0.5	0.8	0.7	0.5	0.9
(Min, Max)	(0, 2.1)	(0, 1.8)	(0, 2.9)	(0, 2.2)	(0, 2.2)	(0.3, 2.2)
Eosinophils (%)						
N	97	97	97	74	74	74
Mean (SD)	1.6 (1.1)	1.5 (1.3)	2.5 (1.7)	1.8 (1.2)	1.5 (1.0)	2.6 (2.4)
Median	1.3	1.3	2.0	1.3	1.2	2.1
(Min, Max)	(0.1, 6)	(0, 9)	(0.2, 9)	(0, 5.3)	(0, 4.7)	(0.4, 19)
Lymphocytes (%)						
N	97	97	97	74	74	74
Mean (SD)	25.6 (9.2)	25.0 (8.5)	32.4 (8.4)	26.6 (9.8)	25.8 (10.5)	33.3 (9.8)
Median	24.5	24.6	32.4	25.5	26.3	32.9
(Min, Max)	(3.4, 48.2)	(3.9, 45)	(12.9, 54.5)	(4.6, 54)	(5.3, 55)	(5.6, 57.8)
Monocytes (%)						
N	97	97	97	74	74	74
Mean (SD)	5.2 (1.7)	4.6 (1.4)	5.8 (1.6)	5.4 (2.0)	4.8 (1.8)	6.2 (2.0)
Median	4.9	4.4	5.5	5.2	4.6	6.4
(Min, Max)	(1.9, 13)	(1.5, 10)	(3.2, 10.8)	(1, 12.2)	(1.7, 10.1)	(1.7, 12)
Neutrophils (%)						
N	97	97	97	74	74	74
Mean (SD)	66.8 (10.0)	59.1 (9.0)	67.7 (9.6)	65.4 (11.3)	57.9 (10.7)	66.6 (12.0)
Median	67.9	58.6	68.0	65.6	57.7	65.8
(Min, Max)	(45.9, 93)	(38, 81.1)	(48.4, 92.1)	(34.5, 93.1)	(33.2, 90.6)	(38.3, 90.7)
Platelets (x10^3/mcl)						
N	97	97	97	72	72	72
Mean (SD)	273.4 (59.5)	261.1 (61.2)	293.2 (67.1)	281.0 (59.8)	267.7 (62.6)	299.5 (56.8)
Median	266.0	253.0	284.0	273.0	266.5	287.0
(Min, Max)	(163, 461)	(126, 456)	(171, 494)	(156, 458)	(133, 403)	(195, 465)

Source: iss.pdf, Appendix 4, Table 7.1.1.2

Outliers and marked outliers in hematology parameters

No patients discontinued from the study or were reported as an AE secondary to a change in a hematology parameter. The following table summarizes the number of patients with a shift from normal to abnormal (or a post-baseline value worse than baseline if the baseline value exceeded the cutoff range for normal) in both the pooled Phase 2/3 analysis (I) and the pooled Phase 3 analysis (II).

Table 20 Outliers for hematology parameters in Analysis Populations I and II

Laboratory test	Cutoff	Population I		Population II			
		Ecallantide (N=219)		Ecallantide (N=100)		Placebo (N=81)	
		N[a]	N (%)[b]	N[a]	N (%)[b]	N[a]	N (%)[b]
Hemoglobin	≤10 g/dL	215	3 (1.4)	97	0	74	1 (1.4)
WBC	<3.0 x 10^9/L	215	0	97	0	74	0
WBC	>ULN	215	55 (25.6)	97	13 (13.4)	74	10 (13.5)
Neutrophils	<30%	206	2 (1.0)	97	0	74	0
Lymphocytes	<5%	206	9 (4.4)	97	1 (1.0)	74	0
Platelets	<75.0 x 10^9/L	214	1 (0.5)	97	0	72	0

[a] Number of patients with both a baseline and post-baseline value
[b] Number of patients with a normal → abnormal or worsened value exceeding the normal range
ULN = upper limit of normal
Source: summary-clin-safety.pdf, Table 2.7.4.37

Coagulation parameters

Mean changes in coagulation parameters

In vitro studies demonstrated that ecallantide could prolong activated clotting time (ACT) and aPTT, potentially leading to an anti-hemostatic effect. As a result, aPTT, prothrombin time (PT), and thrombin time (TT) were routinely monitored in the clinical studies. Overall, there were no clinically relevant mean changes in coagulation parameters in the ecallantide group versus the placebo group (Table 21).

Table 21 Mean change in coagulation parameters (Analysis Population II)

Indices	Ecallantide N=100			Placebo N=81		
	Baseline	Post-baseline		Baseline	Post-baseline	
		Lowest	Highest		Lowest	Highest
aPTT (sec)						
N	96	96	96	74	74	74
Mean (SD)	21.3 (4.9)	20.4 (2.0)	23.0 (4.4)	21.5 (5.3)	20.1 (1.6)	22.9 (8.6)
Median	20.6	20.2	22.1	20.7	20.2	21.6
(Min, Max)	(16.2, 54.9)	(15.1, 25.9)	(17.3, 47.2)	(16, 58.7)	(14.7, 23.4)	(15.5, 91.2)
PT (sec)						
N	96	96	96	75	75	75
Mean (SD)	11.2 (1.6)	10.8 (1.0)	11.6 (1.5)	11.4 (1.8)	11.0 (1.0)	12.7 (7.0)
Median	11.0	10.6	11.5	11.4	11.0	11.9
(Min, Max)	(9.4, 20.5)	(9.4, 13.3)	(9.7, 18.9)	(9.4, 21.3)	(9.8, 13.2)	(9.5, 60)
Thrombin time (sec)						
N	95	95	95	73	73	73
Mean (SD)	16.4 (2.2)	15.7 (1.1)	17.5 (4.7)	16.2 (1.3)	15.7 (1.0)	16.9 (2.1)
Median	15.9	15.5	16.5	16.2	15.6	16.4
(Min, Max)	(14, 28.3)	(13.7, 20.3)	(14.3, 52.9)	(13.4, 21.3)	(13, 20.3)	(13.5, 26.4)

Source: iss.pdf, Appendix 4, Table 7.3.1.2

Reviewer's comment: The clinical data do not suggest an increased risk of bleeding associated with ecallantide. The in vitro studies were conducted with ecallantide concentrations of 2 mcg/ml or greater, whereas the maximum observed ecallantide plasma concentration following the 30 mg SC dose is ~0.6 mcg/ml (3-fold lower). At the to-be-marketed dose, ecallantide is

expected to inhibit plasma activity by 10% and any effects on coagulation parameters would likely be transient given the short-half life.

Outliers and marked outliers in coagulation parameters
Data on outliers for coagulation parameters are reported in Table 22. No discontinuations from an HAE study secondary to coagulation abnormalities were reported. No bleeding events were reported for any of these patients. The aPTT elevations as high as 140.8 sec was reported; all aPTT elevations were observed in the IV formulation dosing groups. Seven of the 9 returned to baseline at follow-up. In the remaining 2, follow-up values were not reported. Similarly, in patients with PT elevations, all returned to within normal range at follow-up with the exception of 3 with missing follow-up PT values.

Of the 3 patients in the Analysis Population II reported with elevations in thrombin time, 2 had abnormal results (35.3 and 33.7 sec, respectively) at Follow-up Visit 1 (7 days post-dose) but normal TT at the 4-hour post-dose time point (17.1 and 21.7 sec, respectively) and at a later follow-up (Visit 2).

Table 22 Outliers for coagulation parameters in Analysis Populations I and II

Laboratory test	Cutoff	Population I		Population II			
		Ecallantide (N=219)		Ecallantide (N=100)		Placebo (N=81)	
		N[a]	N (%)[b]	N[a]	N (%)[b]	N[a]	N (%)[b]
aPTT	>1.5 x ULN	213	9 (4.2)	96	0	74	1 (1.4)
PT	>1.5 x ULN	201	7 (3.5)	96	0	75	2 (2.7)
Thrombin time	>30 sec	186	19 (10.2)	95	3 (3.2)	73	0

[a] Number of patients with both a baseline and post-baseline value
[b] Number of patients with a normal → abnormal or worsened value exceeding the normal range
ULN = upper limit of normal
Source: summary-clin-safety.pdf, Table 2.7.4.37

Reviewer's comment: Based on the outlier data, observed changes in coagulation parameters do not appear to correlate with an increased bleeding risk. Although in vitro studies have raised the concern about possible anti- hemostatic effects, there is an additional theoretical concern about hypercoagulability. Ecallantide is highly homologous with Tissue Factor Protein Inhibitor (TFPI). TPFI knockout is a lethal mutation in mouse models due to increased coagulation. Theoretically, neutralizing antibodies against ecallantide could bind endogenous TFPI and potentially lead to hypercoagulability. The clinical safety database does not show any thromboembolic AEs. However, the issue could be further explored by cross-reactivity studies for antibodies against ecallantide and TFPI.

Clinical chemistry

Mean changes in clinical chemistry parameters
Overall, there were no clinically significant mean changes from baseline when comparing clinical chemistry parameters in the ecallantide group to placebo. Results are summarized in Table 23.

Table 23 Mean change in clinical chemistry parameters (Analysis Population II)

Indices	Ecallantide (N=100)			Placebo (N=81)		
	Baseline	Post-baseline		Baseline	Post-baseline	
		Lowest	Highest		Lowest	Highest
AST/SGPT (U/L)						
N	98	98	98	75	76	76
Mean (SD)	27.4 (27.0)	24.5 (32.1)	33.4 (43.0)	25.8 (17.0)	23.9 (18.0)	31.5 (25.4)
Median	19.0	16.0	21.5	22.0	20.5	24.5
(Min, Max)	(7, 183)	(7, 297)	(10, 297)	(7, 134)	(7, 124)	(10, 162)
AST/SGOT (U/L)						
N	98	98	98	75	75	75
Mean (SD)	29.6 (69.9)	20.6 (12.3)	4.7 (97.1)	21.8 (7.2)	20.3 (6.9)	25.1 (10.7)
Median	20.0	18.0	21.0	21.0	19.0	23.0
(Min, Max)	(11, 706)	(9, 116)	(12, 975)	(10, 55)	(10, 52)	(13, 85)
Alk phos (U/L)						
N	98	98	98	77	77	77
Mean (SD)	72.0 (19.7)	67.7 (20.6)	74.2 (20.5)	77.6 (31.4)	72.0 (28.3)	80.1 (32.8)
Median	69.0	64.5	2.5	69.0	66.0	72.0
(Min, Max)	(40, 161)	(34, 175)	(40, 175)	(35, 267)	(33, 220)	(34, 258)
Total bili (mg/dl)						
N	98	98	98	76	76	76
Mean (SD)	0.4 (0.2)	0.3 (0.2)	0.5 (0.2)	0.4 (0.2)	0.4 (0.2)	0.5 (0.2)
Median	0.4	0.3	0.5	0.4	0.3	0.4
(Min, Max)	(0.2, 1.4)	(0.2, 0.8)	(0.2, 1.5)	(0.2, 1.4)	(0.2, 1.1)	(0.2, 1.1)
BUN (mg/dl)						
N	98	98	98	77	77	77
Mean (SD)	12.8 (3.7)	10.8 (3.1)	13.9 (3.7)	13.8 (4.6)	12.0 (3.9)	14.6 (4.5)
Median	13.0	10.5	14.0	13.0	12.0	14.0
(Min, Max)	(5, 22)	(5, 21)	(8, 25)	(5, 29)	(5, 26)	(5, 29)
Creatinine (mg/dl)						
N	98	98	98	77	77	77
Mean (SD)	0.9 (0.2)	0.8 (0.2)	0.9 (0.2)	0.9 (0.2)	0.8 (0.1)	0.9 (0.2)
Median	0.9	0.8	0.9	0.8	0.8	0.9
(Min, Max)	(0.5, 1.2)	(0.4, 1.2)	(0.5, 1.3)	(0.5, 1.3)	(0.6, 1.2)	(0.6, 1.3)
Cr kinase (U/L)						
N	98	98	98	76	76	76
Mean (SD)	413.7 (2888)	87.4 (70.2)	527.2 (3867)	106.4 (67.0)	85.9 (48.9)	134.6 (100.3)
Median	91.5	64.5	96.5	85.0	73.5	101.0
(Min, Max)	(26, 29K)	(25, 569)	(42, 38K)	(24, 275)	(24, 275)	36, 540)
GGT (U/L)						
N	98	98	98	77	77	77
Mean (SD)	23.3 (18.6)	21.1 (17.5)	25.2 (20.1)	25.1 (20.8)	23.1 (19.4)	27.2 (22.7)
Median	17.5	16.0	19.0	19.0	16.0	18.0
(Min, Max)	(8, 123)	(5, 118)	(8, 134)	(5, 104)	(4, 107)	(6, 107)
Glucose (mg/dl)						
N	98	98	98	76	76	76
Mean (SD)	93.9 (18.9)	85.4 (16.2)	110.1 (26.9)	102.0 (34.7)	92.4 (19.2)	111.4 (31.7)
Median	90.5	85.0	106.0	90.5	91.0	103.0
(Min, Max)	(62, 178)	(26, 146)	(71, 269)	(62, 294)	(50, 162)	(75, 260)
LDH (U/L)						
N	97	97	97	76	76	76
Mean (SD)	180.8 (221.7)	145.3 (27.6)	186.1 (233.2)	161.2 (25.1)	147.9 (26.0)	163.3 (28.3)
Median	156.0	144.0	159.0	157.5	144.5	159.5
(Min, Max)	(83, 2323)	(70, 217)	(70, 2435)	(91, 222)	(89, 211)	(89, 222)
Total protein (g/dl)						
N	98	98	98	77	77	77
Mean (SD)	7.1 (0.4)	6.8 (0.5)	7.2 (0.4)	7.1 (0.5)	6.8 (0.5)	7.2 (0.5)
Median	7.1	6.8	7.3	7.1	6.8	7.2
(Min, Max)	(6.1, 8.1)	(5.8, 7.9)	(6.3, 8.3)	(6, 9)	(5.3, 8.8)	(5.7, 9.2)

Source: iss.pdf, Appendix 4, Table 7.2.1.2

Outliers and marked outliers in clinical chemistry parameters

No patients discontinued secondary to abnormal laboratory values. No patients met criteria for Hy's law. The most notable individual abnormalities were observed for creatinine kinase. Both ecallantide and placebo-treated patients appeared to have CK elevations, which may be related to the severity of tissue swelling associated with an HAE attack. In general, values returned to within reference range or near baseline at later follow-up or were normal post-dose but then noted to be elevated at later follow-up 1 week or more later; the time course of these latter cases make it difficult to attribute the lab abnormalities to ecallantide given the drug's short half life. The following cases did not resolve during the specified follow-up period:

- Patient 8814317011 had a total bilirubin of 1.6 mg/dl and had a documented history of Gilbert's syndrome.
- Patient 8805013099 had a total bilirubin of 1.3 mg/dl pre-dose, 1.8 mg/dl at Day 7 and 1.2 at Week 4.
- Patient 8804022001 had an elevated creatinine of 6.2 mg/dl on Day 7 and an LDH of 1145 U/L at Follow-up Visit 2. The patient was a kidney transplant patient with chronic renal failure who died during the study. This death is described in Section 7.3.1.
- Patient 8004009001 had an LDH of 618 U/L at Follow-up Visit 1 which remained elevated at 617 at the 4-week blood draw. Pre-dose value was 403 U/L. Further follow-up is not provided.
- Patient 8804022004 had an LDH of 769 U/L at Follow-up Visit 1 which remained elevated at 507 at the 4-week blood draw. Pre-dose value was 403 U/L. Further follow-up is not provided.
- Patient 8805051099 had an LDH of 816 U/L at Follow-up Visit 1. Baseline level was 608 U/L. Further follow-up is not provided.
- Patient 8805059099 had an LDH of 707 U/L at baseline and 1134 U/L at 4 hours post-dose. Further follow-up is not provided.
- Patient 8820426020 had several lab abnormalities on admission, most notably a CK of 28,650 U/L (negative MB fraction). At follow-up visit 1, the CK was 569 U/L.
- Patient 8804032001 had a pre-dose glucose of 248.8 mg/dl and 429 mg/dl at discharge. The patient was a known diabetic.

Table 24 Outliers for clinical chemistry parameters in Analysis Populations I and II

Laboratory test	Cutoff value	Population I		Population II			
		Ecallantide (N=219)		Ecallantide (N=100)		Placebo (N=81)	
		N[a]	N (%)[b]	N[a]	N (%)[b]	N[a]	N (%)[b]
ALT/SGPT	>2.5 x ULN	217	18 (8.3)	98	4 (4.1)	76	2 (2.6)
AST/SGOT	>2.5 x ULN	217	9 (4.1)	98	2 (2.0)	75	0
Alk phos	>2.5 x ULN	217	1 (0.5)	98	0	77	0
Total bili	>1.5 x ULN	217	4 (1.8)	98	0	76	0
GGT	>2.5 X ULN	213	8 (3.8)	98	1 (1.0)	77	2 (2.6)
LDH	>2.5 x ULN	205	9 (4.4)	97	1 (1.0)	76	0
Creatinine	>1.5 x ULN	217	1 (0.5)	98	0	77	0
BUN	>35 mg/dl	217	1 (0.5)	98	0	77	0
Cr kinase	>ULN	207	39 (18.8)	98	10 (10.2)	76	7 (9.2)
Glucose	<55 mg/dl	217	9 (4.1)	98	2 (2.0)	76	1 (1.3)
Glucose	>210 mg/dl	217	7 (3.2)	98	1 (1.0)	76	1 (1.3)

[a] Number of patients with both a baseline and post-baseline value
[b] Number of patients with a normal → abnormal or worsened value exceeding the normal range
ULN = upper limit of normal
Source: summary-clin-safety.pdf, Table 2.7.4.39

Reviewer's comment: Ecallantide does not appear to have any clear effects on routine chemistry parameters. Creatinine kinase was noted to be elevated in both the ecallantide and placebo populations, perhaps as a nonspecific result of soft tissue swelling from acute HAE attacks.

7.4.3 Vital Signs

Overview of vital sign assessment and selection of studies for drug-control comparisons
Routine vital sign assessment was performed at baseline and at appropriate intervals through each study. The review focuses on the initial 24 hours following dosing given the pharmacokinetics of ecallantide. A detailed schedule of vital sign assessment timepoints for each study is provided in the Individual Study Reviews located in Section 10.

Measures of central tendency, outliers, and marked outliers were reviewed for each vital sign. Baseline is defined as the closest observation prior to dosing. Vital sign changes were not performed by study visit because of the variety of time points used for laboratory assessments across studies. Instead, the most abnormal value from all follow-up visits was selected for analysis. For comparison to placebo control, the review focuses on the Analysis Population II, consisting of the pooled Phase III data. The entire HAE population (Analysis Population I) is also reviewed, particularly in terms of repeat dose data and outliers.

Mean change in vital signs
No clinically meaningful differences in mean change in vital signs were reported between the ecallantide and placebo treatment groups in the Phase 3 program. Although pyrexia was one of the more common AEs reported for ecallantide, mean values for body temperature did not reflect this AE. The changes are summarized in Table 25.

Table 25 Mean change in vital signs (Analysis Population II)

Indices	Ecallantide (N=100)			Placebo (N=81)		
	Baseline	Post-baseline		Baseline	Post-baseline	
		Lowest	Highest		Lowest	Highest
Temperature (ºC)						
N	100	100	100	77	77	77
Mean (SD)	36.6 (0.5)	36.4 (0.3)	36.9 (0.5)	36.6 (0.4)	36.4 (0.3)	36.9 (0.3)
Median	36.6	36.4	36.9	36.6	36.4	36.9
(Min, Max)	(35.5, 38.5)	(35.6, 37.1)	(36.1, 39.3)	(35.6, 38.2)	(35.3, 37.1)	(36.2, 37,8)
Pulse (bpm)						
N	100	100	100	77	77	77
Mean (SD)	80.1 (14.2)	67.2 (10.3)	81.2 (12.4)	80.0 (13.5)	70.5 (10.1)	83.5 (10.3)
Median	80.0	67.0	80.0	79.0	70.0	84.0
(Min, Max)	(51, 123)	(47, 117)	(52, 121)	(54, 114)	(41, 92)	(59, 115)
Systolic BP (mmHg)						
N	100	100	100	77	77	77
Mean (SD)	121.6 (14.7)	113.4 (11.6)	126.5 (12.6)	119.0 (14.9)	111.5 (12.8)	123.1 (13.3)
Median	121.0	115.5	126.0	118.0	110.0	120.0
(Min, Max)	(95. 175)	(87, 139)	(93, 168)	(78, 160)	(87, 140)	(95, 164)
Diastolic BP (mmHg)						
N	100	100	100	77	77	77
Mean (SD)	78.4 (9.2)	70.5 (10.0)	81.9 (8.6)	75.7 (10.8)	69.9 (9.7)	78.8 (10.2)
Median	80.0	70.0	82.0	76.0	70.0	78.0
(Min, Max)	(58, 102)	(48, 95)	(55, 105)	(45, 100)	(45, 92)	(53, 112)

Source: iss.pdf, Appendix 4, Table 8.1.2

Outliers and marked outliers in vital signs

No patients were discontinued from the study secondary to vital sign abnormalities. The total numbers of patients with shifts from normal → abnormal are shown in Table 26. Review of outliers is consistent with the commonly reported AE of pyrexia, with 4 patients reporting temperatures >38ºC after receipt of ecallantide in the Phase 3 program. More patients with decreases in blood pressure and pulse were also reported in the ecallantide group compared to placebo. One patient (Patient 8805051099) experienced hypotension in the setting of an anaphylactic reaction to ecallantide, described in Section 7.3.4.

Table 26 Outliers for vital signs in Analysis Populations I and II

Laboratory test	Cutoff value	Population I		Population II			
		Ecallantide (N=219)		Ecallantide (N=100)		Placebo (N=81)	
		N[a]	N (%)[b]	N[a]	N (%)[b]	N[a]	N (%)[b]
Temperature	≥38ºC	219	10 (4.6)	100	4 (4.0)	77	0
SBP	≥150 mmHg	219	29 (13.2)	100	2 (2.0)	77	2 (2.6)
SBP	>20% decrease	219	50 (22.8)	100	11 (11.0)	77	3 (3.9)
DBP	>20mmHg increase	219	33 (15.1)	100	1 (1.0)	77	3 (3.9)
Pulse	<60bpm	219	76 (34.7)	100	18 (18.0)	77	10 (13.0)
Pulse	>120 bpm	219	9 (4.1)	100	0	77	0

[a] Number of patients with both a baseline and post-baseline value
[b] Number of patients with a normal → abnormal or worsened value exceeding the normal range
ULN = upper limit of normal

Reviewer's comment: There do not appear to be any clear vital sign shifts due to ecallantide. Review of the individual narratives suggest that the observed decrease in blood pressure and pulse in the majority of these cases may have been related to resolution of pain and the acuity of the initial attack, as the these vital sign changes appeared to correlate to some extent with patient reports of improvement. The exception would be in cases of anaphylaxis, where decreased blood pressure and tachycardia were recorded as would be consistent with anaphylactic cardiovascular changes.

7.4.4 Electrocardiograms (ECGs)

No formal QT studies were conducted in the ecallantide program. Given the absence of a preclinical effect and the expected mode and setting of administration, intensive ECG monitoring in EDEMA4 in lieu of a separate formal thorough QT study was performed as discussed with the Division (August 24, 2007 submission). Twelve-lead ECGs were obtained at screening, pre-dose, between 2 and 4 hours post-dose to correspond to the Cmax window, and at Follow-up Visit 1. All ECGs were interpreted by a central reader.

No mean shifts from normal → abnormal were recorded. None of the ecallantide or placebo patients reach a threshold QTc interval of >500msec post-dose in Analysis Population II. The longest QTc interval recorded was 469 msec in an ecallantide patient and 521 msec at baseline in a placebo patient. One ecallantide patient had a >65msec change from baseline noted only at Follow-up Visit 1, making correlation to the drug less likely.

Reviewer's comment: Based on these results, ecallantide does not appear to have an effect on the QTc interval. Aside from supraventricular tachycardia and asymptomatic bradycardia, no arrhythmias were reported as AEs.

7.4.5 Special Safety Studies

Study DX88-102, Rechallenge study

In order to further define hypersensitivity reactions to ecallantide, patients with a history of a hypersensitivity reaction in EDEMA1, EDEMA2, or EDEMA3 were invited to enroll in a rechallenge study. The study consisted of 2 phases: a skin-testing phase and a test-dose phase. For the skin-test phase, escalating doses of ecallantide were administered by skin-prick and intradermal injection and compared to histamine and saline controls. A skin test was considered positive if the difference in the observed erythema or edema was >3mm from the saline control. For the test-dose phase, escalating doses were administered via intravenous infusion. The escalating dose procedure was not intended as a drug desensitization protocol. If any test was positive, the patient could proceed to the next test only with the approval of the Sponsor and the investigator. At the investigator's discretion, patients could also undergo a separate desensitization protocol. Details of the dosing for each phase of rechallenge are found in the Individual Study Summary, Section 10.6.1.

Nine patients underwent the rechallenge testing procedures. Six of the 9 patients successfully completed the test-dosing phase. Four of the 6 patients have since gone on to participate in other

ecallantide studies and have not experienced additional hypersensitivity reactions. Three patients had positive test results:

- Patient 8805019001 was a prior participant in EDEMA2. After the initial dose of 20 mg/m2 IV, the patient developed eye erythema, eye swelling, urticaria of the back and face, nasal congestion, rhinorrhea and sneezing. She tested positive for specific IgE to *P. pastoris* 3 weeks prior to ever receiving study drug. During the rechallenge, she successfully completed the skin testing phase. However, approximately 8 minutes after the start of the 3 mg IV infusion, she developed sneezing, rhinorrhea, nasal congestion, cough, and throat itchiness. She received Benadryl and her symptoms resolved.
- Patient 8805051099 participated in EDEMA2 and received 13 doses of ecallantide without reaction. The patient subsequently enrolled in EDEMA3 and received 7 doses over a 5-month period. After the 7th dose, she developed pruritus and anaphylaxis (hypoxia and hypotension). The patient had positive IgE antibodies to *P. pastoris.* During the rechallenge, the patient developed a positive skin reaction on ID testing at the 1:100,000 dose. The investigator requested permission to administer a 1 mg SC dose. Seven minutes after dosing, the patient developed dyspnea, rash, anxiety, pharyngeal edema, vomiting, diarrhea, urinary incontinence, and hypoxia, consistent with anaphylaxis. The patient was treated with epinephrine and conveyed to the hospital for further observation prior to being discharged home. The patient has not participated in further studies.
- Patient 8814326002 was a participant in EDEMA 3 and received 4 doses of ecallantide. After the 4th injection, the patient experience nausea, pruritus, and injection site pruritus. The patient tested positive for IgE antibodies to *P. pastoris* and non-IgE antibodies to ecallantide. During rechallenge, the patient had a positive ID test at 1:10,000 dilution. The patient did not participate in further studies.

Reviewer's comment: Overall, the rechallenge procedure successfully identified patients who could receive additional ecallantide. None of the patients who had a successful rechallenge who then went on to further dosing have had new AEs suggestive of hypersensitivity. The safety of the rechallenge procedure, performed in the appropriate setting, appears comparable to similar graded challenge procedures for other drug allergies. However, the total number of patients studied was limited, so the generalizability of these results is uncertain. Notably, antibody status was not predictive. While all 3 patients who failed rechallenge and the patient with the most severe reaction, Patient 8805051099, did have positive IgE antibodies to P. pastoris, the application includes information on other patients with positive antibodies who did not have any hypersensitivity reactions, suggesting that the positive predictive value may be limited. The negative predictive value may be higher but this issue has not been systematically addressed.

7.4.6 Immunogenicity

Antibody screening and methodology

Screening for formation of non-IgE and IgE antibodies to ecallantide and IgE antibodies to *P. pastoris* were performed throughout the clinical program. The schedule for antibody testing in each study is provided in the Individual Study Reviews located in Section 10. An ELISA assay was used in EDEMA3 and a more sensitive ECL assay was used for EDEMA4. Serum samples

obtained from EDEMA3 were retested retroactively using the new ECL assay where sample quantity was sufficient. Retesting of sera from older studies (EDEMA0, EDEMA1, and EDEMA2) was not performed because the stability of the older samples was uncertain. Neutralizing antibody assays were performed on samples confirmed positive by ECL assay. Serum samples negative for anti-ecallantide antibodies were presumed to be negative for neutralizing antibodies and were not assayed.

Overall, ELISA and ECL assay results correlated closely per the Applicant. For the purposes of safety analysis, the antibody status of subjects was based on the combined results of both assays. If a sample tested positive to either assay, the sample was considered positive.

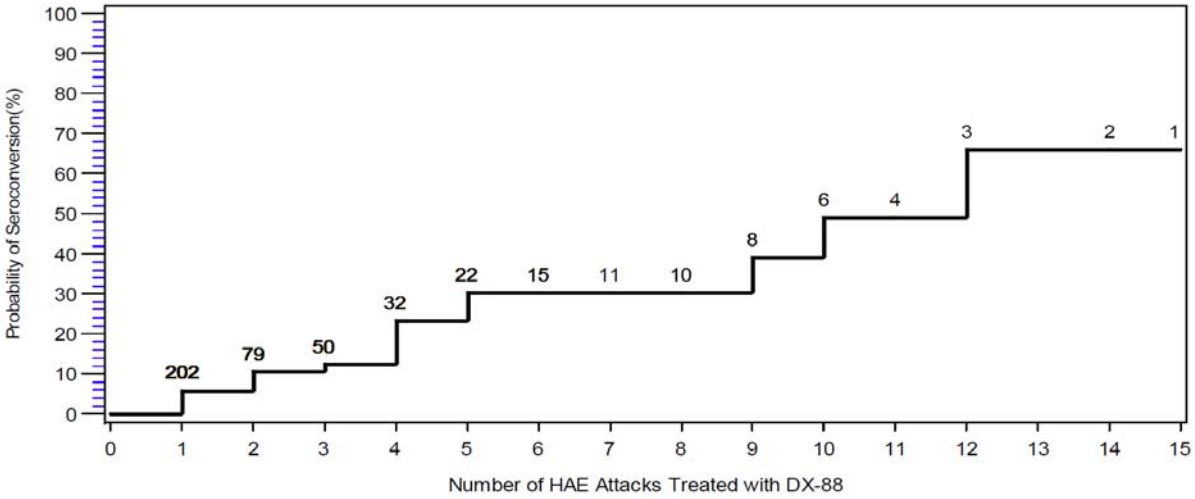
Reviewer's comment: The Agency's review of the immunogenicity assays is pending at the time of this review; however, preliminary review suggests that the IgE assays and neutralizing antibody assays may be limited in sensitivity, resulting in an underestimation of seroconversion. The non-IgE antibody assays appear adequate.

