



**Recombinant Human C1 Esterase Inhibitor (Conestat alfa)
for prophylaxis to prevent attacks in adult and adolescent
patients with hereditary angioedema**

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Title:

Recombinant Human C1 Esterase Inhibitor (Conestat alfa) for prophylaxis to prevent attacks in adult and adolescent patients with hereditary angioedema

For Peer Review Only

Abstract:

Introduction: Hereditary angioedema (HAE) due to C1 inhibitor (C1-INH) deficiency is a debilitating and potentially lethal disease. Management includes on-demand treatment of angioedema and their prophylaxis. Plasma derived C1-INH is an established treatment for both on demand and prophylaxis of HAE. Conestat alfa is a recombinant form of human C1-INH (rhC1-INH) produced in transgenic rabbits. It has granted drug's registration as treatment option for acute HAE attacks in adults and adolescents in Europe, America, and other countries. Long-term prophylaxis with rhC1-INH received recent consideration in clinical trials.

Areas covered: This review will critically appraise available information about rhC1-INH (conestat alfa) prophylactic treatment in adult and adolescent patients with congenital C1-INH deficiency. Results from a phase II randomized placebo-controlled trial for prophylaxis of severe HAE evidenced positive treatment outcomes for its application, both twice or once weekly.

Expert commentary: Phase II clinical studies suggest that rhC1-INH is a viable option for prophylaxis of HAE. Safety and tolerability data are comparable to other available HAE specific drugs, zeroing the possibility for blood-borne viral transmission. Sustainability of modern technologies is granting a practically stable and continuous recombinant production process. With other available options, rhC1-INH facilitates tailoring HAE treatment to patients' needs.

1. Introduction and overview of the market

Hereditary angioedema due to C1 inhibitor deficiency, hereafter HAE, is an autosomal dominant genetic disease characterized by recurrent attacks of subcutaneous and submucosal swellings. The pathophysiological mechanism involves activation of the contact system and release of the vasoactive peptide bradykinin that increases vascular permeability [1-3] (Figure 1). The underlying genetic defect is in SERPING1 gene that codes for C1-INH protein. Mutations in one of the two alleles result in low plasma concentration of C1-INH (HAE Type 1), or in low functional plasma levels, due secretion of an antigenically identical dysfunctional protein product (HAE Type 2). Both types have identical impact on the enzymatic systems controlled by C1-INH and cause identical clinical phenotypes [4]. Mutations reported to be pathogenic for HAE are affecting the C-terminal of the C1 inhibitor molecule [5], and only one missense mutation in the N-terminal can be related to clinical symptoms of angioedema [6].

HAE manifestations can affect different locations: facial and peripheral subcutaneous angioedema attacks which are disabling, disfiguring and painful; and abdominal mucosal swellings which cause severe pain, and could mimic an emergency surgical condition, 'acute abdomen', possibly resulting in unnecessary invasive iatrogenic procedures [7][8,9]. The most serious disease manifestations are the laryngeal attacks, which can lead to death by asphyxiation and are the primary cause for the high mortality rate associated with the disease [10].

Current HAE treatment strategies include 1) on demand therapy (ODT) to revert acute manifestations, 2) long-term prophylaxis (LTP) to avoid/reduce angioedema recurrences and 3) short-term prophylaxis (STP) for attack prevention in high risk

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3 circumstances [11]. Upon diagnosis and lifelong thereafter, all HAE patients should
4
5 be enabled to immediate use of ODT at appearance of angioedema, to avoid
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7 disease mortality and to shorten attack duration. When this approach does not lead
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9 to satisfactory disease control, LTP should be considered to restore normal activities
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11 and improve patients' quality of life (QoL) [11].
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14 Several drugs have specific approval for HAE treatment, although with country
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16 differences in indication and availability. Plasma derived C1-INHs have indications
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18 for both ODT and LTP [12,13]. The bradykinin receptor antagonist icatibant, the
19
20 kallikrein inhibitor kalbitor and the recombinant C1-INH conestat alfa are approved
21
22 just for ODT [14,15]. On the opposite, attenuated androgens are exclusively
23
24 approved for LTP [16]. In several countries, fresh frozen plasma is the only resource
25
26 for ODT. Where multiple options are available, choosing among treatments for ODT
27
28 mainly rely on patient's preferences based on administration route (intravenous or
29
30 subcutaneous) and perceived efficacy. In LTP, benefits expected from a therapy
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32 should receive careful evaluation against side effects: this is particularly difficult
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34 when long-term side effects need to be envisaged from drug characteristics in
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36 absence of epidemiological evidence.
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41 For decades, cost/benefit balance has been the dilemma of attenuated androgens.
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43 Their high clinical efficacy in preventing HAE symptoms, needs to be weight against
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45 a long list of potential side effects in absence of studies confirming any treatment-
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47 related increased morbidity [17]. Ten years ago intravenous pdC1-INH was approved
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49 for LTP. A subcutaneous formulation of pdC1-INH was approved for LPT in U.S. in
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51 2017 and will soon reach Europe [18]. The same route of administration is used by
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53 lanadelumab, a monoclonal antibody blocking kallikrein for several weeks. This
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55 compound successfully completed phase III and is now filing for approval in LTP
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3 [19]. Again targeted to kallikrein is an oral small molecule that is now entering phase
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5 III trial [20]. It is likely that safety shown in other genetic disorders will render gene
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7 therapy an appealing approach to completely revert HAE [21].
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10 Considering the large array of effective treatments, we can see LTP approach to
11
12 HAE aimed at identifying the most physiological and safe drug. Reverting HAE
13
14 patients to normal clinical and biochemical phenotype is the objective of C1-INH
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16 replacing approaches. It has been first attempted using the plasma-derived protein.
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18 Therapies based on plasma-derived proteins started in the seventies when main
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20 problem were purity and blood borne infections [22]. These problems were
21
22 minimized over the years and pdC1-INH preparations are highly purified with an
23
24 excellent safety profile. Both viral safety and purity are guaranteed by accurate
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26 monitoring and a purification process abating different infectious agents and leading
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28 to final product close to 100% purity [23-25]. The clinical experience with the pdC1-
29
30 INH available at present shows no report of infection and high clinical efficacy for
31
32 both ODT and LTP suggesting that the purification process does not affect protein
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34 pharmacodynamics [26]. In the second half of 2017 a shortage in availability of
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36 pdC1-INH was faced worldwide, due to difficulties to rapidly scale production based
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38 on request. This is a well-known problem of plasma based products. As a solution to
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40 this, and other above mentioned problems with plasma derived products,
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42 recombinant proteins as human therapy became an interesting alternative [27]. The
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44 same applies to C1-INH, with the recombinant product (rhC1-INH) conestat alfa,
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46 currently available as a marketed pharmaceutical product for treatment on HAE. It is
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48 produced in the mammary gland cells and secreted into the milk of transgenic rabbits
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50 and then purified for use in humans [28].
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55 Efficacy and safety of rhC1-INH for ODT in HAE patients, have been investigated in
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3 several randomized placebo-controlled trials, as well as in open-label studies [15,29-
4 32]. Conestat alfa (Ruconest[®]) is currently approved for acute HAE treatment in
5 Europe, the US, Israel and South Korea. The product is available on a named-
6 patient basis in other territories where it has not yet obtained marketing
7 authorization. Recently, rhC1-INH has shown to be efficacious and well tolerated as
8 a prophylaxis option in a randomized, placebo-controlled crossover trial [33]. The
9 results of this study supported the initial proof of concept open-label prophylaxis
10 study [34], and sporadic case reports of efficacy in LTP and STP [35].

