Recombinant replacement therapy for hereditary angioedema due to C1 inhibitor deficiency

Hereditary angioedema is a rare genetic condition transmitted as an autosomal dominant trait and characterized most commonly by the production of either inadequate or nonfunctioning C1 esterase inhibitor (C1-INH), a blood protein that regulates proteases in the complement, fibrinolytic and contact systems. Patients with hereditary angioedema suffer from episodic, unpredictable manifestations of edema affecting multiple anatomical locations, including the GI tract, facial tissue, the upper airway, oropharynx, urogenital region and/or the arms and legs. A rational approach to treatment is replacement of C1-INH protein, to normalize the levels of C1-INH activity and halt the progression of the biochemical activation processes underlying the edema formation. Ruconest® (Pharming Group NV, Leiden, The Netherlands) is a highly purified recombinant human C1-INH. This article will focus on the results of ten clinical studies demonstrating the efficacy and safety of Ruconest, which is now approved for use in Europe, Israel and the USA.

Keywords: C1-INH • C1-INH-HAE • hereditary angioedema • recombinant human C1 esterase inhibitor • Ruconest®

Background HAE

Patients with hereditary angioedema (HAE) due to C1 esterase inhibitor (C1-INH) (C1-INH-HAE) experience acute attacks of localized swelling (angioedema) due to mutations in the C1-INH gene, *SERPINE1*, causing either a deficiency and decreased functionality (Type I HAE) or mutations causing a nonfunctionality of the C1-INH protein, which may be quantitatively normal or increased in the blood (Type II HAE). To date, more than 250 genetic mutations (point mutations or wide gene rearrangements) have been described (HAEdb; <http://hae.enzim.hu>) [1]. *De novo* mutations account for approximately 25% of C1-INH-HAE cases [2].

The lack of functional C1-INH results in uncontrolled activation of the contact system, leading to an excessive release of bradykinin causing increased vascular permeability (Figure 1) [3]. The fluid leak from the intravascular compartment into the subcuta-

neous or submucosal space becomes manifest as an acute episode of angioedema, which is the hallmark of an C1-INH-HAE attack.

Clinically, patients with C1-INH-HAE experience recurrent acute attacks of soft tissue swelling that can affect multiple anatomic regions, including the GI tract, facial tissues, upper airway, oropharynx, urogenital region and/or the arms and legs. These acute attacks are associated with considerable morbidity, and they often require hospitalization and immediate medical intervention. Laryngeal attacks can be life threatening, due to obstruction of the upper airway and are associated with significant morbidity and mortality [4]. Sudden onset of intestinal mucosa edema causes severe pain, due to the occlusion of the bowel and may mimic a surgical 'acute abdomen.' In undiagnosed C1-INH-HAE patients, this may lead to unnecessary surgical intervention. Sometimes regarded as clinically insignificant, swelling attacks in the extremities of the body are disabling,

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Figure 1. The role of C1 esterase inhibitor.

B1R: B1 bradykinin receptor; B2R: B2 bradykinin receptor; BK: Bradykinin; FXII: Factor XII; HK: High molecular weight kininogen; PK: Plasma kallikrein; PPK: Plasma prekallikrein.
C1-INH: C1 esterase inhibitor.

disfiguring and painful [5]. Such peripheral attacks account for more than half of the attacks in patients with C1-INH-HAE [6]. Although the majority of the published literature describing C1-INH-HAE attacks focuses on a single anatomical site, attacks can affect one or more anatomical locations simultaneously or start in one area and spread to another site [7].

Despite being a rare condition, C1-INH-HAE is associated with a significant healthcare and economic burden. Over a one year period in the United States, C1-INH-HAE accounted for >5000 emergency department visits, 41% of which resulted in hospitalization [8]. C1-INH-HAE is also associated with psychological problems, anxiety and depression resulting in high rates of absenteeism from work and school [9]. The quality of life (QoL) of C1-INH-HAE patients has been found to be significantly compromised by limited personal autonomy, and its impact on professional and leisure activities [10]. The low prevalence of C1-INH-HAE combined with a clinical presentation of intermittent recurrent abdominal pain and/or episodes of skin swelling, often results in a significant delay in diagnosis of patients, which further results in a decline of their QoL [11]. A recent study reported the average delay to diagnosis to range from 11 to 20 years, with an average age at diagnosis of 13.2 years, despite an onset of symptoms of 5.7 years; however, there is a paucity of epidemiological information in the pediatric population and further epidemiological studies are needed [12].

Current therapies for C1-INH-HAE

Therapy for C1-INH-HAE is targeted at treatment of attacks and prophylactic therapy (Table 1). Current guidelines recommend that once diagnosed, all patients should have immediate 'on demand' access to acute therapy [13–28], because of the serious potential consequences of untreated attacks. Furthermore, early treatment is associated with a more rapid improvement of symptoms than delayed treatment. To accomplish this task, empowering patients to self-administer home therapy is essential [29].