Antibody seroconversion

The number of patients at risk to seroconvert was based on patients with at least 1 post-baseline evaluation. Patients with a missing pre-treatment evaluation were considered negative at baseline; patients who were positive at pre-treatment were excluded. Therefore, the number of seroconversions represents those patients with a negative or missing pre-treatment evaluation and a positive post-treatment evaluation. Based on these criteria, 26 of 202 (12.9%) patients seroconverted to anti-ecallantide antibodies (any class). Four of 195 (2.1%) seroconverted to anti-ecallantide IgE and 14 of 175 (8.0%) seroconverted to anti-*P. pastoris* IgE. Four patients with neutralizing antibodies were identified in the Analysis Population I.

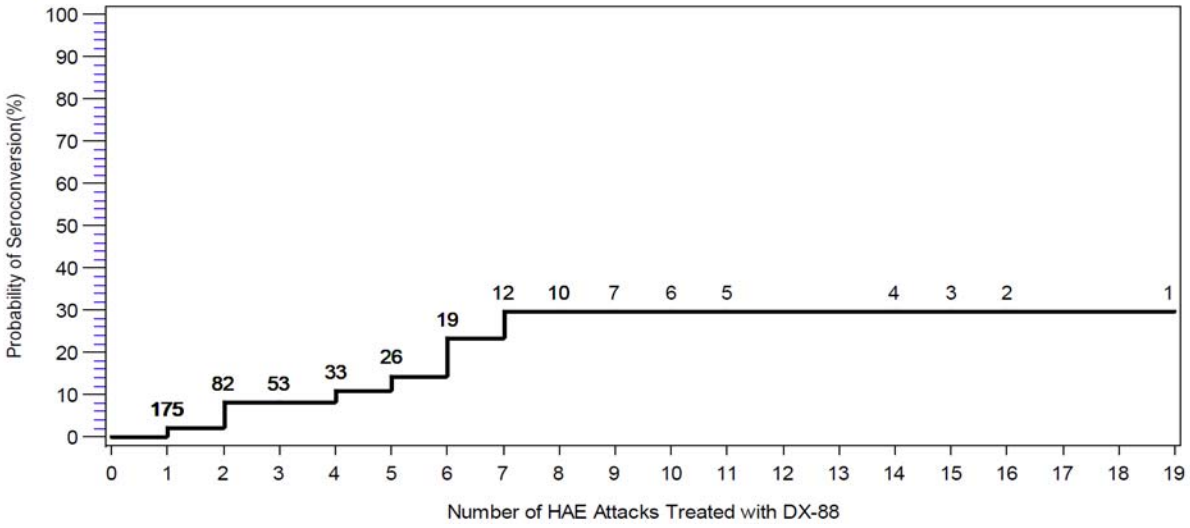
The probability of seroconversion increased with the number of treated episodes through 5 episodes, then seemed to plateau after the 9th episode. There are few patients treated for more than 9 HAE attacks, so extrapolation beyond this point is not possible. Figure 1 displays a Kaplan-Meier analysis of the probability of seroconverserion for both IgE and non-IgE antibodies to ecallantide over the number of treated HAE episode. Within this dataset, seroconversion to IgE anti-ecallantide was not observed until the 4th exposure to ecallantide and the probability of seroconversion after 8 attacks is estimated to be 12%.

Figure 1 Number of ecallantide-treated HAE attacks to seroconversion of IgE and non-IgE antibodies to ecallantide (Analysis Population I)



For *P. pastoris* IgE antibodies, there was an increase in the probability of seroconversion up through the 7th episode and then the rate was estimated at 30% after 7 attacks. These results are summarized in Figure 2.

Figure 2 Number of ecallantide-treated HAE attacks to seroconversion of IgE antibodies to *P. pastoris* (Analysis Population I)



<u>Adverse events by antibody status</u>

Anaphylaxis and other hypersensitivity reactions are discussed separately in Section 7.3.4. In terms of other AEs, there was no apparent difference in the frequency or nature of non-hypersensitivity AEs reported in patients seronegative versus seropositive for IgE and non-IgE to ecallantide and anti-*P. pastoris* IgE for Analysis Population I.

Of the 4 patients who tested positive for neutralizing antibodies, 3 reported an adverse drug reaction. Patients 8805054099, 8805024907, and 8814326002 reported reactions suggestive of drug hypersensitivity. However, the time course between development of neutralizing antibodies and the reactions were not closely correlated, with the two events separated in each of the cases by months to years.

Cross-reactivity with Tissue Factor Protein Inhibitor (TFPI)
As noted in Section 4.1, the Applicant has not made an assessment of potential cross-reactivity with endogenous tissue factor pathway inhibitor (TFPI). Ecallantide shares 88% homology with TFPI. In knock-out mouse models, TFPI deficiency is an embryonic lethal due to hypercoagulability. Based on this literature, TFPI cross-reactivity may theoretically predispose to thrombotic events in humans.

Reviewer's comment: Although thromboembolic AEs were not reported in the HAE program, potential cross-reactivity could be addressed via in vitro assays and monitoring for thromboembolic AEs.

7.5 Other Safety Explorations

7.5.1 Dose Dependency for Adverse Events

There was no apparent dose dependency for AEs but as noted, limited dose-ranging was performed in the clinical development program. In terms of number of doses, the percentage of patients reporting at least one or more adverse events increased with number of exposures. Fifty-two of 108 (48.1%) who received a single dose reported at least one AE compared to 60 of 80 (75.0%) who received 2-4 doses and 18 of 19 (94.7%) who received 5 to 9 doses. All 12 patients who received >9 doses reported at least 1 AE. The nature of the AEs reported did not appear to change, with the exception of hypersensitivity reactions. Although hypersensitivity reactions, including 1 case of anaphylaxis, were observed in patients upon first exposure, the other cases of anaphylaxis occurred in patients who had had multiple exposures to ecallantide.

Reviewer's comment: The increase in percentage of patients reporting an AE with increasing dose exposure is not unexpected, as patients who have had more HAE attacks and treatments have had more opportunities to experience an HAE. Likewise, the occurrence of anaphylaxis with multiple exposures is expected as well.

7.5.2 Time Dependency for Adverse Events

The majority of AEs were reported within the first 24 hours of dosing. There were no AEs consistently associated with a delayed time to onset.

7.5.3 Drug-Demographic Interactions

In general, subgroup analysis was limited by small sample sizes. The percentage of ecallantide-treated patients reporting AEs was similar between male (66.7%) and female (63.9%) patients in the whole HAE population (Analysis Population I). There were no apparent differences in the nature of AEs, with the exception of anaphylaxis, which all occurred in female patients with the exception of 1 case. The number of pediatric (n=18) and geriatric patients (n=4) is too small to draw conclusions about age, as was the case with racial subgroups.

7.5.4 Drug-Disease Interactions

The AEs frequency or profile did not appear to be associated with presenting attack severity, anatomic attack sites, or with the subtype of HAE (Type I vs. Type II).

7.5.5 Drug-Drug Interactions

No formal drug-drug interaction studies were conducted. Ecallantide is a small protein and is not expected to interact with CYP450 enzymes or p-glycoproteins.

7.6 Additional Safety Explorations

7.6.1 Human Carcinogenicity

No carcinogenicity studies were performed for ecallantide. One patient discontinued from study due to a new diagnosis of B-cell lymphoproliferative disorder.

Reviewer's comment: The Pharmacology/Toxicology reviewer has concluded that a formal carcinogenicity study in one species is feasible and would be an appropriate post-marketing commitment if approved.

7.6.2 Human Reproduction and Pregnancy Data

Although appropriate contraception was specified in all the protocols, two patients were exposed to ecallantide with conception estimated to have occurred within 6 days of the last ecallantide dose for 1 patient and within 28 days of the first dose and 15 days prior to the second dose. Both patients were reported to have normal pregnancies with delivery of healthy, full-term infants. An additional ongoing 3rd pregnancy is reported for DX-88/19 (EDEMA4 OLE). No other information on ecallantide use in pregnancy or lactation in humans is available.

7.6.3 Pediatrics and Effect on Growth

No formal studies in pediatrics or effect on growth were conducted for ecallantide. Although the inclusion criteria for EDEMA2, EDEMA3, and EDEMA4 included patients down to the age of 10 years, few pediatric patients were studied in the clinical development program. The nature

and number of AEs observed in children appeared comparable to the adult population but the low number of patients limits conclusions about safety in this subpopulation. The limitations of the safety database in regards to the pediatric population numbers are discussed more fully in Section 7.2.1.

7.6.4 Overdose, Drug Abuse Potential, Withdrawal and Rebound

No data is presented on overdose, drug abuse potential, withdrawal and rebound. In the CTS studies, ecallantide doses of up to 100.8 mg IV have been administered to patients without evidence of added toxicity per the Applicant. Given the expected mode of administration through a healthcare provider and intermittent use for HAE, combined with the short half-life of the drug, overdose, drug abuse, and withdrawal are not anticipated.

7.7 Conclusions

The safety of ecallantide at the proposed 30 mg SC dose is supported by the submitted clinical study data. Safety data showed that ecallantide is most commonly associated with headache, nausea, diarrhea, pyrexia, and injection site reactions. The most concerning adverse events are anaphylaxis and other hypersensitivity reactions. The size of the safety database is somewhat limited due to the rarity of HAE and the difficulty of conducting controlled trials to evaluate unpredictable, acute HAE attacks. However, given the potential severity of HAE and the lack of effective treatment alternatives, the safety profile for the proposed dose is acceptable with appropriate risk management strategies for hypersensitivity reactions.

8 Postmarketing Experience

Ecallantide is currently not marketed for any indication.

9 Literature review and references

The Applicant provided 37 literature references with electronic copies regarding hereditary angioedema, the role of kallikrein in HAE, and anaphylaxis. In addition, the reviewer performed an electronic PubMed search [search term: ecallantide] that yielded 13 literature reports, two of which overlapped with the references provided by the Applicant. These reports were reviewed briefly and did not suggest additional safety concerns.

10 Individual Study Reviews

10.1Individual Study Report: EDEMA0

10.1.1 Study Protocol: DX88/2 (EDEMA0)

10.1.1.1 Administrative information

- Title: Open-label, single ascending IV dose study to assess the tolerability and efficacy of DX88 administered following the onset of peripheral and/or facial edema or abdominal symptoms in patients with angioedema
- Study dates: March 27, 2001 to April 9, 2003
- Study sites: 4 sites (Germany, Italy, Spain)
- Study report date: June 7, 2007

10.1.1.2 Objectives/Rationale

- Assess the tolerability and efficacy of ascending single doses of ecallantide in HAE
- Determine the PK profile of ascending single doses of ecallantide in HAE/AAE patients

10.1.1.3 Study design overview

EDEMA0 was an open-label, single ascending dose study of ecallantide in patients with acute HAE and acquired angioedema (AAE) attacks. Three patients were enrolled at each dose level (10, 40, and 80 mg administered intravenously over 10 minutes) within 10 hours of onset of an HAE/AAE attack. The dose level was increased serially after the safety and efficacy data for the lower preceding dose level had been reviewed. A total of 9 patients (3 per dose group) were enrolled among the dose groups.

10.1.1.4 Study population

Adult patients with HAE or AAE.

<u>Inclusion criteria</u>

- Age 18 years or older
- Previously confirmed diagnosis and history of HAE **OR**
- AAE defined as acquired function C1 INH deficiency with
    - A history of recurrent angioedema
    - Functional deficiency of C1 INH (<50% normal value)
    - Normal or low level of C1q
    - No evidence of genetic disease
- Presentation within 10 hours of onset of attack

<u>Exclusion criteria</u>

- Life-threatening episode of angioedema
- Use of prophylactic aspirin
- Pregnancy or breastfeeding

- Serum creatinine >200mcM/L

10.1.1.5 Study treatments
Single 10-minute IV infusion of 10, 40, or 80 mg ecallantide.

Reviewer's comment: Dose selection was based on PK sampling from Phase 1 data. An 18 mg dose was estimated to achieve a plasma concentration of 500nm, the same concentration estimated for the total amount of circulating pre-kallikrein.

10.1.1.6 Study procedures
The following table summarizes the schedule of procedures and assessments.

Table 27 EDEMA0: Schedule of assessments

		Treatment visit		Post-treatment day*					Post-treatment	
	Screen	Pre-dose Day 0	24-hf post-treatment period	2	3	4	5	6	Wk 1	Wks 4-6
Pregnancy test	X	X							X	
History	X									
Physical exam	X		24 hr						X	
Temperature	X	X	15, 30 min and 1, 2, 4, 8, 12, 24 hr						X	
BP and HR	X	X	15, 30, 45 min and 1, 2, 4, 8, 12, 24 hr						X	
ECG	X	X	2x during first 8 hr then at 24 hr						X	X
Previous and concomitant medications	X	X		X	X	X	X	X	X	X
Angioedema sx assessment	X	X	5, 10, 15, 30 min and 1, 2, 4, 6, 8, 12, 16, 20, 24 hr							
Digital photographs[1]		X	30 min then hourly until attack regression, then hourly for 3 hrs						X	
Investigator pain assessment	X	X	Q15min for first 4 hrs							
McGill Pain Questionnaire[2]	X	X	24 hr							
Abdominal ultrasound[2]	X	X	Once during 24 hr	X					X	
Waist measurement[2]	X	X	Once during 24 hr						X	
PK sampling	X	X	5, 10, 15, 30 min, 1, 2, 4, 6, 8, 12, 24 hr							
Coagulation labs	X	X	1, 4, 24 hr						X	
Special labs[3]	X	X	1, 4, 8, 24 hr	X					X	
Routine labs[4]	X	X	24 hr						X	
Patient diary No.1	Dispense	Collect								
Patient diary No.2		X	Dispense after 24 hr						Collect	
AEs	X	X	X	X	X	X	X	X	X	X

* By telephone or if logistically feasible by visit day
[1] At dosing for all patients, but at follow-up only in cases of peripheral or facial attack
[2] At screening for all patients, but at follow-up only in cases of abdominal attack
[3] C1-INH, C4, kallikrein
[4] Routine chemistry, hematology, and urinanalysis
Source: dx-88-2-csr-body.pdf, Table 9-2

10.1.1.7 Efficacy parameters

- Attack classification and symptom assessment by the investigator and verified against the patient diary
- Digital photography in cases of peripheral or facial attacks
- Visual Analog Scale (VAS) for pain by investigator
- McGill Pain Questionnaire (GI attacks)
- Abdominal ultrasound (GI attacks)
- Waist circumference (GI attacks)
- Patient diaries
    - Attack site
    - Pain, difficulty in motion, appetite, sleep, general function, and global satisfaction (on VAS)

10.1.1.8 Safety parameters

- AEs
- ECG
- Routine clinical laboratory tests (glucose, urea, creatinine, total bilirubin, AST, ALT, GGT, alkaline phosphatase, creatinine kinase, total protein, CBC with differential, urinanalysis)
- Pregnancy test
- Special labs: C1 INH (antigenic and functional), C4, kallikrein and consumption of high molecular weight kininogen (HMWK; surrogate marker)
- Anti-ecallantide non-IgE antibody

10.1.1.9 PK parameters

- C_{max}
- T_{max}
- $T_{½}$
- Terminal elimination rate constant
- AUC

10.1.1.10 Dose Review

A dose review group consisting of the sponsor, its agent (Harrison Clinical Research), and the investigators was to review the safety and efficacy data at each dose level. The original protocol stated that the group would generate a written report for each discussion, but the Applicant states that these reports have not been recovered despite due diligence.

10.1.2 Results

10.1.2.1 Study patients

A total of 48 patients were screened. Treatment was restricted to the first 9 patients who returned for treatment of an acute attack, 3 per dose level. No patients discontinued from the study. Four male and 5 female patients enrolled; 7 had a diagnosis of HAE and 2 patients treated with the 80 mg dose had a diagnosis of AAE. The mean age was 51.8 years (range 31 to 67

years). Three patients presented with facial HAE attacks, 2 patients reported abdominal symptoms predominantly, 2 patients reported peripheral symptoms, and 1 patient reported a mix of peripheral and abdominal involvement.

10.1.2.2 Efficacy endpoint outcomes
The primary efficacy endpoint was the proportion of patients who reported beginning of resolution of attack symptoms by 4 hours post-dose. The beginning or resolution was the time at which the first sign and/or symptom present at dosing was no longer present. Using this definition, 2 patients in the 10 mg reported beginning of attack resolution by 4 hours, compared to 1 patient in the 40 mg group and 1 patient in the 80 mg group.

Reviewer's comment: Given the small numbers of patients and lack of a control, no conclusions can be made about efficacy or relative dose response.

10.1.2.3 Safety outcomes
No deaths were reported in the study. A total of 18 AEs were reported by 4 patients: 4 AEs among 2 patients in the 10 mg group and 14 AEs among 2 patients in the 40 mg group. One AE, cough, was recorded in 2 patients. The other AEs included a range of organ systems: hypertension NOS, injection site reaction, nasopharyngitis, dry mouth, sleep apnea, iron deficiency anemia, pyrexia, hemoglobin decreased, asthenia, breast mass NOS, breast pain, irregular menstruation, and rhinitis NOS.

One SAE, anaphylactoid reaction, was reported in Patient 305 after receipt of the 40 mg dose. The patient initially presented for treatment of acute genital edema. Within 5 minutes of the start of the infusion, she reported pruritus, which rapidly progressed to urticaria, edema, dysphagia, dyspnea, enteritis, and acute abdominal pain with an urge to defecate. She was treated with epinephrine, polaramine IV, and hydrocortisone IV. She was hospitalized overnight for observation prior to discharge without further sequelae. The investigator independently performed immunoblotting and detected both IgE and non-IgE antibodies to ecallantide. A separate ELISA assay performed by the applicant was negative for ecallantide or *P. pastoris* antibodies. No rechallenge procedure was attempted.

10.1.3 Study summary and conclusions
EDEMA0 demonstrated that IV doses of ecallantide up to 80 mg were generally tolerated in a sample of 9 adult patients without major toxicity, but the risk of anaphylaxis was present even upon initial exposure. No conclusions regarding efficacy could be made given the small number of participants and lack of a control arm.

10.2Individual Study Report: EDEMA1

10.2.1 Study Protocol: Study DX88/4 (EDEMA1)

10.2.1.1 Administrative information

- Title: An ascending four dose placebo controlled study to assess the efficacy and tolerability of DX-88 (ecallantide) administered following onset of acute attacks of HAE
- Study Dates: October 22, 2002 to May 4, 2004
- Study sites: 29 sites in the US, 1 site in Belgium, 1 site in Israel
- Study report date: June 20, 2004

10.2.1.2 Objectives/Rationale

- Determine an effective dose of ecallantide in patients experience acute HAE attacks

10.2.1.3 Study design overview

EDEMA1 was a randomized, placebo-controlled, double-blind ascending dose-ranging study of ecallantide in patients ≥10 years of age with acute HAE attacks. The study evaluated 4 dose groups (5, 10, 20, and 40 mg/m^2 IV) of ecallantide compared to placebo. Twelve patients per dose level were treated; 2 assigned to placebo and 10 to ecallantide. Patients received a single dose and were asked to assess their symptoms during a resident period and 2-6 days post dose, with additional follow-up visits at 1, 2, and 4 weeks.

10.2.1.4 Study population

Patients 10 years of age or older presenting within 4 hours of onset of HAE symptoms of at least moderate severity.

<u>Inclusion criteria</u>

- 10 years of age or older
- Confirmed diagnosis of HAE with at least 1 clinical and 1 laboratory criterion:
    - Clinical criteria
        - Recurrent, self-limited, non-inflammatory angioedema lasting more than 12 hours without urticaria
        - Recurrent abdominal pain lasting more than 6 hours without organic disease
        - Recurrent laryngeal edema
        - Familial history
    - Laboratory criteria
        - C1-INH functional level <50% normal
        - Historical documentation of C1-INH mutation

<u>Exclusion criteria</u>

- Serious intercurrent illness or active infection
- Serum creatinine >10% ULN or LFT >2x ULN
- AAE
- Receipt of investigational drug or device within 30 days
- Pregnancy or breastfeeding
- Patients previously treated with ecallantide

10.2.1.5 Study treatments

Single 10 minute infusion of ecallantide (5, 10, 20, or 40 mg/m^2; maximum dose of 100 mg) or placebo.

10.2.1.6 Study procedures
The table below summarizes the schedule of procedures:

Table 28 EDEMA1: Schedule of assessments

	Screen	Pre-dose	Post-treatment evaluation											
			0-1h	1-24 hours post-dose										
				1	2	4	6	8	12	24	Day 2-6[1]	Day 7	Wk 2	Wk 4
Pregnancy test	X	X												
Medical history	X													
Physical exam	X									X		X	X	X
Waist measurement	X		X											
Vital signs	X	X	X	X	X	X	X	X	X	X		X	X	X
ECG	X	X				X								
Symptom record		X	X	X	X	X	X	X	X					
Diary	Issue	revie w												
Study drug			X											
Photograph[2]		X	X	X	X	X	X	X	X					
VAS[3]		X	X	X	X	X								
McGill Pain Questionnaire[3]		X		X		X								
Urinanalysis	X	X	X											
Concomitant meds	X	X	X	X	X	X	X	X	X	X	X	X	X	X
AE	X	X	X	X	X	X	X	X	X	X	X	X	X	X
PK sampling		X	X	X	X	X	X	X	X	X				
Routine labs	X	X							X					
Special labs[4]	X	X		X		X		X		X				
Coagulation labs	X	X		X		X		X		X		X	X	X
Antibody test[5]	X	X											X	X

[1] Phone evaluation
[2] Peripheral attacks only
[3] abdominal attacks only
[4] Special labs: C1-INH, C4, kallikrein, HMWK
[5] Anti-ecallantide non-IgE antibodies
Source: dx-88-4-csr-body.pdf, Table 9-1

10.2.1.7 Efficacy parameters

- Percentage of patients reporting significant improvement at the primary attack location within 4 hours after drug infusion.

10.2.1.8 Safety parameters

- AEs
- ECG

- Routine clinical laboratory tests (glucose, urea, creatinine, total bilirubin, AST, ALT, GGT, alkaline phosphatase, creatinine kinase, total protein, CBC with differential, urinanalysis)
- Pregnancy test
- Special labs: C1 INH (antigenic and functional), C4, kallikrein and consumption of high molecular weight kininogen (HMWK; surrogate marker)
- Anti-ecallantide non-IgE antibody

10.2.1.9 PK parameters
- C_{max}
- T_{max}
- $T_{½}$
- Terminal elimination rate constant
- AUC

10.2.1.10 Data safety monitoring board
An independent DSMB consisting of 3 clinical pharmacologist and/or HAE experts plus a 4th independent member was organized. A blinded DSMB determined whether to proceed to the next dose level at the end of each dose cohort. A decision to terminate the study for reasons of lack of safety and efficacy was part of the review.

10.2.2 Results

10.2.2.1 Study patients

Patient disposition
A total of 140 patients were screened and the first 48 patients returning for treatment of an acute HAE attack were enrolled. The 48th and 49th patients presented at approximately the same time so a total of 49 patients were treated. Forty-three patients completed the full 4 weeks of the study, while 6 patients discontinued early. Of the 6, 3 were lost to follow-up and 2 refused to return for follow-up and were coded as non-compliant. The final patient, Patient 2201 died due to renal failure. The patient was a renal transplant patient who suffered from chronic rejection of the transplant prior to enrollment in the study.

Demographics
Thirty-eight (77.6%) patients were female. The majority (n=43, 87.8%) were Caucasian, 4 (8.2%) were Hispanic, 1 (2.0%) was black, and 1 (2.0%) was categorized as other. The mean age was 32.5 years (range 11-62 years). On average, patients presented within 134 minutes of onset of symptoms. Primary attack locations were reported as follows: n=23 (47%) abdominal, n=22 (45%) peripheral, and n=4 (8%) laryngeal. The various locations were evenly distributed in the ecallantide and placebo treatment groups. Nine patients reported HAE symptoms in other locations in addition to the designated primary attack site.

10.2.2.2 Efficacy endpoint outcomes

The proportion of patients reporting a significant improvement ("successful outcome") for the primary attack location at 4 hours post-dose was the primary efficacy outcome assessed. Overall, in the ecallantide group, 29 of 40 (72.5%) reported significant improvement compared to 2 of 8 patients (25.0%) in the placebo group (p=0.0169). The proportion of successful outcomes by dose level is shown in

Table 29 EDEMA1: Proportion of successful outcomes by dose cohort

Dose level	Ecallantide	Placebo	P
5 mg/m^2	8/10 (80%)	1/2 (50%)	0.454
10 mg/m^2	5/10 (50%)	0/2	0.470
20 mg/m^2	7/10 (70%)	0.2	0.152
40 mg/m^2	9/10 (90%)	1/2	0.318

Source: dx-88-4-csr-body.pdf

Reviewer's comment: The comparison of the pooled ecallantide and placebo groups support the efficacy of ecallantide. The comparisons by dose cohort however are limited by the small sample sizes and do not permit a controlled evaluation of dose response.

10.2.2.3 Safety outcomes

A total of 124 AEs were reported. Thirty-nine of 49 patients (79.6%) reported at least 1 AE. In the ecallantide arm, 32 of 41 (78.1%) reported at least 1 AE compared to 7 of 8 (87.5%) in the placebo group. The most commonly reported AE was headache, reported by 6 patients (14.6%) of the ecallantide group. Other AEs reported in at least patients included the following: diarrhea NOS, vomiting NOS, abdominal pain NOS, nausea NOS, upper respiratory tract infection, cough, and allergic rhinitis.

A total of 5 SAEs were reported for 5 ecallantide patients.

- Patient 1303 (20 mg/m^2) had allergic rhinitis (sneezing, itchy throat, congestion, nasal drainage, and shortness of breath) with throat edema within 3 minutes of start of infusion, The patient was treated with 2 doses of epinephrine and cetirizine. The patient was discharged 8 hours later without further sequelae.
- Patient 501 (10 mg/m^2) was hospitalized for an HAE attack 21 days after treatment with ecallantide.
- Patient 2205 (5 mg/m^2) was treated with ecallantide for an abdominal attack. Twenty-three days later, the patient was hospitalized for swelling of the chest and difficulty breezing. Two days after admission, the patient had seizure. The patient was discharged 2 days after the event without sequelae.
- Patient 2510 (20 mg/m^2) was treated with ecallantide for an abdominal attack. Twenty-seven days later, the patient presented for follow-up and was noted to have an ECG suggestive of ischemic changes. Echocardiogram, angiogram, and repeat ECG showed no sign of cardiac ischemia.
- Patient 2201 was a study death and is described in detail below.

One death was reported. Patient 8804022001 had a history of dual nephrectomy and kidney transplant 1 year prior to enrollment. The patient was reported to have chronic rejection of the transplant and died of chronic renal failure 29 days after administration of ecallantide.

Reviewer's comment: Patient 1303's case description meets diagnostic criteria for anaphylaxis.

10.2.3 Study summary and conclusions
EDEMA1 demonstrated that IV doses of ecallantide up to 40 mg/m^2 were generally tolerated without major toxicity, but the risk of anaphylaxis was present even upon initial exposure. In a pooled analysis of ecallantide versus placebo, ecallantide appears to have efficacy. There was no clear dose response among the 4 doses tested.

10.3Individual Study Report: EDEMA2

10.3.1 Study Protocol: EDEMA2/DX-88/5

10.3.1.1 Administrative information
- Title: Study DX-88/5: EDEMA2: Evaluation of DX-88's effects in mitigating angioedema – An open-label study to assess the efficacy and tolerability of repeated doses of DX-88 (recombinant plasma kallikrein inhibitor) in patients with HAE
- Dates: November 13, 2003 to January 24, 2003
- Multicenter
- Study report date: July 2, 2008

10.3.1.2 Objectives/Rationale
- Assess the safety and efficacy of repeated dosing of DX-88 (ecallantide) in HAE acute attacks

10.3.1.3 Study design overview
EDEMA2 was an open-label, repeat dose study of ecallantide for the treatment of acute HAE attacks. Qualified patients presenting within 4 hours of the onset of an acute attack of at least moderate severity were treated with a single dose of ecallantide (Dose A). If no improvement was noted within 4 hours, a second dose (Dose B) could be administered. Patients could receive a maximum of 20 doses for separate attacks.

10.3.1.4 Study population
The study population was based on planned treatment of 240 attacks, which consisted of 77 patients.

<u>Inclusion criteria</u>
- Age 10 years or older
- Confirmed physician diagnosis of HAE
- Presentation within 4 hours of onset of symptoms
- HAE attack of at least moderate severity

Exclusion criteria

- Serious intercurrent illness or active infection
- Serum creatinine >110% ULN and/or not <50% of calculated Cr clearance or liver transaminases >2x ULN
- Receipt of an investigational drug or device other than ecallantide within 30 days prior to dosing
- Pregnancy or active breastfeeding
- Acquired angioedema
- Patients who had not completed their Day 8 follow-up procedures for a previously treated attack

Reviewer's comment: The diagnostic criteria for HAE and exclusion of AAE are not as rigorous as those specified for the Phase 3 program. For a Phase 2 study focused primarily on safety of repeated doses, these diagnostic criteria are not as critical as in the Phase 3 studies; however, the extent to which EDEMA2 results can be used to support the efficacy of repeated doses is considered secondary to the results from the Phase 3 OLE studies.

10.3.1.5 Study treatments

Escalating IV doses (5 mg/m^2, 10 mg/m^2, or 20 mg/m^2) were administered by sequential dose cohorts. The transition from each dosage cohort to the next was based on the review of safety and efficacy in the EDEMA1 study by the DSMB. For example, once the DSMB had determined the 10 mg/m^2 dose level safe in EDEMA1, patients enrolled in EDEMA2 were then given 10 mg/m^2. Patients were not restricted to a particular dose cohort and could receive repeated doses of ecallantide at a different dose level from the one received previously. From July 2005 to study conclusion, IV infusions were changed to ecallantide 30 mg SC fixed dose. Patients who had an incomplete response were eligible for Dose B.