11
12 In this publication we will critically review the up-to-date available literature, and also
13 try to address the missing points in current knowledge to explain how rhC1-INH is
14 efficacious as a treatment for prophylaxis of HAE.
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20 21 22 23 24 25 26 27 **2. Body of review**

28 29 30 **2.1. Why recombinant human C1-INH from transgenic rabbits?**

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32 We already pointed to the relevance for safe and effective treatment to reverse
33 effects of HAE on patients' life. Knowledge, awareness and existing treatments
34 improved dramatically over the past decades and we are now moving toward HAE
35 personalized therapy.
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41 Human C1 inhibitor (hC1-INH) is a pluripotent plasma glycoprotein with a vast
42 number of biologic effects and a possibly growing number of therapeutic applications
43 [36]. Considering the large spectrum of physiologic functions, it seems reductive to
44 consider genetic C1-INH deficiency just as a condition exposing to angioedema
45 recurrences and limit therapeutic intervention to avoid this symptom. Accordingly,
46 reversal of the genetic deficiency appears as the approach closest to restore
47 physiologic homeostasis. Recombinant approaches for production of C1-INH started
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3 in late 1980s aimed at understanding structure-function correlates of disease
4 causing mutations [37,38]. Recombinant human C1-INH (rhC1-INH) has been
5 produced from mammalian cell culture, bacterial and yeast-based expression
6 systems to be used for research, but these systems were never applied to products
7 for use in humans [39]. This may be explained by rhC1-INH poor expression levels in
8 the culture, its inactivation throughout the production process, and/or the non-
9 mammalian glycosylation profiles of these methods. The expression of rhC1-INH in
10 the milk of lactating transgenic animals provides an alternative to the initially
11 investigated culture-based expression systems. It provides high rhC1-INH
12 expression levels (up to 12 g/l) and mammalian glycosylation profiles as reported for
13 different human proteins [40,41]. The rabbit as a transgenic platform gives some
14 specific advantages as relatively short gestation period and safe environment to
15 minimize adventitious agents in the production process. Translated into therapeutic
16 product, these characteristics predict infectious safety and production easily sized to
17 demand. In terms of biologic characteristics, conestat alfa has protease inhibitory
18 properties identical to those of the human plasma protein and significant
19 pharmacokinetic differences likely depending on species specific peculiarities in
20 glycosylation [42]. We aim at discussing further in detail these aspects. (Table 1)

2.2. Introduction to the drug

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45 Conestat alpha is a rhC1-INH (Ruconest®, Pharming Technologies B.V., Leiden,
46 The Netherlands) obtained through a purification process of transgenic New Zealand
47 white rabbits' milk (*Oryctolagus cuniculus*). The promoter used to drive expression of
48 the hC1-INH transgene is the bovine alpha-S1-casein promoter, which is specific for
49 the secretion of caseins in the milk. The protein content of rabbit milk is
50 approximately 14% of which about 65% consists of various caseins aggregated in

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3 micelles. The remaining proteins in rabbit milk are whey proteins including
4 transferrin, whey acidic protein, immunoglobulins, albumin and lactalbumin. After
5 collection, rabbit milk undergoes series of standard centrifugation, filtration, and
6 chromatography steps that give rhC1-INH, 99% purity as assessed by sodium
7 dodecyl sulfate polyacrylamide gel electrophoresis [43]. The 1% impurities are
8 multimers and N-terminal cleaved C1-INH species. Host-related impurities were
9 analysed (ELISA) and measured to be approximately 10 ppm in the commercial
10 batches and defined to consist of minimal traces of rabbit protein. [43]. The activity of
11 purified rhC1-INH is 6.1 U per mg of protein, the same as pdC1-INH [43]. Protein
12 sequencing analysis of recombinant and plasma proteins reveals identical
13 polypeptide N- and C-terminals for the two molecules. Nevertheless, the two proteins
14 differ in molecular mass and this is explained by differences in glycosylation (21%
15 and 26-28% carbohydrate content respectively) [43,44].

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32 Conestat alfa and pdC1-INH showed kinetics for inhibition of C1s, kallikrein, FXIIa,
33 and FXIa comparable in terms of protein potency and specificity. Observed
34 differences in the various studies can be explained by the different methods used for
35 the experiments, and the differences of the protocol followed for sample collection by
36 the investigators. [25,42,43]. (Table 1)
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44 **2.3. Chemistry and biology: SERPIN vs. Non-SERPIN part of C1-INH**

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47 Sequence analysis demonstrates that the C1 inhibitor is a member of **serin protease**
48 **inhibitors (serpin) superfamily**. The molecule consists of two distinct domains that
49 may be observed by electron microscopy [45]. The serpin domain extends from the
50 carboxy-terminal, contains the reactive site and is responsible for protease inhibitory
51 function. A rod-like amino-terminal of about 100 aa represents the non-serpin
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3 domain. It shows no significant homology with any known protein and its amino
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5 acids' most striking characteristic is the presence of 10 glycosylation sites [46].
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8 As for other serpins, C1-INH consists of alpha-helices and beta-sheets, as well as an
9
10 exposed mobile reactive center loop that is cleaved upon contact with a target
11
12 protease [47]. This reaction results in the formation of a stable, covalent bond.
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14 Protease binding causes dramatic conformational changes in C1-INH, which crushes
15
16 the protease against its lower pole resulting in irreversible inactivation of the enzyme.
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18 This defines serpins to be "suicidal" protease inhibitors. (Figure 2)
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22 C1-INH inactivates a variety of proteases including complement system proteases
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24 (C1r, C1s, MASP2), contact system proteases (factor XII, plasma kallikrein), an
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26 intrinsic coagulation protease (factor XI) and the fibrinolytic proteases (plasmin).
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28 (Figure 1) (For detailed review see Davis) [48].
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31 The highly mobile N-terminal of the protein is comprised of 97 amino-acids, and the
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33 attached sugars probably being of greater biologic importance than its non-compact
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35 polypeptide chain. This N-terminal, elongated domain, probably plays the role of an
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37 "anchor", helping the C1-INH molecule to "attach" to different surfaces. It does not
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39 directly contribute to protease inhibition and establishes non-covalent interactions
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41 with other proteins, and cell surfaces or lipids [36,49,50].
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45 Differences in the N-terminal glycosylation (see Table 1) modify the
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47 pharmacokinetics of the molecule. The removal of sialic acids from C1-INH (asialo-
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49 C1-INH) is found to significantly enhance its clearance from the circulation showing a
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51 half-life of 3 to 5 min in an investigational rabbit model [51]. This effect is presumably
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53 conducted via its binding to asialo-glycoprotein receptors in the liver with enhanced
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55 clearance due to exposure of the penultimate galactosyl residue. Subsequent
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3 removal of the latter reverts the clearance rate up to values similar to that of normal
4 C1-INH [51]. Though, the removal of sialic acid or galactose groups does not impair
5 the protease inhibitory function of C1-INH in vitro [51].
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10 As expected, rhC1-INH produced in transgenic rabbits differs from C1-INH produced
11 in humans in the glycosylation profile. In fact, each animal adds sugar-to-protein with
12 a pattern specific of the species, and furthermore, differences might occur between
13 different tissues within the same species.
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20 a. Pharmacodynamics

