Review: Clinical Trial Outcomes

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CLINICAL INVESTIGATION

Ecallantide for the treatment of acute attacks of hereditary angioedema due to C1-inhibitor deficiency

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Hereditary angioedema (HAE) is a rare, potentially life-threatening disease that is characterized by unpredictable swelling of the subcutaneous tissues and mucosa. Several subtypes of HAE are now recognized, with the majority of cases caused by a deficiency in C1-inhibitor (C1-INH), herein referred to as HAE-C1INH. During an HAE attack, deficiency in C1-INH results in unopposed plasma kallikrein activation and increased levels of bradykinin, which results in the swelling and pain associated with the disease. Ecallantide is a highly specific and potent plasma kallikrein inhibitor approved for the treatment of acute attacks of HAE-C1INH. In two randomized, placebo-controlled, Phase III clinical trials, 30 mg of subcutaneously administered ecallantide demonstrated significant, rapid and durable symptom relief compared with placebo. The main safety concern following ecallantide is hypersensitivity reactions, including anaphylaxis. For this reason, ecallantide should be administered by a healthcare professional with appropriate medical support to manage anaphylaxis and HAE-C1INH.

Keywords: angioedema • clinical trials • ecallantide • plasma kallikrein

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Hereditary angioedema (HAE) is a disease characterized by unpredictable, non-pitting, non-pruritic swelling of subcutaneous tissues and mucosa. The prevalence of HAE is unknown but is estimated to be approximately 1 in 50,000 people (~6000 patients in the USA) [1]. Symptoms typically begin at 10–11 years of age and worsen around puberty; however attacks have been reported in children as young as 6 months of age [2,3]. Swelling episodes can involve the skin, peripheral extremities, GI tract, genitalia, neck, face, pharynx and larynx. Untreated attacks are typically protracted, lasting anywhere from 2–7 days. The frequency of attacks is variable between patients but can range from weekly to once a year or less. Some patients have reported an attack frequency of more than ten attacks per month. Similarly, attack location and severity is highly variable between patients [1]. The triggers for an HAE attack are poorly defined for many patients but may include tissue injury secondary to trauma, medical procedures or surgery, stress, infection, menstruation, and the use of estrogen or ACE inhibitors [4]. In some patients, the onset of an attack is associated with prodromal symptoms such as fatigue, erythematous rashes, muscle ache and abdominal pain; however, patients are frequently unable to identify a precipitating event for an attack [5].

HAE is a potentially life-threatening disease, as swelling of the laryngopharyngeal tissues can result in asphyxiation. It has been reported that >50% of HAE patients will have a laryngeal attack at some point during their life [6] and mortality from these attacks can be three- to nine-fold higher in undiagnosed, untreated patients [7]. HAE is also associated with a significant amount of physical pain and psychological distress, which has a profound effect on the patient's quality of life [8,9]. While

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peripheral attacks may not always be painful, they are frequently disfiguring and interfere with both work and leisure activities. Gastrointestinal (GI) attacks are associated with severe debilitating pain and the similarities betweem presentation of an acute abdominal HAE attack and acute abdomen has led to unnecessary surgeries and hospitalizations [10]. One study reported that up to a third of HAE patients with abdominal attacks had unnecessary abdominal surgery due to misdiagnosis of their disease [11]. A survey on the burden of illness related to HAE revealed that, due to the unpredictable nature and severity of HAE attacks, patients experienced a significant impact on their educational, career development and social activities. In addition, compared with the normal population, HAE patients have a greater prevalence of anxiety and depression, which is similar in magnitude to that reported for other chronic diseases such as inflammatory bowel disease and diabetes [9].

HAE can be divided into two main categories: HAE due to C1-inhibitor (C1-INH) deficiency (HAE-C1INH) and HAE with normal C1-INH (HAEnC1) [12]. As an autosomal dominant disease, the vast majority of HAE patients have a heterozygous mutation; however patients with homozygous mutations have been identified [13,14]. Within the HAE-C1INH category, two subtypes are defined: Type I HAE, observed in 85% of patients, is associated with low C1-INH levels with a concomitant reduction in C1-INH function and Type II HAE, observed in approximately 15% of cases, is associated with normal or sometimes high levels of nonfunctional C1-INH protein.

HAEnC1 was described in 2000 [15,16]. There have since been several descriptions of HAEnC1 with mutations in the factor XII gene, and others where the cause remains unknown (the preponderance of cases occurring in females) [17,18]. As such, there is now consensus that within the category of HAEnC1, two subtypes can be defined: HAEnC1 with factor XII mutation and HAEnC1 of unknown cause [12]. In addition, just as Type I and II HAE are bradykinin-mediated diseases, it is possible that bradykinin plays a role in HAEnC1 [12]. However, because the majority of cases of HAEnC1 are not associated with a known genetic mutation and its underlying pathogenic mechanism is unknown, diagnosis and treatment of this form of HAE remains a significant challenge for the clinician [19].

Despite the hereditary nature of the various HAE subtypes, delay in diagnosis is common [1,20]. As recently as 2005, the average time between symptom onset and diagnosis was still greater than 8–10 years [21,22]. Up to 25% of patients have a *de novo* C1-inhibitor mutation, which contributes to the difficulty in diagnosis [23]. In addition to the familial forms of recurrent angioedema, sporadic forms due to an acquired C1-INH deficiency, ACE-induced and idiopathic angioedema have also been described, which can also complicate diagnosis.

Pathophysiology

C1-INH is the primary regulator of the coagulation, kallikrein–kinin (contact) and complement systems and a minor inhibitor of the fibrinolytic system [24]. While the activation state of the classical complement pathway is clearly altered in HAE-C1INH and is instrumental in guiding diagnosis of the disease, evidence suggests that increased production of bradykinin due to dysfunction in the kallikrein–kinin system pathway (Figure 1) is the primary mediator responsible for HAE-C1INH symptoms [25]. The proteins associated with the kallikrein–kinin system have a number of important physiological functions, including anticoagulation, profibrinolytic, anti-adhesive and proinflammatory activities, making them crucial mediators in vascular biology and inflammatory reactions [26]. Under non-pathological conditions, C1-INH inhibits a number of important enzymatic reactions in this pathway including factor XIIa activation of prekallikrein, plasma kallikrein digestion of high molecular weight kininogen to bradykinin and feedback activation of factor XII by kallikrein (a positive feedback loop) [4]. During an HAE-CINH attack, the lack of C1-INH results in uninhibited activation of the kallikrein–kinin pathway leading to overproduction of bradykinin, which binds to bradykinin-2 receptors on endothelial cells and results in vasodilation with extravasation of fluid, manifesting clinically as swelling and pain. In addition to factor XII activation of the bradykinin-forming cascade, Joseph and colleagues recently presented evidence for factor-XII independent activation that has implications for HAE-C1INH [27]. They demonstrated that in factor XII-deficient plasma, prekallikrein acquires enzymatic activity upon binding to high-molecular weight kininogen, which results in the release of bradykinin, but only in the absence of C1-INH. This intriguing finding provides an alternative mechanism by which attacks may be initiated [27].