10.3.1.6 Study procedures
The following table outlines the schedule of procedures.

Table 30 EDEMA2: Schedule of procedures

EDEMA2	Screen	Enroll	Post-dosing evaluation			Follow-up day		
			Post-dosing (hr)			Days 2-6 (phone)	Day 7	4 wks
			0-1	2	4			
Informed consent	X							
Urine pregnancy test		X						
History, demographics	X							
Physical exam	X				X		X	X
Vital signs	X	X	X	X	X		X	X
ECG	X	X			X		X	X
Urinanalysis	X	X						
Dosing			X					
Digital photograph		X	X	X	X			
VAS		X	X	X	X			
McGill Pain Questionnaire		X						
Concomitant meds	X	X	X	X	X	X	X	X
Adverse events		X	X	X	X	X	X	X
Blood samples								
•*Chemistry*	X	X					X	X
•*CBC/diff*	X	X			X		X	X
•*Coag panel*	X	X			X		X	X
•*Antibody levels*	X	X	X		X			
•*PK test*		X					X	X
		X	X	X	X			

Source: dx-88-5-csr-body.pdf, Table 4

10.3.1.7 Efficacy parameters

Primary efficacy endpoints

- Proportion of successful outcomes (i.e. attack resolution begun by 4 hours after a single dose and maintained for greater than 24 hours after a single dose)
- Proportion of patients who have a partial response (i.e. an initial response to dosing followed by a relapse 4 to 24 hours after the dosing)

Secondary efficacy endpoints

- The proportion of patients who respond to a second dose of ecallantide after an initial partial response
- Time to resolution onset of each acute attack as determined by patient report
- Time to resolution onset of each acute attack as determined by digital photography (optional) or pain scores in abdominal attacks
- Development of ecallantide antibodies
- Relationship between PK and clinical effect

10.3.1.8 Safety parameters

- Adverse events
- Laboratory assessments
- Vital signs
- ECG
- Physical examination

- Development of antibodies to ecallantide or *P. pastoris*

10.3.1.9 Statistical plan
Descriptive statistics were used to summarize patient demographic data and other baseline characteristics. Efficacy analyses were based on the patients' first attack in EDEMA2, in the interest of keeping efficacy comparisons among dose groups independent of one another. The unit of analyses for most endpoints was by treatment episode, not by individual patient.

10.3.2 Results

10.3.2.1 Protocol deviations
A number of protocol deviations were reported. The most common deviation was the administration of study drug outside the protocol window. In 4 cases, dosing assignment was not obtained prior to dose. Patient 1804 received 10mg/m2 ecallantide as prophylaxis prior to jaw surgery as compassionate use.

In addition, 7 patients were granted exception of inclusion criteria in presenting more than 4 hours after onset of HAE attack. Two patients became pregnant during the study: Patient 6299 had her last dose of ecallantide on April 8, 2005 and gave birth to a healthy male infant on July 19, 2006. Patient 6299 received ecallantide on November 2, 2005 but soon after found out she was pregnant despite a negative pregnancy test at screening, with an estimated date of conception in September 2005. She delivered a healthy male infant on June 19, 2006.

Reviewer's comment: The deviations are not likely to significantly impact the results of the study.

10.3.2.2 Datasets analyzed
The ITT and safety analysis are based on all study-treated attacks. The PP population consists of all study-treated attacks with no major protocol violation. The difference between these 2 populations is 1 episode.

10.3.2.3 Study patients
A total of 77 patients from 26 study sites were enrolled and treated for 240 HAE attacks. This population constitutes the ITT population. Twenty of the 77 (26%) had prior exposure to ecallantide.

<u>Baseline demographics</u>

Table 31 EDEMA2: Patient demographics

	5 mg/m^2 N=14	10 mg/m^2 N=40	20 mg/m^2 N=9	30 mg N=14	Overall N=77
Age					
Mean (SD)	34.6 (13.6)	31.7 (15.2)	28.7 (12.4)	38.0 (11.8)	33.0 (14.1)
Range	11-53	13-78	12-52	10-55	10-78
Sex (N,%)					
Male	6 (42.9%)	11 (27.5%)	4 (44.4%)	8 (57.1%)	50 (64.9%)
Female	8 (57.1%)	29 (72.5%)	5 (55.6%)	6 (42.9%)	27 (35.1%)
Race (N,%)					
White	10 (71.4%)	38 (95.0%)	8 (88.9%)	11 (78.6%)	67 (87.0%)
Black	3 (21.4%)	2 (5.0%)	0	0	5 (6.5%)
Hispanic	1 (7.1%)	0	1 (11.1%)	2 (14.3%)	4 (5.2%)
Asian	0	0	0	1 (7.1%)	1 (1.3%)

Source: dx-88-5-csr-body.pdf, Section 11.2.1, Table 7

HAE history

The 77 patients enrolled in EDEMA2 ranged in age from 10 to 78 years. Of the 77 patients, 68 (88%) had a diagnosis of Type I HAE, 8 (10.4%) had Type II HAE, and 1 patient was reported as unknown due to the absence of diagnostic or confirmatory laboratory testing. The 77 patients experienced a mean attack frequency of 2.5 attacks/month. The mean duration of the most recent HAE attack was 47.9 hours (SD 37.9). The most common locations of HAE attack by history was abdominal (48.1%), followed by peripheral (32.5%). One patient reported laryngeal attack as the most common site of attack. Fourteen patients (18.2%) reported that a combination of attack sites was the most common presentation.

The most common concomitant treatments for HAE reported by the patients included attenuated androgens oxandrolone (n=6) and stanozolol (n=4), hydrocodone (n=6), oxycodone (n=2), aminocaproic acid (n=2), and fresh frozen plasma (n=2).

HAE presentation

Peripheral HAE attacks were reported as the first study-treated attacks for 35 (45.5%) patients. Abdominal attacks were reported for 32 (41.6%) patients. Ten (13.0%) patients presented with laryngeal attacks for their first study-treated attack. The total number of study-treated HAE attacks at each dose level and location is shown below.

Table 32 EDEMA2: Attack site of 240 study-treated HAE attacks

	Intravenous			Subcutaneous	
Primary location	5 mg/m^2 N=14	10 mg/m^2 N=40	20 mg/m^2 N=9	30 mg N=14	Overall N=77
Peripheral	14 (58.3%)	57 (40.4%)	5 (33.3%)	17 (28.3%)	93 (38.8%)
Abdominal	10 (41.7%)	65 (46.1%)	5 (33.3%)	33 (55.0%)	113 (47.1%)
Laryngeal	0	19 (13.5%)	5 (33.3%)	10 (16.7%)	34 (14.2%)

Source: dx-88-4-csr-body.pdf, Section 11.2.5.1, Table 13

10.3.2.4 Efficacy endpoint outcomes

Primary efficacy endpoint: Successful and partial outcomes

Successful outcome
A successful outcome was defined as onset of resolution within 4 hours of dosing and continuing for 24 hours after dosing. Of the 240 treated attacks, 165 attacks (68.9%) were reported to have a successful outcome. Among the 4 dosage levels, the 30 mg SC dose had the highest proportion of successful outcomes (49 of 60 attacks, 81.7%), followed by the 10 mg/m^2 IV and 20 mg/m^2 IV doses (68.1% and 60.0%, respectively). The 5 mg/m^2 IV dose had 11 of 24 attacks (45.8%) with successful outcomes.

Reviewer's comment: Based on EDEMA2, the 30 mg SC dose is the most appropriate for study in the Phase 3 program. The 30 mg SC dose corresponds approximately to a 15 mg/m^2 dose in an average-sized adult.

Partial response
Another 41 of 240 attacks (17.1%) were reported as having a partial response, meaning a response to dosing for at least 1 symptom at the primary attack site within 4 hours of treatment followed by a relapse within 24 hours or receipt of Dose B. A partial response was reported for 11.7% of the SC dose-treated attacks, 26.7% for the 20 mg/m^2 IV-treated attacks, 15.6% of the 10 mg/m^2 IV-treated attacks, and 33.3% of the 5 mg/m^2 IV-treated attacks. By attack site, peripheral attacks were reported to have a 23.7% (22 of 93 attacks) partial response rate, followed by 13.3% (15 of 113 attacks) for abdominal attacks, and 11.8% (4 of 34 attacks) for laryngeal attacks.

<u>Secondary efficacy endpoints</u>

Dose B
Of 31 evaluable attacks treated with Dose B, 22 were reported to have a positive response at 4 hours. Data at 24 hours was not collected systematically for Dose B.

Time to beginning of attack resolution by patient report
Time to beginning of attack resolution was defined as the time within 4 hours of the end of ecallantide treatment when the patient first reported relief of symptoms at the primary attack site. Patients receiving emergency intervention were censored at the time of therapy. Overall, the median time to beginning of attack resolution was 43.0 minutes for Attack 1, 38.0 minutes for Attack 2, 37.5 minutes for Attack 3. Attacks treated with the 30 mg SC dose had a median time of 37.5 minutes for Attack 1 and 18 minutes for Attack 3.

Reviewer's comment: The time to beginning of attack resolution does not show a clear dose response among the different dose groups, although the 30 mg SC dose appears to have performed the most consistently. There does not appear to be a decrease in efficacy from the first attack to the 3rd attack, although the number of treated attacks also decreased from 14 to 6, making the comparison less certain. It may be that efficacy is consistent over multiple treatments; alternatively, there may be a core group of responders to drug whereas patients with less pronounced responses may elect not to receive additional doses.

<u>Abdominal attack responses</u>

A number of different instruments were used to assess response to abdominal attacks, including a Visual Analog Scale (VAS) for pain, the McGill Pain Questionnaire, and change in waist girth. According to VAS measurements, pain was reduced by 83.2%, 79.5%, and 66.8% at 4 hours post-dosing for Attacks 1, 2, and 3, respectively. These results corresponded with an average reduction of 2 scale points (total of 0 to 5) on the McGill Pain questionnaire at 4 hours. For Attacks 1 and 2, an average 2 to 4% reduction in waist circumference was measured at 4 hours; for Attack 3, the decrease in average waist circumference was negligible.

Reviewer's comment: These measures of various aspects of abdominal attacks are generally supportive. It is worth noting, however, that neither the VAS nor the McGill Pain Questionnaire are PRO instruments validated for use in HAE, nor is waist circumference a routinely utilized clinical measure.

Plasma ecallantide concentrations at 1, 2, and 4 hours
Plasma concentrations at several timepoints for the different doses are shown in the table below.

Table 33 EDEMA2: Plasma ecallantide concentrations (ng/ml) at 1, 2, and 4 hours post-dose by dosage level (PP population)

Dosage level	1 hour	2 hours	4 hours
5 mg/m^2 IV			
N	23	23	24
Mean (SD)	192.5 (109.6)	135.1 (234.0)	23.0 (22.4)
Median	191.4	84.3	19.1
Range	30.0-402.1	12.1-1165.7	0-66.9
10 mg/m^2 IV			
N	138	138	139
Mean (SD)	602.8 (778.1)	265.2 (217.8)	86.1 (65.8)
Median	415.4	222.0	71.2
Range	0-5438.2	0-1768.5	0-447.8
20 mg/m^2 IV			
N	11	14	14
Mean (SD)	1235.1 (1205.6)	276.2 (121.3)	170.4 (186.1)
Median	729.0	265.7	104.4
Range	594.7-4613.3	104.3-609.3	24.2-672.8
30 mg SC			
N	70	68	70
Mean (SD)	509.7 (281.2)	627.5 (326.7)	473.8 (208.5)
Median	488.2	586.7	477.0
Range	66.1-1323.9	78.5-1623.6	0-1016.5

Source: dx-88-5-csr-body.pdf, Section 11.4.2, Table 26

Reviewer's comment: The pharmacokinetic parameters assessed in EDEMA2 are reviewed in more detail in the Clinical Pharmacology Team's review. Based on the findings here, there appears to be a fair amount of variability in plasma concentration levels, which could potentially result in different degrees of efficacy among individuals. When comparing the different dosage levels, the 30 mg SC dose appears to have the most constant levels over the initial 4 hour period post-dose.

10.3.2.5 Safety outcomes

Drug exposure

As previously mentioned, 20 patients had prior exposure to ecallantide in a previous study. During EDEMA3, 33 patients were treated for 1 attack while another 13 patients were treated for 2 attacks. Twenty-one patients were treated for 3-7 attacks, and 6 patients were treated for 8-12 attacks. A single patient was treated for 13, 14, 15, 16, 17, or 18 attacks each. By dose level, 18 patients were treated with 5 mg/m^2 IV, 55 with 10 mg/m^2 IV, 9 with 20 mg/m^2 IV, and 31 with 30 mg SC. Correspondingly, 24 attacks were treated with 5 mg/m^2 IV, 141 with 10 mg/m^2 IV, 15 with 20 mg/m^2 IV, and 60 with 30 mg SC.

Adverse events

SAEs and deaths

No deaths occurred during the study. Nine patients reported HAE as an SAE. Other SAEs that were reported include the following: ovarian necrosis with abdominal adhesions (Day 25), pancreatitis (onset Day 2), and jaw fracture (Day 1 prior to attack). In addition, 2 patients with hypersensitivity drug reactions were reported as SAEs.

- Patient 2497 had pruritus, tingling, popular rash, flushing, nausea, dizziness, diaphoresis, and faintness during Treatment Episode 6 within 10 minutes of injection with ecallantide 30 mg SC. She was treated with diphenhydramine, IM epinephrine, IV hydrocortisone, cetirizine, and ranitidine. During the episode, her blood pressure decreased from a pre-dose baseline of 102/67 → 87/60 mmHg at 30 minutes post-dose. A serum tryptase level taken at 2 hours post-event was 2.7 ng/ml. The patient did not receive additional doses of ecallantide after the event.
- Patient 5499 developed flushing, hives, and conjunctival redness with tearing with 1 minute of 10mg/m^2 IV infusion for Treatment Episode 6. His heart rate increased from 120 → 172 bpm and blood pressure increased from 122/73 → 152/100 mmHg. The infusion was stopped prior to completion and patient was treated with diphenhydramine. The case narrative notes that serum tryptase levels were drawn but results are not reported. The patient subsequently received 2 additional doses of 30 mg SC in EDEMA2.

Reviewer's comment: Patient 2497's case qualifies as an anaphylactic event. Patient 5499's event is evocative of an allergic reaction but does not meet full criteria for anaphylaxis. The SAEs of HAE reported are likely a reflection of the underlying disease. Based on other efficacy data provided, it does not appear that ecallantide makes an acute attack worse although this possibility cannot be fully excluded. Of the other SAEs, the time courses reported make them less likely to be related to study drug with the exception of the case of pancreatitis. The case of pancreatitis occurred in a 16 year-old female patient with a comorbid diagnosis of lupus. This patient went on to receive 3 additional doses of SC ecallantide without incident.

Discontinuations due to AEs

There were no discontinuations due to AEs.

Common adverse events

A wide range of AEs were reported. The most frequently reported AEs included the following: GI disorders (nausea, diarrhea, abdominal pain, dyspepsia), fatigue, upper respiratory tract infection, and headache. Given the small sample sizes and the varying number of patients in each dosage levels, it is difficult to draw conclusions about specific AEs for particular dosage levels. For the same reason, it is also difficult to draw conclusions about possible dose relationships. Overall, the 30 mg SC dose appears comparable to the 10 mg/m2 and 20 mg/m2 IV doses in terms of proportion of patients reporting at least 1 AE (52%, 51%, and 44%, respectively). The 5 mg/m2 IV dose group appeared to have the smallest proportion (27.8%) of patients reporting at least 1 AE.

Administration site reactions
Eight patient reported local administration site reactions: 2 patients receiving ecallantide 10 mg/m2 IV and 6 patients who received ecallantide 30 mg SC. The reactions were characterized by local pain/soreness and burning. One patient who received a SC dose reported local pruritus as well.

Other allergic drug reactions
In addition to the 2 SAEs described above, a number of other AEs were reported by patients that were suggestive of a potential allergic drug reaction.

- Patient 0701 (2nd dose) reported pruritus and rash. Seronegative for antibodies to ecallantide and *P. pastoris*.
- Patient 1703 (2nd and 4th doses) reported generalized pruritus after the 2nd dose and localized urticaria on the left wrist after the 4th dose. The patient has since received 6 additional doses. Seronegative for antibodies to ecallantide and *P. pastoris*.
- Patient 1901 (13th dose) pruritus 7 hours after treatment. Patient has received multiple doses since the reported reaction.

10.3.3 Study summary and conclusions
EDEMA2 is generally supportive of ecallantide's efficacy for acute attacks of HAE and supports the selection of the 30 mg SC dose. The strength of the efficacy findings for repeat dosing are limited by two main factors: 1) the inclusion criteria (specifically, HAE diagnostic criteria) were not as rigorous as those specified in the Phase 3 program and could have potentially resulted in the inclusion of acquired angioedema (AAE) patients; and 2) the efficacy measurements were based on unvalidated symptom scores that were unrelated to the MSCS and TOS, limiting cross-study comparisons. As a result, although EDEMA2's results are positive, EDEMA2 remains a secondary study in terms of efficacy support. In terms of safety, the primary safety concern is anaphylaxis and other hypersensitivity reactions. Antibody status does not appear to be predictive of these reactions. Reactions on both repeat and first exposure were observed.

10.4Individual Study Report: EDEMA3

10.4.1 Study Protocol: EDEMA3

10.4.1.1 Administrative information

- Title: EDEMA3, Evaluation of DX-88's effects in mitigating angioedema: A double-blind, placebo-controlled study followed by a repeat dosing phase to assess the efficacy and safety of DX-88 (recombinant plasma kallikrein inhibitor) for the treatment of acute attacks of HAE
- Study sites: Multicenter – 25 sites in the US, Canada, Europe, and Israel
- Study dates: December 8, 2005 to February 10, 2007
- Study report date: May 23, 2008

10.4.1.2 Objectives/Rationale

- To assess the efficacy and safety of DX-88 (ecallantide) in the treatment of acute attacks of HAE

10.4.1.3 Study design overview

The study was a Phase 3, randomized, double-blind, placebo-controlled multicenter study. Patients 10 years of age and older presenting within 8 hours of onset of a moderate to severe HAE attack were randomized to receive a single dose of 30 mg SC ecallantide or placebo. Patients were stratified by anatomic attack location (laryngeal vs. other) or by prior enrollment in other ecallantide studies.

Patients were eligible to receive an additional open-label dose of ecallantide if the patient was at risk of *severe upper airway compromise* (SUAC) and the Investigator judged that additional treatment was warranted. Risk of SUAC was defined as the presence of ≥3 of the following 7 findings: appearance or worsening of dyspnea, appearance or worsening of stridor, increased respiratory effort, change or loss of voice, cyanosis, decreased oxygen saturation, or increased $PaCO_2$ and/ or decreased PaO_2.

Patients were observed for a minimum of 4 hours after dosing prior to discharge and up to 3 follow-up visits were scheduled. Total study duration was up to 97 days including screening, enrollment, and the follow-up visits. Alternatively, patients could roll over to the open-label extension (OLE) phase of the study after a minimum of 1 follow-up visit for treatment of new, separate HAE attacks. Once 72 patient treatments were completed in the double-blind part, the repeat dosing OLE was open to all patients regardless of prior participation in the double-blind part. The OLE repeat-dose phase is described separately in Section 10.4.3.

10.4.1.4 Study population

Patients 10 years or older with documented diagnosis of Type I or II HAE were eligible.

<u>Inclusion criteria</u>

- 10 years of age or older
- Documented diagnosis of Type I or II HAE:
    - Clinical history consistent with HAE (SC or mucosal nonpruritic swelling without accompanying urticaria)

  - Function or antigenic C1-INH level below the lower limit of the normal range or up to 15% above the lower limit of the normal range as defined by the reference laboratory
  - C4 level below the lower limit of the normal range or up to 15% above the lower limit of the normal range as defined by the reference laboratory
  - Age of reported onset ≤25 years or documented complement component C1q level at or above the lower limit of the normal range
- Enrollment visit: presentation at the site within 8 hours of patient recognition of an acute HAE attack with at least one moderate to severe symptom complex (patient and investigator must agree that at least one symptom complex is moderate or severe):
  - **Normal** – patient's state absent of an acute HAE attack
  - **Mild** – noticeable symptoms but do not impact activities of daily living
  - **Moderate** – treatment or intervention highly desirable and symptoms impact activities of daily living
  - **Severe** – treatment or intervention required due to inability to perform activities of daily living
- Sexually active and fertile patients required to use at least 2 methods of contraception for the duration of the study

<u>Exclusion criteria</u>

- Receipt of an investigational drug or device other than ecallantide within 30 days prior to study treatment
- Patients who received ecallantide within 7 days of presentation for dosing in the double-blind part of EDEMA3
- Treatment with non-investigationalC1-INH concentrate for angioedema within 7 days prior to enrollment
- Acquired angioedema, estrogen-dependent angioedema, and/or drug-induced angioedema
- Pregnancy or breastfeeding
- Any other condition that may compromise safety or compliance at the discretion of the investigator

Patients could withdraw from the study at any time at their own request or could be withdrawn at the discretion of the investigator or sponsor. Reasons for early withdrawal included adverse event, noncompliance or protocol violation, withdrawn consent, or termination of the study.

10.4.1.5 Study treatments

<u>Treatments administered</u>

- Initial dose
  - Single 30 mg ecallantide administered via 3 x 1cc SC injections to the upper arm, thigh, and abdomen. In the event that an injection site coincided with an attack site, multiple injections could be administered to the same site as long as the injections were separated by a minimum of 5cm.
  - Placebo SC in 3 separate 1 ml injections
- Additional dosing

- Open-label 30 mg ecallantide
- Standard of care

Blinding
Ecallantide and placebo are both clear colorless liquids and are indistinguishable by appearance. Vials were labeled with assigned codes corresponding to the randomization codes. A single statistician was unblinded to assigned study treatments; all other study personnel and patients remained blinded.

Randomization
Patients were randomized 1:1 to ecallantide or placebo. The randomization was stratified by anatomic location of the attack (laryngeal vs. others) as determined by the investigator and by prior participation in an ecallantide clinical study (patients may or may not have received ecallantide in the previous study). A third-party vendor provided a centralized web-based or telephone-based system for generation the randomization assignments to individual patients as they presented at the time of their attacks.

Prior and concomitant therapy
Receipt of certain medications was reason for exclusion, as noted above. The CRF was used to record any additional concomitant medications and emergency treatments administered, if any. Emergency treatments included opioid/pain medication, anti-emetics (5-HT3 receptor antagonists), and HAE alternative therapies, listed as follows:

- Aminocaproic acid
- C1-INH
- Fresh frozen plasma
- Tranexamic acid
- Methylprednisolone
- Oxandrolone
- Danazol
- Prednisone
- Stanozolol
- Dexamethasone
- Dehyroepiandrosterone
- Methyltestosterone

Treatment compliance
All study drugs were administered in clinic. Study drug accountability was verified during on-site monitoring visits conducted by the Sponsor.

10.4.1.6 Study procedures

Table 34 EDEMA3: Schedule of procedures

EDEMA3	Screen	Enroll	Post-dosing evaluation						Follow-up day		
									Visit 1	Visit 2	Visit 3
			Post-dosing (hr)				Discharge	Day 2*	6-10	23-37	83-97
			0-1.5	2	3	4					
Informed consent	X										
Urine pregnancy test	X	X									
History, demographics	X										
Physical exam	X	X					X		X	X	
Vital signs	X	X					X		X	X	
ECG	X	X		X					X	X	
Urinanalysis	X	X							X		
eDiary completion		X	X	X	X	X	X	X	X		
Symptom complex identification		X	X	X	X	X		X			
Assessment of overall response			X	X	X	X		X			
Symptom complex assessment*			X	X	X	X		X			
Severity assessment*		X				X					
Dosing		X									
Open-label DX-88 for incomplete response or relapse						X					
Dosing for severe upper respiratory compromise°			X	X	X	X					
Clinical observations			X	X	X	X	X				
Concomitant meds	X	X	X	X	X	X	X	X	X	X	
Adverse events	X	X	X	X	X	X	X	X	X	X	X
Blood samples											
•*Chemistry*	X	X					X		X	X	
•*CBC/diff*	X	X					X		X	X	
•*Coag panel*	X	X					X		X	X	
•*C1-INH level (if not done)*	X										
•*C4 (if not done before)*	X										
•*Antibody levels*	X	X							X	X	X

* Phone call
Source: dx-88-14db-csr-body.pdf, Section 9.1, Table 2

10.4.1.7 Efficacy parameters

Primary efficacy endpoint: Treatment Outcome Score (TOS) at 4 hours

The primary efficacy endpoint was the **Treatment Outcome Score (TOS)** at 4 hours post-dose in the ecallantide group versus placebo. The TOS is a patient-reported outcome (PRO) symptom-response outcome score:

$$TOS = \frac{\sum symptom\ complex\ score \times symptom\ complex\ weight}{\sum symptom\ complex\ weight}$$

In this equation, symptom complex score = response assessment and symptom complex weight = symptom complex severity at baseline. The TOS has the following components:

- Symptom complex identification. The following symptom complexes were assessed:
  - internal head/neck
  - stomach/GI
  - genital/buttocks
  - external head/neck
  - cutaneous
- Severity assessment of each symptom complex at baseline ("symptom complex weight"); see severity definitions used for MSCS calculation
  - Severe = 3
  - Moderate = 2
  - Mild = 1
  - Normal = 0
- Response assessment of each symptom complex post-dose ("symptom complex score")
  - Significant improvement = 100, " a lot better or resolved"
  - Improvement = 50, "a little better"
  - Same = 0,
  - Worsening = -50, "a little worse"
  - Significant worsening = -100, "a lot worse"

A higher TOS value corresponded to a greater response. Emerging symptom complexes were weighted according to their peak severity assessment and if still present at 4 and/or 24 hours were assigned a response assessment of "significant worsening" (i.e. -100). Emerging symptom complexes that were no longer present at 4 and/or 24 hours were assigned an assessment of "same." Medical interventions that were clearly directed to a specific symptom complex only affected that particular symptom complex response (e.g. anti-nausea medications would be regarded as "significant worsening" for the GI/abdominal complex but would not affect the Cutaneous response assessment). Medical interventions that were not clearly directed to a specific symptom complex affected all symptom complexes.

A sensitivity analysis was performed setting the symptom complex weights to "1" to assess the robustness of the baseline weighting of the severity symptoms used for calculating TOS

<u>Secondary efficacy endpoints</u>

- Change in Mean Symptom Complex Severity (MSCS) at 4 hours
  - The MSCS is the arithmetic mean of the severity grade of the individual symptom complexes, where each symptom complex is assessed a severity grade of severe to normal. A decrease from baseline MSCS corresponds to a reduction in severity.
  - A baseline severity assessment for emerging symptom complexes were considered "normal."

- Medical interventions resulted in an automatic severity assessment of "severe" at 4 and 24 hours. Medical interventions that were clearly directed to a specific symptom complex only affected that particular symptom complex response. Medical interventions that were not clearly directed to a specific symptom complex affected all symptom complexes.
- The use of open-label ecallantide for SUAC resulted in a severity assessment of "severe" at 4 and 24 hours.

Table 35 Severity assessment for MSCS calculation

Severity Assessment	Score	Definition
Severe	3	treatment or intervention required due to inability to perform activities of daily living (e.g. throat swollen/difficulty breathing, lips swollen/cannot eat, feet swollen/cannot walk)
Moderate	2	treatment or intervention highly desirable and symptoms impact activities of daily living (e.g. hands swollen/cannot button shirt, feet swollen/discomfort wearing shoes)
Mild	1	noticeable symptoms but do not impact activities of daily living
Normal	0	patient's state absent of an acute HAE attack

- Time to onset of significant improvement in overall response
  - Based on "overall response" assessment
  - "Significant improvement" defined as an overall response assessment of "a lot better or resolved"
  - Patients who did not report significant improvement through 4 hours post-dosing were censored at 240 minutes
  - Patients that received additional therapy were censored at the time of medical intervention

<u>Tertiary efficacy endpoints</u>

- Durability of response/TOS at 24 hours post-dosing
- TOS at 4 hours as determined by the investigator
- Proportion of responders at 4 hours
  - TOS ≥70
- Time to onset of sustained improvement
  - Sustained response defined as any positive overall response assessment for a continuous duration ≥45 minutes
- Proportion of patients receiving medical intervention
- Assessment of open-label treatment with ecallantide for SUAC
- Change in clinical laboratory measures

10.4.1.8 Safety parameters

<u>Adverse events</u>

All AEs were reported from enrollment (Study day 1) through the conclusion of follow-up visit 2. Any AEs that were suspected to be related to study procedures were reported from time of informed consent through enrollment and from follow-up visit 2 to 3. Investigators used NCI CTC criteria for grading AE severity. AE coding was performed using the MedDRA coding dictionary (Version 6.0).

Physical exam
Routine exams were conducted at screening and/or enrollment prior to dosing, study discharge, follow-up visits 1 and 2.

Vital signs
Body temperature, heart rate, blood pressure, and weight were recorded at screening and/or enrollment prior to dosing, study discharge, follow-up visits 1 and 2.

Electrocardiogram
A 12-lead ECG was obtained for each patient at screening, enrollment prior to dosing, 2 hours post-dose, follow-up visits 1 and 2. In situations where an ECG could not be taken immediately due to the severity of the patient's attack site, the ECG screening from baseline was used as the baseline.

Clinical laboratory parameters
A CBC with differential and platelet count, serum chemistries, and coagulation tests were obtained at screening and/or enrollment prior to dosing, study discharge, follow-up visits 1 and 2. A routine urinanalysis was obtained at screening and/or enrollment prior to dosing, study discharge, and follow-up visit 1. A urine pregnancy test was performed at screening and at enrollment.

Antibody testing
Samples for serum antibody testing were collected at screening and/or enrollment prior to dosing, follow-up visits 1 (Study day 6 to 10), 2 (Study day 23 to 37), and 3 (Study day 83 to 97). Antibody testing was performed for detection of development of IgE and non-IgE antibodies to ecallantide and IgE antibodies to *P. pastoris*.

10.4.1.9 Statistical plan
The sample size of 62 was calculated to provide 85% power, based on the assumption that 72.5% of ecallantide patients would have significant improvement by 4 hours compared to 25% of placebo. The sample size was later increased to 72 to ensure a sufficient number of patients used the eDiary to aid the validation of the PRO measures.

All analyses were based on the intent-to-treat population. Additional analyses based on the per-protocol population and as-treated populations were also performed for comparison. The as-treated population analysis was performed because after conclusion of the study, the Applicant discovered that 2 patients were randomized on the same day at the same study center and received incorrect treatment. One patient randomized to receive ecallantide received placebo instead and the second patient assigned to placebo received ecallantide.

The primary and secondary efficacy analyses on TOS at 4 hours and change from baseline MSCS were performed using a Wilcoxon Rank Sum Test, assuming a non-normal distribution of results. Imputations were used for emerging symptom complexes and medical interventions in the primary analysis. Demographic data and safety data were presented using descriptive statistics.