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22 Genetically low C1-INH function, lessens the control of the esterase activity that
23 generates upon spontaneous formation of the C1 complex. Such an inefficient
24 control allows cleavage of the natural substrates of C1, C4. In HAE patients, neo-
25 synthesis of C4 cannot compensate consumption resulting in characteristically low
26 plasma levels. Reversal of C4 consumption with normalization of plasma levels has
27 been used as biomarker for therapies target to C1-INH replacement. Accordingly,
28 pharmacokinetic curve of conestat alfa at doses of 100 and 50 U/Kg, shows a dose-
29 dependent restoration of C4 consumption measured by decrease of C4 break-down
30 products (C4b/c) and parallel increase of C4 plasma levels [42]. C4 peaks
31 approximately 12 hours post-infusion and then gradually reverts to baseline levels.
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33 When post-infusion C1-INH functional plasma fall below 0.7 U/ml, C4 consumption
34 resumes. Doses below 25 U/kg have minimal effect on C4 plasma levels.
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48 Pharmacodynamics effect of rhC1-INH is measured as reduction in the amount of
49 cleaved high-molecular-weight kininogen (CHK), indirect marker of bradykinin
50 formation. Cleaved HK increases during attacks in HAE and in inter-critical periods
51 discriminates frequently symptomatic patients from those suffering rare angioedema
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3 symptoms [52]. Conestat alfa in doses of 50 U/Kg significantly reduces cHK [53].
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6 **b. Pharmacokinetics and metabolism of rhC1-INH**

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8 Plasma levels of C1-INH in the normal population range from 70 to 130% (0.7–1.3
9 U/ml). In a large HAE population, functional levels of C1-INH ranged between 10%
10 and 30% [54]. In the open-label escalation study with conestat alfa in asymptomatic
11 HAE patients (doses from 6.25–100 U/ kg) there is a dose-dependent increase in
12 functional C1-INH activity up to normal for doses of 50 and 100 U/kg [42]. Elimination
13 half-life derived from this study is 1.6 and 2 hrs, which is clearly shorter compared to
14 plasma C1-INH. The fractional catabolic rate of radiolabelled C1-INH purified from
15 normal plasma, is 0.025 of the plasma pool/hour in normal subjects and 0.035 in
16 HAE patients [55]. These catabolic rates correspond to a half-life 20 and 14 hours
17 respectively. When pdC1-INH for therapeutic use substitutes the radiolabelled
18 protein, the estimated post-infusion half-life shows great variability among studies.
19 Using Berinert, it is 39.1 hrs, with Cinryze 56 hrs [12,56]. The conditions of these
20 studies are extremely different and head to head comparison among preparations is
21 not appropriate. Nevertheless, the strikingly shorter half-life of the recombinant
22 protein is evident. It is explained by the glycosylation profile of the recombinant
23 product that enhances binding to asialoglycoprotein and mannose receptors that are
24 abundant in the liver and rapidly clear the protein from the circulation. The dose-
25 dependent increase of the half-life of rhC1-INH is ascribed to saturation of these
26 receptors [42]. However, the clinical efficacy of conestat alfa does not appear to be
27 affected by the shorter plasma life. A meta-analysis of controlled studies of efficacy
28 of on demand pdC1-INH and rhC1-INH, shows a dose dependent increase, which is
29 independent from the pharmacokinetic characteristics of the different products [57].
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31 A model of population pharmacokinetic (PPK) analysis used data from 120 subjects
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3 who received 214 administrations of rhC1-INH during 6 clinical studies [58]. Using
4 the Michaelis-Menten elimination kinetics model, a single dose of rhC1-INH of 50
5 U/kg would restore normal functional C1-INH levels in more than 94% of C1-INH-
6 HAE patients. This suggests that doses below 50U/Kg prevent a vast number of the
7 patients from achieving C1-INH levels within the normal range [57]. The PPK model
8 confirms the importance of a weight-based dosing of rhC1-INH up to a dose of 4200
9 U (two commercial vials). The PPK model was also used to investigate differences in
10 peak C1-INH activity levels after initial administration compared to repeated
11 administrations for subsequent HAE attacks, and indicated no such differences [58].
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23 **d) Clinical development of conestat alfa**

24 **Phase I studies – an open-label dose-escalation study**

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26 The study protocol for this open-label dose-escalation study was conducted in 12
27 asymptomatic subjects with a typical medical history of HAE with functional C1-INH
28 and C4 levels of less than 40% of normal. All subjects were symptom free for a
29 period of at least 2 weeks before rhC1-INH was administered for the purpose of the
30 study. The patients were divided into 4 groups of 3 patients (starting from 6.25, 12.5,
31 25, and 50 U/kg, respectively) and each patient was given 2 doses of rhC1-INH with
32 a washout period of at least 5 weeks before the dose was escalated up to 100 U/kg.
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34 The course of functional C1-INH in plasma showed a full initial recovery and a dose-
35 dependent clearance of rhC1-INH. The observed safety profile and biologic activity
36 of rhC1-INH suggested further clinical studies to assess its efficacy in treating acute
37 HAE attacks [42].
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52 **On demand treatment of acute attacks**

