# Recombinant human C1 esterase inhibitor for prophylaxis of hereditary angio-oedema: a phase 2, multicentre, randomised, double-blind, placebo-controlled crossover trial

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## Summary

**Background** Hereditary angio-oedema is a recurrent, oedematous disorder caused by deficiency of functional C1 inhibitor. Infusions of plasma-derived C1 esterase inhibitor deter attacks of hereditary angio-oedema, but the prophylactic effect of recombinant human C1 esterase inhibitor has not been rigorously studied. We aimed to assess the efficacy of recombinant human C1 esterase inhibitor for prophylaxis of hereditary angio-oedema.

**Methods** We conducted this phase 2, multicentre, randomised, double-blind, placebo-controlled crossover trial at ten centres in Canada, the Czech Republic, Israel, Italy, Macedonia, Romania, Serbia, and the USA. We enrolled patients aged 13 years or older with functional C1-inhibitor concentrations of less than 50% of normal and a history of four or more attacks of hereditary angio-oedema per month for at least 3 months before study initiation. Patients were randomly assigned centrally (1:1:1:1:1:1), via an interactive response technology system with fixed allocation, to receive one of six treatment sequences. During each sequence, patients received intravenous recombinant human C1 esterase inhibitor (50 IU/kg; maximum 4200 IU) twice weekly, recombinant human C1 esterase inhibitor once weekly and placebo once weekly, and placebo twice weekly, each for 4 weeks with a 1 week washout period between crossover. All patients, investigators, and study personnel who participated in patient care were masked to group allocation during the study. The primary efficacy endpoint was the number of attacks of hereditary angio-oedema observed in each 4 week treatment period. Attack symptoms were recorded daily. The primary efficacy analysis was done in the intention-to-treat population. Safety was assessed in all patients who received at least one injection of study medication. This study is registered with ClinicalTrials.gov, number NCT02247739.

**Findings** Between Dec 29, 2014, and May 3, 2016, we enrolled 35 patients, of whom 32 (91%) underwent randomisation (intention-to-treat population) and 26 (81%) completed the study. The mean number of attacks of hereditary angio-oedema over 4 weeks was significantly reduced with recombinant human C1 esterase inhibitor twice weekly (2·7 attacks [SD 2·4]) and once weekly (4·4 attacks [3·2]) versus placebo (7·2 attacks [3·6]), with mean differences of −4·4 attacks (p<0·0001) and −2·8 attacks (p=0·0004), respectively. We recorded adverse events in ten (34%) of 29 patients given twice-weekly recombinant human C1 esterase inhibitor, 13 (45%) of 29 patients given the once-weekly regimen, and eight (29%) of 28 patients given placebo. Headache (twice-weekly treatment) and nasopharyngitis (once-weekly treatment) were the most common adverse events. Two (7%) adverse events (fatigue and headache) were deemed possibly related to treatment with recombinant human C1 esterase inhibitor, but both resolved without additional treatment. No thrombotic or thromboembolic events, systemic allergic reactions (including anaphylaxis), or neutralising antibodies were reported.

**Interpretation** Prophylaxis with recombinant human C1 esterase inhibitor provided clinically relevant reductions in frequency of hereditary angio-oedema attacks and was well tolerated. In view of the pharmacokinetic profile of recombinant human C1 esterase inhibitor, our results suggest that efficacy of C1-inhibitor replacement therapy might not be a direct function of plasma trough concentrations of C1 inhibitor.

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## Introduction

Hereditary angio-oedema is a rare genetic disorder characterised by unpredictable recurrence of self-limiting cutaneous and mucosal oedema.[1] The disorder is caused by deficiency of functional C1 inhibitor attributable to mutations within the C1-inihibitor gene.[2] Insufficient amounts of functionally active C1 inhibitor fail to inhibit essential complement,[3] contact,[4] and fibrinolysis protease[4] cascades, which leads to production of crucial vasoactive mediators (eg, bradykinin) that facilitate angio-oedema.[5] Hereditary angio-oedema can elicit oedema in multiple anatomical locations, including the genitourinary tract, abdomen, upper and lower extremities (hands, feet, arms, and legs), and the oropharyngeal–laryngeal region.[6,7]

Attacks of hereditary angio-oedema impair quality of life[6] and are potentially life-threatening when they occur in the larynx.[8] Prophylactic treatments for these attacks have included synthetic 17-α-alkylated androgen derived from

## Research in context

### Evidence before this study

Infusion of recombinant human C1 esterase inhibitor effectively resolves acute attacks in patients with hereditary angio-oedema. Data from a historical control trial suggested that recombinant human C1 esterase inhibitor might also prevent attacks of hereditary angio-oedema despite its relatively short plasma half-life (about 2·5 h). We searched PubMed for clinical trials of hereditary angio-oedema prophylaxis published in English up to June 30, 2014, using the MeSH search terms "angioedemas, hereditary" AND "prophylaxis." We identified ten articles. We examined the articles and identified an unmet clinical need for a non-plasma-derived source of human C1 esterase inhibitor that is safe, efficacious, and well tolerated for the prevention of hereditary angio-oedema attacks.

### Added value of this study

Findings from our randomised, double-blind, crossover study show that prophylaxis with recombinant human C1 esterase inhibitor provided clinically relevant reductions in frequency of hereditary angio-oedema and was well tolerated.