While significant progress has been made in understanding some of the fundamental aspects of the plasma kallikrein–kinin cascade in HAE-C1INH, much remains unknown. For example, the positive feedback of plasma kallikrein on factor XII activation has long been recognized but the importance of this loop in attack progression and/or duration is still poorly elucidated [28]. Within this cascade, differences in the temporal dynamics of autoactivation versus kallikrein activation of factor XII (2000-fold faster for kallikrein activation [29]), suggests the potential importance of plasma kallikrein activation in mediating the

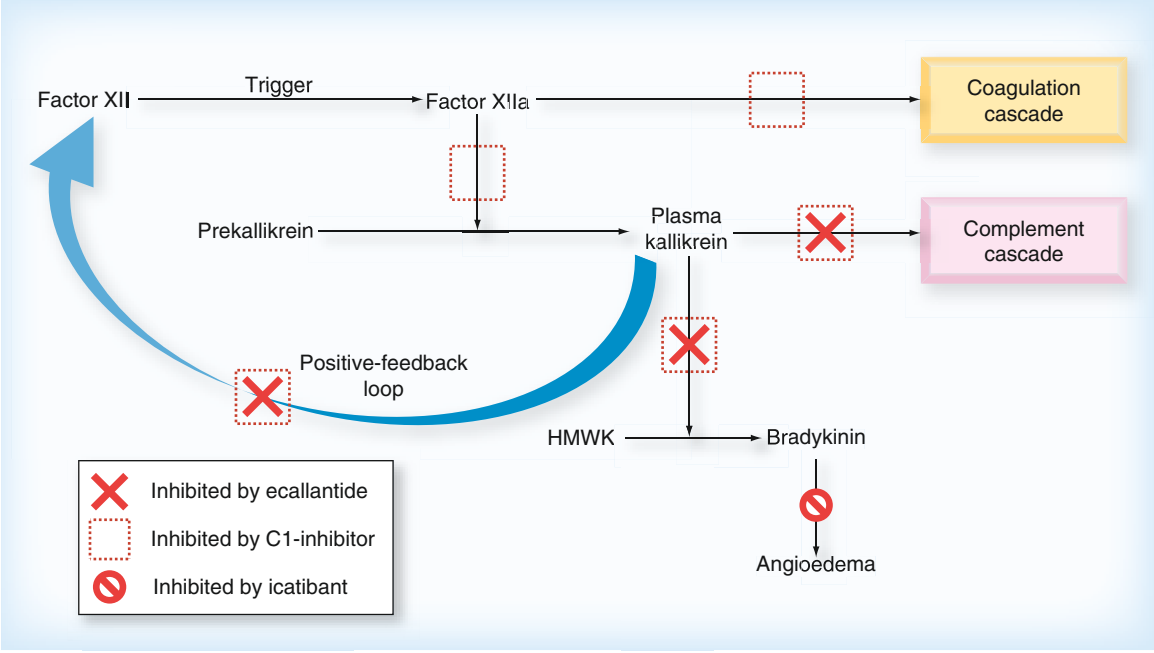

Figure 1. Kallikrein–kinin pathway and its involvement in an acute attack of hereditary angioedema. During an hereditary angioedema attack, factor XII is activated to factor XIIa and converts prekallikrein to kallikrein. Plasma kallikrein then digests HMWK to release bradykinin. Bradykinin acts at bradykinin 2 receptor (and possibly bradykinin 1 receptor and C1q receptor) to cause vasodilation, inflammation and edema.
HMWK: High molecular weight kininogen.
Adapted from Kaplan and Joseph [4].

inherent instability of the contact system in HAE-C1INH patients. That is, in HAE-C1INH patients where C1-INH function is diminished, the rapidity of kallikrein activation could quickly consume what little C1-INH is available for system homeostasis leading to the progression of an acute angioedema attack. In addition, factors that determine duration or severity of an attack remain elusive. Answering this question is complicated because plasma C1-INH levels do not correlate with attack severity and there is no known association between the duration or severity of an attack and other components of the cascade, such as plasma kallikrein or bradykinin. In addition, plasma kallikrein and bradykinin are very difficult to measure *in vivo*. However, there is evidence that differences in plasma levels of aminopeptidase P, an important enzyme in the catabolism of kinins, may contribute to the severity of attacks [30]. Involvement of the B1 bradykinin receptor, C1q receptor, polymorphisms in the bradykinin receptors, degrading enzymes on cell surfaces or contact-system proteins may also play an integral role, but these are not completely understood yet [1,31]. Nevertheless, significant advances in both the diagnosis and treatment of HAE have occurred due to our current understanding of HAE pathophysiology.

Diagnosis & treatment of HAE

In recent years a number of guidelines and consensus documents have provided both extensive diagnosis algorithms, as well as evidence-based treatment recommendations for patients with the various subtypes of HAE [12,32–34]. These documents also provide guidelines for the treatment of patient populations that may present unique challenges, such as children and pregnant or lactating women [33,34].

■ Diagnosis

An understanding of the pathophysiology of HAE-C1INH has allowed for the development of laboratory tests to aid in disease diagnosis and differentiation of HAE-CINH from other forms of angioedema. HAE-C1INH should be suspected if patients report with:

- Recurrent attacks of non-pitting, non-pruritic swelling without urticaria;
- Family history of angioedema;
- Onset of symptoms in childhood/adolescence;
- Recurrent abdominal attacks;

- Occurrences of upper airway edema;
- Failure to respond to antihistamines, glucocorticoids or epinephrine; and
- Prodromal symptoms before swelling.

Laboratory tests for blood levels of C1-INH protein and C1-INH function are important for confirming a diagnosis of HAE-C1INH Type I and II versus other forms of angioedema [33]. C1-INH function will be reduced in both Type I and II, whereas C1-INH protein levels will only be reduced in Type I. Differential diagnosis of HAE-CINH Type I/II from other causes of angioedema (HAEnC1, acquired C1-INH deficiency, ACE-inhibitor induced angioedema and idiopathic angioedema) typically involves meeting a combination of criteria: abnormal C1-INH and complement protein assays, family history, age of symptom onset and the absence of urticaria. An accurate diagnosis is essential as therapeutic options will differ depending on the specific type of angioedema [33].

Treatment options

The therapeutic options available for treatment of acute HAE-C1INH attacks have increased dramatically in recent years. As a result, it is now recommended that all attacks of HAE-C1INH should be considered for treatment. Attacks of the upper airway are considered to require mandatory treatment due to the potential for increased mortality [33].

Both prophylactic and acute treatments are available but access differs depending on the marketing authorization of the various treatments [35]. In addition, the choice of treatment strategy – that is, short- versus long-term prophylaxis or acute treatment – must be individualized based on the patient's situation. For example, all patients are candidates for short-term prophylaxis when they are likely to encounter situations known to trigger attacks, such as significant dental work or surgical procedures [36]. Alternatively, long-term prophylaxis may be appropriate in patients with a high frequency of attacks.

Attenuated androgens

Attenuated androgens (methyltestosterone, danazol, oxymetholone or stanozolol) have been used in the prophylactic treatment of HAE-C1INH in the USA and Europe for many years. Until recently, they were the primary therapeutic approach used in the USA for short- and long-term prophylaxis [37]. The benefits of attenuated androgens include their effectiveness, low-cost and oral availability, but they can have a high rate of adverse effects such as hair growth, virilization, weight gain, hepatic necrosis, hepatic neoplasms, hypertension and abnormal lipoprotein metabolism [37]. These side-effects can be minimized by titration to the lowest dose necessary to maintain symptom control. They are contraindicated in pregnancy, lactation, prostate cancer and childhood. Potential damage to the liver and alterations in lipid metabolism requires vigilant monitoring of liver function and lipid profiles.

Solvent detergent-treated plasma & fresh frozen plasma

With the availability of more targeted therapeutic options, solvent detergent-treated and fresh frozen plasma (FFP) are currently considered the treatment of last resort for acute attacks of HAE-C1INH and should only be considered when other specific treatments are not available [33]. FFP is administered intravenously (iv.) and contains C1-INH but also contains contact-system proteins, which could theoretically exacerbate an attack by providing additional substrate for the generation of bradykinin [1]. Plasma also carries the risk of blood-borne infectious agents due to limited treatment (solvent/detergent) to ensure viral safety. No controlled studies have been conducted using FFP but there are reports of its effectiveness in the treatment of acute attacks of HAE-C1INH, with little evidence of worsening a preexisting attack [38].