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54 The efficacy of rhC1-INH for the treatment of HAE patients during acute angioedema
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3 attacks has been demonstrated in several studies, including three double-blind,
4 placebo-controlled efficacy studies [15,29] and five open-label studies where patients
5 maintained treatment of subsequent attacks [29,30,32,34,59]. No relapse or rebound
6 episodes were reported, suggesting that conestat alfa mechanism of action and
7 specific pharmacodynamics, lead to sustained protein activity, rather than its
8 relatively short elimination plasma half-life. In the largest of these studies (n = 224
9 attacks treated), 96% of attacks were treated with a single dose of rhC1-INH of 50
10 U/kg (max 4200 U) [30]. Moreover, administration of an additional treatment dose
11 was not reported for subsequent attacks.
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Prophylaxis

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25 The efficacy of rhC1-INH for prophylactic treatment of patients with HAE to prevent
26 onset of angioedema attacks has been investigated in two studies, one open label
27 pilot study and one double-blind, randomized, placebo-controlled study [33,34].
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31 Extension for the indication has been submitted and waiting for approval, expected in
32 the third quarter of 2018 [60].
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Open-label pilot prophylaxis study in HAE

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39 An open-label, uncontrolled, pilot study to test efficacy and safety of prophylactic
40 once-weekly administration of conestat alfa at 50 U/kg was conducted over an 8-
41 weeks period in patients with high frequency of monthly attacks [34]. The study
42 enrolled 25 patients with a mean attack-rate of 0.9 attacks per week (median of 0.6
43 attacks, range 0.4 to 4.5 attacks per week). The mean breakthrough attack rate
44 during the treatment period was 0.4 attacks per week (median of 0.3, range from 0.0
45 to 1.5 attacks per week), which showed to be significantly lower than the reported
46 historical average attack rate.
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Randomized placebo-controlled trial for prophylaxis of HAE

Recently, the results from a phase II multi-centre, randomized, double-blind, placebo-controlled, 3-period cross-over study were published [33]. The study aimed at evaluating the efficacy and safety of conestat alfa in prophylaxis of angioedema attacks within adult and adolescent patients with severe course of C1-INH-HAE.

Eligible patients were those above the age of 13 years with a clinical history of frequent HAE attacks (>4 attacks per month). Each patient received three 4-week periods of treatment, twice weekly, with a 1-week washout between different treatment periods. The study was conducted in 32 (ITT) patients (26 completed the study) who received intravenously in a double-blind fashion injection of conestat alfa 50 U/kg (to a maximum of 4200 U for patients ≥ 84 kg), either once weekly or twice weekly. The outcomes reported by the authors were that the mean number of attacks of hereditary angioedema over 4 weeks was significantly reduced with conestat alfa twice weekly, and once weekly, versus placebo, with mean differences of -4.4 attacks ($p < 0.0001$) and -2.8 attacks ($p = 0.0004$), respectively [33].

In addition, prophylaxis with conestat alfa for HAE has been reported in sporadic case report series, both used in long-term prophylaxis, and as a successful choice in short-term prophylaxis before deemed invasive procedures [35]. (Table 2)

e) Post marketing surveillance

The database following safety and tolerability of conestat alfa includes clinical and laboratory data arising from almost 1600 administrations of rhC1-INH in twelve completed clinical development program studies. [53]

Adverse events:

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3 The safety data analyses demonstrate that rhC1-INH at doses of 50 IU/kg and 100
4 IU/kg is generally safe and well tolerated when administered for treatment and
5 prevention of HAE attacks. The adverse event profile found in the randomized,
6 placebo-controlled studies was similar for patients treated in the rhC1-INH and saline
7 treatment groups. There was no increase in the incidence of treatment-emergent
8 adverse events with higher rhC1-INH dose, administration of additional rhC1-INH
9 doses for an attack, or with repeated treatment of subsequent attacks [30]. The most
10 common adverse reactions ($\geq 2\%$) reported in all clinical trials were headache,
11 nausea and diarrhea.
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23 Important for clinical practice is to avoid using the product in patients who are rabbit
24 allergic due to potentially serious allergic reactions, which has happened within three
25 minutes after administration of conestat alfa in only a single healthy volunteer with a
26 pre-existent (retrospectively known), non-disclosed rabbit dander allergy [42].
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32 Throughout the clinical development of the drug, no safety issue related to
33 hematology, biochemistry, coagulation, urinalysis, vital signs, or ECG parameters
34 was noted.
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39 **Thrombogenicity:**

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42 Concerns regarding a thrombogenic risk of conestat alfa, or regarding a clinically
43 meaningful effect on activation of coagulation or fibrinolysis (due to its effect on
44 proteases of the contact/coagulation system and previous reports in high-dose
45 treatment with plasma-derived C1-INH) have not been recorded during clinical
46 development and clinical studies. Conestat alfa did not show effect on activation of
47 coagulation and fibrinolysis [61]. In fact, D-dimer levels, which are considered
48 biomarkers of thrombosis were followed during acute treatment with conestat alfa
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3 and treatment was not associated with thrombotic events [62].
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6 **Immunogenicity:**

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8 Immunogenicity of rhC1-INH was extensively tested throughout the clinical
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10 development programs, supporting that conestat alfa has low potential to induce
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12 anti-C1-INH antibodies or anti-host-related impurities (HRI) response.
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14 Confirmed antibodies against C1-INH or HRI were observed infrequently and were
15
16 not associated with clinical symptoms indicating hypersensitivity or changes in rhC1-
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18 INH efficacy [63].
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21 There was no association between treatment-emergent adverse events or new acute
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23 HAE attacks and the presence of any confirmed anti-C1-INH or anti-HRI antibodies.
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25 No patients were tested positive for neutralizing antibodies to endogenous C1-INH or
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27 rhC1-INH. No anti-rabbit IgE antibodies were reported elevated with treatment with
28
29 conestat alfa and moreover, no severe hypersensitivity reactions were reported
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31 beyond the single case in a Phase 1 study and in the post-approval phase as of the
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33 date of the manuscript preparation. (Table 3)
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36 37 **f) Regulatory affairs**

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40 RhC1-INH started to be investigated as a potential pharmacological agent in the
41
42 treatment in HAE with various clinical studies conducted since the late 1980s
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44 [43,64,65]. Subsequently, the registration of the drug conestat alfa (Ruconest®) was
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46 secured in 2010 in Europe, and following in 2014 in the USA and Israel.
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50 The European Commission has granted rhC1-INH (Ruconest®) a marketing
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52 authorization for the treatment of acute angioedema attacks in adults and
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3 adolescents with HAE due to C1-INH deficiency. Currently, it is now approved for
4 use in all the 28 EU member countries plus Norway, Iceland and Liechtenstein.

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7 The United States' Food and Drug Administration (FDA) approved rhC1-INH
8 (Ruconest®) for the treatment of acute angioedema attacks in adult and adolescent
9 patients with C1-INH-HAE. Because of the limited number of patients with laryngeal
10 attacks, effectiveness was not established in HAE patients with laryngeal attacks by
11 the FDA.
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18 Conestat alfa is approved in Israel for the treatment of acute angioedema attacks in
19 adults with hereditary angioedema (HAE) due to C1 esterase inhibitor deficiency.