### Implications of all the available evidence

Recombinant human C1 esterase inhibitor, which is not constrained by concerns regarding supply and viral transmission associated with plasma-derived treatments, might be an effective prophylactic treatment option for patients with hereditary angio-oedema. Furthermore, we have provided evidence to support the possibility of an alternate mechanism of action of C1 esterase inhibitor replacement therapies.

testosterone, such as danazol and stanozolol.[9,10] However, these treatments have been associated with dose-related adverse events and detrimental hormonal effects, which hinder their use in many individuals, particularly children and women.[9,10] Plasma-derived C1 esterase inhibitor (Cinryze; Shire ViroPharma, Lexington, MA, USA) is indicated for routine prophylaxis of hereditary angio-oedema attacks.[11,12] Findings from a phase 3 registration study[11] showed that intravenous plasma-derived C1 esterase inhibitor (1000 IU twice weekly) reduced attack frequency by roughly 50% compared with placebo. Results from an open-label extension study[12] highlighted individual response variability and the potential need for dose adjustment to reduce breakthrough hereditary angio-oedema attacks. Furthermore, a subcutaneous preparation of plasma-derived C1 esterase inhibitor was approved by the US Food and Drug Administration in June, 2017. A population pharmacokinetic model[13] predicted that the mean trough plasma concentration of C1-inhibitor functional activity was 40·2% after 40 IU/kg of twice-weekly subcutaneous C1 esterase inhibitor and 48·0% after 60 IU/kg twice weekly. A 2017 double-blind study[14] showed that mean difference in monthly attack rate versus placebo was –2·42 attacks (95% CI –3·38 to –1·46) with 40 IU/kg and –3·51 attacks (–4·21 to –2·81) with 60 IU/kg. On the basis of these data, plasma concentrations of functional C1 inhibitor might predict efficacy of a prophylactic treatment.

Recombinant human C1 esterase inhibitor (Ruconest; Pharming Technologies BV, Leiden, the Netherlands) is approved for the treatment of acute attacks of hereditary angio-oedema in adults and adolescents. Intravenous administration of this product restores concentrations of functional C1 inhibitor to physiologically normal levels, but the plasma half-life of the recombinant protein is 3 h (ie, at least eight times shorter than that of plasma-derived C1 esterase inhibitor products).[15] Recombinant human C1 esterase inhibitor is approved for the treatment of hereditary angio-oedema attacks at a dose of 50 IU/kg, to a maximum of 4200 IU. In a review by Hack and colleagues,[16] an analysis of placebo-controlled studies of various plasma and recombinant C1 esterase inhibitor preparations for treatment of acute attacks of hereditary angio-oedema showed no difference in efficacy and attack relapse rates among the products, despite their different half-lives. In a phase 2, exploratory, open-label, 8 week trial[17] in 25 patients with hereditary angio-oedema, once-weekly administration of recombinant human C1 esterase inhibitor 50 IU/kg reduced the median number of attacks by 50% compared with patient-reported attacks occurring during the previous 2 years. Historical comparisons lack the rigor of randomised controlled trials, and data for the optimal frequency of administration of recombinant human C1 esterase inhibitor for prophylaxis (ie, once or twice a week) are insufficient. This study was designed to assess the efficacy of recombinant human C1 esterase inhibitor for prophylaxis of hereditary angio-oedema.

## Methods

### Study design and participants

We did this phase 2, multicentre, randomised, double-blind, placebo-controlled crossover trial at ten centres in Canada, the Czech Republic, Israel, Italy, Macedonia, Romania, Serbia, and the USA. We enrolled patients aged 13 years or older with functional concentrations of C1 inhibitor of less than 50% of normal, and a history of frequent attacks of hereditary angio-oedema (four or more attacks per month for at least 3 consecutive months before study initiation). We excluded patients with an allergy to rabbits or a diagnosis of acquired angio-oedema, pregnant or breastfeeding mothers, and patients receiving angiotensin-converting enzyme inhibitors. Patients could continue stable doses of any prophylactic androgen or antifibrinolytic treatments if they met the criteria for frequent attacks. Patients who were using plasma-derived C1 esterase inhibitor for prophylaxis or treatment of acute

attacks were required to discontinue this treatment for at least 7 days before study initiation.

Approval was obtained from appropriate institutional review boards at each participating institution, and the study was done in accordance with the Declaration of Helsinki and Good Clinical Practice regulations. All patients or legal guardians provided written informed consent.

### Randomisation and masking

Patients were randomly assigned centrally (1:1:1:1:1:1), via an interactive response technology system with fixed allocation (Williams Latin Square design), to receive one of six treatment sequences. Each treatment sequence consisted of three 4 week treatment periods separated by a 1 week washout period before crossover. Within each treatment sequence, patients received recombinant human C1 esterase inhibitor (50 IU/kg for patients <84 kg or 4200 IU for patients ≥84 kg) twice weekly, recombinant human C1 esterase inhibitor once weekly and placebo (saline) once weekly, and placebo (saline) twice weekly (figure 1A). All patients, investigators, and study personnel who participated in patient care (eg, drug administration and assessments) were masked to group allocation during the study.