Table 1 summarizes current treatment options for the management of acute attacks [13,30–40]. Strategies include increasing C1-INH plasma levels with plasma derived or recombinant C1-INH replacement therapy, inhibition of kallikrein and blockade of bradykinin 2 receptors.

Long-term prophylaxis is recommended for patients who experience >12 moderate-to-severe attacks per year or when they are affected with symptoms on more than 24 days per year despite on demand treatment [16]. Approved therapies for prophylaxis include plasma-derived C1-INH (e.g., Cinryze® [Shire]), and androgens (e.g., Danazol® [Sanofi-Synthelabo]). A randomized, double-blind clinical trial evaluating Ruconest for C1-INH-HAE prophylaxis is ongoing (NCT02247739).

Short-term prophylaxis using these agents is recommended for patients undergoing surgical or other invasive, traumatic procedures (dental work) especially when they have a history of swelling with physical trauma [12,15,17,19,41,42].

Rationale for recombinant human C1-INH

C1-INH replacement therapy was developed after the discovery in 1963 by Donaldson and Evans that patients with HAE had a specific inherited defect in the plasma protein C1-INH [43].

Until recently, all C1-INH products developed have been derived from plasma. Initially, there were no dedicated virus inactivation methods incorporated in its production so there was a significant potential risk for transmission of blood-borne pathogens [44]. Indeed, in one series, 86% of patients were infected with hepatitis C virus (HCV) following treatment with a plasma-derived (pd) C1-INH product prior to the introduction of virucidal methods [45]. Hepatitis G transmission has also been documented in patients receiving pdC1-INH despite the use of heat treatment virucidal methods [46].

Stringent donor selection criteria, and other precautionary measures, including vaccination against hepatitis A and hepatitis B were then introduced to further reduce any potential risk of viral transmis-

Table 1. Available treatment options for the management of acute attacks.

Indication	Dosage	Considerations	Pregnancy category (US labeling)	Most common adverse events	
Replacement C1-INH					
rhC1-INH					
Ruconest® [33,35]	Treatment of acute attacks in adult and adolescent patients (license limited to adult patients in EU)	50 U/kg up to a max of 4200 U. Option for repeat dose, based on clinical response	Administered iv. and licensed for self-administration (US only) Contra-indicated in patients with a known or suspected allergy to rabbits	B	Headache, nausea, diarrhea
pdC1-INH					
Berinert™ [31,36]	Treatment of acute abdominal, facial, or laryngeal attacks in adult and adolescent patients	20 U/kg	Administered iv. and licensed for self-administration	C	Headache, nausea, dysgeusia
Cinryze® [34,37]	Routine prophylaxis against angioedema attacks in adolescent and adult patients Treatment and preprocedure prevention of angioedema attacks in adults and adolescents (EU only)	1000 U 1000 U. Option for repeat dose, based on clinical response	Administered iv. and licensed for self-administration	C	Headache, nausea, rash, vomiting
Bradykinin B ₂ receptor blockade					
Icatibant (Firazyr®) [30,32,38,39]	Treatment of acute attacks in adults	30 mg. Option for up to two repeat doses, based on clinical response	Administered subcutaneously. Licensed for self-administration	C	Worsening or recurrence of the HAE attack within 48 h. Injection site reactions
Kallikrein inhibitor					
Ecallantide (Kalbitor®) [30,40]	Treatment of acute attacks in patients 12 years and older	30 mg	Administered subcutaneously. Not licensed for self-administration. Risk of anaphylaxis. Not licensed in EU	C	Headache, nausea, diarrhea
C1-INH: C1 esterase inhibitor; HAE: Hereditary angioedema.					

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sion [47]. The pdC1-INH products available today (Cinryze and Berinert™) now all undergo purification followed by pasteurization and nanofiltration to remove bacterial and viral pathogens. However, despite donor selection and current dedicated virus inactivation steps, plasma-derived products can still transmit nonenveloped viruses, such as the Parvovirus PB19V [48]. Other viruses with similar characteristics, such as picornavirus, circovirus and other parvoviruses (PARV4), continue to extend the list of known and unknown possible transmissible human pathogens [49]. These developments add to the grow-

ing body of evidence that viral inactivation steps effective against these types of viruses are urgently needed to improve the safety of all plasma-derived therapeutic agents [48].

Advances in gene technology in the early 2000s, facilitated the development of recombinant products in order to reduce the potential risks of viral transmission, increase the purity of the final product (contaminating proteins) and provide the prospect for an indefinite supply of product. Recombinant products produced in mammalian cells are inherently free of blood-borne human pathogens, if no human-derived

Table 2. Specific activity and purity of commercially available C1 esterase inhibitor products.			
	Berinert™	Cinryze®	Ruconest®
Specific activity (U/mg)	11.57	10.41	12.13
Purity (%)	97.0	89.5	98.6

Adapted from Feussner *et al* [58].

proteins are added during the upstream and downstream processing. Key challenges include the prevention of virus entry into the biologic manufacturing process, which could be achieved by avoiding the use of animal- and human-derived proteins, running the production process in a closed system, ensuring that nutrients for the cell culture system are pathogen free and implementing vigorous testing for adventitious viruses. Similar to plasma-derived products, robust virus inactivation/elimination steps have been implemented to reduce the theoretical risk of cross-species infection [50].

