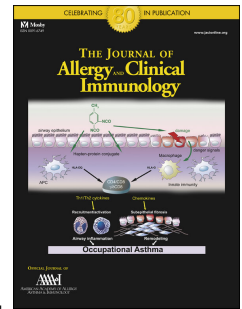


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Allergenicity and Safety of rhC1INH in Patients with Allergy to Rabbit or Cow's Milk

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TITLE PAGE**Allergenicity and Safety of rhC1INH in Patients with Allergy to Rabbit or Cow's Milk**

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ABSTRACT

Background

Recombinant human C1 inhibitor (rhC1INH) for on demand treatment of hereditary angioedema, is purified from milk of transgenic rabbits. It contains low amounts ($<0.002\%$) of host-related impurities (HRI), which could trigger hypersensitivity reactions in patients with rabbit allergy (RA) and/or cow's milk allergy (CMA).

Objectives

Assessment of allergenicity and safety of rhC1INH in RA and/or CMA patients.

Methods

Patients with CMA and/or RA underwent skin prick test (SPT), intracutaneous test (ICT), and, when both were negative, subcutaneous (SC) challenge with up to 2100U (14 mL) rhC1INH. The negative predictive value (NPV) of the skin test protocol was calculated, defined as the ratio of patients without systemic symptoms of hypersensitivity following SC challenge, over the number of patients having tested negative for both the SPT and ICT. Adverse events after exposure to rhC1INH were recorded.

Results

Twenty-six patients with RA and/or CMA were enrolled. Twenty-four had negative SPT and ICT for rhC1INH, whereas 2 had negative SPT, but positive ICT to rhC1INH (only the highest concentration). Twenty-two patients with negative SPT and ICT underwent SC challenge. None developed allergic symptoms. Local treatment-emergent adverse events (TEAEs) occurred in 7 patients (32%) after SC challenge. In 5 these were considered drug related. All were mild.

Conclusion

None of the patients with negative SPT and ICT for rhC1INH had allergic symptoms during rhC1INH challenge. The NPV of the combination of SPT and ICT for the outcome of the SC challenge was 100% (95% CI: 84.6% ; 100%). SC administration of rhC1INH was well-tolerated.

Clinical Implications

Negative SPT and ICT with undiluted rhC1INH, in subjects with cow's milk and/or rabbit allergy, most likely rules out an allergy to rhC1INH.

Capsule summary

Patients with an allergy to rabbit or cow's milk underwent a skin test protocol with rhC1INH. In those with a negative SPT and ICT, none had an immediate type hypersensitivity reaction during subcutaneous drug challenge.

Key words:

Hereditary angioedema; Recombinant human C1 esterase inhibitor; allergenicity; rabbit allergy; cow's milk allergy; skin prick test; intracutaneous test; intradermal test; subcutaneous administration; subcutaneous challenge

Abbreviations

AE: adverse event, BAT: basophil activation test, C1INH: C1 esterase inhibitor, C1INH-HAE: hereditary angioedema with C1 esterase inhibitor deficiency, CMA: cow's milk allergy, DBPCFC: double-blind placebo controlled food challenge, HAE: hereditary angioedema, HRI: host-related impurities, ICT: intracutaneous test, NPV: negative predictive value, RA: rabbit allergy, rhC1INH: recombinant human C1INH, SAE: serious adverse event, SC: subcutaneous, sIgE: specific Immunoglobulin E, SPT: skin prick test, TEAE: treatment-emergent adverse event, TESAE: treatment-emergent serious adverse event