Reviewer's comment: The imputation rules were intended for a conservative measure of the TOS and MSCS. The statistical reviewer has noted that the imputations favor study drug if there are more emerging symptom complexes or medical interventions in the placebo arm. However, the clinical review notes that this statistical result would be consistent with the clinical interpretation of a greater number of emerging symptom complexes and medical interventions in the placebo arm, i.e. ecallantide is more efficacious than placebo.

10.4.2 Results

10.4.1.1 Protocol amendments

- Amendment 1, September 26, 2006 – increased the sample size from 62 to 72 patients to facilitate PRO validation and changed the statistical analysis of the primary efficacy endpoint to a more conservative test, the Wilcoxon Signed Rank test.
- Amendments 1.1 (June 18, 2007) and 1.2 (July 17, 2007) – updated administrative information (personnel and address changes).

10.4.2.2 Study patient disposition

Seventy-two patients were randomized; 36 in the ecallantide arm and 36 in the placebo arm. Patient 361004 was not included in the per-protocol population due to an eDiary malfunction that prevented completion of the baseline and 4-hour post-dose assessment. The disposition of the patients is shown in Table 48.

Table 36 EDEMA 3: Patient disposition

	Ecallantide N=36 N(%)	Placebo N=36 N (%)	Total N=72 N (%)
Intent to treat population[a]	36 (100.0)	36 (100.0)	72 (100.0)
Per protocol population[b]	35 (97.2)	36 (100.0)	71 (98.6)
Safety population[c]	36 (100.0)	36 (100.0)	72 (100.0)
Patients completing double-blind phase	35 (97.2)	36 (100.0)	71 (98.6)
Patients rolling over to continuation study[d]	21 (58.3))	27 (75.0)	48 (66.7)
Patients withdrawing from study	1 (2.8)	0	1 (1.4)
Adverse event	0	0	0
Noncompliance or protocol violation	0	0	0
Withdrawal of consent	0	0	0
Lost to follow-up	1 (2.8)	0	1 (1.4)
Investigator discretion	0	0	0
Left study site against medical advice	0	0	0

[a] Patients who received any amount of study drug and completed the 4-hour follow-up

[b] Patients who received a complete dose of study drug with no major protocol violations and completed the 4-hour follow-up

[c] Patients who received any amount of study drug
[d] All patients were eligible to enroll in the open-label extension study.
Source: dx-88-14b-csr-body.pdf, Section 10.1, Table 3

10.4.2.3 Protocol deviations
A complete listing is provided in Appendix 16.2.2.1.0 of the full study report. The major protocol deviation was the administration of incorrect study medication to two patients, as described in Section 10.4.1.9. There were also deviations related to study entry criteria: 1 patient failed to have a pregnancy test at screening and 2 patients had C1-INH levels verified post-dose rather than prior to treatment. Other protocol deviations related to the use of a paper diary rather than eDiary was reported for 8 patients.

Reviewer's comment: The incorrect administration of study treatment appears to have impacted the study results. The Applicant has provided an alternative as-treated analysis to demonstrate that statistically significant results would be achieved if these two patients were included in the analysis under the received rather than assigned treatment group. The other protocol violations do not seem likely to have impacted the overall results, although given the small sample size, such effects cannot be ruled out.

10.4.2.4 Treatment compliance
All study drug was administered subcutaneously by study personnel.

10.4.2.5 Datasets analyzed
As described in the Statistical Analysis section, 3 populations were analyzed: ITT-as-randomized, ITT-as-treated, and Per Protocol.

10.4.2.6 Demographics and baseline characteristics

<u>Patient demographics</u>
The demographics at baseline are shown in Table 6. A higher proportion of patients in the placebo group (11 of 36, 31%) had received prior treatment with ecallantide in previous studies compared to the ecallantide group (8 of 36; 22%). The majority of patients with prior exposure were treated in EDEMA2 with open-label ecallantide.

Table 37 EDEMA3: Patient demographics			
	Ecallantide N=36	**Placebo N=36**	**Total N=72**
Age			
Mean (SD)	38.5 (14.6)	32.2 (13.8)	35.4 (14.5)
Range	18-77	11-57	13-77
Sex (N,%)			
Male	12 (33.3)	13 (36.1)	25 (34.7)
Female	24 (66.7)	23 (63.9)	47 (65.3)
Race (N,%)			
White	33 (91.7)	32 (88.9)	65 (90.3)
Black	1 (2.8)	4 (11.1)	5 (6.9)
Hispanic	2 (5.6)	0	2 (2.8)

Source: dx-88-14db-csr-body.pdf, Section 11.2.1, Table 4

Reviewer's comment: The treatment groups appear comparable in terms of age and racial distribution, but the ecallantide group has a higher number of female patients compared to the placebo arm. The significance of this gender imbalance is uncertain as HAE occurs in males and females at the same rate. The difference in prior exposure to ecallantide is not likely to have weighted the treatment group with more responders, since a greater number of patients in the placebo group had a history of ecallantide exposure.

Patient HAE history

Table 38 EDEMA3: HAE attack history		
	Ecallantide N=36	**Placebo N=36**
Age at first HAE symptom onset		
Mean (SD)	12.1 (6.5)	10.3 (6.9)
Range	1-32	1-25
Lowest historical functional C1-INH		
Mean % (SD	18.7 (20.4)	22.8 (24.8)
Range	0-59	0-97
Lowest historical antigenic C1-INH, mg/dl		
Mean (SD)	22.4 (24.0)	18.4 (21.8)
Range	3-79	0-80
Lowest historical C4, mg/dl		
Mean (SD)	10.6 (12.9)	9.9 (13.5)
Range	0-55	0-56
Most common prior HAE symptom complex (N,%)		
Laryngeal	3 (8.3)	2 (5.6)
Stomach/GI	22 (61.1)	21 (58.3)
Genital/buttocks	4 (11.1)	1 (2.8)
External head and neck	3 (8.3)	0
Cutaneous	20 (55.6)	17 (4.2)

Source: dx-14db-csr-body.pdf, Section 11.2.3 Table 6 and 7

Reviewer's comment: The treatment groups appear fairly comparable in terms of age of onset, historical laboratory values, and prior attack site history. In terms of historical function C1-INH levels, the range in the placebo group is as high as 97%, which would be well within normal.

However, upon review of individual line listings, patients with a functional level within the normal range appeared to have documented antigenic levels below normal. The range of normal for antigenic levels varies by reference laboratory. For most labs, the upper cutoff for normal antigenic level is ~40-50 mg/dl.

Previous and concomitant medications

The majority of patients had taken androgens as prior prophylactic therapy for HAE: danazol and stanozolol in 58.3% and 47.2% in the ecallantide group compared to 38.9% and 33.3%, respectively, in the placebo group. Aminocaproic acid, fresh frozen plasma, diphenhydramine, C1-inhibitor replacement, prednisone, and hydroxyzine were also reported by several patients as commonly used acute treatments in the past.

At screening, all patients reported taking concomitant medications. The most commonly listed medication was danazol (11 of 36 in the ecallantide arm, 5 of 36 in the placebo arm). Other commonly used medications included stanozolol, systemic antihistamines, acetaminophen, levothyroxine, lorazepam, and ibuprofen.

Reviewer's comment: There appears to have been an imbalance in the number of patients taking danazol between the two treatment arms. The impact of this discrepancy is unclear, although the severity of presenting attacks appears comparable between the two groups.

Presenting symptom complex severity

Each randomized patient presented with at least one symptom complex that was moderate to severe. Patients could report multiple symptom complexes. The most commonly reported symptom complexes in the ecallantide group were cutaneous (n=21) and stomach/GI (n=20). In the placebo group, 14 patients reported cutaneous symptoms and 21 reported stomach/GI symptoms. Laryngeal attacks were reported in 9 ecallantide patients and 4 placebo patients. The patient-reported severity of the symptom complexes is displayed below.

Table 39 EDEMA3: Severity of symptom complexes at baseline		
	Ecallantide N=36 N, %	**Placebo N=36 N, %**
Internal head/neck symptoms (including laryngeal)		
Mild	1 (2.8)	1 (2.8)
Moderate	7 (19.4)	1 (2.8)
Severe	1 (2.8)	2 (5.6)
Stomach/GI		
Mild	1 (2.8)	1 (2.8)
Moderate	14 (38.9)	13 (36.1)
Severe	5 (13.9)	7 (19.4)
Genital/buttocks		
Mild	0	0
Moderate	1 (2.8)	3 (8.3)
Severe	1 (2.8)	1 (2.8)
External head/neck		
Mild	2 (5.6)	3 (8.3)
Moderate	1 (2.8)	3 (8.3)
Severe	1 (2.8)	3 (8.3)
Cutaneous		
Mild	4 (11.1)	1 (2.8)
Moderate	13 (36.1)	11 (30.6)
Severe	4 (11.1)	2 (5.6)

Source: dc-88-14db-csr-body.pdf, Section 11.2.5, Table 13

Reviewer's comment: The distribution of symptom complexes and severity at baseline appears comparable between the two treatment arms.

10.4.2.7 Efficacy endpoint outcomes

Primary efficacy endpoint: TOS at 4 hours

Based on the pre-specified analysis, the study failed to demonstrate a statistically significant difference between ecallantide and placebo. Numerically, the results favored ecallantide over placebo. When re-analyzed used the as-treated population, the results show a statistically significant benefit for ecallantide over placebo. The Per Protocol results confirm the As-Treated results.

Table 40 EDEMA3: TOS at 4 hours						
Statistic	**ITT-as-randomized**			**ITT-as-treated**		
	Ecallantide N=36	**Placebo N=36**	**P**	**Ecallantide N=36**	**Placebo N=36**	**P**
Mean	46.8	21.3	0.100	49.5	18.5	0.037
Median	50.0	0		50.0	0	
Std Dev	59.3	69.0		59.43	67.8	
Min, Max	(-100, 100)	(-100,100)		(-100,100)	(-100,100)	
25th, 75th	(0,100)	(0, 100)		(0,100)	(0, 100	

Source: dx-88-14db-csr-body.pdf, Section 11.4.1, Table 14

Reviewer's comment: The success of the study is altered by the dosing mistake described in Protocol Deviations, where two patients erroneously received the wrong medication. These

results suggest that ecallantide has some efficacy, although the results do not appear to be robust and the limitations of a small sample size are apparent. In regards to the primary efficacy variable, the numerical value of the TOS is difficult to understand. While a positive value denotes improvement, the multipliers included in the formula are not intuitive. For example, the clinical relevance of a difference between a mean value of 46.8 and 21.3 is unclear. Also, the standard deviations appear to be quite large, suggesting a fair amount of variability in the dataset.

Secondary efficacy endpoints

Change in MSCS from Baseline
Although numerically favorable, the study did not show a statistically significant benefit for ecallantide over placebo for the efficacy endpoint, change in MSCS from baseline at 4 hours. When reanalyzed using the as-treated population, the results are statistically significant (-0.9 vs. -0.48; p=0.044).

Table 41 EDEMA3: Primary efficacy endpoint, Change from baseline MSCS at 4 hours post-dose

	Baseline MSCS	Change from baseline at 4h	P
Ecallantide (N=36)	2.2 (0.5)	-0.9 (1.1)	0.09
Placebo (N=36)	2.3 (1.0)	-0.5 (0.7)	

Time to Significant Improvement
Based on patients' overall response assessments, the median time to significant improvement was 165.0 minutes for ecallantide. The estimated median for placebo was not reached by 240 minutes, but the difference was not statistically significant (p=0.136). The results were not altered using the as-treated dataset, but were statistically significant in favor of ecallantide when based on the per protocol dataset (p=0.045).

Tertiary efficacy endpoints

TOS at 24 hours post-dosing
The median (IQR) TOS at 24 hours postdose was 75.0 (0, 100) in the ecallantide group compared to 0 (-100,100) in the placebo group (p=0.044).

Reviewer's comment: The TOS at 24 hours supports a durable improvement in symptoms.

Change in MSCS from baseline at 24 hours
The mean change in MSCS at 24 hours was -0.87 (SD 1.0) in the ecallantide group and -0.46 (SD 1.1) in the placebo group (p=0.142). Similar results were obtained for the as-treated population analysis.

Reviewer's comment: These results are comparable with the change from MSCS observed at 4 hours post-dose. While numerically favorable, the results are not statistically significant.

Time to onset of sustained improvement in overall response
The mean time to onset of sustained improvement was 79 minutes in the ecallantide group and 113 minutes in the placebo group (p=0.075). When assessed using the as-treated population, the mean times are 77 and 116 minutes, respectively (p=0.023).

Proportion of successful response assessment at 4 hours post-dosing (TOS≥70)
Fifteen patients (42%) in the ecallantide group compared to 12 (33.3%) in the placebo group had a TOS≥70 at 4 hours (p=0.47). No statistically significant differences were observed when adjusted for attack location or prior use of ecallantide.

Proportion of patients receiving medical intervention
Five patients (14%) in the ecallantide group compared to 13 (36%) of placebo received medical intervention. The most commonly administered interventions were emergency medications such as opioids for pain control and anti-emetics. No patients required intubation or urgent surgical decompression. In both treatment groups, fewer patients with peripheral attacks required intervention than patients with a laryngeal attack (p=0.014).

Open-label experience due to SUAC
One patient (311016) in the placebo group and 2 patients (326012 and 361004) in the ecallantide group received open-label ecallantide for SUAC that occurred soon after dosing with the randomized study drug.

- Patient 311016 initially presented with laryngeal edema and reported worsening dyspnea, increased respiration, and change/loss voice almost immediately after receipt of placebo. Within 15 minutes of receipt of open-label ecallantide, she reported symptoms as "a little better." Her symptom assessment remained "a little better" up to 4 hours post-dose. At 24 hours, she reported symptoms as "a lot better or resolved" along with self-administration of diphenhydramine and epinephrine SC for the attack.
- Patient 326012 presented with mild external head/neck symptoms and moderate internal head/neck symptoms. She did not report any symptom improvement 45 minutes after the initial dose and subsequently developed appearance or worsening of stridor, change/loss of voice, and increased respiratory effort. Thirty minutes after the second, open-label SUAC dose, the patient reported symptoms as "a lot better or resolved." No other medical interventions were recorded.
- Patient 361004 presented with laryngeal edema. At 1 hour 45 minutes after the initial ecallantide dose, the patient reported symptoms as "a little worse." Thirty minutes after receipt of a second, open-label dose, the symptoms were reported as "a little better." An updated overall response assessment at 24 hours was not recorded for this patient but per the case narrative, the patient had recovered without sequelae by that time.

10.4.2.8 Safety outcomes

<u>Extent of exposure</u>
A total of 36 patients received one 30 mg dose of ecallantide. Two of these 36 received a second 30 mg dose for SUAC. One placebo patient also received an open-label 30 mg dose for SUAC.

Adverse events

Deaths and serious adverse events (SAE)
No deaths were reported in the study. Three cases of HAE in the ecallantide arm and 2 cases in placebo were reported as SAEs.

- Patient 322002 (ecallantide) was hospitalized for an acute HAE attack of peripheral edema 4 days after treatment with ecallantide for a separate abdominal HAE attack. The patient was discharged without sequelae.
- Patient 334001 (ecallantide) initially presented with laryngeal edema and was treated with ecallantide before being hospitalized later that same day for a GI HAE attack. The patient was discharged without sequelae.
- Patient 361004 (ecallantide) was treated at 9:40 am for laryngeal edema. The patient was later hospitalized that same day for SUAC and treated with a second ecallantide dose at 12:06pm. The patient was discharged the next day and recovered without sequelae.
- Patient 304004 (placebo) was hospitalized for an acute peripheral HAE attack of the right hand 6 days after receipt of placebo for an acute external head/neck HAE attack. The patient was discharged the next day without sequelae.
- Patient 326003 (placebo) was hospitalized with an acute stomach/GI HAE attack 1 day after treatment with placebo for an acute stomach/GI attack. The patient was treated with normal saline, ketorolac, and ondansetron and recovered without sequelae.

Study discontinuation due to AE
No early discontinuations from the study due to an AE were reported.

Common adverse events
The most common adverse events are shown in Table 42. HAE was reported as an AE in 3 patients in the ecallantide arm and 4 patients in the placebo arm. Local injection site reactions were reported in 1 patient in the ecallantide group and 1 patient in the placebo group.

Table 42 EDEMA3: Adverse events occurring in ≥2 patients in the ecallantide group and greater than in the placebo group

Adverse event	Ecallantide N=36 N,%	Placebo N=36 N,%
Patients with 1 or more AEs	20 (55.6)	12 (33.3)
Patients with no AEs	16 (44.4)	24 (66.7)
Headache	4 (11.1)	2 (5.6)
Diarrhea	3 (8.3)	0
Pyrexia	3 (8.3)	0
Nasopharyngitis	2 (5.6)	1 (2.8)
Nasal congestion	2 (5.6)	0
Tachycardia NOS	2 (5.6)	1 (2.8)

Reviewer's comment: The overall AE event profile appears consistent with AEs reported in previous trials and in EDEMA4. No major bleeding or thrombotic events were reported as AEs

during the DB phase. No anaphylaxis was reported in the DB phase, but this AE was observed in the OLE and is described in Section 10.4.3.

Laboratory evaluations
No clinically significant alterations in mean routine laboratory tests, including coagulation parameters, were reported for either treatment group. Two patients in the ecallantide group had a transient rise in thrombin time at 4 hours. One ecallantide patient also experienced anemia 3 days after dosing but was reported as recovered 1 week later. Another ecallantide patient was reported as having a blood glucose level of 26 mg/dl (normal 70 -115 mg/dl) at 4 hours post-dose. The hypoglycemia resolved and values within normal range were reported at follow-up visits.

Antibody testing
No IgE antibodies to ecallantide were detected. Two patients with prior exposure to ecallantide tested positive for non-IgE ecallantide antibodies prior to dosing in EDEMA 3 and also at Follow-up Visit 1. Both patients were reported as having improved symptoms as measured by the TOS at 4 hours.

Seven ecallantide patients and 4 placebo patients tested positive for IgE antibodies to *P. pastoris.* No hypersensitivity reactions were reported in these 11 patients.

Reviewer's comment: The study duration for the double-blind phase of EDEMA3 was up to 97 days in duration if patients completed all follow-up visits. Additional antibody information was collected from patients who rolled over to the open-label extension phase, so not all patient data is represented from the double-blind phase alone.

Vital signs
No clinically significant alterations in mean vital signs were observed in either treatment group.

Tachycardia NOS was noted in two patients in the ecallantide group. Patient 301008 had a baseline heart rate of 124 bpm → 110 bpm at 2 hours post-dose → 76 bpm at the first follow-up visit. Patient 313003 had a baseline heart rate of 101 bpm → 105 bpm at 2 hours post-dose → 60 bpm at first follow-up.

Three patients were recorded as having pyrexia. Patient 305001 reported a fever 1 day after ecallantide that resolved with a 325 mg dose of aspirin. Patient 317002 also reported a fever 1 day after ecallantide that resolved with 650 mg acetaminophen and acetaminophen/codeine. Patient 318002 was recorded as being febrile 2 hours after ecallantide. The patient recovered after 1000mg acetaminophen. The patient also reported an influenza-like illness and fatigue.

Reviewer's comment: The tachycardia does not appear to be treatment-related, given the documentation prior to ecallantide administration. Fever may potentially be related given the time course and absence of other evident fever sources.

Physical exams

The majority of physical exam findings reported were signs and symptoms related to the presenting HAE attack. No notable abnormalities were otherwise reported.

ECGs
No mean changes in ECG parameters were recorded for either treatment group. Both tachycardia and bradycardia were observed in several individuals. Patient 315003 was noted to have sinus bradycardia at screening of 54 bpm → 47 bpm at 2 hours post-dose. No follow-up information is available on this patient.

Reviewer's comment: Overall, the safety profile for ecallantide in EDEMA3 appears acceptable. No SAEs were recorded besides HAE, which most likely reflects the underlying condition since more patients in the placebo group were noted to have this HAE. Hypersensitivity reactions remain a concern for this biologic product, although the rate of events would be expected to be quite low in a single-dose study. The open-label phase with repeat doses is more likely to yield information on antibody responses and hypersensitivity reactions.

10.4.3 EDEMA3 Open-label extension study
10.4.3.1 Administrative information

- Study period: December 28, 2005 (first patient began treatment in repeat-dose phase) to September 21, 2007 (last patient completed)
- Study report date: August 6, 2008
- Study sites: multicenter, 24 sites in the US, Canada, Belgium, Italy, and Israel

10.4.3.2 Study design and conduct
Patients previously enrolled in the double-blind phase of EDEMA3 could enroll in the open-label phase once the Follow-up Visit 1 had been completed. Once the double-blind phase was closed, all patients who had qualified were eligible for participating in the repeat-dosing open-label phase. Patients 10 years and older with new attacks were eligible for repeat doses in this phase. A new attack was defined as an HAE attack that presented after a return to normal state following a previous acute attack. Patients were required to present to the study site within 8 hours of onset of an acute attack with the same symptom complexes outlined in the double-blind phase. Qualified patients received 30 mg ecallantide SC. If patients had an incomplete response to treatment, Dose B of study drug could be given anytime from 4 hours through 24 hours post-dosing. Dose B consisted of randomized study drug (1:1 ecallantide:placebo). Incomplete response was defined as a reoccurrence of an attack between 4 and 24 hours after initial improvement after dosing or as not achieving "significant improvement" within 4 hours following some improvement after dosing. Patients who showed no response to the initial dose of ecallantide were not eligible for Dose B treatment with study drug. After administration of study drug, patients were discharged at 4 hours post-dose with 1 follow-up phone call and up to 3 planned follow-up visits at Days 6-10, 23-47, and 83-97 after treatment. Patients could be treated for a maximum of 20 attacks at an interval of 8 days or more.

The TOS and MSCS were recorded as efficacy variables. Safety was assessed through AEs, laboratory test evaluations, physical exams, ECGs, antibodies to ecallantide and *P. pastoris*, and

vital signs. Antibody testing was performed at screening if not done during the double-blind phase, enrollment, and at each follow-up visit.

10.4.3.3 Results

Patient disposition

From the double-blind phase, 22 ecallantide and 26 placebo patients received at least 1 dose of ecallantide in the OLE phase. Another 19 new patients also joined the study, for a total of 67 patients in the safety population. One new patient (365004) was excluded from the ITT dataset due to missing data at the 4-hour post-dose assessment. Three patients (4.5%) had an incomplete response and received blinded Dose B. Of the 3, 1 patient received ecallantide and 2 patients received placebo. Patient 301002 withdrew due to an AE of lymphoproliferative disorder. Another patient, Patient 305001 experienced an anaphylactic reaction during Treatment Episode 7 and did not receive further medication but was not formally withdrawn from the study.

Table 43 EDEMA 3 OLE: Patient disposition

	Ecallantide N=22 N (%)	Placebo N=26 N (%)	New patients N=19 N (%)	Total N=72 N (%)
Intent to treat population[a]	22 (100.0)	26 (100.0)	18 (94.7)	66 (98.5)
Per protocol population[b]	21 (95.5)	26 (100.0)	18 (94.7)	65 (97.0)
Safety population[c]	22 (100.0)	26 (100.0)	19 (100.0)	67 (100.0)
Patients receiving Dose B	1 (4.5)	1 (3.8)	1 (5.4)	3 (4.5)
Patients withdrawing from study	4 (18.2)	1 (3.8)	5 (26.3)	10 (14.9)
Adverse event	1 (4.5)	0	0	1 (1.5)
Lost to follow-up	2 (9.1)	0	2 (10.5)	4 (6.0)
Voluntary withdrawal	0	1 (3.8)	1 (5.3)	2 (3.0)
*Other**	1 (4.5)	0	2 (10.5)	3 (.5)

Source: dx-88-14rd-csr-body.pdf, Section 10.1, Table 3
[a] Patients who received any amount of study drug
[b] Patients who received a complete dose of study drug with no major protocol violations
[c] Patients who received any amount of study drug
* 1 patient enrolled in EDEMA4; 2 other patients withdrawn due to Sponsor's decision to discontinue the study.

Patient exposure

In addition to 22 patients from the ecallantide arm in the double-blind phase and 1 patient in the placebo arm that received ecallantide for SUAC, 17 patients (25.8%) had had prior exposure to ecallantide as part of EDEMA1 (n=4) and EDEMA2 (n=15). A total of 160 attacks were treated during the OLE. The majority of patients were treated for 1 attack during the OLE; 1 patient was treated for 13 attacks. The exposure is summarized in Table 44.

Table 44 EDEMA3 OLE: Patient exposure

HAE attack number	Ecallantide N=22 N (%)	Placebo N=26 N (%)	New patients N=19 N (%)	Total N=72 N (%)
1*	0	0	18 (100.0)	18 (27.3)
2	22 (100.0)	26 (100.0)	3 (16.7)	51 (77.3)
3	13 (59.1)	17 (65.4)	0	30 (45.5)
4	6 (27.3)	15 (57.7)	0	21 (31.8)
5	5 (22.7)	6 (23.1)	0	11 (16.7)
6	4 (18.2)	5 (19.2)	0	9 (13.6)
7	2 (9.1)	1 (3.8)	0	3 (4.5)
8	1 (4.5)	0	0	1 (1.5)
9	2 (9.1)	1 (3.8)	0	3 (4.5)
10	0	1 (3.8)	0	1 (1.5)
11	1 (4.5)	1 (3.8)	0	2 (3.0)
12	1 (4.5)	0	0	1 (1.5)
13	1 (4.5)	0	0	1 (1.5)
14	1 (4.5)	0	0	1 (1.5)

* Includes attack treated during the double-blind phase
Source: dx-88-14rd-csr-body.pdf, Section 10.1, Table 4

Sixty-five of 153 treated attacks in the ITT population involved multiple symptom complexes. Thirty-three attacks had laryngeal involvement. The Applicant reports heterogeneity in individual patients, both in attack site and in severity, from one attack to the next.

Reviewer's comment: The repeat exposure data is limited, given that the number of patients who received more than 2 doses total is so few. The OLE was almost 2 years in duration. While enrollment was ongoing and not all patients were in the study for the entire duration, it is still somewhat surprising that the patients did not present for treatment more frequently. Moderate-to-severe qualifying attacks may have been relatively infrequent. Alternatively, patients may have sought treatment elsewhere for subsequent attacks. The observation of heterogeneity in attack site and severity is consistent with other reports in the literature.

Demographics

The participants in the OLE phase were comparable in terms of age, gender, ethnicity, and HAE history to those patients in the double-blind phase. The OLE included 11 patients who were ≤18 years of age and 7 patients ≤16 years of age.

Efficacy results

The TOS at 4 hours and the change in MSCS from baseline at 4 hours varied by treatment episode. The first treatment episode only includes new patients who did not participate in the double-blind phase. The following tables summarize these results.

Table 45 EDEMA3 OLE: TOS at 4 hours by treatment episode

Treatment episode	N	Median (IQR)	Mean (SD)
1	18	68.8 (50, 100)	71.3 (28.9)
2	51	100 (50, 100)	73.3 (44.9)
3	30	100 (70, 100)	81.9 (28.5)
4	21	100 (38, 100)	81.2 (24.5)
5	11	100 (0, 100)	48.5 (68.5)
6	9	60 (50, 100)	60.4 (49.3)

Source: dx-88-14rd-csr-body.pdf, Section 11.4.1.1, Table 15

Change in MSCS at 4 hours

Table 46 EDEMA3 OLE: Mean change in MSCS at 4 hours by treatment episode

Treatment episode	N	Median (IQR)	Mean (SD)
1	17	-1.0 (-1.5, -1.0)	-1.2 (0.9)
2	51	-1.0 (-1.8, -0.5)	-1.1 (0.9)
3	30	-1.0 (-2.0, -1.0)	-1.3 (0.9)
4	21	-2.0 (-2.0, -1.0)	-1.4 (0.8)
5	11	-1.0 (-1.3, 0)	-0.9 (0.7)
6	9	-1.0 (-1.0, -0.3)	-0.9 (0.8)

Source: dx-88-14rd-csr-body.pdf, Section 11.4.1.1, Table 16

Only 3 patients received Dose B, limiting analysis. Of the 2 patients who received placebo as Dose B, both patients reported symptoms to be "a lot better or resolved" at the 4- and 24-hour assessments. The third patient who received ecallantide as Dose B reported symptoms to be the "same" and did not receive further treatment in the study.

Reviewer's comment: The TOS values suggest efficacy over repeated doses, although the number of patients upon which the TOS is based decreases with each episode. This may be a function of the underlying rate of attacks; alternatively, these results could be due to self-selection of responders vs. non-responders. The MSCS scores appear consistent with the TOS, which is expected as the MSCS is included in the calculation of the TOS. In the absence of a control, these results are difficult to interpret as the natural course of an HAE attack is gradual improvement. Numerically, the MSCS results appear comparable to those observed for the ecallantide arm in the double-blind phase.

Safety endpoints

Common adverse events

Overall, 40 patients (59.7%) reported at least 1 AE during the OLE. Similar AEs as those observed during the double-blind phase were reported. The most common events included headache (n=10), nausea (n=6), HAE (n=6), URI NOS (n=6), and nasopharyngitis (n=5). There was no clear correlation between the nature or frequency of these events with treatment episode. The majority of AEs were reported by 1 patient each.

SAEs

Seven patients reported a total of 18 SAEs, including Patient 305001 who reported a total of 9 SAEs and subsequently withdrew from the treatment episode and did not receive further treatment in the study.

- Patient 305001 experienced anaphylaxis during her 7th treatment episode. Following the event, the patient skin tested positive to ecallantide. She underwent a rechallenge procedure with 1 mg ecallantide SC and developed pharyngeal edema, hypoxia, dyspnea, generalized rash, urinary incontinence, vomiting, anxiety/sense of impending doom, and diarrhea. The patient received 2 doses of epinephrine and was observed in a hospital emergency room for an additional 5 hours prior to discharge home. The patient tested positive for non-IgE antibodies to ecallantide and IgE antibodies to *P. pastoris.* Patient 305001 was previously enrolled in EDEMA2 and had received 13 injections of ecallantide for 12 separate HAE attacks.
- Patient 301002 discontinued from the study due to a diagnosis of lymphoproliferative disease made 16 days after the second follow-up visit for the 11th treatment episode.
- The other SAEs included concussion and laceration sustained during a motor vehicle accident, infectious diarrhea with hematochezia, colitis NOS, and 2 hospitalizations due to HAE.

Laboratory and vital sign evaluations

No consistent patterns or persistent changes in laboratory parameters were observed, both in terms of individual values or mean values. Similarly, no consistent changes in vital sign parameters were observed.