Although no biological product can guarantee the absence of risk, the current absence of cases reporting transmission of any infectious agent by recombinant products supports the demonstration of viral safety. For many diseases treated with plasma-derived products, recombinant products are now recommended the product of choice by the Medical and Scientific Advisory Council (MASAC) [51], the Italian Association of Haemophilia Centres (AICE) [49] as well as other national medical and regulatory advisories; they are also considered by the US FDA to be inherently superior to plasma-derived products with respect to transmission of infectious agents [52].

Introduction to Ruconest®

Ruconest (Pharming) was approved in Europe in 2010 and in the USA and Israel in 2014 for the treatment of acute angioedema attacks in patients with C1-INH-HAE [53–55]. Transgenic New Zealand White (NZW) rabbits, with mammary gland-specific expression vectors from the casein gene fused to genomic human C1-INH sequences, were obtained by a micro-injection technique. The NZW rabbits as transgenic production platform have the advantage of a high expression rate of recombinant human C1-INH, which is poorly expressed in cell-based systems, easy to scale up due to the relatively short breeding time compared with other mammals, and is a highly controlled closed environment minimizing the risk of pathogen entry [56,57]. The amino acid sequence of the recombinant C1-INH is identical to that of human C1-INH, with only differences in glycosylation but with the same inhibitory specificity of target proteases (e.g., activated C1s, kallikrein, FXIIa and FXIa) [57].

Table 2 shows the specific activity and purity of three commercially available C1-INH protein concentrates. Ruconest has the highest specific activity and purity, containing fewer nontherapeutic proteins compared with the plasma-derived C1-INH products. Whether these impurities (like the identified immunoglobulin heavy chain in Berinert) also have immunomodulatory effects, as described for similar intermediate purity type plasma-derived concentrates, remains to be determined [58,59].

Clinical pharmacology

Mechanism of action

C1-INH, which is a normal constituent of human blood, is one of the serine protease inhibitors (serpins); its normal plasma level is approximately 275 µg/ml (about 2.5 µM). C1-INH inhibits several target proteases of complement and regulatory points of the contact and coagulation systems [60,61]. The formation of covalent complexes between the target protease and C1-INH leads to their inactivation.

Pharmacokinetics & pharmacodynamics

Pharmacokinetics (PK)

The normal range of C1-INH activity in the general population is 0.7–1.3 U/ml (70–130% activity). The median plasma level of C1-INH activity in patients with HAE is approximately 0.2 U/ml, or approximately 20% of the normal amount [62]. Administration of escalating doses of Ruconest (6.25–100 U/kg) to asymptomatic HAE patients resulted in dose-dependent increases in functional C1-INH activity [63]. Doses of 50 and 100 U/kg restored C1-INH activity to normal levels. A C_{max} of 1.36 U/ml was observed, the volume of distribution was found to be approximately 3 l and the elimination half-life was 2.4 and 2.7 h, respectively (Table 3) [63,64]. The plasma half-life of pdC1-INH is longer than that of Ruconest and is reported to vary significantly (10–96 h) in patients with C1-INH-HAE. However, the clinical efficacy is not affected by the short life and additional/other mechanism of action models need to be investigated to explain the observation [65,66].

A population PK (PPK) analysis was conducted on PK data from a total of 120 subjects who received a combined total of 214 administrations of Ruconest across six clinical studies [61]. A one compartment

Table 3. Baseline corrected pharmacokinetics parameters (mean $\pm$ SD) following administration of 50 and 100 U/kg Ruconest® to asymptomatic hereditary angioedema patients [64].

Parameters	50 U/kg	100 U/kg
C _{baseline} (U/ml)	0.18 $\pm$ 0.12	0.14 $\pm$ 0.08
C _{max} (U/ml)	1.2 $\pm$ 0.2	2.3 $\pm$ 0.2
T _{max} (h)	0.31 $\pm$ 0.10	0.31 $\pm$ 0.10
AUC (U $\times$ h/ml)	3.3 $\pm$ 1.0	10.6 $\pm$ 2.5
CL (ml/h)	1207 $\pm$ 414	781 $\pm$ 147
Half-life (h)	2.4 $\pm$ 0.6	2.7 $\pm$ 0.3
V _{ss} (l)	3.0 $\pm$ 0.9	2.4 $\pm$ 0.5

model with Michaelis-Menten elimination kinetics provided the best overall description of the available data. Simulations performed on these data indicated that administration of a single dose of Ruconest 50 U/kg would result in >94% of C1-INH-HAE patients achieving functional C1-INH levels of at least 0.7 U/ml (the lower limit of the normal range), whereas many patients would not achieve C1-INH levels within the normal range following administration of lower doses.