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22 Conestat alfa is approved in South Korea for the treatment of acute angioedema
23 attacks, except oro-pharyngeal & laryngeal locations in adult patients with hereditary
24 angioedema.
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29 In the pediatric population (2-13 years old), the study has been completed and
30 submitted for approval, awaiting to expand the indication in this subpopulation
31 ([https://www.pharming.com/pharming-announces-positive-data-from-paediatric-
32 clinical-trial-with-ruconest/](https://www.pharming.com/pharming-announces-positive-data-from-paediatric-clinical-trial-with-ruconest/)).

37 **3. Conclusion**

38
39 Hereditary angioedema (HAE) is a rare autosomal dominant disease characterized
40 by recurrent swellings of the extremities, oro-facial-pharyngeal zones, upper airways,
41 genitalia, often severely disrupting patients' lives. HAE is a potentially life threatening
42 disease, with 1-5% of all angioedema episodes locate to the larynx. In absence of
43 appropriate diagnosis and treatment 25% of patients die for laryngeal edema [10].
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51 The burden of HAE has huge geographical discrepancy due to differences in
52 treatment availability. In the recent years, new drugs for C1-INH-HAE were approved
53 in Europe and North America. Available treatments can be seen as optimal to avoid
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3 mortality granted unrestricted availability and education for appropriate use. In terms
4
5 of minimizing morbidity, on demand approach is insufficient for frequently
6
7 symptomatic patients; attenuated androgens are limited by side effects and
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9 intravenous pdC1-INH by administration route and empiric optimal dosing. Raising
10
11 demand for optimal prophylaxis, pushed towards development of novel hi-technology
12
13 approaches based on different platforms. Subcutaneous pdC1-INH, already
14
15 available in U.S., and upcoming prophylactic approaches tackling plasma kallikrein,
16
17 should further improve treatment efficacy and customization. Using transgenic
18
19 animal technology, rhC1-INH aims at providing replacement therapy with a human
20
21 protein produced by recombinant technology that is free from blood borne human
22
23 infectious agents and in transgenic animal that allows to scale production based
24
25 upon request. The recently published phase II study demonstrates that the short
26
27 half-life of the rhC1-INH does not lessen efficacy as prophylactic therapy. Safety,
28
29 tolerability and efficacy on repeated infusions, relay on the long development
30
31 program that brought rhC1-INH registration in Europe, USA, Israel, and other
32
33 countries for treatment of acute HAE attacks in adult and adolescent patients. The
34
35 same intravenous formulation was used to obtain evidence for effective in
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37 prophylaxis. In the presence of subcutaneous and oral competitors prophylactic
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39 therapy administered intravenously, cannot be successful. A concentrated
40
41 formulation of conestat alfa for subcutaneous or intramuscular administration is
42
43 expected to bring this product into the market for HAE prophylaxis. When such a
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45 low-volume formulation will prove as effective as competitors in large number of
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47 patients followed for several months, the compound will become an interesting
48
49 additional alternative for patients with HAE.
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54 55 **4. Expert commentary** 56 57 58 59 60

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3 C1-INH is an acute-phase protein that for structure and inhibitory mechanism
4 belongs to the superfamily of serine-protease inhibitors. Soon after discovery that
5 genetic C1-INH deficiency was the cause of HAE, C1-INH derived from human
6 plasma became the life-saving drug for these patients. Technological evolution and
7 improved safety expanded the indication of plasma derived products are now used
8 as replacement therapy in genetic defects. This is true also for C1-INH, established
9 treatment for long-term prophylaxis of HAE. Need for large supply of safe proteins,
10 prompted to develop the recombinant technology in transgenic animals. Conestat
11 alfa, the recombinant human C1-INH purified from milk of transgenic rabbits, was
12 initially approved as on demand intravenous therapy. When long-term replacement
13 of C1-INH deficiency by the plasma derived protein became a standard approach to
14 the disease, conestat alfa was considered for analogous use. Main drawback to
15 foresee this product as successful for prophylaxis was its short half-life. Six hours
16 after infusion conestat alfa is not detectable in patients' plasma. Nevertheless,
17 analysis of data from on demand trials showed that clinical efficacy of plasma and
18 recombinant C1-INH was dependent on the initial dose and not influenced by the
19 pharmacokinetic profile. Moreover, an animal model of stroke, showed rhC1-INH to
20 be superior to the pdC1-INH in protecting from extension of the ischemic brain injury
21 [66]. Such a difference appeared to be explained by the stronger affinity of rhC1-INH
22 for mannan binding lectin (MBL) and thus better inhibit the lectin pathway of
23 complement: rhC1-INH binds MBL with a relatively high affinity
24 (230nM), whereas pdC1-INH does not show any binding up to 40um [67]. Thus, the
25 possibility that clearance from the circulation did not necessarily mean protein
26 catabolism was considered. Normal C1-INH binds to endothelial cells maintaining
27 intact protease inhibitory activity [68]. C1-INH binding characteristics have been
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3 investigated to explain other potential anti-inflammatory effects of human C1-INH
4 [49]. These effects do not appear to require protease inhibition and depend on non-
5 covalent interactions with other proteins, cell surfaces or lipids. C1-INH is a multi-
6
7 faceted anti-inflammatory protein that acts through a variety of mechanisms including
8
9 protease inhibition in addition to several different non-covalent interactions, not
10
11 related to its anti-protease activity.
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15 Thus, efficacy of conestat alfa, administered once or twice per week, in HAE
16
17 prophylaxis was tested on the hypothesis that rhC1-INH is still biologically active
18
19 after disappearance from the circulation. The promising results of the two studies
20
21 where conestat alfa was tested as prophylactic agent suggest that the hypothesis is
22
23 valid. In order to become an alternative for replacement therapy in HAE patients, a
24
25 low-volume subcutaneous or intramuscular formulation of conestat alfa needs to
26
27 demonstrate to be as effective as the plasma-derived protein.
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30 The possibility that rhC1-INH has biologic activity independent from plasma levels
31
32 opens to the possibility of indications outside from HAE. Plasma derived C1-INH and
33
34 rhC1-INH have been studied in a variety of animal models of diseases involving
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36 contact and complement system. Among others, pancreatitis, sepsis, thermal injury,
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38 xenotransplantation, and various models of ischemia-reperfusion injury (e.g.,
39
40 myocardial infarction, stroke, delayed graft function in transplantation)
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42 [69,70][71,72][73][74][75,76][77]. The efficacy of C1-INH in humans with these
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44 disease states needs to be assessed by appropriate studies. When such studies will
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46 be designed the different efficacy of plasma and recombinant products will have to
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48 be considered taking into account the findings in the animal model of stroke.
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52 53 **5. Five-year view**