### Procedures

A vial of lyophilised recombinant human C1 esterase inhibitor powder (2100 IU) was reconstituted in 14 mL of sterile water for injection. Sterile water was slowly added to each vial and mixed gently. The resulting reconstituted mixture was used immediately. If more than one vial was needed, vials were reconstituted separately then mixed together before injection. All doses were prepared in identical total volumes per administration by study personnel aware of treatment allocation (ie, study delegated pharmacists). Doses were provided to study drug administration personnel in a concealed fashion in opaque syringes, and administered intravenously to the patients over roughly 5 min (≤10 min).

Patients who had acute attacks of hereditary angio-oedema could receive medications specific to hereditary angio-oedema (ie, open-label recombinant human C1 esterase inhibitor, plasma-derived C1 esterase inhibitor [for laryngeal attacks only], icatibant, ecallantide) or symptomatic medications (eg, analgesics, narcotics, antiemetics). If patients received open-label recombinant human C1 esterase inhibitor for an acute attack on the day scheduled for administration of prophylactic recombinant human C1 esterase inhibitor, administration was deferred for 24 h.

The location, duration, severity, and type of treatment administered for any attacks of hereditary angio-oedema were recorded daily in patient diaries. Patients were to record this information whether or not study treatment was actually administered. Attacks that progressed from one anatomical location to another without complete

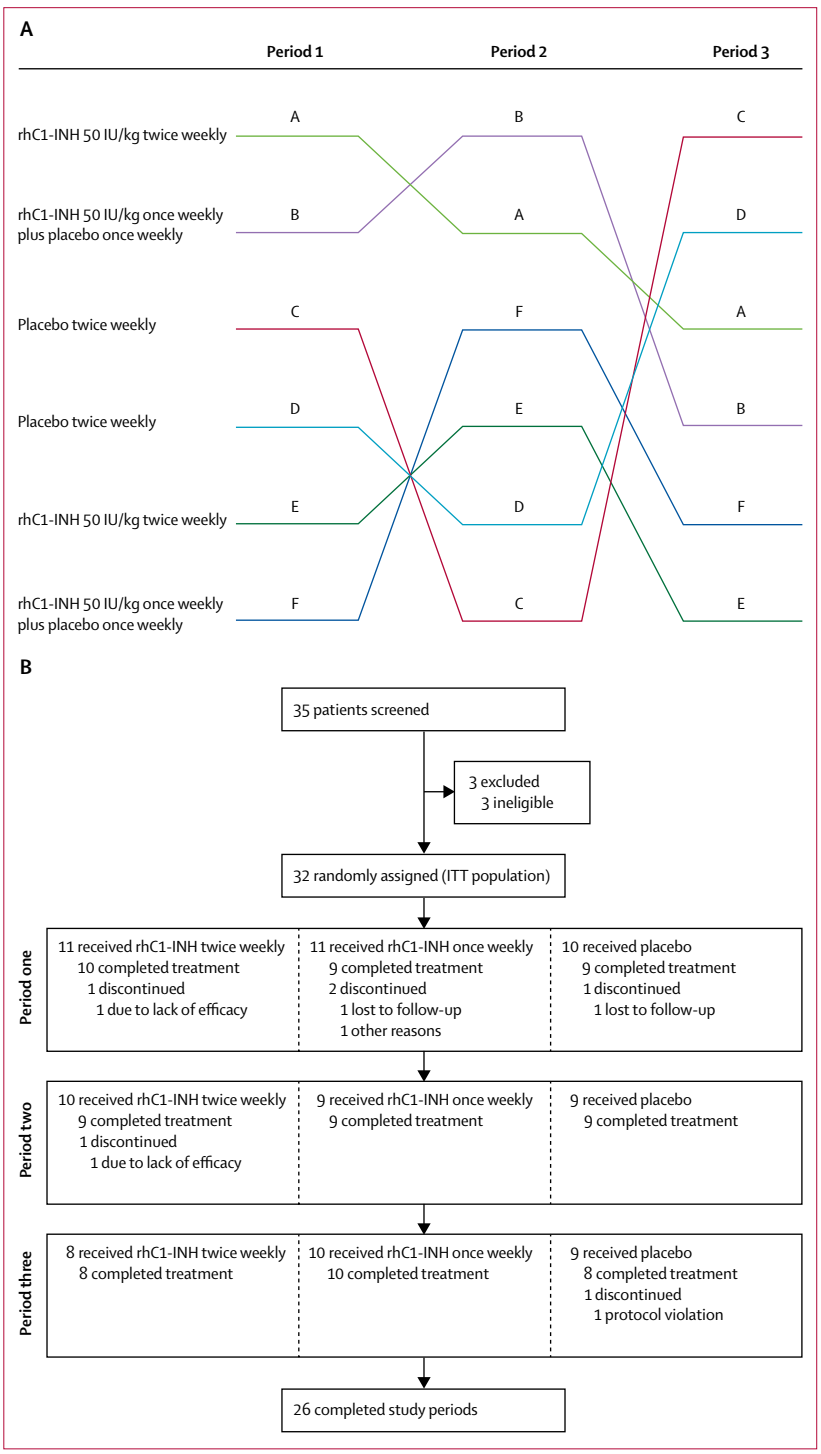


***Figure 1:*** **Study design and patient disposition**

(A) Treatment sequences. Each capital letter within the figure represents a single treatment sequence. Each of the three periods represents 4 week administration of rhC1-INH 50 IU/kg (or 4200 IU for patients ≥84 kg) twice weekly, rhC1-INH 50 IU/kg (or 4200 IU for patients ≥84 kg) once weekly and placebo once weekly, and placebo twice weekly. Each treatment period was separated by a 1 week washout period. (B) Trial profile. Patient numbers do not flow vertically through each period (ie, rhC1-INH once weekly: period one, n=11; period two, n=9; period three, n=10) because patients were randomly assigned to different treatment schedules. For example, some patients who received rhC1-INH twice weekly during period one crossed over to receive rhC1-INH once weekly during period two (treatment sequence A), whereas others received placebo during period two (treatment sequence E). ITT=intention-to-treat. rhC1-INH=recombinant human C1 esterase inhibitor.