Antibody testing and hypersensitivity reactions

Fifteen of 67 patients (22.4%) had at least 1 serum sample test positive for antibodies to *P. pastoris* or ecallantide. Two patients had positive samples for non-IgE antibodies to ecallantide and IgE to *P. pastoris*. Four patients (6.0%) tested positive for non-IgE antibodies to ecallantide. Nine (13.4%) tested positive for IgE to *P. pastoris*. One patient tested positive for IgE to ecallantide (Patient 326002) at the first follow-up visit for treatment episode #4, but tested negative on subsequent follow-up visits. No hypersensitivity reaction is reported for the patient, but on the 4th treatment episode, the patient reported generalized pruritus and nausea occurring approximately 10 minutes after injection that last for 25 minutes, followed by injection site pruritus 6.5 hours after injection.

Of the 11 patients with positive IgE to *P. pastoris,* 1 patient had an anaphylactic event and positive rechallenge (Patient 305001, described above) and 1 patient had generalized pruritus and nausea (Patient 326002, described above). A third patient, Patient 317005, developed urticaria approximately 2 hours after receipt of ecallantide. The other nine patients do not have any AEs reported to suggest an allergic reaction (search terms: urticaria, pruritus, rash, wheezing, bronchospasm, syncope, dizziness, lightheadedness, diaphoresis, injection site reaction, drug reaction, allergy).

In addition to patients with positive serologies, 1 patient reported abdominal itching 4.5 hours after receipt of ecallantide while another patient reported itching “similar to allergies” although

the time-course is not specified in this case. Several cases of rash are reported but the time-courses are not specified.

Six patients reported some type of injection site reaction, including Patient 326002 described above with the suspected hypersensitivity reaction.

The individual efficacy results over time do not suggest a potential neutralizing effect from non-IgE antibodies to ecallantide, but the data is limited to 6 patients and would depend to some extent on the effect size of the drug and the severity of the specific attack.

Reviewer's comment: Hypersensitivity reactions, including anaphylaxis, appear to be the most serious of the adverse events recorded and the most clearly related to drug administration. A frequency is somewhat difficult to calculate give the unequal exposures to the drug among individual patients.

10.4.4 Study summary and conclusions
EDEMA3 is generally supportive of ecallantide's efficacy in the treatment of acute HAE attacks but the study did not demonstrate a statistically significant difference between ecallantide and placebo. The Applicant attributes the non-significant results to the accidental administration of placebo to 1 patient assigned to ecallantide and ecallantide to 1 patient assigned to placebo. When the data is analyzed using an as-treated dataset to correct for this error, the results are statistically significant. While this sensitivity analysis along with secondary and tertiary endpoints suggest efficacy, these results are not robust and confirmatory results from the second placebo-controlled trial, EDEMA4, are needed.

10.5 Individual Study Report: EDEMA4

10.5.1 Study Protocol: EDEMA4 (DX-88/20)

10.5.1.1 Administrative information
- Title: EDEMA4, A randomized, double-blind, placebo-controlled, multicenter study to assess the efficacy and safety of DX-88 (ecallantide) for the treatment of acute attacks of HAE
- Study sites: Multicenter, US and Canada
- Study dates: April 16, 2007 to June 26, 2008
- Study report date: September 1, 2008

10.5.1.2 Objectives/Rationale
- Assess the efficacy and safety of 30 mg SC ecallantide vs. placebo in the treatment of moderate to severe acute HAE attacks

10.5.1.3 Study design overview
The study was a Phase 3, randomized, double-blind, placebo-controlled multicenter study conducted under SPA. Patients presenting within 8 hours of onset of a moderate to severe HAE

attack were randomized to receive a single dose of 30 mg SC ecallantide or placebo. Patients were stratified by anatomic attack location (laryngeal vs. other).

Patients were eligible to receive an additional open-label dose of ecallantide if the patient was at risk of *severe upper airway compromise* (SUAC) within 4 hours after dosing. Risk of SUAC was defined as the presence of ≥3 of the following 7 findings: appearance or worsening of dyspnea, appearance or worsening of stridor, increased respiratory effort, change or loss of voice, cyanosis, decreased oxygen saturation, or increased $PaCO_2$ and/ or decreased PaO_2. A single additional dose could also be administered if symptoms had failed to resolve or if an attack relapsed from 4 to 24 hours post-first-dose. *Failure to respond* was defined as not achieving "beginning of improvement" within 4 hours post-initial-dose. *Incomplete response* was defined as not achieving "significant improvement" within 4 hours post-dose. Relapse was defined as a reoccurrence of an attack between 4 and 24 hours post-dose.

After treatment, patients were rolled over to the extension phase of the study for treatment with open-label ecallantide for new, separate HAE attacks.

10.5.1.4 Study population
Patients 10 years or older with documented diagnosis of Type I or II HAE were eligible.

Inclusion criteria

- 10 years of age or older
- Documented diagnosis of Type I or II HAE:
    - Clinical history consistent with HAE (SC or mucosal nonpruritis swelling without accompanying urticaria)
    - Function or antigenic C1-INH level below the lower limit of the normal range or up to 15% above the lower limit of the normal range as defined by the reference laboratory
    - C4 level below the lower limit of the normal range or up to 15% above the lower limit of the normal range as defined by the reference laboratory
    - Age of reported onset ≤25 years or documented complement component C1q level at or above the lower limit of the normal range
- Enrollment visit: presentation at the site within 8 hours of patient recognition of an acute HAE attack with at least one moderate to severe symptom complex (patient and investigator must agree that at least one symptom complex is moderate or severe):
    - **Normal** – patient's state absent of an acute HAE attack
    - **Mild** – noticeable symptoms but do not impact activities of daily living
    - **Moderate** – treatment or intervention highly desirable and symptoms impact activities of daily living
    - **Severe** – treatment or intervention required due to inability to perform activities of daily living
- Sexually active and fertile patients required to use at least 2 methods of contraception for the duration of the study

Exclusion criteria

- Receipt of an investigational drug or device within 30 days prior to study treatment with the exception of:
    - C1-INH concentrate for angioedema within 7 days
    - Ecallantide within 3 days
- Acquired angioedema, estrogen-dependent angioedema, and/or drug-induced angioedema
- Pregnancy or breastfeeding
- Any other condition that may compromise safety or compliance at the discretion of the investigator

Patients could withdraw from the study at any time at their own request or could be withdrawn at the discretion of the investigator or sponsor. Reasons for early withdrawal included adverse event, noncompliance or protocol violation, withdrawn consent, or termination of the study.

10.5.1.5 Study treatments

Treatments administered

- Initial dose
    - Single 30 mg ecallantide administered via 3 x 1cc SC injections to the upper arm, thigh, and abdomen. In the event that an injection site coincided with an attack site, multiple injections could be administered to the same site as long as the injections were separated by a minimum of 5cm.
    - Placebo
- Additional dosing
    - Open-label 30 mg ecallantide
    - Standard of care

Blinding

Ecallantide and placebo are both clear colorless liquids and are indistinguishable by appearance. Vials were labeled with assigned codes corresponding to the randomization codes. A single statistician was unblinded to assigned study treatments; all other study personnel and patients remained blinded.

Randomization

Patients were randomized 1:1 to ecallantide or placebo. The randomization was stratified by anatomic location of the attack (laryngeal vs. others) as determined by the investigator and by prior participation in an ecallantide clinical study (patients may or may not have received ecallantide in the previous study). A third-party vendor provided a centralized web-based or telephone-based system for generation the randomization assignments to individual patients as they presented at the time of their attacks.

Prior and concomitant therapy

Receipt of certain medications was reason for exclusion, as noted above. The CRF was used to record any additional concomitant medications and emergency treatments administered, if any. Emergency treatments included opioid/pain medication, anti-emetics (5-HT3 receptor antagonists), and HAE alternative therapies, listed as follows:

- Aminocaproic acid
- C1-INH
- Fresh frozen plasma
- Tranexamic acid
- Methylprednisolone
- Oxandrolone
- Danazol
- Prednisone
- Stanozolol
- Dexamethasone
- Dehyroepiandrosterone
- Methyltestosterone

Treatment compliance
All study drugs were administered in clinic. Study drug accountability was verified during on-site monitoring visits conducted by the Sponsor.

10.5.1.6 Study procedures

Table 47 EDEMA4: Schedule of procedures

EDEMA4	Screen	Enroll	Post-dosing evaluation						
			0-4 hrs				Discharge (if ≥5 hrs post-dose)	Day 2	FU Visit 1 Day 7
			0-1	2	3	4			
Informed consent	X								X†
Urine pregnancy test	X	X							
History, demographics	X								
Physical exam	X	X				X	X		X
Vital signs	X	X				X	X		X
ECG	X	X		X		X	X		X
Urinanalysis	X	X							X
eDiary training	X					X			
eDiary completion		X	X	X	X	X			X
Symptom complex categorization*		X	X	X	X	X			
Assessment of overall well-being*			X	X	X	X			
Symptom complex assessment*			X	X	X	X			
Severity assessment*		X				X			
Dosing		X							
Open-label DX-88 for incomplete response or relapse						X			
Dosing for severe upper respiratory compromise°			X	X	X	X			
Clinical observations			X	X	X	X	X		
Concomitant meds	X	X	X	X	X	X	X		X
Adverse events	X	X	X	X	X	X	X		X
Blood samples									
•*Chemistry*	X	X				X			X
•*CBC/diff*	X	X				X			X
•*Coag panel*	X	X				X			X
•*C1-INH level (if not done)*	X								
•*C4 (if not done before)*									
•*Antibody levels*		X							x
Phone F/U								X	

† For DX-88/19 (open-label extension study)
* via eDiary
° Can occur at any time

10.5.1.7 Efficacy parameters

Primary efficacy endpoint: Change from baseline in MSCS

The primary efficacy endpoint was the change from baseline in **Mean Symptom Complex Score (MSCS)** at 4 hours post-dosing. The MSCS is the arithmetic mean of the severity grade of the individual symptom complexes, where each symptom complex is assessed a severity grade of severe to normal:

Table 48 Severity assessment for MSCS calculation

Severity Assessment	Score	Definition
Severe	3	treatment or intervention required due to inability to perform activities of daily living (e.g. throat swollen/difficulty breathing, lips swollen/cannot eat, feet swollen/cannot walk)
Moderate	2	treatment or intervention highly desirable and symptoms impact activities of daily living (e.g. hands swollen/cannot button shirt, feet swollen/discomfort wearing shoes)
Mild	1	noticeable symptoms but do not impact activities of daily living
Normal	0	patient's state absent of an acute HAE attack

A decrease from baseline MSCS corresponds to a reduction in severity. The following symptom complexes were assessed:

- internal head/neck
- stomach/GI
- genital/buttocks
- external head/neck
- cutaneous

No imputations were made for the primary analysis. Sensitivity analyses performed to assess the effects of emerging symptom complexes and medical interventions were performed using the following imputations: Emerging symptom complexes were included in the MSCS calculation if present at the 4-hour and 24-hour MSCS assessment timepoints. If medical interventions were performed during an attack, the affected symptom complex(es) were assigned a severity of "severe" at 4 and/or 24 hours.

<u>Secondary efficacy endpoints</u>

- **Treatment Outcome Score (TOS)** at 4 hours post-dose. The TOS has the following components:
  - Symptom complex identification (same complexes assessed for the MSCS)
  - Severity assessment of each symptom complex at baseline ("symptom complex weight")
    - Severe = 3
    - Moderate = 2
    - Mild = 1
    - Normal = 0
  - Response assessment of each symptom complex post-dose ("symptom complex score")
    - Significant improvement = 100, " a lot better or resolved"
    - Improvement = 50, "a little better"
    - Same = 0,
    - Worsening = -50, "a little worse"
    - Significant worsening = -100, "a lot worse"

  - TOS = ∑(response assessment x severity at baseline) ÷ ∑ severity at baseline

    - A higher TOS value corresponds to a greater response
    - Imputations for sensitivity analyses:
        - Emerging symptom complexes were weighted at the peak severity assessment. If the emerging complex was still present at 4 hours and/or 24 hours, an assignment of "significant worsening" was made. If not present at those timepoints, an assignment of "same" was made.
        - If medical intervention during an attack was performed, a response assessment of "significant worsening" and a severity assessment of "severe" were given at 4 and/or 24 hours.
- Time to "significant improvement" in **Overall Response Assessment**, based on period of 15 minutes post-dose to 4 hours post-dose
    - Global symptom assessment by patient; not based on MSCS or TOS
        - Significant improvement = 100, " a lot better or resolved"
        - Improvement = 50, "a little better"
        - Same = 0,
        - Worsening = -50, "a little worse"
        - Significant worsening = -100, "a lot worse"
    - Assessed at 15, 30, 45, 60, 75, 90, and 105 minutes, 2, 2.5, 3, 2.5, and 4 hours
- Proportion of patients maintaining a significant improvement ("a lot better or resolved") in overall response continuously during the 24-period after dosing
- Proportion of responders at 4 hours post-dose
    - Improvement in existing laryngeal symptoms (not based on changes in individual symptom complex scores but on the overall MSCS)
    - Stabilization of existing peripheral/stomach/GI symptom complexes (4-hour score no worse than baseline)
    - Decrease in MSCS in 4 hours

<u>Tertiary efficacy endpoints</u>

- Durability of response at 24 hours post dose based on MSCS
- Durability of response at 24 hours post-dose based on TOS
- Proportion of responders at 4 hours post-dose based on TOS≥70
- Proportion of responders at 4 hours post-dose based on TOS≥50
- Time to onset of sustained improvement in overall response assessment
- Proportion of patients receiving medical intervention
- Assessment of response to open-label dosing for failed or incomplete response or for relapse baseline on the change from baseline MSCS at 4 hours post Dose B
- Assessment of response to open-label dosing for SUAC based on change from baseline MSCS to 4 hours post-SUAC dose

10.5.1.8 Safety parameters

<u>Adverse events</u>

AEs were recorded at enrollment (Study Day 1) through to the follow-up Visit 1 (Study day 7). AE severity was graded using the NCI CTCAE Version 3.0 criteria. Coding of AEs was done

using MedDRA Version 10.0 and tabulated by SOC, HLT, and PT. A new and different HAE symptom was recorded as an emerging symptom but was not to be reported as an AE.

Reviewer's comment: The applicability of these severity grading criteria, which were developed for use in cancer patients, to HAE patients is undetermined.

Physical examination
Physical exams were conducted at screening, enrollment (predose), 4 hours post-dosing, and at Follow-up Visit 1 (Study day 7). If discharge was delayed by 1 hour or more, an exam was repeated.

Vital signs
Body temperature, heart rate, respiratory rate, and sitting blood pressure were assessed at screening, enrollment (predose), 2 hours post-dose, 4 hours post-dose, and at Follow-up Visit 1 (Study day 7). If discharge was delayed by 1 hour or more, vital signs were repeated.

ECG
A 12-lead ECG was performed at screening, enrollment (predose), 2 hours post-dose, 4 hours post-dose, and at Follow-up. If discharge was delayed by 1 hour or more, an ECG was repeated. In cases where an ECG could not be performed immediately due to the severity of the attack, the ECG taken at screening was utilized as baseline. All ECGs were read by a central reading facility (The Brigham and Women's Hospital, Boston, MA) that was blinded to patient treatment assignment.

Reviewer's comment: In previous discussion with the Division, the applicant proposed intensive ECG monitoring in EDEMA4 in lieu of a designated thorough QT study.

Clinical laboratory evaluations
Samples for lab evaluations were collected at screening, enrollment (predose), 4 hours post-dose, and at follow-up visit. Lab evaluations included the following: CBC with differential, routine serum chemistry, and coagulations tests. Urinanalysis was performed at screening, enrollment, and at Follow-up Visit 1.

Antibody testing
Testing for all classes of antibodies to ecallantide and IgE antibodies to *P pastoris* were performed at enrollment and Follow-up Visit 1 (Study Day 7).

10.5.1.9 Statistical plan
The primary efficacy analysis was conducted on the ITT population, using the Wilcoxon rank sum test blocked by the stratification used for randomization (attack location and prior enrollment in an ecallantide study). No data imputation was performed. Additional sensitivity analyses were performed to assess the effects of emerging symptoms and medical interventions, as described above in 10.5.1.7.

Safety analysis was based on all patients who received any amount of drug. Tabulations and descriptive statistics were used to represent the safety data.

A sample size of 96 patients was calculated to give the study 80% power to detect a probability of 66.6% that an observation in the placebo treated group was less than an observation in the ecallantide treated group using a Wilcoxon rank sum test with a 0.05 two-sided significance level, assuming a 43% effect size. The effect size was approximated from EDEMA3 results, which showed a change in MSCS at 4 hours in the ecallantide arm was -1.10 and -0.63 for placebo (ITT-as-treated population).

10.5.2 Results

10.5.2.1 Protocol amendments

- Protocol Amendment 0.1, February 21, 2007 – updated administrative information
- Protocol Amendment 2, December 3, 2007 – increased study size from 52 to 96 patients. Allowed option of paper diary in instances where an eDiary could not be administered.

10.5.2.2 Study patients

Patient disposition

Ninety-six patients were enrolled; 48 in the ecallantide arm and 48 in the placebo arm. The disposition of the patients is shown in Table 10. In the ITT population, a total of 36 patients (17 in the ecallantide arm and 19 in the placebo arm) had previously participated in another ecallantide study. In the ecallantide group, 16 patients participated in EDEMA3, 3 patients in EDEMA1, and 4 patients in EDEMA4. In the placebo group, 15 patients were in EDEMA3, 2 patients in EDEMA1, and 8 patients in EDEMA2.

Table 49 EDEMA 4: Patient disposition

	Ecallantide N=48 N(%)	Placebo N=48 N (%)	Total N=96 N (%)
Intent to treat population[a]	48 (100.0)	48 (100.0)	96 (100.0)
Per protocol population[b]	47 (97.9)	48 (100.0)	95 (99.0)
Safety population[c]	48 (100.0)	48 (100.0)	96 (100.0)
Patients rolling over to continuation study[d]	47 (97.9)	46 (95.8)	93 (96.9)
Patients withdrawing from study		1 (2.1)	1 (1.0)
Adverse event	0	0	0
Noncompliance or protocol violation	0	0	0
Withdrawal of consent	0	0	0
Lost to follow-up	0	0	0
Investigator discretion	0	0	0
Left study site against medical advice	0	1 (2.1)	1 (1.0)

[a] Patients who received any amount of study drug

[b] Patients who received a complete dose of study drug with no major protocol violations

[c] Patients who received any amount of study drug

[d] All patients were intended to roll over to the open-label extension study (DX-88/19) for follow-up safety assessments. A total of 2 patients (1 in the ecallantide arm and 1 in the placebo arm) declined further participation.

An additional patient in the placebo arm left the study site against medical advice and was not enrolled in the follow up study.
Source: dx-88-20-csr-body.pdf, Section 10.1, Table 2

10.5.2.3 Protocol deviations
Protocol violations and deviations are summarized in Section 10.2 of the applicant's study report and in Appendix 16.2.2. The majority of violations were due to incomplete e-Diary assessments. In addition, several protocol violated related to patient inclusion criteria were recorded.

- Patient 403019 did not have a documented low C4.
- Patient 407003 did not have historical laboratory levels for C1-INH and C1. Blood samples were taken later.
- Patient 443002 had onset of HAE symptoms at >25 years and did not have a documented C1q level. A blood sample taken prior to dosing later showed a low C1q, which would be more consistent with acquired angioedema (AAE).

10.5.2.4 Treatment compliance
All patients received 30 mg (3 vials) of study drug. In addition, 3 patients in the placebo group and 1 patient in the ecallantide group received open-label ecallantide for SUAC, and 14 patients in the ecallantide group and 20 patients in the placebo group received 30 mg ecallantide as Dose B.

10.5.2.5 Datasets analyzed
Analyses were based on the intention-to-treat (ITT) population unless otherwise specified. Additional analyses with the per-protocol (PP) population were also performed.

10.5.2.6 Demographics and baseline characteristics

Patient demographics

Table 50 EDEMA4: Patient demographics

	Ecallantide N=48	Placebo N=48	Total N=96
Age			
Mean (SD)	37.0 (13.1)	38.0 (12.2)	37.5 (12.6)
Range	15-72	13-72	13-72
Sex (N,%)			
Male	11 (22.9)	20 (41.7)	31 (32.3)
Female	37 (77.1)	28 (58.3)	65 (67.7)
Race (N,%)			
White	39 (81.3)	43 (89.6)	82 (85.4)
Black	3 (6.3)	3 (6.3)	6 (6.3)
Asian	1 (2.1)	1 (2.1)	2 (2.1)
Hispanic	4 (8.3)	1 (2.1)	5 (5.2)
Other	1 (2.1)	0	1 (1.0)

Source: dx-88-csr-body.pdf, Section 11.2.1, Table 4

Reviewer's comment: The treatment groups appear comparable in terms of age and racial distribution, but the ecallantide group has a higher number of female patients compared to the placebo arm. The significance of this gender imbalance is uncertain.

Patient HAE history

Table 51 EDEMA4: Patient HAE history

	Ecallantide N=48	Placebo N=48	Total N=96
Age at first HAE symptom onset			
Mean (SD)	13.4 (7.4)	13.0 (9.5)	13.2 (8.5)
Range	0-44	1-59	0-59
Lowest historical functional C1-INH			
Mean % (SD	31.8 (20.1)	22.7 (19.6)	27.3 (20.2)
Range	0.1-78.0	0-61.0	0-78.0
Lowest historical antigenic C1-INH, mg/dl			
Mean (SD)	10.2 (17.1)	12.7 (23.2)	11.6 (20.5)
Range	0-80.0	2.4-90.0	0-90.0
Lowest historical C4, mg/dl			
Mean (SD)	8.8 (13.2)	10.0 (10.9)	9.4 (12.0)
Range	0-59.0	1.3-60.0	0-60.0
Most common prior HAE symptom complex (N,%)			
Laryngeal	3 (6.3)	2 (4.2)	5 (5.2)
Stomach/GI	21 (43.8)	26 (54.2)	47 (49.0)
Genital/buttocks	2 (4.2)	2 (4.2)	4 (4.2)
External head and neck	2 (4.2)	2 (4.2)	4 (4.2)
Cutaneous	26 (54.2)	23 (47.9)	49 (51.0)

Source: dx-88-csr-body.pdf, Section 11.2.3 Table 6 and 7

Reviewer's comment: The treatment groups appear fairly comparable in terms of age of onset, historical laboratory values, and prior attack site history.

Concomitant medications

The majority of patients, 83 of 96, reported taking concomitant medications at screening (42 in the ecallantide arm, 41 in placebo arm). The most common medications used were sex hormones, taken in similar proportions by both treatment arms. Notable differences between the treatment groups were the following:

- Antihistamines: 18.8% ecallantide vs. 35.4% placebo
- Medications for obstructive airway disease: 4.2% ecallantide vs. 18.8% placebo
- Psychoanaleptics (primarily SSRI antidepressants): 29.2% ecallantide vs. 8.5% placebo

Reviewer's comment: The significance of these differences in concomitant medications is unclear. These particular medications are not known to have a specific efficacious or exacerbating effect in HAE, although both antihistamines and psychoanaleptics are occasionally used to treat urticaria and non-hereditary angioedema.

Presenting symptom complex severity

Each randomized patient presented with at least one symptom complex that was moderate to severe. Patients could report multiple symptom complexes. In the ecallantide group, the most

commonly reported moderate-severe symptom complex was cutaneous. The placebo arm had a larger number of patients reporting moderate-severe GI symptoms in comparison.

Table 52 EDEMA4: Patient-reported symptom complex severity at baseline

	Ecallantide N=48 N, %	Placebo N=48 N, %	Total N=96 N, %
Number of symptom complexes at baseline	80	75	155
Internal head/neck symptoms (including laryngeal)			
Mild	0	6 (12.8)	6 (6.3)
Moderate	6 (12.5)	6 (12.8)	12 (12.6)
Severe	2 (4.2)	1 (2.1)	3 (3.2)
Stomach/GI			
Mild	5 (10.4)	1 (2.1)	6 (6.3)
Moderate	9 (18.8)	20 (42.6)	29 (30.5)
Severe	4 (8.3)	6 (12.8)	10 (10.5)
Genital/buttocks			
Mild	0	1 (2.1)	1 (1.1)
Moderate	4 (8.3)	3 (6.4)	7 (7.4)
Severe	2 (4.2)	1 (2.1)	3 (3.2)
External head/neck			
Mild	4 (8.3)	0	4 (4.2)
Moderate	8 (16.7)	9 (19.1)	17 (17.9)
Severe	2 (4.2)	0	2 (2.1)
Cutaneous			
Mild	2 (4.2)	4 (8.5)	6 (6.3)
Moderate	23 (47.9)	17 (36.2)	40 (42.1)
Severe	9 (18.8)	0	9 (9.5)

Source: dx-88-20-csr.body.pdf, Section 11.2.5, Table 11

Reviewer comment: The distribution of attack sites is not equal, with cutaneous attacks predominating in the ecallantide group versus stomach/GI attacks in the placebo group. This uneven distribution could impact efficacy findings, if ecallantide works better on cutaneous symptoms, for example, or if the PRO instruments do not assess different attack site symptoms similarly. However, the literature and the PRO validation studies actually suggest the opposite, that GI symptoms, primarily pain, tend to resolve more rapidly than peripheral symptoms in most HAE attacks. In terms of laryngeal involvement, the groups are comparable.

10.5.2.7 Efficacy endpoint outcomes

<u>Primary efficacy endpoint: Change from baseline MSCS at 4 hours</u>
Results from the primary efficacy analysis are shown below. The treatment arms had comparable baseline MSCS scores. A statistically significant greater decrease in MSCS from baseline was observed in the ecallantide group compared to the placebo arm. Similar results were observed for the per-protocol population analysis as well (p=0.011).

Table 53 EDEMA4: Primary efficacy endpoint, Mean change from baseline MSCS at 4 hours post-dose			
	Baseline MSCS	Change from baseline at 4h	P
Ecallantide	2.2 (0.5)	-0.8 (0.6)	0.01
Placebo	2.0 (0.4)	-0.4 (0.8)	

Source: dx-88-20-csr.pdf, Section 11.4.1.1, Table 14

Imputations for emerging symptom complexes and medical interventions were also performed. These results are displayed in Table 13 EDEMA4: Primary efficacy endpoint sensitivity analyses.

Table 54 EDEMA4: Primary efficacy endpoint sensitivity analyses			
	Mean change from baseline MSCS at 4 hours		
	Ecallantide (N=47)	Placebo (N=48)	P
Imputation for emerging symptoms	-0.8 (0.6)	-0.2 (0.9)	<0.001
Imputation for emerging symptoms and medical intervention	-0.8 (0.7)	-0.1 (0.9)	<0.001

Source: dx-88-20-csr.pdf, Summary tables 14.2.3.2.1 and 14.2.3.2.2

Secondary efficacy endpoints

TOS at 4 hours
The TOS at 4 hours was the primary efficacy endpoint in the other pivotal Phase 3 trial, EDEMA3. A statistically significant difference between the ecallantide group (mean TOS 53.4, SD 49.7) and the placebo group (mean TOS 8.1, SD 63.2) was observed (p=0.003). Similar results were reported for the PP population. A positive TOS represents symptom improvement.

Time to significant improvement in overall response
Although a greater proportion of ecallantide patients reported significant improvement that placebo (22 vs. 12 patients), no statistically significant differences were noted in the time to significant improvement in overall response between ecallantide and placebo (184.3 vs. 154.3 minutes; p=0.117). Results were censored at 4 hours.

Proportion of patients with a successful response at 4 hours based on MSCS
A "successful response" was defined as improvement in an existing laryngeal symptom complex, stabilization of an existing peripheral symptom complex, or a decrease in MSCS of at least -1.0. Using this definition, 45 of 48 (93.8%) of ecallantide patients were responders versus 28 of 47 (59.6%) of placebo patients (p<0.001). When assessed by logistic regression models, anatomic site of attack was also predictive of a successful response, meaning the odds of having a successful response within 4 hours was 8.49 times higher for non-laryngeal attack patients compared to laryngeal attacks (p=0.022). Prior exposure to ecallantide was not a predictor of successful response.

Maintenance of significant improvement in overall response

"Maintenance" was defined as achieving and maintaining an assessment of "a lot better or resolved" through 24 hours after dosing. Twenty-one of 48 (43.8%) ecallantide patients compared to 10 of 47 (21.3%) placebo patients reported maintenance (p=0.022). Attack site location and prior exposure to ecallantide were not determinants of response.

Tertiary efficacy endpoints

Durability of response at 24 hours post-dosing based on MSCS

Table 55 EDEMA4: Change from baseline MSCS at 24 hours

	Baseline MSCS	Change from baseline at 24h	P
Ecallantide	2.2 (0.5)	-1.5 (0.6)	0.039
Placebo	2.0 (0.4)	-1.1 (0.8)	

Source: dx-88-20-csr.pdf, Section 11.4.3.1, Table 24

Durability of response at 24 hours post-dosing based on TOS

The mean TOS at 24 hours was 88.8 in the ecallantide group vs. 55.1 in the placebo group (p=0.029). Sensitivity analyses performed for emerging symptoms and medical intervention were consistent with a more durable response in the ecallantide arm versus placebo (p=0.019 and 0.041).

Proportion of responders at 4 hours based on TOS≥70 and TOS≥50

Using a TOS cutoff of 70, more ecallantide patients (22 of 48, 45.8%) qualified as responders compared to the placebo arm (9 of 47, 19.1%) [p=0.011]. When a similar analysis is performed using a TOS cutoff of 50, similar results are obtained (68.8% vs. 27.7%, respectively; p=<0.001). Attack location and prior exposure to ecallantide were not significant predictors for either cutoff point.

Patients receiving medical intervention during attack

Fewer patients in the ecallantide group (16 of 48, 33.3%) received medical intervention than in the placebo group (24 of 48, 50.0%). No patients required urgent surgical decompression or intubation. The most common medical interventions administered were emergency medications, consisting of 5-HT3 receptor antagonists, opioids, anti-emetics. One patient in the placebo group received open-label ecallantide for SUAC. One placebo patient also received C1-INH replacement therapy.