Plasma-derived C1-INH

In Europe and Canada, plasma derived C1-INH replacement therapy has been available for prophylaxis and treatment of acute HAE-C1INH attacks for a number of years. The use of human plasma-derived C1-INH products carries a risk of thrombotic events and blood-borne infectious agents [39–41]. However, no cases of blood-borne infectious disease have been described with the use of nanofiltered plasma-derived C1-INH products to date.

Cinryze® (ViroPharma, Exton, PA, USA) is a pasteurized, nanofiltered, plasma-derived C1-inhibitor (human) indicated for routine (short- and long-term) prophylaxis against angioedema attacks in adolescent and adult patients with HAE-C1INH [41]. Cinryze has been available in the USA since 2008 for the prevention of HAE-C1INH attacks, and was approved in Europe in 2011 for the acute treatment and prevention of HAE-C1INH attacks [35]. Cinryze is iv. administered and is approved for self-administration [41].

Berinert® (CSL Behring, Marburg, Germany) is a pasteurized, nanofiltered, plasma-derived C1-inhibitor (human) indicated for the treatment of acute abdominal, facial or laryngeal attacks of HAE-C1INH in adult and adolescent patients [40]. Berinert has been available for treatment of acute attacks of HAE-C1INH in Europe since 1979 and received approval in the USA in 2009 [35]. Berinert was also recently approved (2013) in

Europe for short-term prophylaxis in adult and pediatric patients. Berinert is iv. administered and is approved for self-administration [40].

Recombinant C1-INH

Ruconest™/Rhucin® (conestat alfa; Pharming Group NV, Leiden, The Netherlands) is an analog of the human C1-inhibitor (rhC1INH) produced by recombinant DNA technology in the milk of transgenic rabbits [42]. Ruconest received approval in Europe in 2010 for the treatment of acute attacks of HAE-C1INH in adults and approval in the USA is anticipated. Due to a risk of allergic reaction, Ruconest is contra-indicated in all patients with known or suspected rabbit allergy, or positive serum IgE antibodies against rabbit dander [35]. Ruconest is iv. administered.

Plasma kallikrein inhibitor

Kalbitor® (ecallantide; Dyax Corp., Burlington, MA, USA) is a potent and specific plasma kallikrein inhibitor indicated for treatment of acute attacks of HAE-C1INH in patients 16 years of age and older [43]. Kalbitor received approval in the USA in 2009 for the treatment of acute attacks of HAE-C1INH but is unavailable in Europe. Kalbitor is subcutaneously (sc.)administered. Potentially serious hypersensitivity reactions, including anaphylaxis, have occurred in patients treated with Kalbitor and the Kalbitor label contains a box warning for anaphylaxis [43].

Bradykinin receptor antagonist

Firazyr® (icatibant; Shire Orphan Therapies, Inc., Lexington, MA, USA) is a bradykinin B2 receptor antagonist indicated for treatment of acute attacks of HAE-C1INH in adults 18 years of age and older [44]. Firazyr is approved in both Europe (2008) and the USA (2011) for the treatment of acute attacks of HAE-C1INH. Firazyr is sc. administered and is approved for self-administration. Its major side effect is moderate pain localized to the site of injection.

Ecallantide mechanism of action, pharmacokinetics & clinical development

Ecallantide is a potent (K_i = 25 pM), specific, and reversible inhibitor of plasma kallikrein [43,45]. It is a 60-amino-acid recombinant protein developed using phage display technology and produced by expression in the yeast *Pichia pastoris* [46]. Ecallantide is more potent than C1-INH in its ability to inhibit plasma kallikrein, has low affinity for other proteases and is more selective in its inhibition than C1-INH [46]. As a plasma kallikrein inhibitor, ecallantide should inhibit both the cleavage of high-molecular-weight kininogen to bradykinin, as well as the positive feedback of plasma kallikrein on Factor XII (Figure 1).

Ecallantide is formulated as a colorless, clear, sterile, preservative-free and nonpyrogenic solution that is sc. administered and supplied as three vials, each containing 1 ml of 10 mg/ml ecallantide (30 mg total dose). Due to the chance of hypersensitivity reactions, including anaphylaxis, ecallantide should be administered by a healthcare professional with appropriate medical support to manage anaphylaxis and hereditary angioedema [43].

The pharmacokinetic (PK) parameters of ecallantide in healthy subjects following a single 30 mg sc. dose are presented in Table 1. A mean (± SD) maximum plasma concentration of 586 ± 106 ng/ml was observed approximately 2–3 h postdose. The population pharmacokinetic parameters of ecallantide were characterized in 35 HAE-CINH patients and 62 healthy subjects. Based on this population analysis, no differences in PK parameters were noted between healthy subjects and HAE-C1INH patients. Patient age, sex or weight also had no significant effect on ecallantide exposure. The clearance of ecallantide was 7.56 l/h with a volume distribution at steady state of 15.1 l and an effective half-life of 0.8–4.5 h [47]. Repeat-dose and single-dose pharmacokinetics were similar and accumulation did not occur with once-daily repeated-dose administration [47].

The involvement of the plasma kallikrein–kinin system in the modulation of the intrinsic coagulation pathway suggests that ecallantide could affect coagulation. As part of the safety assessment of ecallantide, activated partial thromboplastin time (aPTT), prothrombin time (PT) and thrombin time were monitored. In both HAE-C1INH patients and healthy volunteers, sc. ecallantide was not associated with clinically relevant aPTT prolongation and safety signals associated with bleeding have not been observed [48]. This result is in line with the finding that individuals with Fletcher Factor Deficiency, who lack prekallikrein, do not have hemostatic abnormalities [49].

Table 1. Pharmacokinetic parameters following 30 mg subcutaneous dose of ecallantide in healthy volunteers.

Parameter	Mean ± SD
T_{max} (h)	2–3
C_{max} (ng·ml)	586 ± 106
AUC (ng·h/ml)	3017 ± 402
Plasma elimination half-life (h)	2.0 ± 0.5
Plasma clearance (ml/min)	153 ± 20
Volume of distribution (l)	26.4 ± 7.8

C_{max}: Maximum plasma concentration; T_{max}: Time to maximum plasma concentration.
Adapted from Stolz and Horn [34].

Ecallantide clinical development program

The clinical development program for ecallantide in the treatment of acute attacks of HAE-C1INH involved ten clinical trials, including four Phase I trials in healthy subjects (DX-88/1, DX-88/6, DX-88/13 and DX-88/15); three Phase II EDEMA trials in HAE patients (EDEMA0℠, EDEMA1® and EDEMA2®); two Phase III trials in HAE patients (EDEMA3®, EDEMA4®) and an open-label Phase III extension study (DX-88/19). EDEMA3 included both a randomized, double-blind, placebo-controlled study (EDEMA3-DB) and an open-label, repeat-dosing, extension study (EDEMA3-RD) [45,47]. Table 2 summarizes clinical studies conducted in patients with HAE.

■ Efficacy: EDEMA3 & EDEMA4 integrated analysis

The safety and efficacy of 30 mg sc. ecallantide for the treatment of moderate-to-severe acute attacks of HAE-C1INH was evaluated in two double-blind, multicenter, placebo-controlled Phase III studies, EDEMA3-DB and EDEMA4. EDEMA3-DB included 72 patients (36 per treatment group) and EDEMA4 included 96 patients (48 per treatment group); 25 patients enrolled in both studies. Patients were considered eligible for study participation if they were age ≥10 years of age and presented to the study site within 8 h of a moderate-to-severe HAE-C1INH attack at any anatomic location. Eligible patients were randomized 1:1 to receive 30 mg of sc. ecallantide or placebo. Randomization was stratified based on anatomic location of the attack (laryngeal, abdominal or peripheral) and prior exposure to ecallantide [50,51].