MANUSCRIPT**Introduction**

Acute attacks of hereditary angioedema (HAE) due to C1 esterase inhibitor (C1INH) deficiency (C1INH-HAE) can be treated by replacing C1INH. Several C1INH drugs are approved for this indication, including recombinant human C1INH (rhC1INH, conestat alfa, Ruconest).¹ The active constituent rhC1INH is secreted in the milk of transgenic New Zealand White rabbits expressing the human gene for C1INH, and has an amino-acid sequence identical to that of endogenous human C1INH.²⁻⁴ rhC1INH is a highly-purified final product which contains <0.002% of host-related impurities (HRI)^{2,3,5} consisting primarily of milk or dander proteins. It is unclear whether these impurities are able to trigger allergic reactions in patients with a pre-existing allergy to rabbit dander exposed to rhC1INH. Additionally, although cow's milk proteins differ from rabbit milk proteins, rabbit milk impurities in rhC1INH may theoretically elicit an allergic reaction in patients with cow's milk allergy (CMA) due to cross-reactivity of involved IgE with HRIs in rhC1INH.³

rhC1INH is contraindicated in patients with known or suspected allergy to rabbits (labeling EU) or rabbit-derived products (labeling USA). This is largely based on the occurrence of a single allergic reaction in a healthy study subject with a previously undisclosed rabbit allergy in a safety study conducted in 2005.³ This study subject developed an itchy rash and wheezing upon first exposure to 100 U/kg rhC1INH shortly after the start of the intravenous infusion. Subsequently, an elevated anti-rabbit epithelium IgE value of 39.6 kU/L was found. Several HAE patients included in the clinical program with rhC1INH who also had elevated anti-rabbit IgE did not develop allergic reactions to rhC1INH, including after repeated exposure.³

96 To further investigate whether rhC1INH is safe in subjects with rabbit allergy (RA) or CMA,
97 we used a skin test protocol to assess the allergic potential of rhC1INH in patients with RA
98 and/or CMA without C1INH deficiency. The primary objective of the study was to assess the
99 allergenicity of rhC1INH, using the negative predictive value (NPV) of a combination of skin
100 prick test (SPT) and intracutaneous test (ICT) for development of allergic reactions during a
101 subcutaneous (SC) challenge with rhC1INH in patients with clinical RA and/or CMA.
102 Furthermore, the safety of the SC challenge was assessed.

Methods

A prospective study was carried out to evaluate the allergenicity and safety of rhC1INH in patients with RA and/or CMA.

Study population

Patients with an age between 18 and 65 years, with clinically confirmed RA and/or CMA were contacted for participation in the study. RA and CMA were defined as a suggestive history with acute symptoms after exposure to rabbit or after ingestion of cow's milk respectively, in combination with sensitization by positive SPT or by the presence of serum IgE > 0.35 kU/L for rabbit dander in case of RA, or for cow's milk extract or for at least one of the known cow's milk allergens in case of CMA. IgE and SPT results were calculated only for those with a positive SPT to the specific allergen. In case of CMA, diagnosis could additionally be confirmed by double-blind placebo controlled food challenge (DBPCFC) as part of standard care, prior to study inclusion. Main exclusion criteria were the presence of severe dermographism, and pregnancy. In female participants with childbearing potential, a urine pregnancy test was performed.

This study was approved by the local Ethics committee (UMC Utrecht, protocol number 11-345), written informed consent was obtained from all participants.

Skin testing and order of proceedings

To assess the allergenicity of rhC1INH, a skin test protocol was carried out. The skin test protocol consisted of three subsequent types of skin testing with increasing dosages of rhC1INH: a SPT, ICT, and SC challenge. Patients could only proceed to the next skin test when the previous test was negative. Antihistamines or other drugs that could interfere with skin testing were temporarily discontinued according to predefined washout periods including

but not limited to 7 days for systemic or topical steroids, and 4 days for most antihistamines. All SPT and ICT tests were negatively and positively controlled with saline and histamine, respectively. rhC1INH was diluted with water for injection. All tests were performed with an observation period of up to 2 hours between each injection, and with an observation time up to 3 hours before discharge. All patients were contacted by phone by the site personnel within 24 hours after all exposures to rhC1INH to enquire of any adverse effects.