symptom resolution, or regressed and then became worse before complete resolution, were considered a single attack. Adverse events were monitored throughout the study. Immunogenicity of recombinant human C1 esterase inhibitor (IgG and IgM antibodies against recombinant human C1 esterase inhibitor, antibodies against host-related impurities) was assessed at screening, before the first administration of the study drug during each 4 week treatment period, and 30 days after the last administration of the final treatment period.

Plasma was collected for assessment of C1-inhibitor concentrations before and roughly 30 min after injections at study visits 1 (week 0), 8 (week 4), 9 (week 5), 16 (week 9), 17 (week 10), and 24 (week 14). A chromogenic assay was used to measure functional C1-inhibitor concentrations. Samples were incubated with an excess of active complement component 1s for 5–15 min, and residual activity was measured by addition of a chromogenic substrate. 1 U/mL represented the concentration of functional C1 inhibitor in pooled normal human plasma. Values below the limit of quantification (0·24 U/mL) were estimated as 0·12 U/mL (ie, half the lower limit of detection).

### Outcomes

The primary efficacy endpoint was the number of attacks of hereditary angio-oedema observed in each 4 week treatment period. The secondary efficacy endpoint was the proportion of patients with a clinical response, defined as a reduction of 50% or more in the number of attacks, with active treatment versus placebo.

### Statistical analyses

With a two-sided α level of 0·05 and 80% power, and in the assumption of a 20% discontinuation rate and a mean number of four attacks per 4 week period, based on randomised controlled trial data,[18] enrolment of 30 patients would be needed to detect a 30% reduction in attacks with recombinant human C1 esterase inhibitor versus placebo.

| | Patients (n=32) |
|---|---|
| Age (years) | |
| Mean (SD) | 45·9 (14·5) |
| Median (range) | 46·3 (16·9–73·5) |
| Sex | |
| Female | 26 (81%) |
| Male | 6 (19%) |
| White race | 32 (100%) |
| Previous use of prophylaxis | 6 (19%) |
| Attacks within last 3 months | |
| Mean (SD) | 17·9 (7·2) |
| Median (range) | 14·5 (12–33) |

Data are n (%), unless otherwise specified.

*Table 1:* **Baseline characteristics (intention-to-treat population)**

We assessed primary and secondary efficacy endpoints in the intention-to-treat (all patients randomly assigned to treatment) and per-protocol (patients in the intention-to-treat population who completed the study and had no major protocol violations) populations. Subgroup analyses (by age group [<18 years, 18–65 years, and >65 years], sex, and previous use of C1 esterase inhibitor prophylaxis [naive or prior use]) were also examined for the primary efficacy endpoint. Safety was assessed in all patients who received at least one injection of study medication according to the treatment received. Analysis of the primary efficacy endpoint was done hierarchically by use of generalised estimating equations for repeated measures analysis. On the basis of goodness-of-fit criteria, we selected the negative binomial distribution with autoregressive correlation structure, with a negative binomial attack distribution and treatment group, period, and sequence as factors.

We first tested the null hypothesis for the primary endpoint—ie, that the number of attacks of hereditary angio-oedema normalised to a 4 week period would be statistically similar ($p>0·05$) between twice-weekly recombinant human C1 esterase inhibitor and twice-weekly placebo. If the null hypothesis was rejected (ie, twice-weekly recombinant human C1 esterase inhibitor significantly reduced the number of attacks versus placebo), then we determined statistical differences ($p\leq0·05$) between the once-weekly regimen and placebo. Because the analysis with generalised estimating equations assumed data were missing at random, we did a sensitivity analysis of the primary endpoint using a last observation carried forward imputation to assess the possibility that missing data did not occur at random. The null hypothesis for the secondary endpoint was that the clinical response rate in either group would be greater than 0·5. We analysed this hypothesis using a two-sided test without multiplicity adjustments.

We used SAS (version 9.3 or higher) for all statistical analyses. This study is registered with ClinicalTrials.gov, number NCT02247739.

### Role of the funding source

The sponsor of the study had a role in study design, data collection, data interpretation, data analysis, and writing of the report. All authors had full access to all the data in the study, approved the final published manuscript, and affirmed the integrity of the data and analyses. The corresponding author had final responsibility for the decision to submit for publication.