A single dose of open-label 30 mg ecallantide was made available to patients who experienced respiratory distress (referred to as the severe upper airway compromise [SUAC] dose) within 4 h of the initial dose. In EDEMA4, an open-label dose (Dose B) was available between 4 and 24 h if patients had no or incomplete response, or relapse (reoccurrence of attack symptoms) following the initial dose.

Efficacy in both EDEMA3 and EDEMA4 was evaluated using two HAE-specific, patient reported outcome measures: the Mean Symptom Complex Severity (MSCS) score and the Treatment Outcome Score (TOS). The psychometric properties of these tests, including reliability, validity and minimally important difference, were characterized by Vernon *et al.* using pooled data from a randomized controlled trial [52]. Change in MSCS score at 4 h postdosing was the primary end point in EDEMA4 and a secondary end point in EDEMA3-DB. TOS 4 h postdosing was the primary end point in EDEMA3-DB and a secondary end point in EDEMA4. For both MSCS score and TOS, patients identified the location of the attack based on five symptom complexes: internal head/neck (referred to as laryngeal); stomach/GI (GI); and genital/buttocks, external head/neck or cutaneous (collectively referred to as peripheral).

MSCS score is a comprehensive point-in-time measure of symptom severity. On presentation, patients identified all active symptom complexes and rated the severity of each on a three-point scale (1: mild; 2: moderate; 3: severe). At 4 and 24 h after dosing, patients again rated the severity of all symptom complexes identified at baseline, as well as any emergent symptom complexes (0: no symptoms [applicable only to symptoms present at baseline]; 1: mild; 2: moderate; 3: severe). The ratings from all presenting and emerging symptom complexes were averaged to generate the MSCS score. A decrease in MSCS score from baseline reflects symptom improvement; the minimally important difference (MID) for change in MSCS score from baseline was estimated to be -0.30 [52].

TOS is a comprehensive response to treatment. At 1, 2, 3, 4 and 24 h postdosing, patients' assessment of treatment response compared with baseline was recorded on a categorical scale as follows: significant improvement = 100, improvement = 50, same = 0, worsening = -50, significant worsening = -100. The

Table 2. Summary of clinical studies of ecallantide.

Study	Study design	Dose	Phase	Patients (n)
EDEMA0	Open-label, single-dose	10 and 40 mg iv.	II	9
EDEMA1	Double-blind, placebo-controlled, single-dose	80 mg iv.	II	49
EDEMA2	Open-label, repeat-dose	5, 10, 20, 40 mg/m^2 or placebo iv. or 30 mg sc.	II	77[†]
EDEMA3-DB	Double-blind, placebo-controlled, single-dose	30 mg sc. or placebo sc.	III	72
EDEMA3-RD	Open-label, repeat-dose	30 mg sc.	III	67
EDEMA4	Double-blind, placebo-controlled, single-dose	30 mg sc. or placebo sc.	III	96
DX88/19 (continuation)	Open-label, repeat-dose	30 mg sc.	III	147

[†]Any individual patient may have been treated at more than one dose level and for more than one attack at each dose.
iv.: Intravenous; sc.: Subcutaneous.

TOS is weighted by baseline severity; the MID for TOS was estimated to be +30 [52].

The similarity in the design of EDEMA3-DB and EDEMA4 justified an integrated analysis of the data. For purposes of this review, results from the integrated analysis are included as the data are representative of a larger population sample and may more adequately summarize the expected clinical experience of ecallantide use ([53]; see [50,51] for separate analysis of EDEMA3-DB and EDEMA4, respectively). We note that individually, both studies reached statistical significance in their primary end points [50,51]. The integrated analysis included 143 unique patients. Baseline demographics were similar between both studies and were well matched between treatment groups for age, sex and race.

Figure 2A shows the change from baseline in MSCS score 4 and 24 h postdosing in the integrated analysis (n = 143). The mean change in MSCS score was significantly greater for the ecallantide group than for placebo at 4 h (ecallantide [mean ± SD]: -0.97 ± 0.78; placebo: -0.47 ± 0.71; p < 0.001) and 24 h (ecallantide [mean ± SD]: -1.37 ± 0.78; placebo: -1.04 ± 0.73; p = 0.028). In addition, 74.3% of ecallantide-treated patients compared with 49.3% of placebo-treated patients, experienced a reduction in symptom severity of at least 0.30 (MID) by 4 h [53].

Figure 2B shows the TOS at 4 and 24 h after dosing in the integrated analysis (n = 143). At 4 h, TOS was significantly higher for ecallantide-treated patients than for placebo-treated controls (ecallantide [mean ± SD], 55.5 ± 46.5; placebo 20.0 ± 58.9; p < 0.001). A significant higher TOS was also observed at 24 h in ecallantide-treated patients compared with placebo-treated patients (ecallantide [mean ± SD], 75.5 ± 40.4; placebo 51.4.0 ± 59.6; p = 0.039). In addition, 70.0% of ecallantide treated patients, compared with 38% of placebo-treated patients, experienced an improvement of at least 30.0 (MID) by 4 h [53].

The significantly greater improvements in the MSCS and TOS measures in ecallantide versus placebo treated patients demonstrate the efficacy of ecallantide for the treatment of moderate and severe acute attacks of HAE.

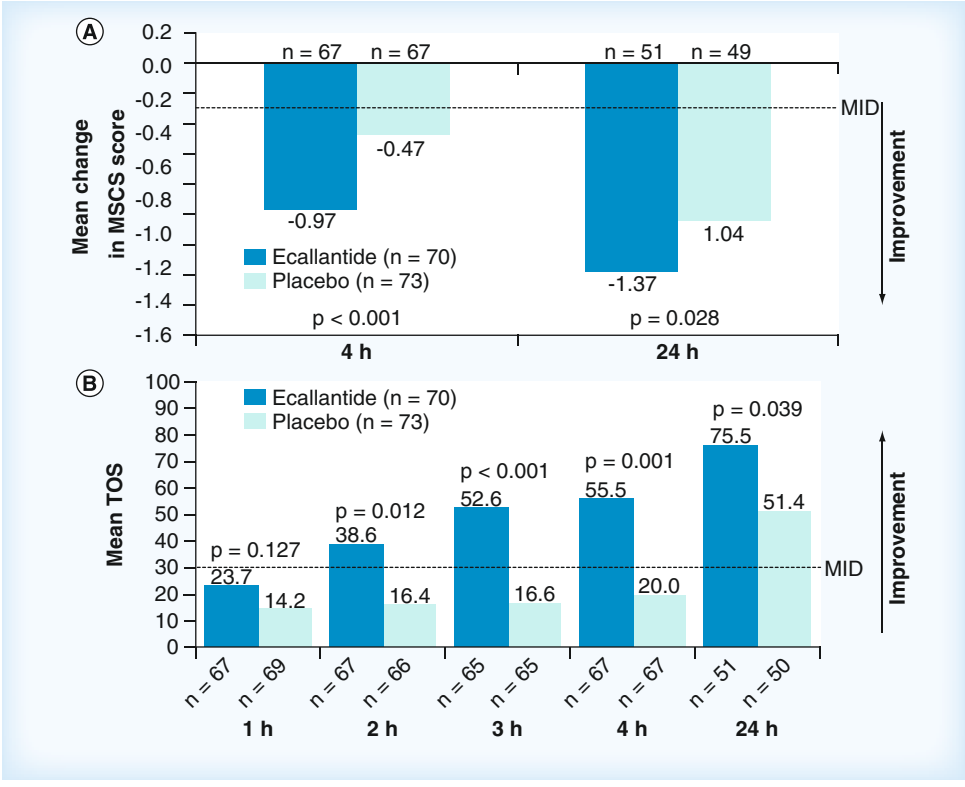

Figure 2. Efficacy outcomes from integrated analysis of EDEMA3-DB and EDEMA4. (A) Mean change in MSCS score at 4 and 24 h post-treatment with ecallantide or placebo. Negative values indicate improvement. p-values are from a Wilcoxon rank sum test. **(B)** Mean change in TOS at 1, 2, 3, 4 and 24 h post-treatment with ecallantide or placebo. p-values are from exact Wilcoxon two-sample test with Monte Carlo simulation.
MID: Minimum Important Difference (MSCS score = -0.30; TOS = 30.0); MSCS: Mean Symptom Complex Severity; TOS: Treatment Outcome Score.