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3 In a five-year period, we can easily envisage that HAE prophylaxis with androgens
4 will be marginal in countries where new treatments are available. On demand
5 treatment and prophylaxis will still co-exist due to the high variability in symptom
6 frequency. In the field of prophylaxis two approaches will compete: replacing the
7 genetic defect versus silencing the generation of the angioedema mediator. Both
8 appear to provide nearly complete prevention of symptoms and decision will rely on
9 expected long term benefit and patient preference. C1-INH replacement appears
10 more physiologic and some long-term experience is already available to confirm
11 absence of side effects. Theoretical positive aspect for C1-INH is the possibility to
12 prevent, along with angioedema, the consumption of classical pathway complement
13 components. Genetic defects of the components of this pathway are associated with
14 reduced apoptosis and autoimmunity [78]. We still lack data showing whether C1-
15 INH deficiency exposes to diseases other than angioedema. In a short, while HAE
16 registries will make this information available facilitating therapies tailored on
17 patients' needs. This is part of a general trend towards personalized/precision
18 medicine aimed at restoring the physiologic state in respect of personal needs. Non-
19 trivial to this is treatment administration modality. In 5 years, we will still have HAE
20 patients controlling the disease with an on demand approach or others with
21 prophylaxis. For the last approach there will be subcutaneously injectable treatments
22 delivered every few days or every month, as well as daily tablets. Choosing between
23 the different options depends mainly on patients' attitude. There are patients who
24 just want to forget the disease and others, who prefer to maintain an active control.
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26 The different options will likely satisfy most of these needs.
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3 To close, we can predict that in 5 years extended information on disease variability
4 and a large array of treatment options will render HAE the prototype of successful
5 precision medicine.
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10 **6. Key issues**

- 11 • Hereditary angioedema (HAE) results from an autosomal dominant hereditary
12 deficiency of the plasma protein C1 inhibitor (C1-INH-HAE) and is
13 characterized by the occurrence of acute attacks of angioedema that can be
14 life-threatening, disfiguring and/or disabling.
15
16 • Recombinant human C1-INH (rhC1-INH) is approved for the treatment of
17 acute angioedema in adult and adolescent patients with HAE.
18
19 • A recent publication from a phase 2 randomized double-blind, placebo
20 controlled, cross-over trial has presented data about rhC1-INH as an
21 efficacious agent for prophylactic treatment of HAE in adult and adolescent
22 patients, which confirms the previously reported data from an open label pilot
23 study and sporadic case reports.
24
25 • C1-INH is a human plasma protein involved in the regulation of the
26 complement, coagulation, fibrinolysis, and contact systems. It is a powerful
27 controlling protein in early stages of these bio-amplification cascades, and in
28 that way, a crucial regulating factor of the innate immune response.
29
30 Therefore, its role could be further investigated as a potential controller agent
31 in various cases of abnormal inflammation.
32
33 • Recombinant human C1 inhibitor (rhC1-INH) has some distinguishing
34 features, when compared to plasma-derived C1-INH, probably related to the
35 difference in the glycosylation of the non-serpin N-terminal of the molecule.
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37 These specificities account for the differences in its pharmacokinetic
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3 behaviour (e.g. shorter plasma half-life), as well as its stronger affinity to
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5 suppress the lectin complement pathway.

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7 • Further studies are needed to explain rhC1-INH behaviour in the human body,
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9 as the drug's plasma half-life of clearance is not a sufficient predictor of its
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11 efficacy.

12 13 **7. Information resources**

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16 Pubmed: <https://www.ncbi.nlm.nih.gov/pubmed/>

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18 Clinical trials.gov registry and database of clinical studies of human participants:
19 <https://clinicaltrials.gov>

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21 Pharma companies' websites: www.ruconest.com, www.cinryze.com,
22 www.berinert.com, www.haegarda.com

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25 Regulatory affairs: www.ema.europa.eu, www.fda.gov

Legends: Figures and Tables

Figures:

1. Figure 1: Bio-amplification plasma cascades and C1-INH.
2. Figure 2: C1-INH: mechanism of action with proteases

Tables

3. Table 1: Characteristics of recombinant human and plasma-derived C1-INHs
4. Table 2: Clinical efficacy: studies with rhC1-INH for prophylaxis
5. Table 3. Safety and tolerability of rhC1-INH and competitors for prophylaxis of HAE

1. Table 1: Characteristics of recombinant human and plasma-derived C1-INH

Characteristics of C1-INH		
Classification	Serine protease inhibitor, serpin	
Synonyms	C1-esterase inhibitor, C1s-inhibitor, C1-inactivator, alpha2-neuramino-glycoprotein, Serpin Family G member 1	
Activity/Function	C1-inhibitor controls activation of the C1 complex and the generation of kinins. It inhibits the target proteases by the formation of a proteolytically inactive stoichiometric complex. It regulates proteases in complement, contact, coagulation and fibrinolytic systems.	
Cofactors	Glycosaminoglycans	
Characteristics of commercially available C1-INH for the treatment of HAE		
Properties	Recombinant human C1 inhibitor (conestat alfa)	Plasma derived C1 inhibitors
Target proteases	C1s, C1r, aFXIIa, bFXIIa, kallikrein, FXIa, plasmin, MASP-1, MASP-2	
Mature polypeptide sequence	Identical, 478 amino acids	
Apparent Molecular Weight* kDa (SDS-PAGE)	98 [25]	105 [25]
Molecular Weight** kDa (mass spectrometric analysis)	67 [43]	74-71[44] [43]
Carbohydrate contents (w/w)	21% [43]	28-26% [43,44]
Glycosylated amino-acids (mature protein)	Not reported	Multiple O-linked and 6 N-linked glycans: Six N-glycosylation sites at Asn-3, -47, -59, -216, -231, and -330. Seven O-glycosylation sites at Ser-42 and Thr-26, -49, -61, -66, -70, and -74[44]. Another study reported 24 O-glycosylation sites [46]
Type of sugar residues	N-glycans of oligomannose-, hybrid-, and complex-type structures. 25% are neutral and the remaining are sialylated (N-acetylneuraminic acid only). Of these, 67% are monocharged of the hybrid-, mono- or diantennary complex type. Part are (a1-6)-core-fucosylated or (a1-3)-fucosylated in the lower or upper antenna (Lewis x epitope). Small core 1-type O-glycans with the usual (a2-3)- and (a2-6)-sialylation pattern of O-glycoproteins of nonmucinous origin [28]	(a2-3)-disialylated and (a2-6)-disialylated complex-type diantennary N-glycans (molar ratio, 2:1). (a2-3)- monosialylated core 1-type O-glycan. The N-glycans are (a1-6)-core-fucosylated for 30%, and the only sialic acid found is N-acetylneuraminic acid. Lower amounts of sialylated tri- and tetraantennary structures are also reported [44]
Plasma half-life of clearance (hours)	1.6 (50 U/kg) [42] 2.9 (100 U/kg) [42]	10 – 96 [79-81]
Activity (concentration) U/mL	150 [25]	50-100 [25]
Genetics	Human C1-INH gene sequence through a bovine alpha-S1-casein promoter, specific for the secretion of caseins in the milk, introduced via micro-injection transgenic technique in New Zealand white rabbits (<i>Oryctolagus cuniculus</i>)	17-kilobase single-copy gene on human chromosome 11, which consists of eight exons
Synthesis	New Zealand white rabbits mammary gland epithelial cells and secreted in the milk	Human hepatocytes, fibroblasts, monocytes, macrophages, endothelial cells, amnionic epithelial cells, and perhaps cells in the microglia
Final purity of the commercial drug		
Purity	99% [25]	89 – 97 % [25]
Impurities	1% rhC1-INH multimers and N-terminal cleaved C1-INH species [25,43]	3 – 11 % low and high-molecular weight proteins [25]
Host related impurities	10 ppm, traces of rabbit protein; No endogenous rabbit C1-INH [25]	Human α 1-antichymotrypsin and ceruloplasmin, C3, immunoglobulin heavy constant mu [25]
Kinetics, second-order rate constant K_{on} ($M^{-1}s^{-1}$) (higher values correspond to higher inhibitory capacity)		
C1s	6.1 x 10x4 [43]	5.1 x 10x4 [43] 4.8 x 10x4 [64] 6.2 x 10x4 [39]
Factor XIa	9.8 x 10x2 [43]	9.0 x 10x2 [43] 1.8 x 10x3 [82] 3.9 x 10x2 [39]
Factor XIIa	6.9 x 10x3 [43]	5.7 x 10x3 [43] 9 x 10x3 [82] 4.5 x 10x3 [39]
Kallikrein	9.1 x 10x3 [43]	7.6 x 10x3 [43] 2.6 x 10x4 [82] 7.8 x 10x3 [39]