The PPK simulations support capping the weight-based dosing of Ruconest at 4200 U (two vials) maintaining the concentration of functional C1-INH within an acceptable range for patients in any weight category. The PPK model did not indicate any significant differences in peak C1-INH activity levels after initial administration versus repeated administrations for subsequent attacks [61].

Pharmacodynamics

In the setting of low functional C1-INH, C1 activation causes cleavage of complement component 4 (C4). The pharmacodynamics (PD) activity of Ruconest was demonstrated in asymptomatic HAE patients by a dose-dependent decrease in the formation of C4b/c, the activation cleavage product of C4 [63]. Doses of 50 and 100 U/kg decreased the formation of C4b/c and increased mean normalized levels of C4 relative to baseline. Increased activation of C4 resumed once functional C1-INH levels fell below 0.7 U/ml. Doses $\leq$ 25 U/kg produced only a mild effect on C4 activation and a temporary, minimal elevation of C4 levels relative to baseline.

In addition to complement inhibition, Ruconest is able to prevent the cleavage of high-molecular-weight kininogen (HMWK), and thereby prevent the formation of bradykinin, the mediator of edema in C1-INH-HAE. **Figure 2** shows that during an acute C1-INH-HAE attack HMWK is completely cleaved; infusion of 50 U/Kg of Ruconest almost completely prevent HMWK cleavage.

Clinical efficacy

Attack treatment

The efficacy of Ruconest for the treatment of C1-INH-HAE patients experiencing acute angioedema attacks has been demonstrated in several studies, including three double-blind, placebo-controlled efficacy studies where patients were treated for a single C1-INH-HAE attack [33,35] and five open-label studies where patients could be treated for multiple attacks (**Table 4**) [67–70,74].

A Phase II, open-label study of Ruconest reported outcomes in nine patients with C1-INH-HAE who were treated with an infusion of 100 U/kg for 13 angioedema attacks [67]. Six of the attacks involved the abdominal area, four peripheral extremities, two facial and one urogenital. The median time to relief of symptoms based on both patient and Investigator assessments was 60 min and the median time to minimal symptoms was 4 to 8 h.

Following the demonstration of efficacy in this open-label study [67], three randomized, double-blind, placebo-controlled studies were conducted to evaluate the safety and efficacy of Ruconest for the treatment of acute angioedema attacks [33,35]. The studies were similar in design, inclusion/exclusion criteria and endpoints. Doses of 50 and 100 U/kg were tested in the studies. Efficacy endpoints were the time from drug administration to the initial relief of symptoms and time to minimal symptoms. Each of the three studies showed statistically significant, superior efficacy of Ruconest compared with saline in reducing the time to symptom relief in patients with C1-INH-HAE attacks (**Table 4**). Zuraw *et al.* reported a pooled analysis of two studies showing a significant reduction in time to the beginning of relief of symptoms compared with saline ($n = 29$): median, 66 (95% CI, 61–122) min (100 U/kg), 122 (72–136) min (50 U/kg) and 495 (245–520) min (saline), $p < 0.001$ and $p = 0.013$, respectively, at 100 ($n = 29$) and 50 ($n = 12$) U/kg body weight [33]. Patients treated with Ruconest had statistically and clinically significantly, shorter time to beginning of relief and the evidence for this finding was supported

Figure 2. SDS-PAGE showing HMWK cleavage before and after Ruconest® administration in a patient with an acute hereditary angioedema attack.

Figure courtesy of Mignon van den Elzen and Coen Maas, University Medical Center Utrecht, The Netherlands.

HMWK: High-molecular-weight kininogen.

by multiple sensitivity analyses of the primary efficacy endpoint in addition to the secondary and exploratory efficacy results.

These findings were confirmed in a larger randomized controlled trial (RCT) with 75 patients randomized (3:2) to Ruconest 50 U/kg (max 4200 U) or saline which demonstrated that Ruconest is superior to saline (placebo) in reducing the time to beginning of symptom relief (median times: 90.0 and 152.0 min, respectively; $p = 0.031$) [35].

The efficacy was maintained with treatment of subsequent attacks in the Open Label Extension phases [67–70]. No relapse or rebound episodes were reported for Ruconest, suggesting that its mechanism of action and PD properties, rather than its relatively short half-life, lead to sustained plasma activity. In the largest of these studies ($n = 224$ attacks treated), 96% of attacks were treated with a single dose of Ruconest 50 U/kg (max 4200 U) [68]. Furthermore, no increase in the administration of an additional treatment dose was reported for subsequent attacks.

The results from the clinical studies therefore support the approved dosing of Ruconest at 50 U/kg with a maximum of 4200 U, with an option for one second dose administration within 24 h [54].