The sustained improvements in both of these scores at 24 h also demonstrate the durability of ecallantide in the treatment of HAE attacks.

Time to response

In addition to MSCS score and TOS, time-to-response data were collected in EDEMA3 and EDEMA4 using a global improvement measure. Three distinct time-to-response end points were subsequently characterized: time to beginning of improvement, time to onset of sustained improvement and time to significant improvement (Figure 3) [54]. For these end points, patients completed the overall response assessment every 15 min for the first 2 h, every 30 min for hours 3 and 4, and again at 24 h. Time to beginning of improvement was defined as the first time after dosing that a patient reported a response of 'a little better' or 'a lot better or resolved' within 4 h of treatment. Time to onset of sustained improvement was defined as the first time after dosing that the patient reported a response of 'a little better' or 'a lot better or resolved' that endured

for at least 45 min. Time to significant overall improvement was defined as the first time after dosing that the patient reported a response of 'a lot better or resolved', reflecting complete or near-complete symptom resolution. The speed of effect was also analyzed by primary attack location (abdominal, laryngeal and peripheral).

A greater percentage of ecallantide-treated patients (72.9%) than placebo-treated patients (57.5%) met the criteria for beginning of improvement within 4 h. The median time (interquartile range; IQR) to beginning of improvement was 67.0 min (37.0–225.0) for ecallantide and 105.0 min (37.0–not reached within 4 h [NR]) for placebo-treated patients. However, while displaying a trend in favor of ecallantide-treatment, the distribution of these curves did not reach statistical significance [54].

Similar to the beginning of improvement results, a greater percentage of ecallantide-treated patients reported both onset of sustained improvement (68.6% ecallantide vs 41.1% placebo), and significant overall improvement of symptoms (47.1% ecallantide vs 26.0% placebo) compared with placebo-treated patients within 4 h of dosing. Ecallantide-treated patients experienced more rapid onset of sustained improvement (median [IQR] 98.0 min [52.0–NR] for ecallantide vs NR [52.0–NR] for placebo; $P_{log\ rank}$ = 0.005). The median time to significant overall improvement was not reached by 4 h in either group. However, the curves began to diverge approximately 60 min after dosing and the distributions of the curves were significantly different ($P_{log\ rank}$ = 0.02) [54].

Time-to-response analyses by attack location demonstrate that abdominal attacks responded most rapidly, followed by laryngeal attacks, while peripheral attacks were the slowest to respond [54,55]. A slower response of peripheral attacks following treatment is a common clinical experience and may be due to the capacity of mucosal tissue to more readily resorb extravasated fluid [54,56]. Given the significant pain, disability and concern of possible asphyxiation experienced during an HAE attack, a rapid, consistent and sustained resolution of symptoms is a primary goal of management. Patients treated with ecallantide reported a more rapid, durable and robust symptom resolution when compared with placebo. Indeed, statistically significant improvement in sustained symptom relief (by 2 h) and onset of overall improvement (by 90 min) was observed following ecallantide treatment [54].

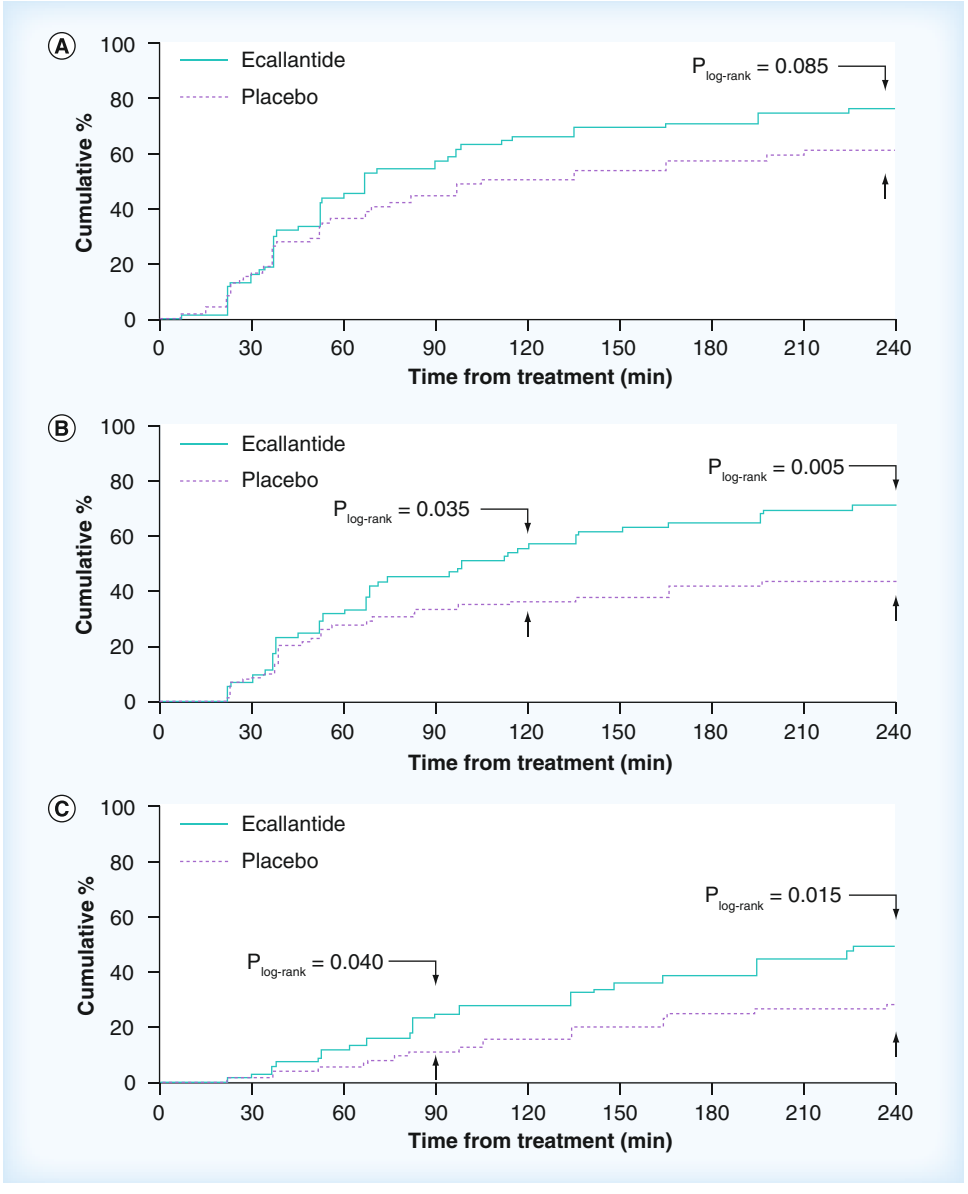

Figure 3. Kaplan–Meier analyses of time-to-response end point for the overall analysis population by treatment group. (A) Time to beginning of improvement, **(B)** time to onset of sustained improvement and **(C)** time to significant improvement.
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Efficacy of ecallantide after repeated use

Patients receiving treatment for acute attacks of HAE-C1INH will

receive repeated doses of drug throughout their lifetime due to the recurrent nature of attacks. Therefore, it is important to examine whether the efficacy and/or safety profile of the treatment changes with repeated use. The efficacy of ecallantide following repeat dosing was examined in two Phase III open-label studies; EDEMA3-RD and DX-88/19.