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3 Legend: C1-INH, C1 esterase inhibitor; rhC1-INH, recombinant human C1 inhibitor; pdC1-
4 INH, plasma-derived C1 inhibitor; GAGs, glycosaminoglycans, HAE, hereditary angioedema;
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6 C1s, complement C1s; C1r, complement C1r; FXII, factor XII; FXI, factor XI; MASP1/2,
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8 mannan-binding lectin serine protease 1/2; C-terminal, protein carboxyl-terminus; N-
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10 terminal, protein amino-terminus; C3, complement C3.
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13 * Measured by Sodium Dodecyl-Sulfate polyacrylamide gel electrophoresis
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16 ** Measured by fine neutron scattering
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Table 2: Clinical efficacy: studies with rhC1-INH for prophylaxis

Study Number	Phase of study	Subjects	Therapeutic regimen	Results	Reference
NCT00851409	Pilot phase II open-label prophylaxis study	Adult C1-INH-HAE patients with history of at least 2 attacks/month N=25 (ITT)	Weekly administration of 50 U/kg conestat alfa	Mean weekly attack rate decreased during the study to 0.4 attacks/week, as compared with the pre-study attack-per-week rate of 0.9	[34]
NCT02247739	Phase II multicenter, randomized, double-blind, placebo-controlled, 3-period cross-over study	≥13 years older C1-INH-HAE patients with history of at least 4 attacks/monthly in the last 3 months prior to study enrollment N=32 (ITT)	Three 4-week-periods of intravenous injection of conestat alfa 50 U/kg (to a maximum of 4200 U for patients ≥84 kg), either once or twice weekly with a 1-week washout between treatment periods	The number of attacks of hereditary angioedema over 4 weeks was significantly reduced with conestat alfa twice weekly, and once weekly, versus placebo, mean differences of -4.4 attacks (p<0.0001) and -2.8 attacks (p=0.0004), respectively	[33]

C1-INH-HAE, hereditary angioedema due to C1 inhibitor deficiency;

Table 3. Safety and tolerability of rhC1-INH and competitors for prophylaxis of HAE

Drug (molecule)	Commercial name, Company	Subjects and therapeutic regimen	Adverse events	Warnings and precautions	References
rhC1-INH	Ruconest, Pharming B.V.	Adults and adolescents; 50 IU/kg, twice weekly, I.V. (up to 4200 IU for ≥ 84 kg)	Headache, nausea and diarrhea	Potentially serious allergic reactions in rabbit allergic patients. Potential immunogenicity.	[15,29-31,34,42,55,63,83,84] SPC of the product
pdC1-INH	Cinryze, Shire	Adults and adolescents; 1000 IU, twice weekly, I.V. (up to 2500 IU, but no more than 100 IU/kg)	Headache, nausea, rash, vomiting and fever	Potential infectious agents' transmission. Thrombotic and thromboembolic events. Allergic reactions. Potential immunogenicity.	[13,22,85,86] SPC of the product
Low-volume pdC1-INH	HAEGARDA, CSL Behring	Adults and adolescents; 60 IU/kg, twice weekly, S.C.	Injection site reactions, hypersensitivity, nasopharyngitis, and dizziness	Potential infectious agents' transmission. Potential thrombotic events. Potential tachyphylaxis.	[18,87] SPC of the product NCT01576523 NCT01912456 NCT02316353
Rh mAb targeting plasma kallikrein	Lanadelumab, Shire	Adults and adolescents; 150 mg, once monthly, S.C. or 300 mg, once or twice monthly, S.C.	Injection site reactions, headache, nasopharyngitis, rash, and dizziness	Potential immunogenicity. Theoretical precaution for cardiovascular accidents, bleeding and autoimmune disorders	[19,33,88] NCT02586805 Ongoing Phase III

rhC1-INH, recombinant human C1 inhibitor; pdC1-INH, plasma-derived C1 inhibitor; HAE, hereditary angioedema; I.V., intravenously; S.C., subcutaneously; SPC, summary of product characteristics; rh, recombinant human; mAb, monoclonal antibody.