Consistent with the PD findings reported by van Doorn *et al.* [63], a review of the efficacy and PK data from published controlled studies in the treatment of C1-INH-HAE attacks suggests that efficacy of C1-INH therapy is optimal when C1-INH activity

levels are restored to the normal range [65]. Analysis of resolution of symptoms in the same way revealed that pdC1-INH at doses of 20 or 25 U/kg reduced time to resolution by approximately 50%, whereas Ruconest at 50 or 100 U/kg reduced time to resolution by about 80% [65].

Efficacy across anatomical locations

Given the various clinical presentations of C1-INH-HAE, the efficacy of Ruconest in treating different anatomical locations of C1-INH-HAE has been reviewed.

An integrated analysis across four clinical studies showed that 83–99% of the Ruconest-treated abdominal attacks responded within 4 h compared with 45% of the attacks treated with placebo [71]. These findings were confirmed in a Phase III study showing a median time to onset of relief of 44–84 min for a patient's first five successively treated attacks [35].

Efficacy for Ruconest in treating peripheral angioedema attacks was similarly established an integrated review. Peripheral attacks had onset of relief in 95% of the Ruconest-treated patients within 4 h, whereas only 21% of saline-treated patients had relief in the same time period [5].

Although abdominal and peripheral attacks are the most common clinical manifestation of C1-INH-HAE, the most serious is laryngeal angioedema. Moldovan *et al.* demonstrated the efficacy of Ruconest in treating 53 potentially life-threatening upper airway angioedema attacks in 35 C1-INH-HAE patients. Onset of relief in these serious attacks was consistent with the treatment results of other attack locations. No use of rescue medication or intubation was required following treatment of attacks with Ruconest [72].

Efficacy in subpopulations

Symptoms of HAE often increase around the time of puberty, possibly due to hormonal influences. The efficacy of Ruconest was reviewed in 16 adolescent C1-INH-HAE patients treated for 50 attacks. Ruconest increased functional C1-INH levels into the normal range (>0.7 U/ml) for 96% of the treated attacks. Also similar to the findings in the adult population, 90% of attacks responded within 4 h after treatment with Ruconest [35].

There are no clinical studies of Ruconest in pregnancy. Despite the availability of animal data at doses up to 12.5-times the human dose, since animal data may not predict outcomes in pregnant women the product labeling indicates that Ruconest should only be used in pregnancy if clearly needed. A single case report was found in the literature reporting the use of Ruconest during pregnancy [73]. In this case, a 22-year-old woman was treated with Ruconest during

her pregnancy for eight potentially life-threatening upper airway attacks. All attacks responded within 4 h after administration of Ruconest. There were no reported delivery complications and the newborn was healthy.

C1-INH-HAE attack prophylaxis

An open-label, uncontrolled, pilot study evaluating the prophylaxis of C1-INH-HAE attacks with once-weekly administration of Ruconest at 50 U/kg was conducted over 8 weeks. The aim of the study was to

Table 4. Clinical studies evaluating the efficacy of Ruconest®.

Study	Design, patient population	Treatments (dose)	Exposure	Primary results†	Ref.
Randomized controlled studies					
Riedl <i>et al.</i> , 2014.	Randomized, double-blind, placebo-controlled study in HAE patients ≥13 years of age experiencing an acute angioedema attack	Ruconest 50 U/kg, up to 4200 U or saline. Option for rescue with open-label dose of Ruconest.	Ruconest: 43 patients Saline: 31 patients five patients randomized to Ruconest and 13 patients randomized to saline received rescue with an open-label dose of Ruconest	Time to beginning of relief of symptoms statistically significantly shorter in patients treated with Ruconest (90 min) compared with patients treated with saline (152 min; $p = 0.031$)	[35]
Zuraw <i>et al.</i> , 2010	Randomized, double-blind, placebo-controlled study in HAE patients ≥12 years of age experiencing an acute angioedema attack	Ruconest 50 U/kg, Ruconest 100 U/kg, or saline	Ruconest 50 U/kg: 12 patients Ruconest 100 U/kg: 13 patients saline: 13 patients	Time to beginning of relief of symptoms statistically significantly shorter in patients treated with Ruconest 50 U/kg (122 min; $p < 0.001$) or Ruconest 100 U/kg (68 min; $p = 0.001$) compared with patients treated with saline (258)	[33]
Zuraw <i>et al.</i> , 2010	Randomized, double-blind, placebo-controlled study in HAE patients ≥16 years of age experiencing an acute angioedema attack	Ruconest 100 U/kg or saline	Ruconest: 16 patients saline: 16 patients	Time to beginning of relief of symptoms statistically significantly shorter in patients treated with Ruconest (61.5 min) compared with patients treated with saline (508 min; $p = 0.003$)	[33]
Open-label studies					
Li <i>et al.</i> , 2015	Open-label study in HAE patients ≥13 years of age experiencing an acute angioedema attack, with option to treat multiple attacks	Ruconest 50 U/kg up to 4200 U. Optional for one additional dose based on clinical response	44 patients treated for 224 attacks. 215/224 (96%) attacks treated with single dose; 9/224 (4%) attacks treated with two doses	Time to beginning of relief of symptoms for the first 5 attacks ranged from 63 to 134 min	[68]
Riedl <i>et al.</i> , 2013	Open-label study in HAE patients ≥12 years of age with an acute angioedema attack, with option to treat multiple attacks	Ruconest 50 U/kg. Optional for one additional dose based on clinical response	62 patients treated for 168 attacks. 151/168 (90%) attacks treated with single dose; 17/168 (10%) attacks treated with two doses	Time to beginning of relief of symptoms for the first 5 attacks ranged from 37 to 67 min	[70]
Reshef <i>et al.</i> , 2013	Open-label prophylaxis study in HAE patients with history of at least 2 attacks/month	Ruconest 50 U/kg once weekly for 8 weeks	25 patients treated	Mean weekly attack rate decreased during the study to 0.4 attacks/week, as compared with the prestudy rate of 0.9 attacks/week	[74]