## Results

Between Dec 29, 2014, and May 3, 2016, we enrolled 35 patients, of whom 32 (91%) underwent randomisation (intention-to-treat population; table 1) and 26 (81%) completed the study (figure 1B). Most (n=26) patients had not previously received any type of prophylactic medication for attacks of hereditary angio-oedema. No patients took oestrogen-containing medications during the study. Although stable doses of androgens or tranexamic acid

were permitted per protocol, no patient received androgens during the study, and only one (3%) patient received tranexamic acid (500 mg twice daily). The per-protocol population comprised 23 patients after exclusion of six patients who withdrew during the study, two patients who received plasma-derived C1 esterase inhibitor, and one patient who received the wrong treatment.

Recombinant human C1 esterase inhibitor significantly reduced the mean number of attacks of hereditary angio-oedema over 4 weeks when given both twice weekly (2·7 attacks [SD 2·4]) and once weekly (4·4 attacks [3·2]) versus placebo (7·2 attacks [3·6]; appendix), with mean differences of –4·4 attacks (p<0·0001) and –2·8 attacks (p=0·0004), respectively (appendix). Findings from the sensitivity analysis using last observation carried forward likewise showed a significant reduction in the mean number of attacks with twice-weekly (2·7 attacks [SD 2·4]) and once-weekly (4·4 attacks [3·2]) dosing with recombinant human C1 esterase inhibitor compared with placebo (8·0 attacks [4·9]), with mean differences of 5·2 attacks (p<0·0001) and 3·6 attacks (p<0·0001). There was no evidence for either a significant sequence effect (missing at random analysis; p=0·72) or period effect (missing at random analysis; p=0·64) with recombinant human C1 esterase inhibitor (appendix).

Subgroup analyses were done. No conclusions could be drawn for age group comparisons because of the small number of patients in the groups younger than 18 years (n=1 per treatment group) and older than 65 years (n≤3 per treatment group). For men and women, similar patterns in the mean number of hereditary angio-oedema attacks were observed within each category, with the largest number of attacks in the placebo group, followed by the once-weekly group, and the smallest number in the twice-weekly treatment group. A generally similar pattern was also observed for the patients with and without previous use of prophylaxis with C1 esterase inhibitor.

Attack frequency was reduced with twice-weekly recombinant human C1 esterase inhibitor by up to 63·3% in the intention-to-treat population and 72·1% in the per-protocol population, and with the once-weekly regimen by up to 34·9% and 44·4%, respectively (figure 2A). A reduction of 50% or more in the number of attacks that occurred during active versus placebo treatment (clinical response) was observed in most patients who received twice-weekly recombinant human C1 esterase inhibitor in the intention-to-treat population (n=23 of 31 [74%, 95% CI 56·8–86·3]) and per-protocol population (n=22 of 23 [96%, 79·0–99·2]; figure 2B). Clinical response was also observed in patients who received once-weekly recombinant human C1 esterase inhibitor in the intention-to-treat population (n=13 of 31 [42%, 95% CI 26·4–59·2]) and the per-protocol population (n=13 of 23 [57%, 36·8–74·4]; figure 2B). Although both recombinant human C1 esterase inhibitor regimens reduced attacks of hereditary angio-oedema, twice-weekly dosing more consistently provided a reduction in attacks of 50% or more (figures 2C, D).