*Other prophylaxis options as androgens and tranexamic acid were not included as the data has been previously summarized in other review articles [89]

Annotated Bibliography:

* - of interest

** - of considerable interest

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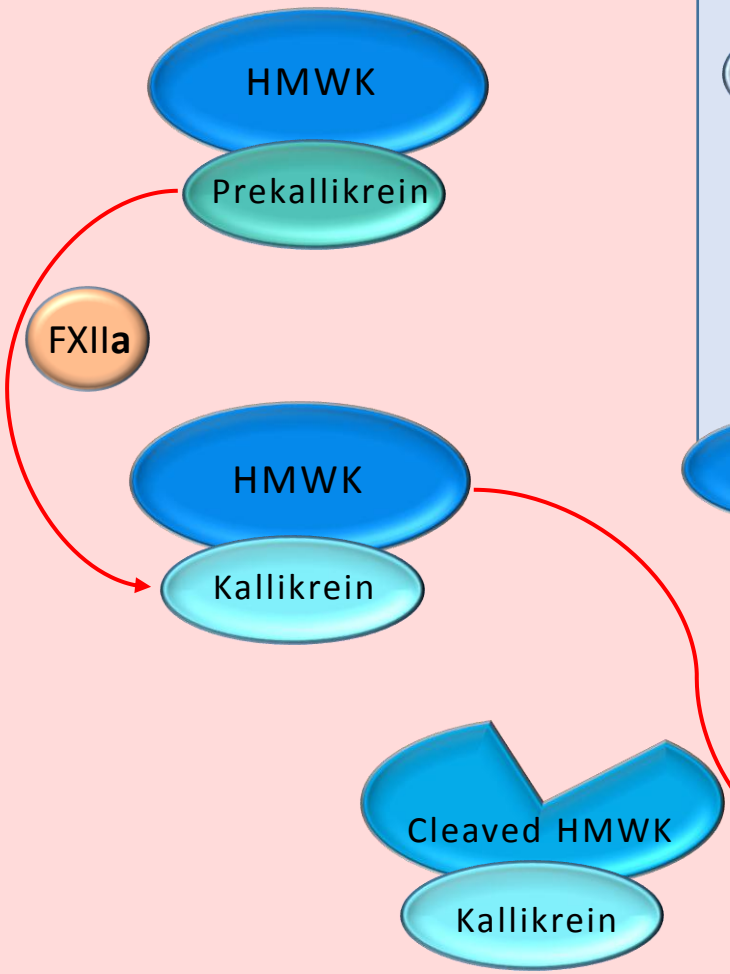
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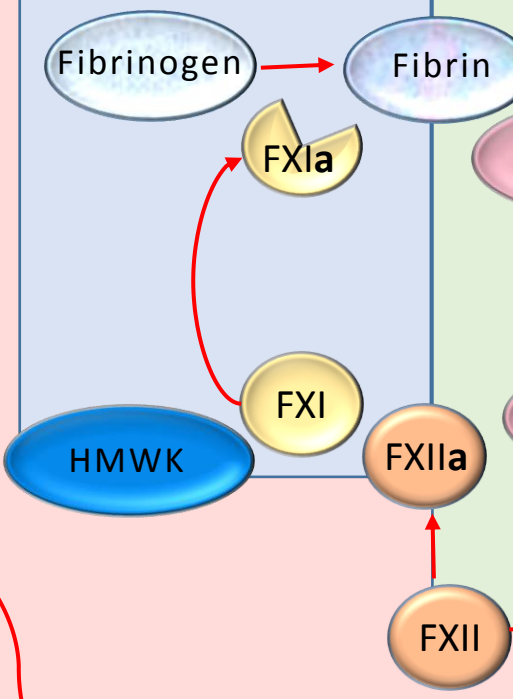
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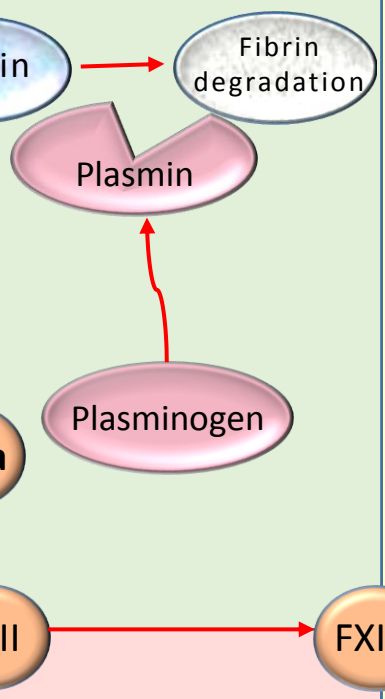
CONTACT SYSTEM



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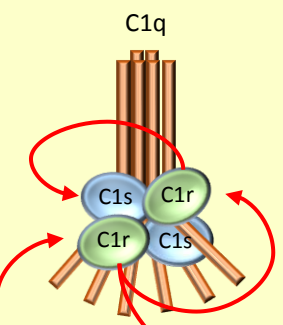
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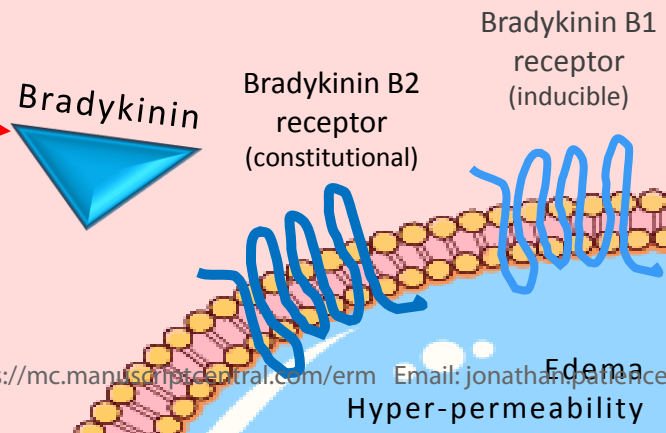
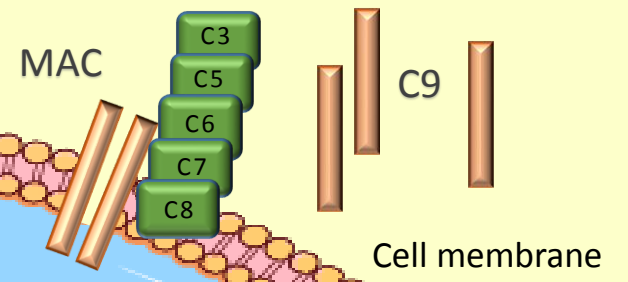
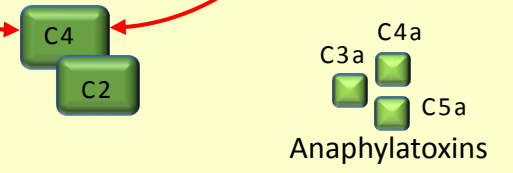
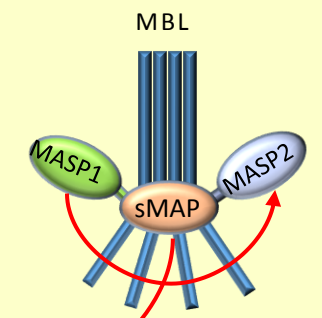
COMPLEMENT SYSTEM

→ Processes regulated by C1-INH

Classical pathway



Lectin pathway



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