10.5.2.8 Safety outcomes

Extent of exposure

Forty-eight patients received double-blinded ecallantide; an equal number received double-blinded placebo. In addition to the double-blinded dose, 1 patient (2.1%) in the ecallantide arm and 3 patients in the placebo arm received an open-label ecallantide dose for SUAC. Another 14 of 48 patients (29.2%) in the ecallantide group and 20 of 48 (41.7%) in the placebo group received open-label ecallantide as Dose B for failure to resolve to relapsing symptoms. One of the 3 patients in the placebo group who received an SUAC dose also received a Dose B of

ecallantide (2 doses of 30 mg ecallantide total in addition to the double-blinded placebo dose). Overall, 70 of 96 patients (72.9%) received at least 1 dose of 30 mg ecallantide and 16 patients received 2 doses of 30 mg ecallantide during the study.

Reviewer's comment: The use of open-label dosing for SUAC and Dose B complicates the safety assessment, since only 10 patients received placebo alone. Any patient who received a Dose B for airway compromise or incomplete response/relapse was analyzed as a placebo-treated patient up to the time of the open-label dose and as an ecallantide-treated patient from the time of ecallantide to the study conclusion.

Adverse events

Deaths and Serious Adverse Events (SAE)
No deaths or life-threatening AEs were reported during the double-blind treatment portion. A total of 3 SAEs were reported. During the double-blind portion, 2 patients in the placebo arm reported an SAE of HAE. No patients in the ecallantide arm reported an SAE during the double-blind portion. For patients who received open-label ecallantide for SUAC or Dose B, 1 patient reported an SAE of worsening HAE requiring hospitalization.

Discontinuations due to AEs
No discontinuations due to AEs were reported, although 1 patient in the ecallantide arm and 2 patients in the placebo arm declined to enroll in the subsequent open-label extension study (DX-88/19).

Common adverse events
Table 56 displays AEs occurring in 2 or more patients during double-blind treatment. Any placebo patient who received a Dose B for airway compromise or incomplete response/relapse who received a Dose B for airway compromise or incomplete response/relapse was analyzed as a placebo-treated patient up to the time of the open-label dose and as an open-label ecallantide-treated patient from the time of ecallantide to the study conclusion. Overall, 8 of 48 (16.7%) ecallantide patients reported at least 1 AE compared to 19 of 48 (35.1%) in the placebo arm. Among the 37 patients receiving at least 1 dose of open-label ecallantide, 13 (35.1%) reported at least 1 AE.

Table 56 EDEMA4: Adverse events occurring in 2 or more patients

Adverse event	Ecallantide N=48 N (%)	Placebo N=48 N (%)
Any AE	8 (16.7)	19 (39.6)
Headache	2 (4.2)	5 (10.4)
Nausea	3 (6.3)	1 (2.1)
Dizziness	2 (4.2)	1 (2.1)
Vomiting	0	3 (6.3)
Diarrhea	0	3 (6.3)
Abdominal pain	1 (2.1)	2 (4.2)
HAE	0	3 (6.3)

Source: dx-88-20-csr-body.pdf, Section 12.2.1

Among patients who received open-label ecallantide for SUAC or Dose B, 2 of 37 patients (5.4%) reported a local injection site reaction. These reactions were described as transient and were characterized by local erythema and swelling. These reactions were not accompanied by pruritus, urticaria, or other symptoms suggestive of hypersensitivity. Local injection site reaction was reported in 1 placebo patient in the double-blind portion of the study. No other AEs were reported in more than 1 patient during the open-label portion.

Reviewer's comment: Given the low sample size, the assessment of common adverse events is limited. The most commonly reported symptoms in the ecallantide group could also be attributed to HAE. Of note, the overall reporting rate for HAE as an AE is much lower in this study than the rate reported in EDEMA3. In both studies, HAE was not to be reported as an AE but the Applicant reports that this guideline may not have been followed in EDEMA3.

Laboratory testing
No clinically significant alterations in mean routine laboratory tests, including coagulation parameters, were reported. In individual patients, 9 of 44 ecallantide patients (20.5%) had a shift from euglycemia to hyperglycemia at the 4 hour mark.

In terms of antibody testing, one patient (403019) developed new anti-ecallantide antibodies during the study after a single dose. Three patients in the ecallantide arm tested positive at the lower limit of detection (titer of 5 or less) at study entry (438001, 417002, and 452004); 2 of these 4 had no prior exposure to ecallantide. A 4th patient (404004) had titers well above 5 and had previously participated in EDEMA3 and had received 2 doses of ecallantide. This patient also tested positive during EDEMA3. In the placebo group, 2 patients tested positive at screening and follow-up, while 2 more were negative at study entry before seroconverting at follow-up. No patients developed IgE antibodies to ecallantide to *P pastoris* within the 7 day follow-up period.

Reviewer's comment: The antibody testing was extended to 28 day follow-up as part of the open-label extension study, DX-88/19. Those results are not included in the submission.

Vital signs
No clinically significant mean changes in vital sign parameters were reported. One patient (428004) in the ecallantide group reported pyrexia on Day 3 of double-blind treatment accompanied by pharyngolaryngeal pain that resolved by the next day without treatment.

Physical examinations
The majority of physical exam findings reported were signs and symptoms related to the presenting HAE attack. No notable abnormalities were otherwise reported.

Electrocardiograms
In the ecallantide group, the mean change in QTc interval from baseline was 2.5, 3.5, and -6.2 msec at 2 hours, 4 hours, and 7 days post-dose, respectively. In the placebo group for the same

time points, the mean changes were -0.3, 2.0, and -8.3 msec, respectively. No patients in either treatment group had a QTc value >500 msec during double-blind treatment. No significant individual shifts from normal to abnormal were reported. Shifts of 30-60 msec and QTc>450 msec were similar between treatment groups. No clinically relevant mean changes were observed for the ST segment, PR or QRS intervals.

Reviewer's comment: In lieu of a formal QT study, the Applicant performed more intensive ECG monitoring during EDEMA4 to address any potential QTc effects. The intervals selected for ECG monitoring were previously discussed with the Division.

10.5.4 Study summary and conclusions
EDEMA4 provides efficacy and safety support for ecallantide as a treatment of acute HAE attacks. Subgroup analysis by attack site was not performed by the applicant but based on individual case narratives there does not appear to have been a significant difference in efficacy by attack site. The study addresses single doses primarily; the safety of extended repeat dosing is not addressed.

10.6Individual Study Report: Rechallenge study

10.6.1 Study Protocol: Study DX88-102

10.6.1.1 Administrative information
- Title: DX88-102, Clinical report of the DX-88 (ecallantide) rechallenge testing procedures
- Study site: Multiple sites in the US
- Study dates:
- Study report date: July 30, 2008

10.6.1.2 Objectives/Rationale
- Evaluate the sensitivity to ecallantide in patients with prior hypersensitivity reactions in EDEMA1, EDEMA2, or EDEMA3 clinical studies

10.6.1.3 Study design overview
In order to further define hypersensitivity reactions to ecallantide, patients with a history of a reaction in EDEMA1, EDEMA2, or EDEMA3 were invited to enroll in a rechallenge study. The study consisted of 2 phases: a skin-testing phase and a test-dose phase. For the skin-test phase, escalating doses of ecallantide were administered by skin-prick and intradermal injection and compared to histamine and saline controls. A skin test was considered positive if the difference in the observed erythema or edema was >3mm from the saline control. For the test-dose phase, escalating doses were administered via intravenous infusion. No subcutaneous injections were administered and the escalating dose procedure was not intended as a drug desensitization protocol.

If any test was positive, the patient could proceed to the next test only with the approval of the Sponsor and the investigator. At the investigator's discretion, patients could also undergo a separate desensitization protocol.

10.6.1.4 Study population
Patients with a history of prior hypersensitivity reaction to ecallantide during EDEMA1, EDEMA2, and EDEMA3 were eligible to participate. The reaction had to be assessed as moderate or severe in intensity by the investigator or medical monitor and have characteristics of an immune-mediated, acute hypersensitivity reaction (e.g. bronchospasm, hypotension, urticaria, etc.).

Inclusion criteria
- >10 years of age
- 2 barrier methods of contraception for the duration of the rechallenge up through 28 days after the last dose of ecallantide if sexually active and fertile

Exclusion criteria
- Undocumented, ongoing acute allergic symptoms
- Pregnancy or breastfeeding
- Antihistamine use 48 hours prior to skin testing
- Current alcohol or drug abuse
- Receipt of an investigational drug or device other than ecallantide within 30 days prior to rechallenge dosing
- Other conditions which may compromise safety or compliance per the investigator

10.6.1.5 Study treatments and procedures

Rechallenge phase

Skin-testing phase

- Skin prick testing
  - Low host-cell-protein (HCP, <5ng/ml) ecallantide
    - 1:100
    - 1:10
    - Full strength (10mg/ml)
  - High HCP (23.5 ng/ml) ecallantide
    - 1:100
    - 1:10
    - Full strength (10mg/ml)
  - Saline negative control
  - Histamine positive control
- Intradermal testing
  - Both low and high HCP
    - 1:100,000
    - 1:10,000
    - 1:1,000
    - 1:100
    - 1:10
    - Full-strength (10 mg/ml)
  - Histamine
  - Saline

Test-dose phase

If all skin testing was negative, patients could enter the test dose phase.

- Stage 1 (low HCP; all doses administered over 3 minutes via IV at an interval of 30 minutes)
  - 3 mg
  - 4.5 mg
  - 7.5 mg
  - 15 mg
- Stage 2 (no sooner than 72 hours after Stage 1)
  - 30 mg ecallantide (20 ml over 30 minutes)

If Stage 2 completed successfully, patient could re-enroll in regular study.

Desensitization

If positive results observed during skin testing or test-dosing, the investigator could design a unique desensitization procedure for the patient pending approval by the Sponsor. In the end, no desensitizations were performed.

10.6.1.6 Efficacy parameters

No formal efficacy assessments were made.

10.6.1.7 Safety parameters

Routine safety assessments included the following:

- Adverse events
- Vital signs

- Physical exams
- Tryptase levels at screening, prior to skin testing and test dosing, and following each test dose

The schedule of procedures is shown below.

Procedure	Screening for Rechallenge Procedures	Visit 1	Visit 2
Informed Consent Form	X		
Vital Signs[a]	X	X	X
Physical Exam	X	X	X
Medical History	X		
Concomitant Medications[b]	X		
PST		X	
Intradermal Skin Test		X	
Intravenous Testing Phase 1		X	
Intravenous Testing Phase 2			X
Adverse Events		X	X
Tryptase Levels[c]	X	X	X
Antibody Collection[c]	X	X	X

[a] Heart rate, blood pressure, temperature.
[b] Concomitant medications were reviewed prior to skin testing.
[c] Not consistently collected.

10.6.1.8 Statistical plan
No formal statistical analysis was planned.

10.6.2 Results
10.6.2.1 Study patients

Nine patients underwent the rechallenge testing procedures. Two of the 9 had had a hypersensitivity reaction in EDEMA1, 5 patients were from EDEMA2, and 2 patients were from the repeat-dosing phase of EDEMA3. Six of the 9 patients were female and all were Caucasian. The mean age was 30 years.

10.6.2.2 Outcomes

The following table summarizes the outcome of rechallenge for all 9 patients. Six of the 9 patients successfully completed the test-dosing phase. Four of the 6 patients have since gone on to participate in other ecallantide studies and have not experienced additional hypersensitivity reactions. Three patients had positive test results:

- Patient 8805019001 was a prior participant in EDEMA2. After the initial dose of 20 mg/m2 IV, the patient developed eye erythema, eye swelling, urticaria of the back and face, nasal congestion, rhinorrhea and sneezing. She tested positive for specific IgE to *P pastoris* 3 weeks prior to ever receiving study drug. During the rechallenge, she successfully completed the skin testing phase. However, approximately 8 minutes after the start of the 3 mg IV infusion, she developed sneezing, rhinorrhea, nasal congestion, cough, and throat itchiness. She received Benadryl and her symptoms resolved.

- Patient 8805051099 participated in EDEMA2 and received 13 doses of ecallantide without reaction. The patient subsequently enrolled in EDEMA3 and received 7 doses over a 5-month period. After the 7th dose, she developed pruritus and anaphylaxis (hypoxia and hypotension). The patient had positive IgE antibodies to *P. pastoris.* During the rechallenge, the patient developed a positive skin reaction on ID testing at the 1:100,000 dose. The investigator requested permission to administer a 1 mg SC dose. Seven minutes after dosing, the patient developed dyspnea, rash, anxiety, pharyngeal edema, vomiting, diarrhea, urinary incontinence, and hypoxia, consistent with anaphylaxis. The patient was treated with epinephrine and conveyed to the hospital for further observation prior to being discharged home. The patient has not participated in further studies.
- Patient 8814326002 was a participant in EDEMA 3 and received 4 doses of ecallantide. After the 4th injection, the patient experience nausea, pruritus, and injection site pruritus. The patient tested positive for IgE antibodies to *P. pastoris* and non-IgE antibodies to ecallantide. During rechallenge, the patient had a positive ID test at 1:10,000 dilution. The patient did not participate in further studies.

Results of the rechallenge procedure for all 9 patients is summarized below.

Patient	Skin-Testing Phase		Test-Dosing Phase		Eligible for Future Treatment with Ecallantide
	PST	ID	Stage 1	Stage 2	
8804013002	Completed	Completed	Completed	Completed	Yes
8804013003	Completed	Completed	Completed	Completed	Yes
8804013007	Completed	Completed	Completed	Completed	Yes
8804013011	Completed	Completed	Completed	Completed	Yes
8805019001	Complete	Complete	Received 3 mg dose prior to hypersensitivity AEs	Not done	No
8805024097	Completed	Completed	Completed	Completed	Yes
8805054099	Completed	Completed	Completed	Completed	Yes
8805051099	Completed	Received 1:100,000 dilution prior to hypersensitivity AEs	Not done*	Not done	No
8814326002	Completed	Received: up to 1:1000 dilution prior to positive skin reaction	Not done	Not done	No

* Patient 8805051099 did not advance to the Test-Dosing phase of the rechallenge, but instead received an additional 1 mg SC rechallenge dose of ecallantide.

10.6.4 Study summary and conclusions

Overall, the rechallenge procedure successfully identified patients who could receive additional ecallantide. None of the patients who had a successful rechallenge who then went on to further dosing have had new AEs suggestive of hypersensitivity. The safety of the rechallenge procedure, performed in the appropriate setting, appears comparable to similar graded challenge procedures for other drug allergies. However, the total number of patients studied is limited, so the generalizability of these results is uncertain.

Notably, it is not possible to predict on the basis of the case narratives of the original hypersensitivity reactions which patients may fail or pass a graded challenge. The case narratives are similar enough that history alone would be insufficient to make this prediction. Antibody status also is not clearly predictive. While all 3 patients who failed rechallenge and the patient with the most severe reaction, Patient 8805051099, did have positive IgE antibodies to *P. pastoris*, the application includes information on other patients with positive antibodies who did not have any hypersensitivity reactions, suggesting that the positive predictive value may be limited. The negative predictive value may be higher but this issue has not been systematically addressed.

U.S. Department of Health and Human Services
Food and Drug Administration
Center for Drug Evaluation and Research
Office of Pharmacoepidemiology and Statistical Science
Office of Biostatistics

STATISTICAL REVIEW AND EVALUATION

CLINICAL STUDIES

BLA/Serial Number:	125277/0002
Drug Name:	Kalbitor (ecallantide)
Indication(s):	Treatment of hereditary angioedema (HAE)
Applicant:	Dyax Corp.
Date(s):	Received Sep. 23, 2008
Review Priority:	Priority
Biometrics Division:	Division of Biometrics II / Office of Biostatistics
Statistical Reviewer:	Dongmei Liu, Ph.D.
Concurring Reviewers:	Qian H. Li, Sc.D., Team Leader Thomas Permutt, Ph.D., Division Director
Medical Division:	Division of Pulmonary and Allergy Products
Clinical Team:	Susan Limb, M.D., Medical Reviewer Sally Seymour, M.D., Team Leader Badrul A. Chowdhury, M.D. Ph.D., Medical Division Director
Project Manager:	Colette Jackson

Keywords: BLA review, Clinical studies, Data imputation

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1. EXECUTIVE SUMMARY

1.1 Conclusions

Dyax Corp. proposes ecallantide for the treatment of acute attacks of hereditary angioedema in patients who are 10 years of age and older. Ecallantide is a plasama kallikrein inhibitor intended for subcutaneous injection. The applicant conducted two phase 3 studies to support the efficacy and safety of ecallantide and claimed that with the recommended dose of 30mg (3.0mL) administered in three 1 mL injections, ecallantide eliminates or reduces signs and symptoms of HAE attacks and offers a significant benefit over available treatments. Issues identified in the phase 3 studies suggest that there is a lack of consistent and substantial evidence to support the efficacy claim of ecallantide. The issues are summarized here.

The main issue identified in one phase 3 study is the significant interaction between the treatment effect and enrollment pre and post sample size change. The efficacy result of this study was largely driven by the enrollment after the decision of sample size adjustment. In the other phase 3 study, statistical significance is only confirmed for intention to treat (ITT) as treated population and per protocol population, but not in ITT as randomized population. The difference between ITT as randomized and ITT as treated population is due to two patients who received wrong drugs.

The primary efficacy end points used in the clinical studies are patient report outcome (PRO) measures --- Treatment Outcome Score (TOS) and Mean Symptom Complex Severity (MSCS). The endpoint using MSCS was changes at 4 hours post-dose from baseline. This change from baseline uses the evaluation of MSCS at two time points (baseline and 4 hours post-dose) and ignored the change pattern in between. We are concerned the adequacy of the endpoint as it does not capture additional efficacy information such as how soon the change starts. For example, for patients whose symptoms completely disappear before 4 hour post-dose, the recovery may occur at 1 hour post-dose, or 2 hours post-dose. Therefore more frequent symptom assessments may provide more complete efficacy information.

The data imputations used by the sponsor in this application are not conservative in assessing treatment differences. The data imputations tend to favor ecallantide. Alternative imputation rules or methods should be considered.

Another deficiency in this submission is the adequacy of number of patients in the age group between 10 to 18 years of age. The applicant proposes the treatment for patients who are 10 years of age and older. However, only 14 patients (8% of the sample size) in the study were less than 18 years old, and of these, only 4 received ecallantide. There are not enough data to support the efficacy and safety for pediatric group.

1.2 Brief Overview of Clinical Studies

The applicant conducted two phase 3, double-blinded, placebo-controlled, parallel arm, multi-center studies comparing ecallantide to placebo. The studies were similar in design. The first phase 3 study (EDEMA3) has a sample size of 72 with patient randomized into the two arms in 1:1 ratio. The second study (EDEMA4) has a larger sample size, 96, with the same randomization ratio. In both studies, patients recruited were age of 10 years old or above. At enrollment, patient presented to the study center within 8 hours of recognition of an acute attack of HAE with symptom complexes assessed as moderate or severe. After initial dosing, responses to the treatment were recorded through either an electronic diary or paper diary. Symptom complex severity assessment was performed by patients at enrollment (baseline) and at 4 and 24 hours post-dose. Response assessment for the individual symptom complexes was performed by patients at 1, 2, 3, 4, and 24 hours post-dose. Patients were discharged at 4 hours post-dose. Follow up visit or phone calls were scheduled during the study participation. In EDEMA3, after double blind phase, all patients including the ones in the placebo arm advanced to open label repeat dosing phase.

The primary efficacy endpoint for EDEMA3 was Treatment Outcome Score (TOS). The secondary efficacy endpoints for EDEMA3 included change of Mean Symptom Complex Severity (MSCS) at 4 hours post-dose from baseline and time to report of significant improvement in overall responses. Followed by the recommendation of FDA, the primary efficacy end point for EDMA4 changed to change of MSCS at 4 hours post-dose from baseline. Except TOS at 4 hours post-dose and time to report of significant improvement in overall responses, there were two additional secondary efficacy end points for EDEMA4 --- proportion of patients maintaining a significant improvement in overall response and proportion of responders at 4 hours based on change from baseline in MSCS.

1.3 Statistical Issues and Findings

Majority of the patients completed the study. Only one patient in the ecallantide arm in each of the two phase 3 studies was lost to follow up. In both studies, most of the demographic and baseline characteristics were balanced in the two study arms. The only exceptions were gender ratio and the percentage of patients with the primary HAE attack locations classified as cutaneous and GI/abdominal in EDEMA4. The results from analyses based on ITT as treated populations in both studies showed patients in the ecallantide arm had statistically significantly greater reduction in MSCS at 4 hours post-dose from baseline, as well as higher TOS at 4 hours post-dose compared to patients in the placebo arm. In both studies, patients treated with ecallantide reached significant improvement earlier than the placebo group, but there was no statistically significant difference. The difference in proportions of patients with response, based on change of MSCS at 4 hours post-dose from baseline <= -1, was only 16% in EDEMA3, while as the difference was 30% in EDEMA4. The difference in EDEMA4 is statistically significant, but the difference in EDEMA3 is not.

The main statistical issues for this application are the interaction between treatment effect and enrollment period (pre and post sample size change) in one of the efficacy study and data imputation in both studies.

In the study which has confirmed statistical significance, there was significant interaction between the treatment effect and enrollment period (pre and post sample size change). If the trial was conducted the same way before and after sample size change, the chance to see such an interaction is very small. The statistical significance of the study was driven by the post sample size change enrollment. Without replicated study to demonstrate the same large treatment effect observed in the post sample size change enrollment, it is hard to accept the evidence in efficacy results due to the small probability to make this observation.

For data imputation, since there were more emerging symptom complexes and medical interventions in the placebo arm than in the ecallantide arm, more data were imputed in the placebo arm than in the ecallantide arm. The imputation rules proposed by the sponsor increased the difference of treatment effect between the ecallantide arm and the placebo arm. To have a balanced assessment of the treatment robustness, alternative imputation rules that are relatively conservative in assessing treatment differences are explored in this review.

We sent out enquiry on a few minor issues to the applicant and are waiting for responses. The questions include a) discrepancy between definition of ITT population and completeness of data, b) clarification on corrections/updates the applicant made on datasets after application was submitted, c) additional analyses using relatively conservative imputation rules, d) list of patients who recorded data by electronic diaries and patients who recorded data by paper diaries.

2. INTRODUCTION

2.1 Overview

2.1.1 Class and Indication

Ecallantide is a plasma kallikrein inhibitor. The applicant is requesting approval for use of ecallantide to treat patients who are 10 years of age and older with acute attacks of hereditary engioedema (HAE). The proposed dose is 30mg (3.0mL) administered in three 1 mL by subcutaneous injections. HAE is a rare and sometimes life-threatening disease. There is presently no marketed or approved treatment for acute attacks or cure for HAE in the United States.

2.1.2 History of Drug Development

BBIND 10426 (CBER) opened for the drug development on ecallantide as intended treatment for HAE on [redacted]. On [redacted], orphan drug designation was granted. On [redacted], [redacted], initial application for fast track designation was submitted and denied by CBER on the grounds that the application did not focus on severe, life-threatening aspects of HAE attacks nor addressed unmet medical needs. In the meeting with sponsor on [redacted], dosing, efficacy endpoints, long-term safety data requirement, and correction on indications were discussed. In the end of phase 2 meeting with sponsor on [redacted], agreement on efficacy end points was reached. There was further discussion on study design and number of clinical trials needed for the efficacy and safety evaluation. On [redacted], request for Special Protocol Assessment (SPA) was made for EDEMA4. FDA recommended change of the primary efficacy end point. Fast track designation was granted on [redacted]. The original protocol for EDEMA4 was submitted on [redacted]. Protocol amendment was made on [redacted] to increase sample size and to allow use of paper diaries. Rolling review was granted on [redacted]. The final rolling portion of BLA was submitted on [redacted].

2.1.3 Specific Studies Reviewed

The summary of all clinical studies the applicant submitted to support this application was given in second 5.2 (Tabular listing of all clinical studies) of the study report. My statistical review focuses on the double blind part of the two phase 3 studies designed for efficacy evaluation --- EDEMA3 and EDEMA4. EDEMA3 was conducted in US, Canada, Europe and Israel. EDEMA4 was conducted only in North America.

2.2 Data Sources

All data was supplied by the applicant to the CBER electronic data room in SAS transport format. The data and final study report for the electronic submission were archived under the network path location <\\cbsap58\M\eCTD_Submissions\STN125277\125277.enx>. The information needed for this review was contained in modules 1, 2.5, 2.7, and 5.3.5.

3. STATISTICAL EVALUATION

3.1 Evaluation of Efficacy

3.1.1 Study Design of EDEMA3

General Design

EDEMA3 is a phase 3, double-blinded, placebo-controlled, parallel arm, multi-center study followed by an open-label repeat dosing phase. The objective of the study was to assess the efficacy and safety of ecallantide (30mg liquid administered by subcutaneous injection) for the treatment of acute attacks of hereditary angioedema (HAE). The study was conducted in 25 sites in US, Canada, Europe, and Israel. The double blinded part was done from December 2005 to February 2007. The open label repeat dosing phase was completed in September 2007.

At enrollment, eligible patients who presented to the study center within 8 hours of recognition of an acute attack of HAE with symptom complexes assessed as moderate or severe were randomized in a 1:1 ratio to receive either a treatment of ecallantide or a matching placebo by subcutaneous injection. Randomization followed a block design, stratified according to prior use of ecallantide and attack locations (laryngeal vs. abdominal vs. peripheral). After initial dosing, responses to the treatment were recorded through an eDiary. Symptom complex severity assessment was performed by patients at enrollment (baseline) and at 4 and 24 hours post-dose. Response assessment for the individual symptom complexes was performed by patient at 1, 2, 3, 4, and 24 hours post-dose. Patients were discharged at 4 hours after receiving the injections, with 3 follow-up visits planned. After a minimum of 1 follow-up visit, patients continued to the open label stage. In special circumstances, i.e. after the initial dosing with study drug if the patient was at risk for severe upper airway compromise (SUAC), a single dose of ecallantide 30mg SC (referred to as a SUAC dose) could have been administered within 0 to 4 hours of the study drug treatment. Total duration of study participation was up to 97 days including the follow-up visits.

Efficacy Endpoints

The efficacy was measured by patient reported outcomes (PRO). The applicant stated that the motivation of using PRO measures was due to the highly variable constellation of HAE symptoms. PRO instruments developed in this study evaluate all signs and symptoms of an HAE attack at any anatomical site, as well as capture severity and change in severity of each symptom across anatomical sites in response to treatment for the full constellation of symptoms. The primary end point of this study was the Treatment Outcome Score (TOS) at 4 hours pose-dosing. The definition of TOS is as follows:

$$TOS = \frac{\sum symptom \quad complex \quad score \quad X \quad symptom \quad comlex \quad weight}{\sum symptom \quad complex \quad weight},$$

where symptom complex score was recorded on a 5-category scale (significant improvement [100], improvement [50], same [0], worsening [-50], and significant worsening [-100]) and

symptom complex weight was recorded on a 4-category scale (normal [0], mild [1], moderate [2], severe [3]). In this study, applicant defined that a clinically meaningful improvement was indicated by a TOS of 30 or above.

One secondary end point was the change of Mean Symptom Complex Severity (MSCS) at 4 hours post-dose from baseline. MSCS is defined as

$$MSCS = \frac{\sum symptom \quad complex \quad severity}{Number \quad of \quad symptom \quad complexes}.$$

MSCS score is a point-in-time global measure of symptom severity. Patient's assessment of severity on each individual symptom complex was recorded on a 0 to 3 categorical scale (normal [0], mild [1], moderate [2], and severe [3]) for 5 symptom complexes (Oropharyngeal Head/Neck, GI/Abdominal, Genital/Buttocks, Non-oropharyngeal head/Neck, and Cutaneous). A decrease in score reflects improvement in symptoms. In this study, applicant defined that a clinically meaningful improvement was indicated by a reduction of 0.3 or greater.

Another secondary endpoint is the time to report of significant improvement in overall responses. It was defined as the first time (in minutes) post-dose that the patient reported the overall assessment as "a lot better or resolved." Patients not reporting the overall assessment as "a lot better or resolved" from 15 minutes through 4 hours post-dose were censored at 240 minutes. Patients who received additional HAE therapy within 4 hours were censored at the time of the medical intervention.

Analysis Populations

Analysis of the primary and secondary efficacy endpoints was conducted on Intent-to-Treat (ITT) population and the Per Protocol (PP) population. The ITT population consisted of all patients who received any amount of study drug and who completed their 4 hour follow-up assessment. Since two patients received the wrong study drug (one patient randomized to ecallantide received placebo and one patient randomized to placebo received ecallantide), ITT population was further defined as ITT-as-randomized and ITT-as-treated. The Per Protocol population consisted of all patients who received a complete dose of study drug and completed their 4 hour follow-up assessment with no major protocol deviations.

Patient Disposition

A total of 72 patients were randomized in a 1:1 ratio to the two arms. Only one patient didn't complete the double-blinded study and it was due to lost to follow-up. The summary of patient disposition is given in Table 1.

Table 1 Summary of patient disposition for EDEAM3

	Ecallantide	Placebo
Randomized	36	36
ITT as randomized population	36	36
ITT as treated population	36	36
Per Protocol population	35	36
Discontinued after study drug was administered	1*	0

* Due to lost to follow-up.

Patient Demographics and Baseline Characteristics

The patient demographics and baseline characteristics are summarized for the ITT-as-randomized population in Table 2. The two study arms were well balanced with respect to age, gender, race, and the stratification factors (prior use of ecallantide and attack locations) applied in randomization. Majority of symptom complexes reported at baseline were stomach/GI symptoms and cutaneous symptoms.

Table 2 Patient demographics and baseline characteristics (ITT-as-randomized population)

		Ecallantide (N=36)	Placebo (N=36)
Age	Mean	38.5	32.2
	Median	37.4	30.4
	Std. Dev.	14.6	13.8
	Range (Min, Max)	(18, 77)	(11, 57)
Gender	Male	12 (33.3%)	13 (36.1%)
	Female	24 (66.7%)	23 (63.9%)
Race	White	33 (91.7%)	32 (88.9%)
	Black	1 (2.8%)	4 (11.1%)
	Hispanic	2 (5.6%)	0 (0%)
Prior use of ecallantide	Yes	8 (22.2%)	11 (30.6%)
	No	28 (77.8%)	25 (69.4%)
Attack location	Oropharyngeal Head/Neck	9 (25%)	4 (11.1%)
	GI/Abdominal	20 (76.9%)	21 (58.3%)
	Genital/Buttocks	2 (5.6%)	4 (11.1%)
	Non-oropharyngeal head/Neck	4 (11.1%)	9 (25%)
	Cutaneous	21 (58.3%)	14 (38.9%)

3.1.2 Study Design of EDEMA4

General Design

The design of EDEMA4 was similar to the design of EDEMA3 with a few exceptions. EDEMA4 was conducted in 30 sites in US and Canada. The study period of EDEMA4 was from April 2007 to June 2008. There was no open label repeat dosing phase in EDEMA4.