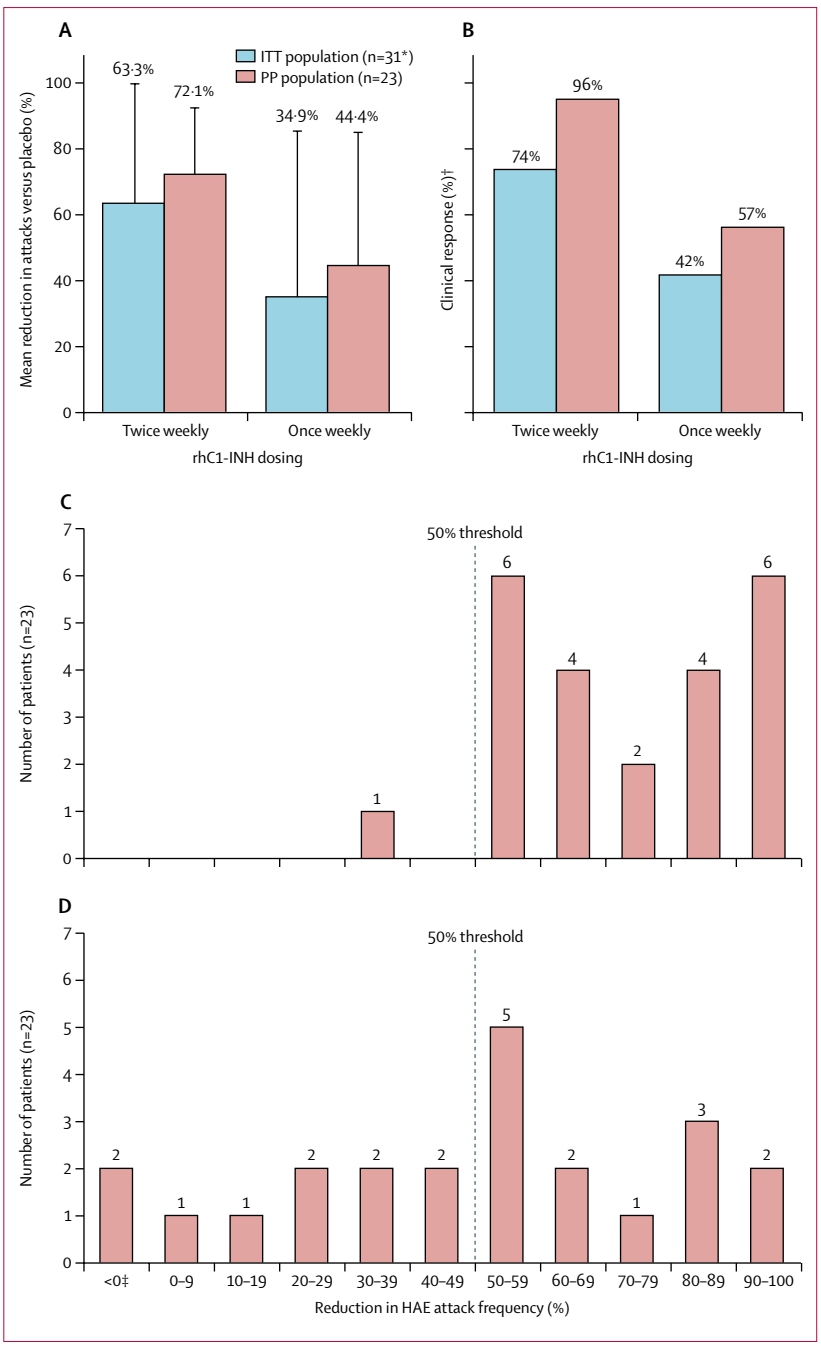


***Figure 2*: Primary and secondary endpoints**

(A) Percentage reduction in attacks. (B) Clinical response. rhC1-INH twice-weekly (C) and once-weekly (D) administration distributions of percentage reduction in clinical response (PP population). ITT=intention-to-treat. PP=per-protocol. rhC1-INH=recombinant human C1 esterase inhibitor. HAE=hereditary angio-oedema. *With exclusion of one patient who was randomly assigned, but did not receive study medication. †Defined as a reduction of 50% or more in the number of attacks that occurred during rhC1-INH treatment versus attacks that occurred during placebo treatment. ‡Two (9%) patients had an increase in attack frequency while receiving once-weekly rhC1-INH (one patient had an increase of 40% and one patient had an increase of 62·5%).

Two (9%) patients had an increase in attack frequency while receiving once-weekly recombinant human C1 esterase inhibitor (one patient had an increase of 40% and one patient had an increase of 62·5%; figure 2D).

See **Online** for appendix

| | rhC1-INH twice weekly | rhC1-INH once weekly | Placebo |
|---|---|---|---|
| **First injection (week 0)** | | | |
| Pre-injection | 12·0% (12–122*) | 12·0% (12–34) | 12·0% (12–32) |
| Post-injection | 128·5% (12–184) | 123·0% (12–160) | 12·0% (12–114) |
| **Eighth injection (week 4)†** | | | |
| Pre-injection | 12·0% (12–12) | 12·0% (12–28) | 12·0% (12–39) |
| Post-injection | 123·5% (87–456) | 12·0% (12–35)‡ | 12·0% (12–36) |

Data are median (range) of functional C1-INH (percent of total detectable). C1-INH concentrations below the lower limit of quantification (24%) were estimated as 12%. C1-INH=C1 esterase inhibitor. rhC1-INH=recombinant human C1 esterase inhibitor. *At the first study visit, one patient had a pre-injection value of 122% and a post-injection value of less than 24%. This patient was randomly assigned to receive rhC1-INH twice weekly at study visit one. It is suspected that the pre-injection and post-injection samples were switched for this timepoint, but this could not be confirmed. All other pre-injection values for this patient during the study were less than 24%. †By the eighth injection, patients randomly assigned to the twice-weekly rhC1-INH group had received eight injections of rhC1-INH, those in the once-weekly rhC1-INH group had received four rhC1-INH injections, and those in the placebo group had received no injections of active drug (rhC1-INH). ‡The final rhC1-INH injection for patients in the once-weekly group occurred on week 3 (seventh injection); therefore, the eighth injection for the rhC1-INH once weekly group was placebo.

***Table 2:*** **Plasma concentrations of functional C1-INH (intention-to-treat population)**

| | rhC1-INH twice weekly (n=29) | rhC1-INH once weekly (n=29) | Placebo (n=28) |
|---|---|---|---|
| Any AE | 10 (34%) | 13 (45%) | 8 (29%) |
| Serious AE | 1 (3%)* | .. | .. |
| Treatment-related AE | 2 (7%)† | .. | .. |
| AEs occurring in ≥5% of patients | | | |
| Headache | 5 (17%) | 2 (7%) | .. |
| Nasopharyngitis | .. | 3 (10%) | 2 (7%) |
| Anxiety | .. | 2 (7%) | .. |

Data are n (%). rhC1-INH=recombinant human C1 esterase inhibitor. AE=adverse event. *Patient underwent a urological procedure for pre-existing phimosis. †Fatigue (n=1) and headache (n=1).

***Table 3:*** **Adverse events (safety population)**

Post-infusion concentrations of C1-inhibitor activity increased above normal (table 2). Consistent with the plasma half-life of recombinant human C1 esterase inhibitor, plasma concentrations of functional C1 inhibitor were not increased in either the twice-weekly or once-weekly treatment groups before the last injection at week 4 (ie, about 3–4 days after the previous injection for the twice-weekly group and about 1 week after the previous injection for the once-weekly group; table 2).