†All times are reported as the median value.

Table 4. Clinical studies evaluating the efficacy of Ruconest®.

Study	Design, patient population	Treatments (dose)	Exposure	Primary results†	Ref.
Moldovan <i>et al.</i> , 2012	Open-label study in HAE patients ≥16 years of age with an acute angioedema attack, with option to treat multiple attacks	Ruconest 2100 U, with option for additional dose of 2100–4200 U, based on clinical response	57 patients treated for 194 attacks. 110/194 (57%) attacks treated with single dose; 84/194 (43%) attacks treated with two doses	Time to beginning of relief of symptoms for the first five attacks ranged from 60 to 120 min	[69]
Choi <i>et al.</i> , 2007	Open-label study in HAE patients ≥16 years of age with an acute angioedema attack, with option to treat multiple attacks	Ruconest 100 U/kg	14 patients treated for 21 attacks	Time to beginning of relief of symptoms based on both patient and Investigator assessments: ≤60 min	[67]

†All times are reported as the median value.

explore the effects, safety and PK profile of repeated administration of Ruconest in patients with a high frequency of attacks/month. Twenty-five patients, with a mean of 0.9 attacks per week and a median of 0.6 attacks per week (range 0.4 to 4.5 attacks per week), were enrolled. The mean breakthrough attack rate during the treatment period was 0.4 attacks per week, with a median of 0.3 attacks per week (range, 0.0 to 1.5 attacks per week), which was significantly lower than the reported historical average attack rate [74].

A randomized, double-blind, placebo-controlled, crossover study is currently ongoing to evaluate the efficacy further (Clinical trials number: NCT02247739).

In addition to the above studies for long-term prophylaxis of C1-INH-HAE, Farkas *et al.* have reported the first successful use of Ruconest for short-term prophylaxis in an C1-INH-HAE patient undergoing a dental procedure [75].

Safety & tolerability
Adverse events

In the ten completed clinical studies, a total of 236 subjects have received a total of 994 administrations of Ruconest [33–35,60–64,67–70,74]. Safety data analyses demonstrated that Ruconest at doses of up to 100 U/kg is generally safe and well tolerated (Table 5). The adverse event profile found in the randomized, placebo-controlled studies was similar for patients treated with Ruconest and placebo treatments [33,35]. No increase in the incidence of treatment-emergent adverse events with a higher Ruconest dose, either administered as additional doses for an acute attack, or as repeated treatment for subsequent attacks [68,69,76]. Most treatment-emergent adverse events were mild to moderate in severity and unrelated to Ruconest administration. No safety signals related to hematology, biochemistry, urinalysis, vital signs or ECG parameters were noted.

No thromboembolic events or increased risk of thromboembolism were reported. No patients experienced a treatment-emergent adverse event that required discontinuation of medication.

The most common adverse reactions (≥ 2%) reported in all clinical trials were headache, nausea and diarrhea (Table 5).

Thrombogenicity

Thrombotic events were reported with the use of high-dose (300–500 U/kg) pd-C1-INH administration in neonates undergoing cardiovascular surgery for congenital heart disease [68,77]. In an open-label study with prophylactic Cinryze use (1000 U twice weekly), 5/146 patients (3.4%) had thrombotic events [37]. Gandhi *et al.* extracted case reports of thrombotic events of patients on pdC1-INH products from the FDA’s Adverse Event Reporting System database [78]. Ten cases of thrombotic events were associated with the use of Cinryze. Based on these findings, the risk for thrombotic events is included in the Warnings and Precautions sections of the US labels for both these pdC1-INHs [79,80]. A recommendation to monitor patients with known risk factors is specified.

To date, no thromboembolic events following administration of Ruconest up to 100 U/kg have been reported in C1-INH-HAE patients and healthy volunteers in the Ruconest clinical development program or in postmarketing experience. An analysis of the effects of Ruconest on coagulation and fibrinolysis has also been reported which found no evidence of a prothrombic effect of treatment with Ruconest [56]. These findings were corroborated by Reshef *et al.* who evaluated D-dimer levels, which are consider biomarkers of thrombosis [81]. They reported that treatment with Ruconest did not affect D-dimer levels and was not associated with thrombotic events [82].