There were six major differences in the design of the two studies. Firstly, randomization in EDEMA4 was stratified based on prior use of ecallantide and anatomic locations of HAE attack categorized in 2 strata, laryngeal vs. all other locations; while in EDEMA3, randomization was stratified based on prior use of ecallantide and attack locations categorized in 3 strata, laryngeal vs. abdominal vs. peripheral.

Secondly, in addition to the SUAC dose, in EDEMA4, if patient's symptoms failed to improve or resolve incompletely at 4 hours after initial dosing, or if an attack relapsed within 24 hours after initial dosing, a single open-label dose of 30 mg SC ecallantide (referred to as Dose B) or standard care was administered. Patients were discharged at 4 hours after the initial dosing as well. Total duration of the study participation in EDEMA4 was up to 7 days including the follow-up visits.

Thirdly, the primary efficacy end point in EDEMA4 was the change of MSCS at 4 hours post-dose from baseline. The primary efficacy end point in EDEMA3, TOS at 4 hours post-dose, was used as the secondary efficacy end point in EDEMA4. This change was recommended by FDA, because MSCS was considered a more straightforward measure of response to treatment than TOS. Two more secondary efficacy endpoints, proportion of patients maintaining a significant improvement in overall response and proportion of patients with successful response at 4 hours post-dose based on change from baseline in MSCS, were added to EDEMA4 by a special protocol assessment (SPA). Maintenance of significant improvement was defined as achieving and maintaining a significant improvement in overall response (i.e. maintaining an assessment of "a lot better or resolved") through 24 hours after dosing. A successful response was defined as improvement in existing laryngeal symptom complex, stabilization of an existing peripheral symptom complex, or a change from baseline in the MSCS score at 4 hours of at least -1.0.

Fourthly, no data imputations were employed for the primary and secondary analyses in EDEMA4. In EDEMA3, TOS and MSCS were imputed for emerging symptom complexes and medical interventions that may have an effect on drug assessment. In both studies, sensitivity analyses were performed using imputations for emerging symptoms and medical interventions to test the robustness of the study conclusions. In this review, to make comparison between the two studies on consistent basis, all the analysis, except the results presented in section of data imputation, were based on unimputed data.

Fifthly, in EDEMA4, no patient received wrong drug, so there was no further classification of ITT-as-randomized and ITT-as-treated. Prior to unblinding, the statistical analysis plan was amended with new definitions of ITT and PP populations. ITT population for EDEMA4 was redefined as patients who received any amount of drug regardless of whether there was a 4-hour assessment. Per Protocol population was defined as all patients who received a complete dose of study drug with no major protocol deviation.

Lastly, a protocol amendment was made on Dec. 3, 2008 to increase the sample size of 52 in the original protocol to 96. Another modification of the protocol was allowing the use of paper diaries. When the protocol amendment was granted, FDA requested that upon BLA submission, assessment on the treatment differences before and after the sample size increase should be performed to ensure that the sample size change has no impact on treatment effect. The applicant failed to submit the required analysis.

Patient Disposition

A total of 96 patients enrolled in EDEMA4. Ninety-five patients completed the study with only one patient in the placebo group withdrew from the study after enrollment. The patient voluntarily left the study site against medical advice. The summary of patient disposition for EDEMA4 is given in Table 3.

Table 3 Summary of patient disposition for EDEMA4

	Ecallantide	Placebo
Randomized	48	48
Intent-to-treat population	48	48
Per protocol population	47	48
Patients withdrew from study	1*	0

* Left study site against medical advice.

Patient Demographics and Baseline Characteristics

In EDEMA4, the demographic and baseline characteristics were similar in the ecallantide and the placebo arms except for gender ratio and attack locations. A higher proportion of females (77.1%) were in the ecallantide group than in the placebo group (58.3%). A higher proportion of patients in the ecallantide group (70.8%) entered the study with cutaneous symptom complexes compared to patients in the placebo group (43.8%), whereas a higher proportion of patients in the placebo group entered with GI/abdominal symptom complexes (56.2%) compared to patients in the ecallandtide group (37.5%). The summary of patient demographics and baseline characteristics is given in Table 4.

Table 4 Patient demographics and baseline characteristics (ITT population)

		Ecallantide (N=48)	Placebo (N=48)
Age	Mean	30.7	38.0
	Median	34.5	38.6
	Std. Dev.	13.12	12.19
	Range (Min, Max)	(15.98, 72.77)	(13.64, 72.37)
Gender	Male	11 (22.9%)	20 (41.7%)
	Female	37 (77.1%)	28 (58.3%)
Race	White	39 (81.3%)	43 (89.6%)
	Black	3 (6.3%)	3 (6.3%)
	Hispanic	4 (8.3%)	1 (2.1%)
	Asian	1 (2.1%)	1 (2.1%)
	Other	1 (2.1%)	0 (0%)
Prior use of ecallantide	Yes	17 (53.4%)	19 (39.6%)
	No	31 (64.6%)	29 (60.4%)
Attack location	Oropharyngeal Head/Neck	8 (16.7%)	13 (27.1%)
	GI/Abdominal	18 (37.5%)	27 (56.2%)
	Genital/Buttocks	6 (12.5%)	5 (10.4%)
	Non-oropharyngeal head/Neck	14 (29.2%)	9 (18.7%)
	Cutaneous	34 (70.8%)	21 (43.8%)

3.1.3 Statistical Methods

Non-parametric Wilcoxon rank sum test was applied to analyses of Treatment Outcome Score (TOS) and change of Mean Symptom Complex Severity (MSCS). Log-rank test was used to compare the time to report of significant improvement in overall responses. Logistic regression was applied to analysis of proportion of patients with responses.

3.1.4 Efficacy Results of EDEMA3 and EDEMA4

The summary of analysis on TOS and MSCS are given in Table 5 and Table 6 respectively. The results reported in this section were from analysis based on ITT-as-treated population. The results show that patients in the ecallantide arm had statistically significant greater reduction in MSCS at 4 hours post-dose from baseline, as well as higher TOS at 4 hours post-dose, compared to patients in the placebo arm. However, the analysis result of EDEAM3 based on ITT-as-randomized population doesn't give a significant P value (p=0.138). The difference between ITT-as-randomized and ITT-as-treated population is only due to two patients who received wrong drugs, one patient who was randomized to the placebo arm received ecallantide and the other patient who was randomized to the ecallantide arm received placebo. Data from two patients are enough to change the study conclusion indicates that the treatment difference was not robust in EDEMA3. This is one of the concerns this reviewer has on the efficacy results of EDEMA3.

There was some minor update on data from EDEMA3 after the application was submitted. The efficacy results of EDEMA3 based on updated data are slightly different from the reported results in submission. Data from EDEMA4 remain the same.

Table 5 Summary of analyses results on TOS at 4 hours post-dose for EDEMA3 and EDEMA4 (ITT-as treated population)

	EDEMA3		EDEMA4	
	Ecallantide (N=36)	Placebo (N=36)	Ecallantide (N=48)	Placebo (N=48)
Mean	62.75	35.83	53.40	8.11
Std. Dev.	39.15	54.15	49.70	63.18
Median	50.00	50.00	50.00	0.00
IQR	(50, 100)	(0, 100)	(0, 100)	(-50, 50)
P value	0.045		0.003	

Table 6 Summary of analyses results on change of MSCS at 4 hours post-dose from baseline for both EDEMA3 and EDEMA4 (ITT-as-treated population)

	EDEMA3		EDEMA4	
	Ecallantide (N=36)	Placebo (N=36)	Ecallantide (N=48)	Placebo (N=48)
Mean	-1.10	-0.64	-0.81	-0.37
Std. Dev.	0.89	0.57	0.63	0.82
Median	-1.00	-1.00	-1.00	0.00
IQR	(-1.5, -0.5)	(-1, 0)	(-1, 0)	(-1, 0)
P value	0.041		0.01	

Since both TOS and MSCS were analyzed by non-parametric Wilcoxon rank sum test, the reviewer has the concern on difference between statistical significance and meaningful clinical difference. Particular attention was paid to a secondary efficacy end point, proportion of patients with successful responses at 4 hours post-dose based on change of MSCS <= -1. This secondary efficacy end point was only in the statistical analysis plan for EDEMA4. The reviewer applied similar analysis to EDEMA3 and compared the results from the two studies. The summary is given in Table 7. As shown in Table 7, the difference in proportion of patients with response at 4 hours was only 16% in EDEMA3, while the difference was 30% in EDEMA4.

Table 7 Proportion of patients with successful responses based on change of MSCS at 4 hours post-dose from baseline less than or equal to 1 (ITT-as-treated population)

	EDEMA3		EDEMA4	
	Ecallantide (n=36)	Placebo (n=36)	Ecallantide (n=48)	Placebo (n=48)
Yes	22 (66.1%)	18 (50%)	29 (60.4%)	14 (29.2%)
No	14 (38.9%)	18 (50%)	19 (39.6%)	34 (70.8%)
P value	0.344		0.003	

To gain better understanding in the treatment difference, the reviewer conducted additional analysis based on different definitions of responder. The definition used for this analysis was only based on cut offs of TOS and change in MSCS regardless of HAE attack locations. The results are summarized in Table 8. Results of EDEAM4 are robust. Regardless of cut offs applied to the definition of successful responses, significant difference between the placebo arm and the ecallantide arm are confirmed by all tests. The results of EDEMA3 are variable. The treatment differences for all the responder definitions were relatively small.

Table 8 Summary results of proportion of patient with successful response based on different definitions (ITT-as-treated population)

		EDEMA3			EDEMA4		
		Ecallantide	Placebo	P value	Ecallantide	Placebo	P value
TOS	≥70	44.4%	30.6%	0.226	45.8%	18.8%	0.006
	≥50	75.0%	50.0%	0.031	68.8%	27.1%	<0.001
	≥30	75.0%	50.0%	0.031	68.8%	27.1%	<0.001
MSCS	≤-1	61.1%	50.0%	0.344	60.4%	29.2%	0.003
	≤-0.3	77.8%	61.1%	0.129	68.8%	37.5%	0.003

3.1.5 Comparison of the EDEMA4 efficacy results between pre and post sample size change

The study period of EDEMA4 was from April 2007 to June 2008. The original protocol for EDEMA4 was submitted on February 21, 2007. Protocol amendment was made on December 3, 2007 to increase sample size and allow use of paper diaries. Before the protocol amendment, electronic diaries had been required. No change on patient selection or conduction of study was made.

The sponsor provided the summary of baseline and disease characteristics for patients who enrolled before and after sample size change. The detail is given in the appendices. In a brief summary, the proportion of females in the pre sample size change enrollment was lower than the proportion of female in the post sample size change enrollment; there was also a difference in the relative distribution of patients with stomach/GI symptoms between the pre and post sample size change enrollment.

To assess whether sample size change had impact on treatment effect, comparison of the efficacy results between pre and post sample size change enrollment was conducted. The results are summarized in Table 9, Table 10, and Table 11.

Table 9 summarizes the efficacy results on change of MSCS at 4 hours post-dose pre and post sample size change. The results show that the treatment difference between the ecallantide arm and the placebo arm was -0.09 with P value of 0.826 in pre sample size change enrollment and was -0.88 with P value less than 0.001 in post sample size change enrollment. Before sample size change, there was merely no difference between the two arms; after sample size change, the treatment difference was enlarged significantly.

Table 9 Summary of change in MSCS at 4 hours post-dose in EDEMA4 pre and post sample size change enrollment (ITT population)

	Pre sample size change		Post sample size change	
	Ecallantide (N=28)	Placebo (N=24)	Ecallantide (N=20)	Placebo (N=24)
Mean	-0.71	-0.62	-0.94	-0.06
Std. Dev.	0.59	0.79	0.67	0.77
Median	-1.00	-0.33	-1.00	0.00
IQR	(-1,0)	(-1,0)	(-1.33,-0.33)	(-0.5,0.33)
P value	0.826		<0.001	

The results on TOS at 4 hours post-dose in Table 10 are similar to the results on change of MSCS. Again, the treatment difference between the two arms was 24.08 with P value of 0.24 before the sample size change; it increased to 72.39 with P value of 0.006 after sample size change.

Table 10 Summary of TOS at 4 hours post-dose in EDEMA4 pre and post sample size change enrollment (ITT populations)

	Pre sample size change		Post sample size change	
	Ecallantide (N=28)	Placebo (N=24)	Ecallantide (N=20)	Placebo (N=24)
Mean	43.27	19.19	67.08	-5.31
Std. Dev.	47.06	57.99	51.05	68.08
Median	50.00	0.00	100.00	0.00
IQR	(0,100)	(-28.57,100)	(50,100)	(-66.67,50)
P value	0.24		0.006	

The proportion of responders based on different cut offs on TOS and change in MSCS in Table 11 gives the similar conclusions to Table 9 and Table 10.

Table 11 Summary of proportions of responders based on cut offs on TOS and change in MSCS in EDEMA4 pre and post sample size change enrollment (ITT population)

		Pre sample size change			Post sample size change		
		Ecallantide (N=28)	Placebo (N=24)	P value	Ecallantide (N=20)	Placebo (N=24)	P value
TOS	≥70	32.1%	25.0%	0.572	65.0%	12.5%	0.001
	≥50	57.1%	33.3%	0.089	85.0%	20.8%	<0.001
	≥30	57.1%	33.3%	0.089	85.0%	25.0%	<0.001
MSCS	≤-1	53.6%	45.8%	0.578	70.0%	12.5%	<0.001
	≤-0.3	64.3%	54.2%	0.459	75.0%	20.8%	0.001

To further clarify the problem, the reviewer made scatter plot on change of MSCS at 4 hours post-dose vs. enrollment time (Figure 1). Each point indicates a patient. Y axis is change of MSCS at 4 hours post-dose; X axis is the enrollment date; the red dots indicate patients in the ecallantide arm; the black dots indicate patients in the placebo arm; the green dotted line shows

when the protocol amendment was granted; the black dotted line shows where the population was split into pre and post sample size change enrollment. Six patients in the placebo arm enrolled after sample size change performed very poorly, i.e. change of MSCS at 4 hours post-dose from baseline was greater than 0; while no patients enrolled before sample size change performed the same. The pattern observed before sample size change is similar to the pattern observed in EDEMA3 where change of MSCS at 4 hours post-dose from baseline for all patients were negative except one patient in the ecallantide arm (whose change of MSCS at 4 hours post-dose was 0.5).

This raised the reviewer's concern on an interaction between treatment effect and enrollment period in EDEMA4. The reviewer conducted logistic regression on proportion of responders based on change of MSCS <=-1 with treatment effect, enrollment period (categorized as pre and post sample size change), and the interaction between treatment effect and enrollment period as covariates. The model is

$$Responder = \beta_0 + \beta_1\ treatment + \beta_2\ enrollment.period + \beta_3 * treatment{:}enrollment.period.$$

The results are summarized in Table 12. Significant interaction effect was detected with P value of 0.04. This indicates that if the trial was conducted exactly the same way before and after sample size change, the chance to observe such a strong interaction effect is very small.

Since the statistical significance in EDEMA4 efficacy results was mainly driven by the large treatment difference in post sample size change enrollment (44 patients) and similar level of treatment difference was not observed in EDEMA3, the reviewer has the concern on the replicability of the EDEMA4 post sample size change results.

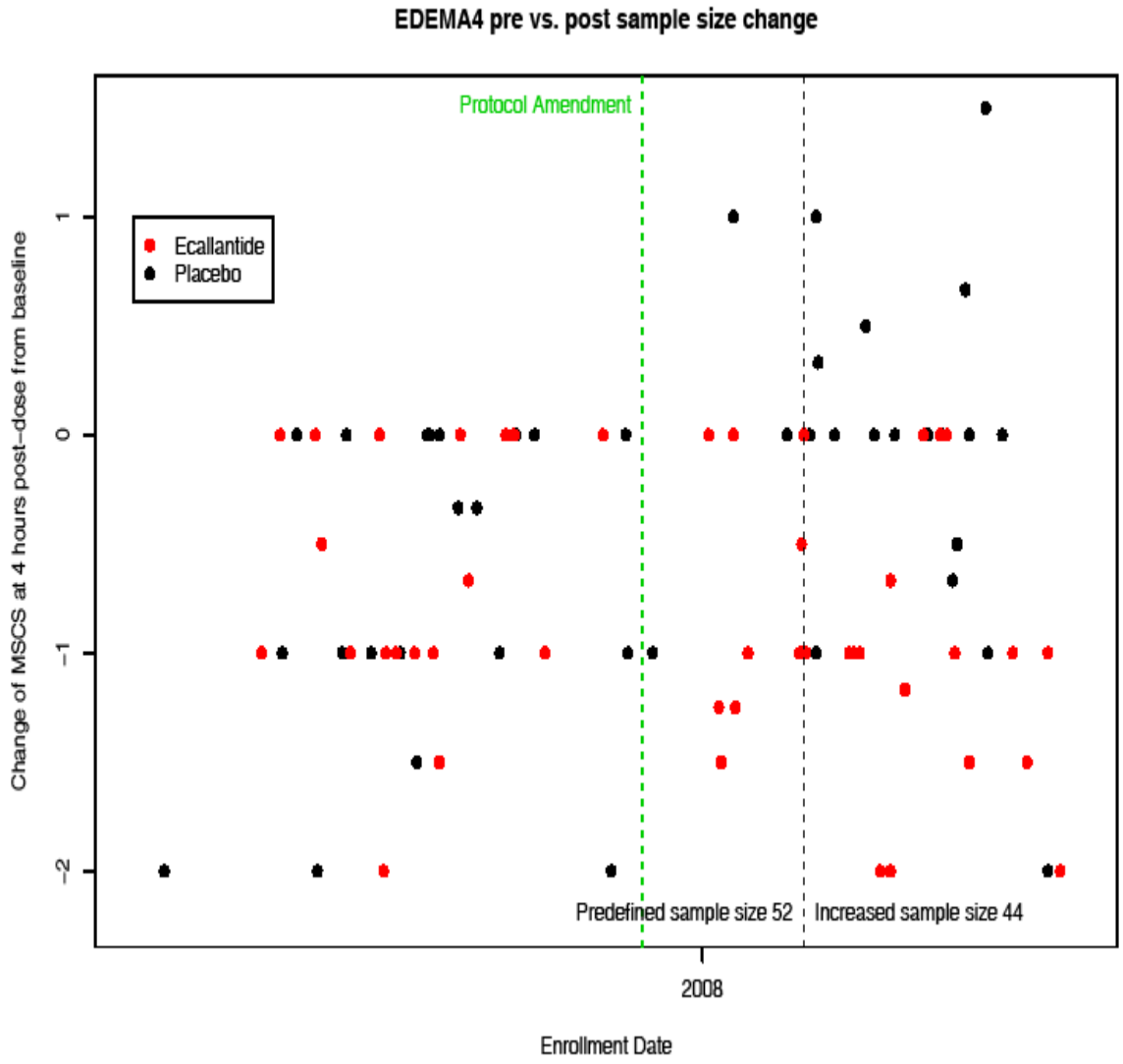

Figure 1 Scattor plot of change in MSCS at 4 hours post-dose vs. enrollment date in EDEMA4 (ITT population)

Table 12 Summary of interaction between treatment effect and enrollment period in EDEMA4 (ITT population)

	Estimate	Std. Error	P value
Intercept	-1.10	0.47	0.02
Treatment	1.53	0.61	0.01
Enrollment period	-2.04	1.12	0.07
Interaction	2.70	1.30	0.04

3.1.6 Data Imputation

The occurrence of emerging symptom complexes (i.e. any new symptom complex that occurred after dosing with study drug and was classified outside of symptom complexes identified at baseline) and medical interventions during an attack affects the evaluation of Treatment Outcome Score (TOS) and change of Mean Symptom Complex Severity (MSCS) at 4-hour and 24-hour post-dose. In the BLA submission, data used for the primary and secondary analyses in EDEMA3 were imputed, data used for the primary and secondary analyses in EDEMA4 were not. Sensitivity analysis on data with and without imputation was conducted for TOS and change in MSCS in EDEMA3, EDEMA4, and integrated summary of efficacy (ISE) to check the robustness of results.

The detail rules for data imputation proposed by the sponsor are available in appendices. Here is a brief summary of it. When there was emerging symptom complex, the baseline severity for the emerging symptom was classified as “normal”. If the emerging symptom was still present at 4/24 hours post-dose, its severity was used to calculate the MSCS at these time points. If the emerging symptom was not present at the evaluation time point, its severity was classified as “normal”. For TOS, the emerging symptom complex was weighted according to its peak severity assessment. If the emerging symptom was still present at 4/24 hours post-dose, the response assessment was assigned as “significant worsening”, otherwise “normal”. When there was medical intervention during an attack before unblinding, for MSCS, symptom complexes that were potentially affected were given a severity assessment of “severe”; for TOS, symptom complexes that were potentially affected were given a response assessment of “significant worsening”. If medical intervention was not clearly directed to a specific symptom complex, all symptom complexes were affected in MSCS and TOS calculations.

The imputation rules proposed by the sponsor were designed for a conservative measure on TOS and MSCS. However, it does not guarantee the treatment differences on imputed data lead to a conservative conclusion on efficacy of the study drug. Because there were more emerging symptom complexes and medical interventions in the placebo arm than in the ecallantide arm, more data in the placebo arm were imputed than in the ecallantide arm. This increased the difference in treatment effect between the two arms. Thus the imputation favored the study drug.

Table 13 summarizes the percentage of data imputed in each study. Table 14 gives the corresponding P values from the test on various imputed data. We see that the higher percentage of data was imputed in the placebo arm than in the ecallantide arm, the more significant the result became.

Table 13 Summary of percentage of data imputed in EDEMA3 and EDEMA4 (ITT-as-treated population)

	EDEAM3				EDEMA4			
	TOS		MSCS		TOS		MSCS	
	Ecallantide	Placebo	Ecallantide	Placebo	Ecallantide	Placebo	Ecallantide	Placebo
Unimputed	0%	0%	0%	0%	0%	0%	0%	0%
Imputed for Emg. Symp.	0%	3%	0%	6%	8%	15%	0%	15%
Imputed for Emg. Symp. + Med. Inv	3%	11%	3%	11%	8%	21%	0%	21%

Table 14 Summary of P values resulted from different data imputations in EDEMA3 and EDEMA4 (ITT-as-treated population)

	EDEMA3		EDEMA4	
	TOS	MSCS	TOS	MSCS
Unimputed	0.045	0.041	0.003	0.01
Imputed for emerging symptom complexes	0.033	0.027	0.002	0.001
Imputed for emerging symptom complexes and medical intervention	0.017	0.016	<0.001	<0.001

This raises the concern that data imputation rules proposed by the sponsor may exaggerate the treatment difference.

The so-called unimputed data are in fact imputed as well, since it ignored the information from emerging symptom complex and potential effect on treatment outcome by medical intervention.

Because the imputation rules proposed by the sponsor favored the study drug, alternative imputation rules that are expected to lead to conservative results are necessary to assess the robustness of the study results. Considering there were more emerging symptoms and medical interventions in the placebo arm than in the ecallantide arm, this reviewer suggests reversing the imputation rules proposed by the sponsor and see if statistical significance can still be confirmed by analysis based on data imputed according to the new rules. For example, instead of assigning significant worsen (-100) to emerging symptom in TOS calculation, assign significant improvement (100) to it. These analyses can be considered extreme imputation rules, which may not be reasonable in estimate treatment difference, but provide information in assessing treatment robustness.

As the efficacy assessments were only made at baseline and 4 hours post-dose, MSCS was only evaluated at two time points. It only captures the change between the two time points, but ignores the pathway of changing. The shortcomings of this approach are illustrated in the examples below. In the following discussion, we also discuss the advantages of an alternative efficacy end point for consideration in future studies, which requires more frequent measurements of MSCS and calculates the area under the curve. This efficacy end point will have less issue with emerging symptom complexes.

As an example, in Figure 2, the patient in case 1 starts with a single severe symptom at baseline and gets improved at 3 hours post-dose, the symptom severity reduces to mild. The change of MSCS at 4 hours post-dose from baseline in case 1 is -2. The patient in case 2 also starts with a single severe symptom at baseline, but gets improved at 0.5 hour post-dose, which is much earlier than that in case 1. The severity of symptom also reduces to mild. The change of MSCS at 4 hours post-dose from baseline in case 2 is -2 as well, the same as that in case 1. However, clinically case 2 is much better than case 1, because the treatment shows benefit more quickly. This difference is not captured by change in MSCS at 4 hours post-dose from baseline. A better measure would be proportion of area under the curve (i.e. AUC --- the area labeled in red) of severity path. In case 1, the AUC is 10. Compare to the total area of 12 which we consider as the maximum potential suffering the patient could experience, the proportion of suffering over the period is 10/12=83.3% of the maximum potential suffering. The treatment helps to reduce the suffering by 16.7%. In case 2, the AUC is 5, the proportion is 5/12=41.7%, the treatment helps to reduce the potential suffering by 48.3%. The difference of 16.7% and 41.7% reflects the difference in the treatment effect of the two cases. This measure could be applied to case 3 and case 4 in the same way. In the two cases with emerging symptom complexes, case 3 and case 4, the change of MSCS is 1. However, the patient in case 3 is in a worse case than the patient in case 4. In case 3, the AUC is 6.5, the proportion of AUC is 6.5/12=54.2%, which is the measure of failure of the treatment. In case 4, the AUC is 0.5, the proportion of AUC is 0.5/12=4.2%. The difference of 54.2% and 4.2% reflects the treatment difference in two cases. If we assign the primary end points of the four cases to be -0.167, -0.417, 0.542, and 0.42 respectively, it reflects the idea of change in MSCS, but in a much more effective way.

Furthermore, because AUC is a continuous measure, we can apply test on continuous variables, which is usually more powerful than non-parametric test, to it. It also solves the problem with imputation due to emerging symptom complexes, because it doesn’t require arbitrary symptom severities to be assigned to emerging symptom complexes. If the new efficacy end point is available, there will be less problems in data imputation.

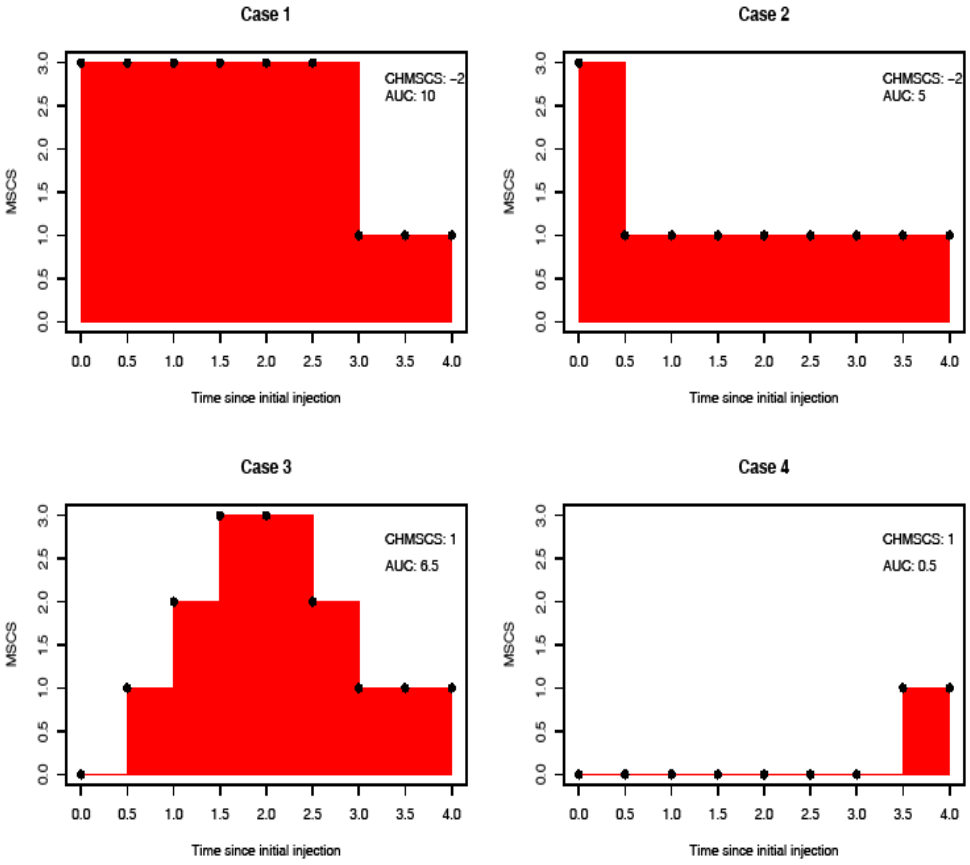

Figure 2 Illustration of an alternative efficacy end point --- AUC.

3.2 Evaluation of Safety

The evaluation of safety was conducted by Dr. Susan Limb. No special analysis on safety evaluation was requested by the clinical review team. Reader is referred to Dr. Susan Limb's review for this section.

4. FINDINGS IN SPECIAL/SUBGROUP POPULATIONS

Subgroup analysis on efficacy end points was done for age, gender, race, prior use of ecallantide, and attack locations of HAE. However, due to the small sample size and majority of patients coming from a single stratum in subgroups, no meaningful conclusions could be drawn from subgroup analysis.

Because the applicant proposes ecallantide for the treatment of acute attacks of hereditary angioedema in patients who are 10 years of age and older, the results of subgroup analysis on age are summarized in Table 15 and Table 16 to show that there were no enough data on pediatric group to support efficacy in patients who are younger than 18 years of age.

Table 15 Summary of results on change of MSCS at 4 hours post-dose from baseline by age group in EDEMA3 and EDEMA4 (ITT as treated population)

	EDEMA3				EDEMA4			
	Pediatric (<18yr)		Adult (>=18yr)		Pediatric (<18yr)		Adult (>=18yr)	
	Ecallantide	Placebo	Ecallantide	Placebo	Ecallantide	Placebo	Ecallantide	Placebo
N	2	7	32	28	2	3	45	39
Mean	-1.00	-0.64	-1.04	-0.46	-1.13	-1.00	-0.79	-0.32
Std. Dev.	0.00	0.48	1.02	0.73	0.18	0.00	0.64	0.83
Median	-1.00	-1.00	-1.00	-0.17	-1.13	-1.00	-1.00	0.00
IQR	(-1,-1)	(-1,0)	(-1.75,-0.5)	(-1,0)	(-1.25,-1)	(-1,-1)	(-1,0)	(-1,0)
P value	0.407		0.022		0.46		0.005	

Table 16 Summary of results on TOS at 4 hours post-dose by age group in EDEMA3 and EDEMA4 (ITT as treated population)

	EDEMA3				EDEMA4			
	Pediatric (<18yr)		Adult (>=18yr)		Pediatric (<18yr)		Adult (>=18yr)	
	Ecallantide	Placebo	Ecallantide	Placebo	Ecallantide	Placebo	Ecallantide	Placebo
N	2	7	34	29	2	3	45	39
Mean	75.00	35.71	47.79	14.37	100.00	58.33	51.33	4.24
Std. Dev.	35.36	47.56	60.42	71.54	0.00	38.19	49.79	63.37
Median	75.00	0.00	50.00	0.00	100.00	50.00	50.00	0.00
IQR	(50,100)	(0,100)	(0,100)	(-25,100)	(100,100)	(25,100)	(0,100)	(-50,50)
P value	0.375		0.052		0.388		0.001	

APPENDICES

1. Demographic and baseline characteristics in the patients enrolled pre and post sample size change.

   Quotation from study report DX-88/20(EDEMA4).