Each test started with the lowest concentration or volume of rhC1INH before proceeding to higher concentrations or volumes. Firstly, a SPT with 1:10 and undiluted rhC1INH was performed. SPT was considered positive when the wheal diameter was ≥ 3 mm over the negative control. Subsequently an ICT was performed with 1:100, 1:10, and undiluted rhC1INH. Since there is no experience with ICT with rhC1INH, and the study population did not have a specific history for rhC1INH, to avoid false positive results the mean erythema diameter size was chosen over mean wheal diameter.^{6,7} ICT was considered positive when the mean erythema diameter was equal to or exceeding the positive control. Lastly, patients underwent a SC challenge with 0.14 mL, 1.4, 4.2, and 8.2 mL rhC1INH (corresponding to 21, 210, 630, and 1230 U rhC1INH, respectively), in total corresponding to the volume of one vial of 14 mL (2100 U) rhC1INH. For the two highest dosages (4.2 and 8.2 mL) a SC infusion kit was used, and SC administration had a duration of up to 10 minutes. SC testing was performed under intense observation and monitoring, in a setting where all precautions were taken and equipment was readily available to treat hypersensitivity reactions, including anaphylaxis. The SC challenge was interpreted as a positive immediate type hypersensitivity reaction if one or more of the typical manifestations occurred: generalized itching, urticaria, angioedema, gastro-intestinal symptoms, dyspnea, wheezing, hypotension.⁸ Local swelling and erythema were measured after predefined observation times, and reported as adverse event (AE) prior to dose escalation.

In vitro testing

In all patients, serum IgE to rabbit dander, cow's milk extract, alpha lactalbumin, beta lactoglobulin, and casein was measured by ImmunoCAP to confirm sensitization (Thermo Fisher Scientific, Uppsala, Sweden).

A direct basophil activation test (BAT) was performed to evaluate the relative contribution of possible allergens, if any, in case an immediate type hypersensitivity reaction to rhC1INH would occur. In the current study several allergens were tested including cow's milk, rabbit dander, rhC1INH and individual allergens from cow's milk and rabbit milk.

Basophil activation was analyzed using a flow cytometry-based assay measuring CD63 expression. Heparinized whole blood was stimulated at 37°C for 30 minutes with allergens diluted in RPMI/IL-3 (2 ng/ml, R&D systems, Minneapolis, MN, USA). Cow's milk and rabbit allergens were diluted to the following concentrations: 0.001, 0.01, 0.1, 1, 10, and 100 µg/ml. rhC1INH was used in the following concentrations: 0.00125, 0.0125, 0.125, 1.25, 12.5, and 25 mg/ml. The reaction was stopped by adding 25 µl of cold PBS/EDTA (20 mM). Cells were stained using PE-conjugated mouse-anti-human CD63, FITC-conjugated mouse-anti-human CD123 (both BioLegend, San Diego, CA, USA), and APC-conjugated mouse-anti-human CD203c (Miltenyi Biotec GmbH, Bergisch Gladbach, Germany). Red cells were lysed using FACS Lysing Solution (BD Biosciences, San Jose, CA, USA). Basophil activation, expressed as percentage of CD63-positive cells within CD203c/CD123-positive cells, was analyzed by flow cytometry using a FACS Canto II (BD Biosciences).

Safety

Since AEs and serious adverse events (SAEs) before any exposure to rhC1INH cannot be related to rhC1INH, only treatment-emergent AEs (TEAEs) and treatment-emergent

serious adverse events (TESAEs) according to Good Clinical Practice guideline SAE definitions are reported.⁹ Safety was evaluated by recording TEAEs and TESAEs, their time of occurrence after exposure to rhC1INH and their intensity and causality to rhC1INH. Causality was reported by the investigators on a 5-point scale, including not related, remote/unlikely, possible, probably or definitely related to the study medication. Subsequently, TEAEs graded as possible, probably or definitely related were considered to be “related” for purposes of reporting. Only TEAEs reported to occur within the first 24 hours after any exposure to rhC1INH could be considered related.