Patients who received one treatment in the placebo-controlled portion of the EDEMA3-DB study were eligible for EDEMA3-RD. In this study, patients received additional doses of ecallantide for subsequent HAE attacks that occurred after 72 h from the initial attack treated in EDEMA3-DB. Most of the patients were treated for one or two additional attacks; however some received treatment for up to six attacks [47]. Both TOS and MSCS scores at 4 h postdosing were consistently improved in favor of ecallantide-treated patients through six treatment episodes [47].

DX-88/19 was an open-label continuation study to evaluate the efficacy and safety of repeated sc. ecallantide for treatment of multiple HAE-C1INH episodes. The primary end point for this study was change in MSCS score at 4 h. Change in MSCS score at 24 h, TOS at 4 and 24 h, and time to response end points were also examined. A total of 147 patients treated with ecallantide were included in the study. Analyses were conducted through 13 qualifying treatment episodes (those episodes with at least 12 treated patients) [57].

Neither MSCS score nor TOS values revealed any decrease in the efficacy of ecallantide across treatment episodes at 4 or 24 h **(Figure 4)**. At 4 h, the mean change in MSCS score from baseline ranged from -1.04 to -1.36 and exceed the MID of -0.30 for all qualifying episodes. In agreement with EDEMA3 and EDEMA4, a large proportion of patients (76.8–93.8%) met the MID for change in MSCS score by 4 h. At 24 h, the mean change from baseline in MSCS score also exceeded the MID for all qualifying treatment episodes (range: -1.31 to -1.99). TOS values at 4 h ranged from 56.2–79.8 across the 13 qualifying treatment episodes and all mean values exceeded the MID of 30. This effect was durable at 24 h postdosing, as all qualifying episodes exceeded the MID (range: 50.0–95.4). Thus, as measured by MSCS score and TOS there is no evidence for a decrease in the efficacy of ecallantide for treatment of HAE with repeated use [57].

Time to response end points further support this conclusion; 43.8–65.2% of patients experienced significant improvement within 4 h of treatment with a median time of 169–240 min in 12 of 13 qualifying treatment episodes. In addition, 69.2–100% of patients experienced onset of sustained improvement within 4 h (median time: 59–113 min). Thus, similar to change in MSCS score and TOS, time to response demonstrates no reduction in efficacy with repeated administration of ecallantide [57].

Additional analyses

Since the FDA approval of ecallantide, several additional analyses have been conducted that provide an updated perspective on the clinical experience to be expected from the use of ecallantide in treatment of HAE-C1INH attacks. These analyses include the assessment of relapse/rebound [58], efficacy of ecallantide based on time to treatment [59], efficacy of ecallantide based on attack location and severity [55], and characteristics of an HAE attack requiring a second dose of ecallantide [60]. Summaries of these studies are presented below and the reader is referred to the associated primary reference for additional information.

Relapse/rebound

While the duration of an HAE attack is highly variable, they typically last for several days [1]. Therefore, a concern in the use of treatments for acute attacks, especially for therapeutics with a short half-life, is that an attack may relapse or rebound following treatment.

To address the potential for relapse and rebound following ecallantide, a *post hoc* analysis was conducted using data from EDEMA3-DB and EDEMA4 (70 ecallantide-treated and 71 placebo-treated patients) [58]. Potential rebound/relapse was identified based on patients' 4 and 24 h efficacy results. Patients were considered to have potential rebound/relapse if they showed improvement in all three efficacy measures at 4 h (MSCS score, TOS and global response) followed by worsening in one or more measure at 24 h. Potential rebound was defined as responses that improved at 4 h but then worsened beyond baseline severity at 24 h. Potential relapse was defined as 4-h improvement that later worsened but not beyond baseline severity at 24 h. Cases were further characterized by the likelihood that the relapse/rebound was clinically relevant. Rebound was considered likely if all three measures showed worsening, possible if two measures worsened, and unlikely if only one measure worsened. Relapse was considered likely if all three measures reached a minimum threshold of worsening, possible if in two measures, and unlikely if one or no measures worsened beyond a minimum threshold [58].

The proportion of patients eligible for relapse/rebound analysis (i.e., those who showed significant improvement in all three efficacy measures at 4 h) was higher for ecallantide treatment then placebo (60 vs 37%, respectively; $p < 0.01$). Of the 42 ecallantide-treated patients eligible for inclusion in the analysis, nine showed signs of worsening at 24 h. Of the 26 placebo-treated patients, seven showed worsening at 24 h [58].