We recorded adverse events in ten (34%) of 29 patients in the twice-weekly dosing group and 13 (45%) of 29 patients in the once-weekly group (table 3). No patient withdrew from the study because of an adverse event. Most adverse events occurred 24 h or more after dosing (n=26 [90%] per group for both recombinant human C1 esterase inhibitor regimens and n=26 [93%] of 28 patients for placebo). Headache (twice-weekly treatment) and nasopharyngitis (once-weekly treatment) were the most commonly reported adverse events with recombinant human C1 esterase inhibitor (table 3). Most adverse events were of mild to moderate intensity; severe nasopharyngitis (once-weekly treatment) and severe abdominal pain (twice-weekly treatment) were reported in one patient each. One (3%) serious adverse event occurred in a patient who had pre-existing phimosis and underwent a urological procedure for this condition (table 3). Two (7%) adverse events (fatigue and headache) were deemed possibly related to treatment with recombinant human C1 esterase inhibitor (table 3), but both resolved without additional treatment. No incidences of hypersensitivity or systemic allergic reactions (including anaphylactic reactions), neutralising antibodies, or thrombotic or thromboembolic events were reported.

## Discussion

Hereditary angio-oedema is a rare and serious genetic disease characterised by painful, debilitating, and unpredictable episodes of swelling.[1] Patients with frequent attacks, in particular, have increased anxiety and sudden feelings of panic with reduced ability to do daily activities, both during and in between attacks.[6] Treatment guidelines from the World Allergy Organization recommend administration of long-term prophylactic medication for severely symptomatic patients whose acute attacks are poorly controlled with on-demand treatment.[19] Nevertheless, long-term prophylaxis poses several potential safety and economic problems related to the possible need, at least for some patients, to maintain such an approach throughout their lifetime.

In this phase 2 trial, intravenous administration of recombinant human C1 esterase inhibitor significantly reduced the number of attacks of hereditary angio-oedema compared with placebo in patients with frequent attacks. Both once-weekly and twice-weekly dosing was well tolerated, with headache being the most frequently reported adverse event. These results support those of an open-label, historical controlled trial[17] that showed a reduction of hereditary angio-oedema attacks with recombinant human C1 esterase inhibitor compared with patients' historical attack rates. Our results also corroborate data from treatment trials of acute attacks of hereditary angio-oedema, which showed a low rate of attack recurrence after treatment with recombinant human C1 esterase inhibitor.[18,20–23]

Replenishment or replacement of plasma concentrations of C1 inhibitor in patients with hereditary angio-oedema (eg, with administration of plasma-derived C1 esterase inhibitor) has been recommended for short-term prophylaxis to prevent attacks during situations likely to elicit an attack (eg, dental surgery),[24] and might also be advantageous as long-term prophylaxis.[11,12] For example, during a 24 week, randomised, double-blind, crossover study[11] in 24 patients with two or more hereditary angio-oedema attacks per month, intravenous administration of plasma-derived C1 esterase inhibitor every 3–4 days (roughly twice weekly) reduced the rate of attacks by about 50% (from 12·7 attacks with placebo to 6·3 attacks with plasma-derived C1 esterase inhibitor; reduction rate of 6·5 attacks), with about 50% of patients

achieving a reduction of 50% or more in the frequency of attacks. Although it is difficult to compare data across clinical trials, in the present study, prophylactic treatment with recombinant human C1 esterase inhibitor (50 IU/kg for patients <84 kg or 4200 IU for patients ≥84 kg) showed improved response rates compared with treatment with plasma-derived C1 esterase inhibitor (1000 IU/10 mL). Twice-weekly administration of recombinant human C1 esterase inhibitor reduced the rate of attacks by up to 72·1%, with up to 96% of patients achieving a reduction of 50% or more in attacks. Both the once-weekly and the twice-weekly regimens significantly reduced the number of attacks; however, achievement of a more than 50% reduction in attacks was obtained more consistently with twice-weekly dosing.

Although C1 esterase inhibitor administration as replacement therapy traditionally has been thought to prevent attacks of hereditary angio-oedema by restoring normal biological control mechanisms for bradykinin release, our results indicate that the plasma half-life of the C1 esterase inhibitor product being administered (and, therefore, maintenance of consistent plasma concentrations of functional plasma C1 inhibitor above the lower level of normal [0·7 U/mL]) might not be solely responsible for the observed efficacy of C1 esterase inhibitor prophylaxis.[16] Indeed, Hack and colleagues[16] suggested that, in view of the absence of attack relapses after administration of recombinant human C1 esterase inhibitor, a temporary increase in plasma concentrations of C1 inhibitor might be sufficient to alleviate acute attack symptoms and possibly prevent future attacks.

Findings from in-vitro studies[25,26] indicate that C1 inhibitor might regulate inflammation through both traditional regulation of complement activation and blockage of leucocyte transmigration during the inflammatory response as a result of binding to selectins—molecules that facilitate leucocyte–endothelial adhesion. Additionally, an animal model of stroke[27] showed that recombinant human C1 esterase inhibitor markedly reduced cerebral damage when administered up to 18 h after transient ischaemia and up to 6 h after permanent ischaemia, a surprisingly wide therapeutic window. The investigators proposed that the advantage of recombinant human C1 esterase inhibitor over the plasma-derived product was related to its greater association with mannose-binding lectin. This binding to mannose-binding lectin might translate into increased inhibition of the complement lectin pathway. The differences in binding between human plasma-derived C1 esterase inhibitor and recombinant human C1 esterase inhibitor might be related to the reduced glycosylation of recombinant human C1 esterase inhibitor on the amino-terminal domain of the protein (ie, the area that does not contain serpin activity), which allows for exposure of the galactose and mannose residues that facilitate lectin binding.