Immunogenicity

Use of any therapeutic protein may lead to the development of antibodies against the protein itself, its plasma counterpart or host-related impurities (HRI). The frequency of these immunological responses depends on particular characteristics of the therapeutic protein such as aggregation and denaturation, and the underlying condition being treated. C1-INH-HAE is an autosomal dominant disorder, since HAE patients have some normal C1-INH in the circulation, although at lower levels: therefore, a negligible immunogenicity can be expected.

For all of the clinical trials, plasma blood samples were collected at time of screening, prior to treatment and at follow up visits before and after administration of Ruconest for antibody testing against C1-INH or against HRI. Screening levels that were above a pre-specified cut-off value underwent confirmation with displacement assays as well as testing for neutralizing anti-C1-INH antibodies. The relationship between the presence of antibodies and the clinical efficacy and safety of Ruconest was also analyzed. Overall, the frequency of screening ELISA results above the cut-off level for antibodies against C1-INH was low and similar between pre- and postexposure samples (<1% and 3%, respectively). Results above the cut-off level tended to be isolated or transient occurrences. No sample tested positive for neutralizing antibodies to pdC1-INH or Ruconest. Anti-HRI antibody results were confirmed by displacement assay for 27/205 patients. Observed anti-C1-INH and anti-HRI antibodies were not associated with adverse clinical findings and had no impact on clinical efficacy [83]. Similarly, Craig *et al.* reported that 11 (19%) of 57 patients treated with the pdC1-INH product Berinert developed anti-pdC1-INH antibodies, without any observed correlation with efficacy or AEs [84]. C1-INH-HAE patients have a heterozygous mutation and produce low levels of endogenous C1-INH, possibly resulting in a tolerance to exogenous C1-INH [85,86].

To reduce the risk for allergic reactions, patients with a past medical history of rabbit allergy were excluded from the clinical development program of Ruconest [35]. One anaphylactic reaction occurred in a healthy adult female volunteer participating in a Phase I study on first exposure to Ruconest [87]. This subject had a clinical history of rabbit allergy that had not been disclosed during the preconsent screening procedure, which would have excluded the subject otherwise. No other anaphylactic reactions have been reported in any of the C1-INH-HAE patients during the Ruconest clinical development program and postmarketing with > 4000 C1-INH-HAE attacks treated to date. The

administration of Ruconest is contraindicated for any individual with known or suspected allergy to rabbits.

To investigate the risk of hypersensitivity reactions further, an analysis was performed of IgE responses to various animal allergens following Ruconest exposure [87]. This analysis found that single and repeated exposure to Ruconest did not induce detectable IgE antibody responses against rabbit or other animal allergens, including cow's milk. Additionally, this review noted four patients who did not have a clinical history of rabbit allergy but had pre-existing antirabbit epithelium IgE antibodies. None of these patients experienced any hypersensitivity-type reaction upon Ruconest exposure. No patients developed IgE antibodies to rabbit dander following treatment with Ruconest.

Although pre-exposure and periodic IgE testing is required in the as per the summary of product characteristics (SPC) in the EU [53], it is not clear what useful information beyond clinical history with regard to the risk of an individual patient developing a hypersensitivity reaction to Ruconest. Of note, no hypersensitivity reactions have been reported since the product was approved in the EU in 2010. It is noteworthy though, that anaphylactic reactions to pdC1-INH have also been reported [80].

Ongoing clinical trials

A number of ongoing clinical trials are ongoing and are summarized below.

NCT01359969 is a multinational, multicenter Phase II trial that is currently enrolling for pediatric patients with HAE, from up to two and including 13 years of age.

This is an open-label, single arm study to evaluate the safety, immunogenicity, PK and efficacy of recombinant human C1 inhibitor at the dose of 50U/kg for the treatment of C1-INH-HAE acute attacks.

After the screening, patients will be eligible for treatment if they present to the clinic with an acute attack of at least moderate severity without signs of spontaneous regression; after the treatment, patients are closely monitored at the site for 4 h to complete efficacy and safety assessments.

Results of this clinical study will help evaluating the clinical benefit of Ruconest in this specific age group.

NCT01397864 is a multinational, multicenter, observational, noninterventional Registry designed to collect data on the safety and immunological profile of Ruconest in the treatment of C1-INH-HAE attacks.

The Registry aims to record adverse events and eventual insufficient therapeutic response, and to assess the immunological profile following single and repeated treatment with Ruconest in 300 patients diagnosed with HAE, in a real-life setting.