During the testing, local allergic reactions could be treated symptomatically with oral antihistamines, and systemic hypersensitivity reactions could be treated with intravenous antihistamines, steroids and/or intramuscular epinephrine.

Analysis

In estimating the sample size for determining the negative predictive value (NPV) of the combination of SPT and ICT for the outcome of the SC challenge, we assumed that at least one patient would have an immediate type hypersensitivity reaction during SC challenge. To obtain a NPV with a 95% confidence interval, in order for the lower limit of the confidence interval to be at least 80%, data needed to be available for 25 evaluable subjects with negative SPT and ICT to rhC1INH.

The study was to be stopped if more than two patients with negative SPT and ICT developed systemic symptoms of hypersensitivity upon subcutaneous challenge with rhC1INH, or if more than 10 patients had a positive skin test (SPT or ICT).

Statistical analysis was performed using SAS software, Version 9.2 for Windows. It consisted of descriptive statistics for the quantitative variables including IgE results and skin test results, and frequency distributions for the ordinal and nominal variables including

demographic, and allergy data. For the serological specific IgE (sIgE) tests regarding RA and CMA as mentioned above, values below or above the limit of detection are reported as 0.00 or >100, respectively, and all sIgE data are described using the median and quartiles.

The NPV of the combination of the SPT and ICT for the outcome of the SC challenge was defined as the ratio of patients without systemic symptoms of hypersensitivity following SC challenge with rhC1INH, over the number of patients having tested negative for both the SPT and ICT.

Results

Study participants and allergy characteristics

In total 26 patients, 15 with RA, 6 with CMA, and 5 with both RA and CMA (Online Repository Table E1) were included in the study (Figure 1). Eleven others were not eligible to participate since RA and CMA could not be confirmed by SPT or IgE. Mean age was 33.7 years (SD 11.4), 17 (65.4%) were female. All 26 included patients were highly atopic and suffered from at least one atopic disease in addition to their RA and/or CMA, a majority of 24 (92.3%) had allergic rhinitis, 18 (69.2%) asthma, and 18 (69.2%) atopic eczema (Table E1).

Symptoms to rabbit exposure consisted of rhinitis, conjunctivitis, sneezing, feeling of swelling in the throat, and in some cases wheezing or dyspnea. CMA patients had symptoms that varied from oral allergy symptoms to anaphylactic shock shortly after ingestion of cow's milk. In 3 patients CMA was confirmed by double-blind placebo controlled food challenge (DBPCFC) as part of standard care. Median sIgE titer to rabbit dander in patients with a positive SPT to rabbit dander was 3.2 kU/L (range 0.16 - 26.3). The median sIgE titer to cow's milk extract in those with a positive SPT to cow's milk was 13.2 kU/L (range 0.32 - >100) (Table E1). All included patients had a positive SPT to rabbit dander and/or cow's milk: 16 were positive to rabbit only, 5 to cow's milk only, and 5 to both. In subjects with only positive SPT to rabbit dander, the median wheal diameter was 6.0 mm (range 3 to 12) and the median difference with the negative control was 5.5 (range 3 to 12). In subjects with only positive SPT to cow's milk (n=5) median wheal diameter was 7.0 mm (range 5 to 15) and the median difference with the negative control was 5.5 mm (range 3 to 12), respectively.

Results of skin prick test and intracutaneous test with rhC1INH

Skin test results are shown in table 1. None (95% CI: 0.0% ; 13.2%) of the subjects had a positive SPT with 1:10 diluted, and undiluted rhC1INH according to the defined criteria. Therefore, all 26 study participants proceeded to ICT. In the 1:100 and 1:10 dilutions of rhC1INH none had erythema exceeding the positive control. Two of the 26 patients had a positive ICT to undiluted rhC1INH (7.7%, 95% CI: 0.9; 25.1) but not to the diluted drug. The two patients had erythema on ICT greater than positive control by 1 mm and 4 mm. They did not experience any immediate type hypersensitivity symptoms as described in the methods section. Both patients were allergic to rabbit dander but not to cow's milk.