On the basis of these observations, we propose that effective C1 esterase inhibitor regimens for attacks of hereditary angio-oedema do not rely exclusively on plasma concentrations of functional C1 inhibitor. Bolstering this hypothesis is the observation that although therapeutic doses of attenuated androgens increase plasma concentrations of C1 inhibitor,[28] lower doses, which do not elevate C1-inhibitor concentrations, also provide effective prophylaxis.[29] Pharmacokinetic and pharmacodynamic modelling based on data from the COMPACT trial showed an exposure–response association between plasma C1-inhibitor concentrations obtained after subcutaneous administration of plasma-derived C1 esterase inhibitor and the degree of hereditary angio-oedema attack risk.[13] The present study suggests that other variables (eg, binding to factor XII, kallikrein, and selectins[25,30,31]) should be considered, in addition to trough plasma concentrations of C1 inhibitor, as possible contributors to the efficacy of C1-inhibitor replacement therapies. For example, the possibility exists that functional C1 inhibitor that is bound to endothelial cells (via interaction with proteins such as mannose-binding lectin) and, therefore, undetectable in plasma, could still be available to inhibit contact activation. Alternatively, or additionally, a brief increase in plasma concentration of C1 inhibitor is sufficient to irreversibly inactivate the target proteases; once inactivated, such proteases do not become activated again because of covalent binding. On the basis of these potential mechanisms, the scheduled administration of C1 esterase inhibitor to patients with frequent hereditary angio-oedema symptoms might reduce attack frequency. This action might occur via regulatory effects early during a subclinical contact activation phase, thus having a prophylactic effect against clinical angio-oedema symptoms.

This study is, to our knowledge, the first randomised, placebo-controlled trial to assess the efficacy and safety of recombinant human C1 esterase inhibitor for prevention of frequent hereditary angio-oedema attacks. Unlike plasma-derived C1 esterase inhibitor, which relies on human blood donation and carries a potential risk of transmission of blood-borne diseases, the supply of recombinant human C1 esterase inhibitor is, theoretically, unlimited and has not been associated with pathogen transmission. Only patients with a history of frequent attacks were included in the present study, which conforms to current treatment recommendations,[19] but does not allow extrapolation of the results to all patients with hereditary angio-oedema or to administration of recombinant human C1 esterase inhibitor for short-term prophylaxis. Moreover, the study is limited by the short, 8 week (two 4 week periods) duration of exposure to recombinant human C1 esterase inhibitor, thereby precluding the study of efficacy and safety over an extended time period; additional studies are needed to explore this possibility.

In summary, recombinant human C1 esterase inhibitor, optimally administered twice a week, was efficacious and well tolerated as replacement therapy for the prevention of acute attacks of hereditary angio-oedema.

### Contributors

MAR, BMG, RFL, ARel, and MC conceived and designed the study. MAR, VG-P, JB, WHY, ARes, SA, RFL, RH, SK, and MC acquired the data. All authors analysed and interpreted the data. MAR, WHY, ARel, and MC wrote the first draft. All authors revised the manuscript for intellectual content. All authors approved the completed article.

### Declaration of interests

MAR has received research grants from BioCryst Pharmaceuticals, CSL Behring, Ionis Pharmaceuticals, Pharming Technologies, and Shire; has served as a consultant for Adverum Biotechnologies, Alnylam Pharmaceuticals, Arrowhead Pharmaceuticals, BioCryst Pharmaceuticals, CSL Behring, Global Blood Therapeutics, Ionis Pharmaceuticals, KalVista Pharmaceuticals, Pharming Technologies, Salix Pharmaceuticals, and Shire; and has served on the speakers' bureaus for CSL Behring, Pharming Technologies, Salix Pharmaceuticals, and Shire. VG-P has served as principal investigator for clinical trials sponsored by Pharming Group. DM has received grants from Swedish Orphan Biovitrum, Pharming Technologies, Shire, and CSL Behring, and personal fees from Pharming Technologies, Shire, Swedish Orphan Biovitrum, and CSL Behring. JB is a researcher for BioCryst Pharmaceuticals, CSL Behring, Dyax, Pharming Technologies, and Shire; has served as a consultant for BioCryst Pharmaceuticals; and has served on the speakers' bureau for Shire. WHY has served as a member of the national and international advisory boards for BioCryst Pharmaceuticals, CSL Behring, and Shire, and has received research or educational grants from BioCryst Pharmaceuticals, CSL Behring, Shire, and Pharming Technologies. BMG is an employee of Pharming Group. ARes has received research grants from Pharming Technologies. RH has received financial support and personal fees from Pharming Group. JRH and ARel are employees of Pharming Healthcare. MC has received grants from Shire and personal fees from Shire, CSL Behring, Pharming Technologies, BioCryst Pharmaceuticals, Alnylam, and KalVista. SA, RFL, and SK declare no competing interests.

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