Table 5. Adverse reactions in ≥ 2% of patients in any Ruconest® group for the integrated dataset combining all seven randomized controlled trials and open-label extension studies in patients experiencing acute hereditary angioedema attacks.	
MedDRA preferred term	All Ruconest® doses†, n = 205 (n; [%])
Headache	19 (9%)
Nausea	5 (2%)
Diarrhea	5 (2%)
In a total of seven randomized controlled trials and open-label extension studies, 205 patients experiencing acute hereditary angioedema attacks were treated with Ruconest for a total of 650 hereditary angioedema attacks.	
†Ruconest doses up to 100 U/kg.	
MedDRA: Medical Dictionary for Regulatory Activities, version 15.0.	

NCT02247739 is a Phase II multicenter, randomized, double-blind, placebo-controlled, 3-period cross-over study to evaluate the efficacy and safety of recombinant human C1 Inhibitor in the prophylaxis of angioedema attacks in patients with C1-INH-HAE. Eligible patients with a history of frequent C1-INH-HAE attacks (>4 attacks per month) are currently enrolled and randomized to a treatment sequence. Each patient will receive three 4-week periods of treatment twice weekly, with a 1-week washout between treatment periods.

Thirty patients will receive in a double-blind fashion intravenous injection of Ruconest 50 U/kg (to a maximum of 4200 U for patients ≥84 kg) either once weekly or twice weekly.

Conclusion & future perspective

Recombinant human C1-INH was developed to offer a safe and effective alternative treatment to pdC1-INH products for acute C1-INH-HAE attacks. In controlled clinical studies, treatment with Ruconest was shown to be safe, with no risk of transmission of human blood-borne pathogens, inhibitory/sensitizing immune responses or thrombotic events reported after 994 administrations in controlled clinical trials. The technology platform ensures a reliable and scalable supply of product of uniform standard quality that is not dependent on human plasma donors.

Ruconest has the same target specificity as the native protein, since it has the same amino acid sequence. It addresses the root cause of the problem in C1-INH-HAE, in other words, C1-INH deficiency, and normalizes C1-INH levels with single dose in >94% of patients. Studies have demonstrated that Ruconest is effective for the treatment of acute HAE attacks. Three randomized clinical trials have clearly demonstrated statistically and clinically relevant results versus placebo in treating acute C1-INH-HAE attacks, and four open-label studies have demonstrated efficacy in treating subsequent attacks. Moreover, a very low rate of symptom recur-

rence (attack relapse) has been observed, and efficacy has been shown across different anatomical locations (abdominal, peripheral and laryngeal). The efficacy of Ruconest has been demonstrated in adolescents, and a study in patients of younger age is ongoing. In addition, an open-label prophylaxis study has suggested positive benefit, and a controlled study is ongoing.

To date, a favorable safety profile of Ruconest has been demonstrated in ten completed clinical studies, with 236 subjects and 994 administrations. No neutralizing antibodies have been detected, and although Ruconest is contraindicated in rabbit-sensitized patients, there is no evidence of induction of IgE responses. There is also no evidence of a prothrombotic effect.

Additional studies are necessary to further explore the mechanism of action of C1-INH, which seems to be independent of C1-INH half-life, and the findings of these studies may help to improve the QoL for the patient by enabling optimization of individual therapy.

Financial & competing interest disclosure

D Moldovan has financial ties with Behring CSL, Pharming Technologies BV, Shire HGT Inc. and Swedish Orphan Biovitrum. JA Bernstein has financial ties to CSL Behring, Dyax, Pharming, Shire and ViroPharma. M Cicardi has financial ties to CSL Behring, Viropharma, Dyax, SOBI, Pharming, BioCryst, Sigma Tau and Shire. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed. In addition to the peer-review process, with the author(s) consent, the manufacturer of the product(s) discussed in this article was given the opportunity to review the manuscript for factual accuracy. Changes were made at the discretion of the author(s) and based on scientific or editorial merit only.

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Executive summary

- Recombinant: there is no risk of transmission of blood-borne pathogens.
- Recombinant: allowing a scalable supply, that is not dependent on plasma donation.
- Ruconest® has the same target specificity as the native protein (since it has the same amino acid sequence).
- Ruconest addresses the root cause of the problem, that is, C1-INH deficiency, and normalizes C1-INH levels with single dose in >94% of patients.
- Three randomized controlled trials clearly demonstrate statistically and clinically relevant results versus placebo in treating acute C1-INH-HAE attacks.
- Four open-label studies demonstrate efficacy in treating repeat attacks.
- Very low rate of symptom recurrence (attack relapse).
- Efficacy across anatomical locations of abdominal, peripheral and laryngeal.
- Efficacy in adolescents shown, younger age study ongoing.
- Open label prophylaxis study suggests positive benefit, controlled study ongoing.
- Safety: ten clinical studies completed, with 236 subjects and 994 administrations, demonstrating a favorable safety profile.
- Immunogenicity: no neutralizing antibodies.
- Allergy: contraindicated in rabbit sensitized patients, but no evidence for induction of IgE responses.
- No evidence for prothrombotic effect.

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