Results of subcutaneous challenge with rhC1INH

The two patients with a positive ICT did not proceed to the SC challenge with rhC1INH. Two others were lost to follow-up or discontinued study participation due to personal reasons (figure 1). The remaining 22 patients underwent SC challenge. Although patients could experience local symptoms, further described in the safety section below, none experienced symptoms suggestive for an immediate type hypersensitivity reaction. The NPV of the combination of SPT and ICT was defined as the ratio of patients without systemic symptoms of hypersensitivity following SC challenge with rhC1INH, over the number of patients having tested negative for both the SPT and ICT. Therefore the NPV of the combination of SPT and ICT for the outcome of the SC challenge was 100% (95% CI: 84.6% ; 100%).

In vitro testing

A BAT was performed in all 26 included patients (Figure 2). When using the clinical definitions of RA and CMA as described above (history and sensitization by SPT and/or IgE)

we could classify 18/26 patients (69%) correctly. One of the RA patients without CMA showed basophil activation after stimulation with cow's milk extract, and vice-versa two CMA patients without RA responded after stimulation with rabbit extract. One of the RA and CMA patients responded only after stimulation with rabbit extract. Thirteen patients (52%) did not reach a plateau in the percentages of CD63-positive cells. Three patients had a negative BAT, and one additional patient showed spontaneous basophil activation resulting in uninterpretable results for these patients. In none of the patients, basophil activation was observed upon stimulation with rhC1INH.

Safety

Local TEAEs within 24 hours after exposure to rhC1INH were observed in 7 patients (32%) after SC challenge (Table 2). In 4 (18%) of them this was considered possibly, probably or definitely related to the study medication. Local TEAEs were considered mild in all patients. Furthermore, 4 patients reported general TEAEs after SC challenge of which 1 case of headache of moderate severity was drug related (4.5%), which is a listed adverse drug reaction of rhC1INH.¹⁰ Local AEs after ICT included painful injection in 15 patients, as is commonly reported for ICTs in daily practice.⁸ No TESAEs were reported during the study.

Discussion

This is the first study that investigates the allergenic potential of rhC1INH in patients diagnosed with an allergy to rabbit dander and/or cow's milk. None of the 22 patients, who had a with negative SPT and ICT to rhC1INH, showed as an immediate type hypersensitivity reaction during the drug challenge with rhC1INH. The NPV of the combination of SPT and ICT for the outcome of the SC challenge was 100% (95% CI: 84.6% ; 100%).

The originally calculated required sample size of 25 patients anticipated that one may have a reaction to the SC challenge. Since this did not occur in any of the included patients, the NPV of the combination of SPT and ICT for the outcome of the SC challenge could be calculated after study completion of 22 patients, without impairing the lower value of the 95%-confidence interval (84.6% ; 100%). A limitation of the study is that rhC1INH was not injected intravenously (i.v) as it is approved for use. For safety reasons we used the subcutaneous administration route as is accepted in diagnostic drug challenges,¹¹ and refrained from i.v. administration. SPT and ICT with immediate readings are commonly used for investigation of immediate hypersensitivity reactions in drug allergy.¹² Plasma-derived C1INH has been administered i.v. for several decades, and recent studies applying the SC route of administration for these preparations indicate that similar safety results can be expected for these two routes of administration.¹³

In a previous study in 130 HAE patients and 14 healthy controls, five subjects had preexisting anti-rabbit epithelium IgE antibodies; one of them was a healthy subject with an undisclosed rabbit allergy, who developed an allergic reaction upon i.v. infusion with rhC1INH. The other four subjects with pre-existing sIgE levels to rabbit epithelium did not have an allergic reaction.³ Since only one of those sensitized developed an allergic reaction, it is questionable whether a test for IgE against rabbit dander provides useful information to predict the risk for allergic reactions to rhC1INH.