Table 12. Summary of Baseline and Disease Characteristics for the First 52 and Last 44 Patients Treated in EDEMA4

	First 52 Enrolled	Last 44 Enrolled
Statistic	**Ecallantide**	
	(N=28)	**(N=20)**
Mean age at informed consent (y)	37.9	35.8
Female, n (%)	20 (71.4)	17 (85.0)
Caucasian, n (%)	25 (89.3)	14 (70.0)
Mean age at first HAE symptom onset (yr)	13.6	13.2
Mean lowest historical functional C1 INH level (%)	30.1	35.4
Mean lowest antigenic C1 INH level (mg/dL)	7.7	15.0
Mean lowest historical C4 level (mg/dL)	6.2	12.9
Moderate/Severe Internal Head/Neck, n (%)	3 (10.7)	5 (25.0)
Moderate/Severe Stomach/GI, n (%)	7 (25.0)	6 (30.0)
Moderate/Severe Genital/Buttocks, n (%)	4 (14.3)	2 (10.0)
Moderate/Severe External Head/Neck, n (%)	6 (21.4)	4 (20.0)
Moderate/Severe Cutaneous, n (%)	18 (64.3)	14 (70.0)
	Placebo	
	(N=24)	**(N=24)**
Mean age at informed consent (y)	36.2	39.8
Female, n (%)	12 (50.0)	16 (66.7)
Caucasian, n (%)	22 (91.7)	21 (87.5)
Mean Age at first attack (y)	10.7	15.3
Mean lowest historical functional C1 INH level (%)	29.4	13.4
Mean lowest antigenic C1 INH level (mg/dL)	14.8	10.8
Mean lowest historical C4 level (mg/dL)	9.9	10.1
Moderate/Severe Internal Head/Neck, n (%)	2 (8.3)	5 (20.8)
Moderate/Severe Stomach/GI, n (%)	16 (66.7)	10 (41.7)
Moderate/Severe Genital/Buttocks, n (%)	4 (16.7)	0
Moderate/Severe External Head/Neck, n (%)	3 (12.5)	6 (25.0)
Moderate/Severe Cutaneous, n (%)	8 (33.3)	9 (37.5)

Source: Summary Tables 14.1.6.1.1, 14.1.6.1.2, 14.1.10.1.1, 14.1.10.1.2, 14.2.1.1.1, 14.2.1.1.2

2. Rules for Data Imputation proposed by the sponsor.

Quotation from clinical study report: DX-88/20 (EDEMA4)

EMERGING SYMPTOM COMPLEXES

Per the SAP, the occurrence of an emerging symptom complex (i.e. any new symptom complex that occurred after dosing with study drug and was classified outside of symptom complexes identified at baseline) affected the MSCS score and the TOS calculations in the sensitivity analyses as follows:

- *MSCS score*
  - *An emerging symptom complex was included in the baseline MSCS score calculation, with its baseline severity classified as "normal."*
  - *An emerging symptom complex was included in the 4-hour and/or 24-hour calculations. If the emerging symptom complex was still present at 4 hours and/or 24 hours, its severity was used to calculate the MSCS score at these times. If the emerging symptom complex was not present at 4 hours and/or 24 hours, its severity was classified as "normal."*
- *TOS*
  - *An emerging symptom complex was weighted according to its peak severity assessment.*
  - *An emerging symptom complex that was still present at 4 hours and/or 24 hours was assigned a response assessment of "significant worsening." An emerging symptom complex that was not present at 4 hours and/or 24 hours was assigned a response assessment of "same."*

MEDICAL INTERVENTION

Per the SAP, patients receiving medical intervention during an attack were to be identified before unblinding, and a medical determination was to be made as to whether the intervention had the potential to affect treatment outcome. Medical intervention that was clearly directed to a specific symptom complex affected only that specific symptom complex in the MSCS score and the TOS calculations; medical intervention that was not clearly directed to a specific symptom complex, as well as open-label dosing with ecallantide for SUAC or as Dose B, affected all symptom complexes in the MSCS score and the TOS calculations. The following was applied to the MSCS score, the TOS, and the overall response assessment calculations:

- *For the MSCS score, symptom complexes that were potentially affected were given a severity assessment of "severe" at 4 hours and/or 24 hours.*
- *For the TOS, symptom complexes that were potentially affected were given a response assessment of "significant worsening" and a severity assessment of "severe" at 4 hours and/or 24 hours.*

• The overall response assessment was classified as "significant worsening" and a severity assessment of "severe" at 4 hours and/or 24 hour

SIGNATURES/DISTRIBUTION LIST

Primary Statistical Reviewer:	Dongmei Liu, Ph.D.
Date:	Jan. 5, 2009
Statistical Team Leader:	Qian H. Li, Sc.D.
Biometrics Division Director:	Thomas Permutt, Ph.D.

CLINICAL PHARMACOLOGY MEMORANDUM

Date: January 9, 2009

From: Yun Xu, MD, PhD
Clinical Pharmacology Reviewer, Office of Clinical Pharmacology
Ping Ji, PhD
Pharmacometrics Reviewer, Office of Clinical Pharmacology

To: Members, Pulmonary-Allergy Drugs Advisory Committee

Subject: Clinical pharmacology overview of the FDA background materials for BLA# 125277, Kalbitor (ecallantide) Injection 30mg, for the treatment of acute attacks of hereditary angioedema (HAE) in patients 10 years of age and older

Absorption, Distribution, Metabolism and Elimination

Following the administration of a single SC dose of ecallantide to healthy subjects, mean maximum plasma concentration was observed approximately 2 to 3 hours after dosing. The bioavailability of the 27.3 mg SC dose is about 90%. No studies on transporter or pH effect on drug absorption were conducted. Following the administration of a single IV dose of ecallantide, the volume of distribution ranged from 5.9 to 18.8 L.

No clinical or preclinical studies were conducted to assess mass balance, route of excretion, or metabolism, as the expected consequence of the metabolism of biotechnology-derived polypeptides is the degradation to small peptides and individual amino acids. As confirmed by population pharmacokinetic modeling, no apparent intrinsic or extrinsic factors appear to be associated with ecallantide pharmacokinetics in a clinical significant manner. However, since the patients with severe hepatic and renal impairment were excluded from the clinical trials, the pharmacokinetics in these patient populations is not characterized based on available information. In addition, only a few patients between age 10 and 18 had drug concentration data. Therefore, the pharmacokinetics for the patients in this age group is not well-characterized because of the small patient number.

Ecallantide is a small polypeptide (7054 Da) and it is expected that elimination is by metabolic catabolism and renal filtration followed by tubular re-absorption. Renal elimination of ecallantide has been confirmed by demonstration of ecallantide activity in urine of treated subjects, indicating the drug is at least partly excreted by kidney. However, the percentage of renal contribution to ecallantide elimination is unclear. Neither human drug-drug interaction studies, nor studies in impaired renal and hepatic patients, have been performed.

Pharmacokinetic parameters of ecallantide in human

The pharmacokinetics of ecallantide in human was evaluated following both intravenous (IV) and subcutaneous (SC) administration. The pharmacokinetics of liquid ecallantide following IV administration was evaluated in 2 studies in healthy subjects (Studies DX-88/1 and DX-88/6) and 3 studies in patients with HAE (Studies DX-88/2 [EDEMA0], DX-88/4 [EDEMA1], and DX-88/5 [EDEMA2] at fixed doses ranging from 10 to 80 mg, or body weight adjusted doses ranging from 5 to 40 mg/m^2. The pharmacokinetics of ecallantide following SC administration was evaluated in 2 studies in healthy subjects (Studies DX-88/13 and DX-88/15) and 1 study in HAE patients (Study DX-88/5). In these studies, ecallantide was administered at nominal doses of 10 mg or 30 mg. In study DX-88/5 and study DX-88/13, ecallantide was administrated subcutaneously in liquid formulation. While in study DX-88/15, ecallantide was administrated subcutaneously in both liquid and lyophilized formulation.

PK analysis from individual studies

Individual pharmacokinetic parameters, were calculated in 3 single-dose studies in healthy subjects (Studies DX-88/1, DX-88/13, and DX- 88/15) and in 1 repeat-dose study in healthy subjects (Study DX-88/6). A description of these studies is summarized in Table 1. Pharmacokinetic parameters in all these 4 studies were derived using traditional methods and plasma concentration data profiles were analyzed either noncompartmentally (Studies DX-88/1, DX-88/13, and DX- 88/15) or using a 2-compartment model (Study DX-88/6). Pharmacokinetic data from Studies DX-88/2, DX-88/4, and DX-88/5 were very sparse and accurate pharmacokinetic parameters could not be derived using traditional methods. Data from these studies, however, were included in the population pharmacokinetic analysis.

Table 1. Description of Pharmacokinetic Studies in Healthy Subjects

Study No. (Country)	Product Desc. (Lot No.)	Study Phase Design	Study Objectives	Regimen, Dose, Route	No. Subj.	Gender (M/F)	Sampling Scheme
DX-88/1 (Scotland)	Liquid (317392)	Phase 1 Double-blind Ascending-dose	Tolerability Pharmacokinetics	Single, 10 mg IV inf. Single, 20 mg IV inf. Single, 40 mg IV inf. Single, 80 mg IV inf.	2 2 4 4	2M/0F 0M/2F 3M/1F 4M/0F	PK: predose, 5, 10, 15, 30, 45 min, 1, 2, 4, 6, 8, 12, and 24 hrs post-dose APTT: predose, 1, 4, and 24 hrs and 7 days post dose Anti-DX-88 antibodies: predose, 35 days post dose
DX-88/6 (UK)	Liquid (493034)	Phase 1 Open-label Repeat-dose	Pharmacokinetics Safety Immunogenicity	Repeat, 20 mg/m^2 IV inf. Q7 days for 4 weeks	8	2M/6F	PK: predose, 15, 30, 45 min, 1, 1.5, 2, 4, 8, 12, 16, and 24 hrs post (10 min inf) **or** predose, 15, 30, 45 min, 1, 1.5, 2, 3, 4, 4.5, 5, 5.5, 6, 6.5, 7.5, 8, 12, 16, and 24 hrs post dose (4 hr inf) APTT: predose, 1, 4, 12, and 24 hrs post each dose Anti-DX-88 antibodies: Days -1, 6, 13, 20, 28, 49
DX-88/13 (UK)	Liquid (227-01-004)	Phase 1 Open-label Three-period crossover	Pharmacokinetics Safety Immunogenicity	Single, 27.3 mg IV inf. Single, 27.3 mg SC inj. Single, 9.1 mg SC inj.	18	8M/10F	PK: predose, 5, 10, 15, 30, 45 min, 1, 1.25, 1.5, 1.75, 2, 2.5, 3, 3.5, 4, 5, 6, 7, 8, 10, 12, 14, 16, 18, 20, 22, 24 hrs post each dose APTT: predose, 15 min 1, 4, and 12 hrs post each dose Non-IgE, IgE anti-DX-88/ IgE anti-P pastoris Antibodies: Days -1, 6, 13, 21, 42
DX-88/15 (UK)	Liquid (227-05-002) Lyophilized (716 2742)	Phase 1 Double-blind Two-period crossover Bioequivalence	Pharmacokinetics Safety Tolerability Immunogenicity	Single, 30 mg SC inj.	24	9M/15F	PK: predose, 5, 10, 15, 30, 45 min, 1, 1.25, 1.5, 1.75, 2, 2.5, 3, 3.5, 4, 5, 6, 7, 8, 10, 12, 14, 16, 18, 20, 22, 24 hrs post each dose APTT: predose, 1, 4, and 12 hours post then Days 15 and 36 Non-IgE, IgE anti-DX-88/ IgE anti-P pastoris Antibodies: Days -1, 7, 15, 36

A summary of the pharmacokinetic parameters after single dose in healthy subjects is presented in Table 2. Following the administration of a single IV dose of ecallantide, Cmax and AUC increased approximately proportional with the dose from 10 to 80 mg. Plasma clearance ranged from 71 to 141 mL per minute and the volume of distribution ranged from 5.9 to 18.8 L. Plasma ecallantide concentration declined rapidly with a mean elimination half-life of 0.6 to 2.0 hours. Following the administration of a single SC dose of ecallantide to healthy subjects, Cmax and AUC increased approximately proportional with the dose from 9.1 to 27.3 mg. A mean maximum plasma concentration was observed approximately 2 to 3 hours after SC dosing. Following SC administration, plasma ecallantide concentration also declined rapidly with an elimination half-life of approximately 2 hours. The absolute bioavailability of the 27.3 mg SC dose is about 90%.

Table 2. Summary of Mean Pharmacokinetic Parameters Following Single-Dose Administration of Ecallantide in Healthy Subjects

Study	Route	Dose (mg)	N	C_{max} (ng/mL)	t_{max} (hr)	AUC (ng*hr/mL)	V_d * (L)	Cl * (mL/min)	$t_{1/2}$ (hr)
DX-88/1	IV	10	2	2000	0.2	1400	5.9	122	0.6
	IV	20	2	4750	0.2	4709	7.4	71	1.2
	IV	40	4	7680	0.2	8823	10.2	76	1.6
	IV	80	4	14800	0.2	17656	11.2	76	1.7
DX-88/6	IV (10 min)	20 mg/m^2	6	6497	n/c	5890	13.2	110	2.0
	IV (4 hr)	20 mg/m^2	6	1170	n/c	5300	11.2	118	1.3
DX-88/13	IV	27.3	16	3741	0.2	3327	18.8	141	1.6
	SC	27.3	17	586	2.7	3017	26.4	153	2.0
	SC	9.1	18	179	2.2	837	29.3	189	1.8
DX-88/15	SC	30 (liquid)	23	995	2.4	4232	23.1	124	2.2

Source: DX-88/1 CSR; DX-88/6 CSR; DX-88/13 CSR; DX-88/15 CSR.
Abbreviations: C_{max}=observed maximum serum concentration after administration, t_{max}=time to reach C_{max}, AUC=area under the concentration time-curve, V_d=volume of distribution, Cl=clearance, $t_{1/2}$=terminal half-life, n/c=not calculated.

* For SC dose, Vd/F and Cl/F is calculated

Study DX-88/6 also assessed pharmacokinetic profiles and safety of ecallantide in healthy subjects following repeat IV dosing (Days 0, 7, 14, 21). Subjects were administered a dose of 20 mg/m^2 ecallantide once weekly for 4 weeks. For the first 3 doses, ecallantide was administered as a 10-minute IV infusion. The final dose was administered as an IV infusion over 4 hours. The pharmacokinetic parameters after each dose were summarized in Table 3. No drug accumulation was observed after repeated weekly IV dose at 20 mg/m^2. Based on the plasma concentration profile, the majority of the administered ecallantide was cleared from the plasma within 6 hours following each dosing.

Table 3. Summary of PK Parameters (Mean ± SD) from Compartmental Models of Plasma Samples Collected from Healthy Volunteers after 10-Minutes and 4-Hour Intravenous Infusion of Ecallantide

Parameter	10-minute Infusion Dose 1 (N=6)	Dose 2 (N=6)	Dose 3 (N=6)	4-h Infusion Dose 4 (N=6)
Dose (mg)	36.5 ± 6.02	36.5 ± 6.02	36.5 ± 6.02	36.5 ± 6.02
AUC (h*μg/mL)	5.06 ± 0.94	5.75 ± 1.26	6.86 ± 2.90	5.25 ± 0.92
Half-life				
Alpha $t_{1/2}$ (h)	0.10 ± 0.04	0.13 ± 0.07	0.13 ± 0.04	0.03 ± 0.01[a]
Beta $t_{1/2}$ (h)	1.18 ± 0.44	1.89 ± 0.80	2.78 ± 2.26	1.27 ± 0.52[b]
C_{max} (μg/mL)	7.02 ± 1.36	6.47 ± 0.67	6.00 ± 0.88	1.17 ± 0.12
CL (mL/h)	7405 ± 1853	6597 ± 1849	5750 ± 1276	7101 ± 1615
Vdss (mL)	9731 ± 3152	13056 ± 3326	16770 ± 9989	11152 ± 1743

Source: Appendix 16.1.13.1 (Tables 3, 4, 5, 6)

Note: AUC = area under the curve, C_{max} = maximum plasma concentration achieved; CL = clearance; SD = standard deviation; Vdss = volume of distribution at steady state

a. (n =2) Plasma concentration data for Subjects 001-0002 and 001-0004 were consistent with a 2-compartment model, whereas data from the other 4 subjects (Subjects 001-0003, 001-0006, 001-0007, and 001-0011) were consistent with a 1-compartment model.

b: This includes terminal half-life estimated from both 1-compartment (Subjects 001-0002 and 001-0004) or 2 compartment (Subjects 001-0003, 001-0006, 001-0007, and 001-0011) modelling.

DX-88/15 evaluated the bioequivalence of liquid and lyophilized formulations of ecallantide in healthy subjects. Subjects were administered 2 SC doses of 30 mg ecallantide at one-week intervals (Days 1 and 8). The test-to-reference ratio (lyophilized/liquid formulation) was summarized in Table 4. The 90% CI for liquid and lyophilized DX-88 Cmax, and AUC0-last ratios were not within 80% to 125% range. Therefore, lyophilized DX-88 formulation was not bioequivalent to the liquid formulation and was not used in later studies.

Table 4. Pharmacokinetic Equivalence Assessment for Liquid and Lyophilized Formulations of Ecallantide

Parameter	Ratio	90% Confidence Interval Lower	Upper
C_{max}	65.6	54.8	78.4
$AUC_{0\text{-last}}$	80.5	74.2	87.4

Population PK analysis

A population pharmacokinetics model was developed to describe the data from all studies with drug concentration measurement. The final pharmacokinetic model with the best fit was a 3-compartment mathematical model.

Based on the population PK analysis, the clearance of ecallantide was 23.5% higher in healthy subjects (9.82 L/h) than in HAE or AAE patients (7.51 L/h). Two covariates affected ecallantide pharmacokinetics: subject weight and assay type. An inverse relationship was observed between the subject body weight and the rate of absorption after SC administration; as weight increased the rate of absorption decreased with no change on the extent of absorption. The assay type affected the central volume of distribution, which was 34% smaller for patients whose samples

were assayed using an LC-MS/MS (LLOQ: 0.473 ug/mL) assay compared to patients whose samples were assayed using an ELISA (LLOQ: 0.156 ng/mL) or LC-MS (LLOQ: 0.5 ug/mL) assay.

Neither patient age nor sex had an effect on ecallantide exposure. However, the relatively small sample distribution of pediatric and elderly population may not allow the labeling recommendation in these two age groups. The whole population PK model dataset (development + validation) consisted of 173 individuals with 3090 concentrations, among which 19 subjects were below 18 yrs of age (191 concentrations, 6%) and 3 subjects were greater than 65 yr of age (16 concentrations, <1%).

Pharmacodynamics

Dose-Response Relationship

One controlled and 2 uncontrolled Phase 2 studies were conducted in HAE patients during early development: EDEMA1, EDEMA2 and EDEMA0, respectively. The final dose selected in the pivotal clinical study, EDEMA3 and EDEMA4, are selected based on the results from these previous studies. In EDEMA0, while a small number of patients were treated and the ecallantide doses used in the study varied, the efficacy data demonstrated that ecallantide had an effect on reducing the duration of attack symptoms. The results of EDEMA1 demonstrated that ecallantide administered at IV doses 5, 10, 20, or 40 mg/m^2 showed clinical activity against attacks at all anatomic locations (abdominal, peripheral, and laryngeal). The 10 mg/m^2 dose (approximately 20 mg, the average human body surface area is about 1.8 m^2) provided significant benefit in mitigating acute signs and symptoms of HAE, and that increasing the dose to a level of 20 mg/m^2 and 40 mg/m^2 provided incremental, although slight, improvement in activity.

A clinical study in healthy subjects (DX-88/13) established comparability between 30 mg IV and 30 mg SC ecallantide doses based on PK parameters, including clearance, elimination half-life, and volume of distribution. As a result, the dose-ranging studies conducted with IV ecallantide (DX-88/1 and DX-88/6) supported ecallantide tolerability and efficacy when administered SC. EDEMA2 evaluated 5, 10, and 20 mg/m^2 IV doses and the 30 mg SC dose in a total of 240 HAE attacks in 77 patients. The study showed a clinical response at each dose level with a more impressive response in the 30 mg SC group compared with the other dose groups. Successful outcome based on improvement of response at 4 hours and maintained for more than 24 hours (the primary endpoint evaluation) was achieved following treatment with 30 mg SC in 49 of 60 (81.7%) of attacks treated, as compared with 11 of 24 (45.8%) of attacks treated at 5 mg/ m^2, 96 of 141 (68.1%) at 10 mg/ m^2, and 9 of 15 (60.0%) at 20 mg/m^2. Time to onset of response was similar across doses. Based on the overall response data, the 30 mg SC dose was deemed an appropriate dose to achieve efficacy. Furthermore, the 30 mg SC dose was studied in HAE patients in EDEMA2 and in healthy subjects in DX-88/13, and found to be well tolerated and showed comparable safety profile to other dose levels. In summary, the 30 mg SC dose showed improved efficacy and comparable safety to other dose levels studied, and was selected as the dose used in the pivotal study.

QT/QTc elongation

In preclinical development, ecallantide was shown to have no direct effects in standard cardiovascular assays, including human ether-a go-go related gene (hERG) assay, isolated Purkinje fiber preparations, inward sodium current (INa), or transient outward potassium current (Ito) in isolated male and female rat cardiomyocytes. For patients taking ecallantide, no clinically significant QT prolongation has been seen or is expected. As agreed with the agency, a thorough QT/QTc study was not conducted. ECG monitoring as proposed in EDEMA4 protocol was accepted as an alternative. In EDEMA4, the randomized, placebo-controlled study to assess 30 mg SC dose vs placebo, 12-lead electrocardiograms (ECGs) were obtained at baseline, around

the Cmax window at 2 hours and 4 hours post-dose, and at follow-up (Day 7). ECGs were evaluated for PR interval, QRS complex, and QTc interval. Ecallantide had no significant effect on the QTc interval, heart rate, cardiac conduction, or any other components of the ECG. Of note, there were no outliers at extremes (>500 msec absolute or >60 msec change from baseline) of QTc in response to treatment with ecallantide at and around the Cmax window of 2 to 4 hours.

Effect on coagulation factors

In vitro enzyme inhibition measurements demonstrated that ecallantide is a potent, selective, and reversible inhibitor of human plasma kallikrein with an equilibrium inhibition constant (Ki) of 25 pM. Enzyme specificity studies demonstrated that ecallantide weakly inhibited 5 additional proteases including neutrophil elastase (Ki =0.75 μM), tissue kallikrein 2 (Ki =0.29μM), pancreatic trypsin (Ki =69 nM), plasmin (Ki =29 nM), and factor Xia (Ki =1.7 nM). Ecallantide demonstrates selectivity for plasma kallikrein over these other enzymes of between 60-fold to 30,000-fold.

In a series of in vitro coagulation studies, ecallantide at 1.0 ug/ml did not inhibit factor XI and only partially (approximately 20%) inhibited plasmin. The maximum ecallantide concentration in HAE patients receiving a 30 mg SC dose is approximately 0.6 to 1 μg/mL. It is therefore less likely that ecallantide would display any clinically meaningful inhibition of plasmin or factor XIa at 30 mg SC dose. In preclinical safety studies, administration of ecallantide results in a dose-dependent, reversible prolongation of activated partial thromboplastin time (aPTT). This is a direct pharmacologic action of ecallantide and is due to the inhibition of kallikrein-mediated activation of factor XII to factor XIIa, which is the initial step in the initiation of the intrinsic clotting cascade. A transient prolongation of aPTT of approximately 2-fold was observed in humans following IV dosing of ecallantide at doses in excess of 20 mg/m^2. However, no clinically significant prolongation in aPTT has been observed in healthy subjects and patients administered ecallantide SC at doses of 30 mg, and no safety signal with respect to bleeding or bruising phenomena has emerged in HAE patients.

Potential bio-analytical issues

The plasma concentration of ecallantide was initially measured using a high performance liquid chromatography method with mass spectral detection (HPLC/MS and HPLC/MS/MS). Due to poor detection limits, a sandwich enzyme-linked immunosorbent assay (ELISA) was then developed with a 100-fold greater sensitivity. Based on preliminary review, some key information was missing from the assay validation report and in-study bio-analytical report. The agency requested the sponsor to provide the missing information. The sponsor provided some of the information and mentioned the rest will be submitted when they receive them from the contract labs who conducted the bio-analysis to measure the drug concentration. Therefore, the pharmacokinetics data above should be considered preliminary before the sponsor submits the complete information.

Immunoassay Summary

Date: January 7, 2009

From: Jack A. Ragheb M.D., Ph.D.

To: Pulmonary Allergy Drugs Advisory Committee

Through: Susan Kirshner, Ph.D., Acting Associate Chief, Laboratory of Immunology, DTP, OBP, CDER, FDA

Amy Rosenberg, M.D., Director, DTP, OBP, CDER, FDA

Subject: STN 125277/0 DX-88 BLA Immunoassay AC Briefing Summary

Product: DX-88 (Ecallantide, Kalbitor, Kallikrein Inhibitor)

Sponsor: Dyax

Indication: Treatment of acute episodes of Hereditary AngioEdema (HAE)

Background, Rationale and Summary

This review covers the immunoassays for detection of Drug Substance (DS) in plasma and the detection of anti-DS binding and neutralizing antibodies in serum, as well as the presence of anti-yeast antibodies.

The proposed indication for this BLA is the treatment of Hereditary Angioedema (HAE), a genetic disorder (autosomal dominant) characterized by acute attacks of localized swelling and inflammation that may be life-threatening. Disease is a result of C1 esterase inhibitor (C1-INH) deficiency. C1-INH has pleotropic affects, with roles in controlling the activation of the complement, kinin-generating, fibrinolytic, and intrinsic clotting pathways. In HAE, diminished inhibition of kallikrein, leading to the dysregulated generation of bradykinin, is thought to be responsible for the attacks of angioedema.

Bradykinin, a member of the kinin family, is a potent vasodilator that increases vascular permeability, resulting in local edema. Bradykinin is generated by the action of the kinin protease kallikrein on high molecular weight kininogen. DX-88 is a 60 amino acid, recombinant, kallikrein inhibitor derived following targeted mutation and reiterative phage display affinity maturation of a peptide encompassing amino acids 10−21 and 31−39 of the first Kunitz domain of the human tissue factor pathway inhibitor (TFPI). DX-88 reversibly binds and inhibits the proteolytic activity of plasma kallikrein with a k_i of 30-40 pM.

Produced in yeast (*Pichia pastoris*), DX-88 has a molecular weight of 7054 Da. It shares 88% identity with TFPI, a.k.a. lipoprotein-associated coagulation inhibitor, between TFPI amino acid residues 59 and 118. Therefore one safety concern for DX-88 is that anti-DX-88 antibodies could potentially cross react with TFPI.

TFPI is a glycosylated protein found predominantly in the vascular endothelium and plasma in both free forms and complexed with plasma lipoproteins. TFPI is a protease inhibitor that regulates the tissue factor (TF)-dependent pathway of blood coagulation. The coagulation process initiates with the formation of a factor VIIa-TF complex, which proteolytically activates additional proteases (factors IX and X) and ultimately leads to the formation of a fibrin clot. TFPI inhibits the activated blood clotting factor X and VIIa-TF proteases in an autoregulatory loop. In addition, TFPI interacts with the proteases trypsin IV and thrombospondin 1, which have inflammatory roles. While not its proposed indication in this BLA, DX-88 is under investigation as a coagulation inhibitor during coronary bypass surgery (IND 10232). However, bleeding diathesises have not been reported during the HAE clinical trials.

The immunoassay methods are generally adequate except for the anti-IgE assays. However, the current validated assay for the anti-DS antibodies was used only in Phase 3 Clinical Studies DX-88/14 and DX-88/20. Thus, the results of immunogenicity assays performed in the Phase 1 and 2 Clinical Studies may not be valid.

A serious deficiency of this BLA is the sponsor's failure to provide any discussion or data on the potential of antibodies directed against the DS to cross-react with endogenous TFPI. Partial deficiency of TFPI is associated with hyper-coaguable states (e.g. venous thrombosis) and the targeted deletion of the TFPI gene is an embryonic lethal mutation in mice. Beyond its clinical implications, such cross-reactivity may interfere with the DX-88 immunoassays, which was not explored by the sponsor. This may be particularly problematic for the immunoassay based PK studies. Such cross-reactivity may also be reflected in the 20% background signal observed in the drug confirmatory ECL assay when results with human serum normal controls (HSNC) are reported as signal/background (S/B) ratios and the need for a relatively high PC antibody concentration (421 ng/mL) to demonstrate selectivity in the neutralizing antibody (Nab) assay.

The assay for both anti-DX-88 and anti-P.pastoris IgE described in the BLA is unexpectedly sensitive for a chromogenic, antigen-specific IgE assay. The extraordinary sensitivity observed for this assay is likely an artifact of the surrogate positive control used to establish the limit of detection and limit of quantitation. Overestimation of assay sensitivity could result in an excess of false negative results when clinical samples are tested. Additionally, the sponsor concluded that cut-point determinations based on normal human serum are not equivalent to those based on serum from treatment naïve HAE patients. However, the sponsor has yet to provided data generated with treatment naïve HAE patient serum or plasma. Collectively, overestimation of assay sensitivity and use of an inappropriately high cutpoint may compound the problem of false negative clinical results for anti-DX-88 and anti-P.pastoris IgE antibodies. This is particularly problematic when interpreting the high prevalence of anaphylaxis associated with DX-88 as it makes it even more difficult to attribute causality to hypersensitivity to the drug, host cell proteins, or both.

Bibliography

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