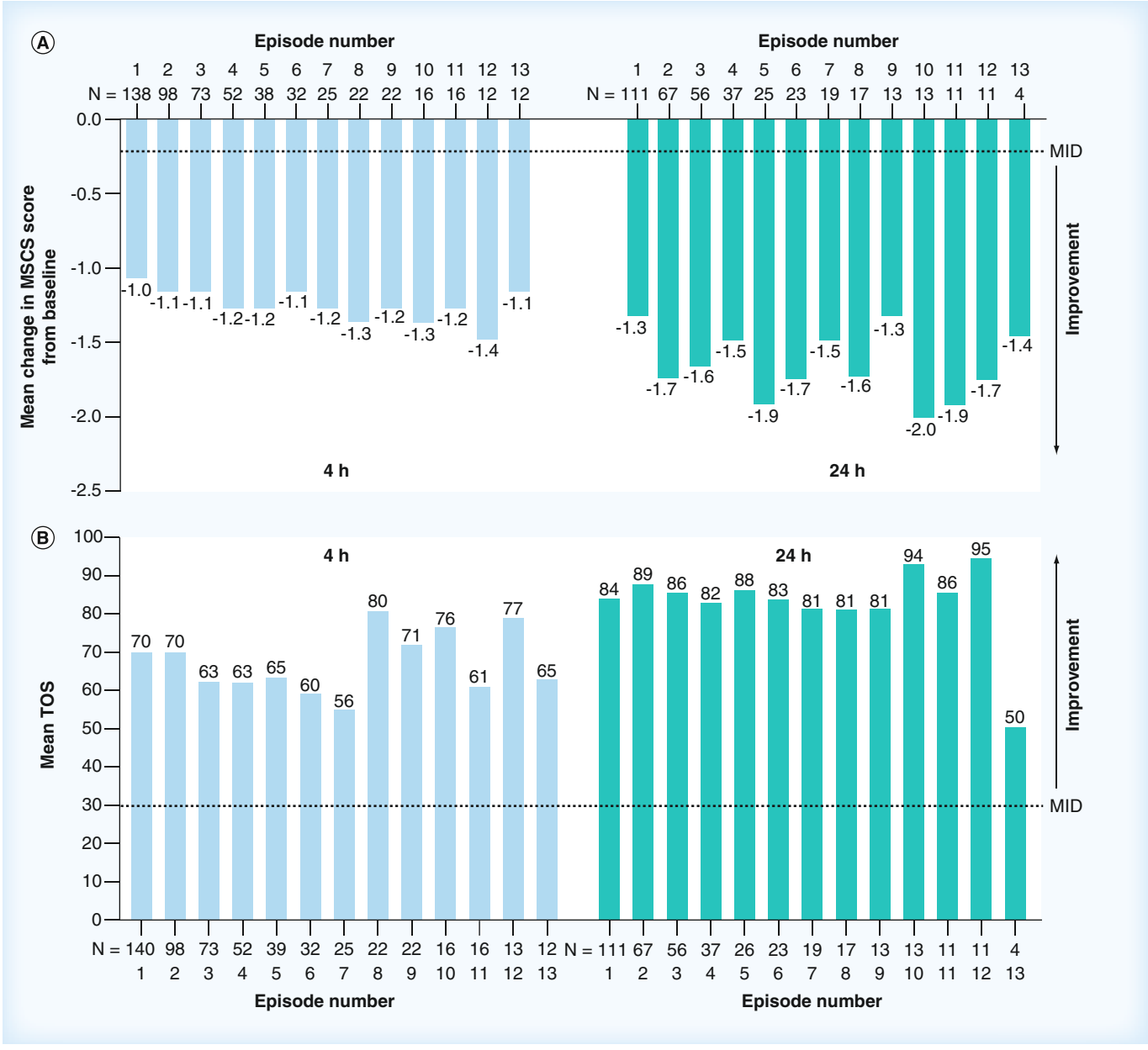

Figure 4. Efficacy outcomes of ecallantide after repeated use. (A) Mean change in MSCS score at 4 and 24 h postdosing by treatment episode. Negative values indicate improvement. **(B)** Mean TOS at 4 and 24 h postdosing by treatment episode. Positive values indicate improvement. Dashed-lines reflect MID (MSCS = -0.30; TOS = 30.0).
MID: Minimally important difference; MSCS: Mean Symptom Complex Severity; TOS: Treatment Outcome Score.
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Of the nine ecallantide-treated patients that showed worsening of symptoms at 24 h, only three patients met the criteria for likely or possible relapse and a single patient for possible rebound. In summary, relapse is only observed in a small proportion of attacks treated with ecallantide and there is little evidence of rebound [58]. These data suggest that a durable response to ecallantide treatment of acute attacks should be expected.

Time from attack to intervention

The amount of time that lapses between the onset of an attack and initiation of treatment could influence symptom improvement, as could patient and attack characteristics such as the anatomic location of the symptom, gender, weight and BMI. Using data from the analysis of EDEMA3-DB and EDEMA4 (70 ecallantide- and 73 placebo-treated patients; n = 143 patients) the impact

of time to intervention [59] and patient characteristics were assessed [55].

Efficacy outcomes were analyzed by time between symptom onset and treatment based on the following cohorts: 0–2, >2–4, >4–6 and >6–8 h. Patients who were treated with ecallantide >2–4 (MSCS score, p = 0.002; TOS, p = 0.003) or >4–6 h (MSCS, p = 0.044; TOS, p = 0.043) from symptom onset demonstrated significantly better outcomes versus placebo for both MSCS score and TOS at 4 h [59]. The number of patients treated in the 0-to-2-h cohort was low; while ecallantide treatment was associated with numerically superior results compared with placebo, these outcomes did not reach statistical significance. Patients in the >6-to-8-h cohort exhibited a decreased response; neither MSCS score nor TOS comparisons between ecallantide and placebo treatment reached statistical significance. For overall response, complete or near-complete resolution was greatest following ecallantide administration in the 0-to-2-h group (71.4%) [59]. Thus, as with other HAE therapies, early administration is optimal and recommended [32,61].

Attack location

A *post hoc* analysis of outcomes by patient and attack characteristics demonstrated that ecallantide was equally effective in males and females and was effective across attack locations and severities [55]. These analyses were based upon the first treatment episode in 143 patients (73 ecallantide- and 70 placebo-treated patients). In regards to anatomic location, both stomach/GI symptoms and internal head/neck symptoms responded well to ecallantide treatment. Both locations demonstrated significant improvement following ecallantide treatment compared with placebo for change in MSCS score (stomach/GI, p = 0.008; internal head/neck, p = 0.04) and TOS (stomach/GI, p = 0.009; internal head/neck, p = 0.02) at 4 h postdosing [55]. Cutaneous and external head/neck symptom locations responded effectively to ecallantide treatment versus placebo when assessed using TOS (cutaneous, p = 0.006; external head/neck, p = 0.02) but did not demonstrate statistical significance when assessed by change in MSCS score [55]. Significantly more patients reported onset of sustained improvement of stomach/GI, internal and external head/neck and cutaneous symptoms following ecallantide treatment than placebo. No significant differences between ecallantide and placebo were observed for attacks of the genitals/buttocks, although the number of evaluable attacks was low [55].

Attack severity

For the analyses of symptom severity, 143 patients (73 ecallantide- and 70 placebo-treated patients) were evaluated for 43 severe, 110 moderate and 30 mild symptoms. For both moderate and severe attacks, a significantly greater proportion of ecallantide- than placebo-treated patients showed onset of sustained improvement within 4 h (moderate, 69.2 vs 46.6%; p = 0.02; severe, 58.3 vs 15.8%; p = 0.006) [55]. Moderate symptoms also demonstrated statistically significant efficacy for both MSCS score (p = 0.007) and TOS (p = 0.001), while severe symptoms showed numerical improvement that did not reach statistical significance [55].

Overall, the data suggest ecallantide is an effective treatment for acute attacks of HAE-C1INH regardless of symptom location or severity. Moreover, additional analysis of 98 patients treated with ecallantide for 220 laryngeal attacks (patients from EDEMA2, EDEMA3, EDEMA4, and DX88/19) demonstrate the efficacy of ecallantide for treatment of the most concerning type of HAE attack [62].

Conversely, excess body weight may influence ecallantide's efficacy. Heavier (>200 lbs) and obese (>30 kg/m^2) ecallantide-treated patients showed a less robust and nonsignificant response compared with placebo for both change in MSCS score and TOS. However, ecallantide was effective for both non-obese patients (MSCS, p = 0.001; TOS, p = 0.001), as well as patients weighing <200 lbs (MSCS, p < 0.001; TOS, p < 0.001). It appears that a standard 30 mg sc. dose may be less effective in obese patients, and a second dose of ecallantide (dose B) can be considered [55].

Factors associated with use of a second dose

As noted, HAE attacks present with highly variable symptoms, severity and duration. As such, certain attacks may require more than one dose of an on-demand treatment and it is recommended that patients have at least two doses available on demand for treatment of acute attacks [32]. An analysis of ecallantide clinical trial data was undertaken in an effort to identify potential factors predictive of needing a second dose [60].

This analysis included 732 ecallantide-treated HAE-C1INH attacks in 179 patients treated in studies (EDEMA2, EDEMA4 and DX-88/19) that allowed a second, open-label dose (Dose B) of ecallantide between 4 and 24 h after the initial dose at the discretion of the investigator for incomplete response, failure to respond (only an option in EDEMA4), or relapse (defined as recurrence of symptoms). Dose B was administered in 88 attacks (12.0%), with 80.5% of Dose B's administered for incomplete response, 12.2% for failure to respond to treatment and 7.3% for relapse [60].

Possible patient and attack characteristics predictive of needing a second dose were analyzed by logistic regression and a multivariate model was built using backward

selection. Univariate analysis suggested baseline severity and peripheral attack were potentially predictive [63]. However, the multivariate model identified only peripheral attacks as significantly correlated ($p < 0.05$) with the necessity of a second dose [63]. It is well documented that peripheral attacks respond more slowly to treatment and the increased rate of a second dose in peripheral attacks described in this analysis is consistent with this finding [54,56].

Efficacy assessments demonstrated that attacks requiring a second dose of ecallantide had minimal improvement at 4 h following the initial dose (MSCS score [mean ± SD]: -0.43 ± 0.67); TOS [mean ± SD]: 27.5 ± 41.5) but symptoms improved following the second dose (MSCS score [mean ± SD]: -1.49 ± 1.23); TOS [mean ± SD]: 65.1 ± 32.5). Thus the second dose of ecallantide provided symptom relief despite the physician's assessment that the initial dose did not provide adequate symptom relief [63].