Of the 22 patients who underwent a SC challenge with rhC1INH, 15 had RA. Their characteristics were comparable to a population described by Liccardi et al.¹⁴ with similar clinical features including age, gender, and symptoms including rhinitis, conjunctivitis, and asthma symptoms, whereas median sIgE titers in that study were somewhat lower: 1.4 kU/L (range < 0.35 to > 100) versus 3.2 kU/L (range 0.16 to 26.3) in the current study. This suggests that our RA population was representative. Our CMA group (11 patients) covered the whole range of mild to severe cow's milk allergic patients, including two patients with a history of anaphylactic shock. Five patients (19%) even suffered both RA and CMA. Taken together, our study population represents the spectrum of RA and CMA patients with emphasis on the severe end of the spectrum.

None of the patients had a positive SPT to rhC1INH. Two RA patients showed a positive ICT (erythema larger than positive control) to undiluted rhC1INH only. Therefore they did not undergo the SC challenge with rhC1INH according to the study protocol. However, the reactions were considered irritative and not allergic, since the ICT was negative in diluted concentrations. Test substances in high concentrations can cause reactions even in healthy individuals. It is essential that non-irritant test concentrations are used and many drugs or drug classes are therefore diluted 1:10 or more for ICT to avoid irritant test concentrations.^{11,12} For rhC1INH the threshold of irritant test concentrations for intradermal testing was not established, since this would require a drug and/or formulation only challenge in skin test positive subjects. This was not the aim of this study and not intended in the study protocol. The assumption of a nonspecific false positive skin test reaction to undiluted rhC1INH is also supported by the fact that unspecific erythema due to ICT was seen in all study subjects and in all diluted test concentrations. In all patients, including patients with a skin reaction only marginally smaller than the positive control, subcutaneous challenges did not result in allergic symptoms to rhC1INH. Furthermore, *in vitro* testing by BAT was

negative for rhC1INH in all patients including both ICT positive patients. One of the patients with positive ICT had the highest IgE level for rabbit extract measured in this study. The healthy study subject with allergic symptoms after injection with rhC1INH as mentioned previously, also had a high sIgE level.³ These numbers, however, are negligible to draw conclusions on this topic.

None of the 22 patients with negative SPT and ICT had an allergic reaction during the SC challenge with rhC1INH, confirming that administration in patients allergic to CM, rabbit, and even to both CM and rabbit, can be considered safe for the large majority of patients. This is supported by the fact that the clinical characteristics and sensitisation profile are representative for the RA and CMA population, probably even in cases with severe allergy. This is further supported by our BAT results. Considering that whey proteins are present in relatively high concentrations in milk of all mammalian species, the basophils of RA and CMA patients (or both) responded, as expected, to rabbit whey proteins but not to rhC1INH.^{15,16} During clinical development and to date since market approval in 2010, there have been no clinical reports of allergic events to rhC1INH in CMA patients. Additionally, in previous safety studies regarding rhC1INH, three subjects with sIgE to cow's milk allergens did not show allergic symptoms after exposure to rhC1INH.³ Although no evidence from daily practice points toward safety concerns in CMA patients, and no evidence for cross reactivity between rabbit milk allergy and cow's milk allergy has been reported, the current study was not powered for a subgroup analysis.

Analysis of adverse events showed that performing SPT and ICT with rhC1INH, as well as subcutaneous administration of rhC1INH, was safe. Only a minority of patients reported adverse events, mostly mild and local, including 3 patients who reported pain during subcutaneous administration of rhC1INH. AEs regarding local swelling, and a local feeling of tightness of the skin, were related to the injected volume. Additionally, despite a prolonged

administration time, the injection technique may be partially responsible for local TEAE due to irritation. The safety of subcutaneous administration of plasma-derived C1INH was shown very recently in case reports^{17,18} and in a phase II trial.¹³ All studies reported similar TEAEs related to the route of drug administration, but in higher percentages.^{19,20} Amongst the other TEAEs related to rhC1INH only known side effects were reported in the current study.