Safety outcomes

Treatment emergent adverse reactions

The safety of ecallantide was evaluated in 255 HAE-C1INH patients, ages 10–78 years (66% female, 86% Caucasian) treated with either iv. or sc. ecallantide. The most common adverse reactions were headache (16.1%), nausea (12.9%), fatigue (11.8%), diarrhea (10.6%), upper respiratory tract infection (8.2%), injection site reactions (7.4%), nasopharyngitis (5.9%), vomiting (5.5%), pruritus (5.1%), upper abdominal pain (5.1%) and pyrexia (4.7%). Anaphylaxis was reported in 3.9% of patients with HAE. Injection site reactions were characterized by local pruritus, erythema, pain, irritation, urticaria, and/or bruising [43].

Table 3 describes the most common treatment emergent adverse effects (TEAE) reported with the use of ecallantide or placebo in EDEMA3-DB and EDEMA4 [43]. The incidence of these reactions is based on 143 unique HAE patients treated with 30 mg sc. ecallantide or placebo. Of the 143 patients, 36.0% experienced a TEAE compared with 34.6% of placebo-treated patients. The TEAEs were defined as any event with onset date/time on or after administration of study drug in the first study in which a patient participated, through 28 days after the last dose for the last study within a given analysis population [47].

Hypersensitivity reactions, including anaphylaxis

Potentially serious hypersensitivity reactions, including anaphylaxis, have occurred in patients treated with ecallantide. Of the 255 HAE-C1INH patients treated with iv. or sc. ecallantide in clinical studies, ten patients (3.9%) experienced anaphylaxis. For the subgroup of patients (n = 187) treated with sc. ecallantide, five patients (2.7%) experienced anaphylaxis. Symptoms associated with these reactions have included chest discomfort, flushing, pharyngeal edema, pruritus, rhinorrhea, sneezing, nasal congestion, throat irritation, urticaria, wheezing and hypotension. These reactions occurred within the first hour after dosing. Other adverse reactions indicative of hypersensitivity reactions included the following: pruritus (5.1%), rash (3.1%), and urticaria (2.0%) [43].

In DX-88/19, eight patients reported potential hypersensitivity reactions. Six (4.1%) of these patients met the definition of anaphylaxis based on the National Institute of Allergy and Infectious Diseases criteria [57]. Three of these cases are included in the current prescribing information and three of the cases are new. All of the cases to date were properly managed and resolved without sequelae. The risk of anaphylaxis led to the inclusion of a boxed warning in the prescribing information for ecallantide and requires that it be administered by a health care professional with appropriate medical support to manage anaphylaxis and HAE [43].

Immunogenicity

The use of any protein-derived therapeutic carries an inherent risk of immunogenicity. For this reason, patients were monitored for the development of anti-ecallantide antibodies in the clinical development program for ecallantide. Based upon Phase III studies EDEMA3 (EDEMA3-DB and EDEMA3-RD) and EDEMA4, 7.4% of patients seroconverted to anti-ecallantide antibodies [43]. Rates of seroconversion increased with exposure over time. In addition, anti-ecallantide and anti-*Pichia pastoris* IgE antibodies were detected [43]. Patients with neutralizing antibodies to ecallantide were determined *in vitro* to be present in 4.7% of patients but the effect of neutralizing antibodies on clinical efficacy is unknown. Patients who seroconvert may be theoretically at a higher risk of hypersensitivity but the long-term effects of antibodies to ecallantide are not known [43].

Future perspective

HAE is a serious disease that profoundly affects patient safety and quality of life. It is generally accepted that the plasma kallikrein–kinin system plays a crucial role in disease pathology and therefore treatment options targeting this pathway are an effective means to manage the disease. Several approved acute ('on-demand') treatment and prophylactic options are currently available but a direct head-to-head comparison of their efficacy and safety profiles has not been undertaken. As all approved products have demonstrated efficacy in randomized, placebo-controlled clinical trials, it is recommended that treatment for HAE be individualized

to patient's needs with the aim to provide optimal care and restore quality of life [32]. As any angioedema attack can become disabling or life-threatening, it is recommended that all patients have access to at least one of the approved 'on-demand' treatments.

Several additional therapies are currently under development for either acute and/or prophylactic treatment of HAE and will continue to expand the therapeutic options available to manage this highly variable disease. Clinical trials are ongoing for the sc. administration of both Cinryze and Berinert [64]. In addition, Ruconest, which is currently available in Europe, has completed Phase III trials in the USA and Santarus, Inc. and Pharming Group NV have announced the submission of a Biologics License Application to the FDA to obtain approval for the treatment of acute angioedema attacks in HAE patients. An orally available small molecule plasma kallikrein inhibitor (BCX4161; BioCryst, Durham, NC, USA) [65], as well as a subcutaneous, long acting, fully human monoclonal antibody that inhibits plasma kallikrein (DX-2930; Dyax, Burlington, MA, USA) are also being developed for use in the treatment of HAE [66].

Despite substantial progress in the treatment and diagnosis of HAE, there remain a number of outstanding research questions. One, provided the number of treatment options available to HAE patients, is it possible to identify biomarkers that could predict an optimal therapeutic strategy? Two, how do we best manage patients diagnosed with HAEnC1? There is anecdotal evidence that currently approved acute therapies for HAE type I/II (C1-INH, icatibant and ecallantide) may be beneficial in HAEnC1 but no randomized or controlled clinical trial has been conducted [12]. Furthermore, while this review has focused on ecallantide for use in Type I and II HAE, the proper management of ACE-induced, acquired and other non-histaminergic angioedemas remains an active area of interest [32]. The development of biomarker assays identifying key molecular players involved in the pathology of each of these diseases will greatly facilitate the development of novel therapeutic strategies for their management.

Table 3. Adverse reactions occurring at ≥3% and higher than placebo in two placebo controlled clinical trials in patients with hereditary angioedema treated with ecallantide.

Adverse reactions	Ecallantide (n; %)†	Placebo (n; %)‡
Headache	8 (8)	6 (7)
Nausea	5 (5)	1 (1)
Diarrhea	4 (4)	3 (4)
Pyrexia	4 (4)	0
Injection site reactions	3 (3)	1 (1)
Nasopharyngitis	3 (3)	0

Patients (n = 25) who participated in both studies and who received a different treatment in each study are counted in both groups. Additionally, patients in the placebo group who received open-label ecallantide for either severe upper airway compromise or as Dose B are counted in the placebo group for adverse events reported prior to the receipt of open-label ecallantide and in the ecallantide group for adverse events reported after the receipt of open-label ecallantide.

†Total n = 100; ‡Total n = 81.

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No writing assistance was utilized in the production of this manuscript.

Executive summary

Background

- Hereditary angioedema with C1-Inhibitor deficiency (HAE-C1INH) is a rare, potentially life-threatening disease that is characterized by unpredictable swelling of subcutaneous tissues and mucosa.

Ecallantide clinical development

- Ecallantide is a potent and specific plasma kallikrein inhibitor with demonstrated efficacy in the treatment of acute attacks of HAE-C1INH at any anatomic location.
- Treatment of acute attacks of HAE-C1INH with ecallantide is associated with rapid, robust and durable symptom relief.
- Repeated use of ecallantide is not associated with a decrease in efficacy.

Ecallantide safety outcomes

- There is a documented safety concern of hypersensitivity reactions and anaphylaxis with use of ecallantide. For this reason, ecallantide should only be administered by a healthcare professional with appropriate medical support to manage anaphylaxis and hereditary angioedema.

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