In conclusion, none of the patients with negative SPT and ICT for rhC1INH had allergic symptoms during rhC1INH challenge. The negative predictive value of the combination of SPT and ICT for the outcome of the SC challenge was 100% (95% CI: 84.6% ; 100%). SC administration of rhC1INH was well-tolerated.

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TABLES**Table 1:**

Results of skin tests with rhC1INH

Table 2:Treatment-emergent adverse events $\leq$ 24 hours after exposure to rhC1INH**Table E1:**

Individual demographic and allergy data

FIGURE LEGENDS

Figure 1: Flowchart of in- and exclusion of patients

SPT: skin prick test, ICT: intracutaneous test, SC challenge: subcutaneous challenge

Figure 2: Basophil Activation Test results

RA: rabbit allergy, CMA; cow's milk allergy, WAP: whey acidic protein. Rabbit extract consist of rabbit dander proteins, whereas Rabbit WAP consist of rabbit whey proteins and therefore represents rabbit milk. BAT results are positive for each patient when the percentage of CD63-positive cells exceeds 5% (dotted line)

Table 1: Results of skin tests with rhC1INH

Patient	Diagnosis	SPT wheal diameter (mm)				Positive control	ICT erythema diameter (mm)				SC challenge Type I hyper-sensitivity reaction
		Negative control	1:10 rhC1INH	undiluted rhC1INH	SPT rhC1INH		1:100 rhC1INH	1:10 rhC1INH	undiluted rhC1INH	ICT rhC1INH	
1	RA	0	0	0	neg	29	10	8	8	neg	no
2	RA	0	0	0	neg	24	23	15	15	neg	no
7	RA	0	0	0	neg	24	9	4	0	neg	no
10	RA	0	0	0	neg	32	8	7	0	neg	lost to FU
11	RA	0	0	0	neg	32	18	18	19	neg	no
17	RA	0	0	0	neg	35	10	13	11	neg	lost to FU
18	RA	0	0	0	neg	42	33	23	16	neg	no
21	RA	0	0	0	neg	33	11	7	14	neg	no
25	RA	0	0	0	neg	42	24	19	29	neg	no
28	RA	0	0	0	neg	29	12	8	17	neg	no
30	RA	0	0	0	neg	36	13	16	37	pos	n.d.
31	RA	0	0	0	neg	23	8	8	11	neg	no
34	RA	2	0	0	neg	29	9	16	18	neg	no
37	RA	0	0	0	neg	39	11	17	17	neg	no
38	RA	0	0	0	neg	27	21	20	31	pos	n.d.
4	CMA	2	0	0	neg	37	10	10	0	neg	no
6	CMA	0	0	2	neg	36	2	6	7	neg	no
8	CMA	0	0	0	neg	38	6	8	16	neg	no
12	CMA	0	2.8	0	neg	21	7	6	10	neg	no
13	CMA	0	0	0	neg	35	0	0	28	neg	no
19	CMA	0	0	0	neg	36	0	10	0	neg	no
5	CMA+RA	0	0	0	neg	32	5	22	20	neg	no
14	CMA+RA	0	0	0	neg	28	9	0	0	neg	no
15	CMA+RA	0	0	0	neg	28	0	0	10	neg	no
23	CMA+RA	0	0	0	neg	25	6	6	0	neg	no
32	CMA+RA	0	0	0	neg	36	27	16	20	neg	no

SPT: skin prick test, ICT: intracutaneous test, SC: subcutaneous, neg: negative, pos: positive, FU: follow-up. N.d.: not done.

SPT positive when wheal diameter ≥ 3 mm over the negative control. ICT positive when mean erythema diameter equal to or exceeding the positive control.

Table 2: Treatment-emergent adverse events ≤ 24 hours after exposure to rhC1INH

	TEAE		Related TEAE	
	n	%	n	%
Total number of patients reporting ≥ 1 TEAE	19	73	14	54
Total number of patients reporting ≥ 1 administration-related (local) TEAE	17	65	14	54
SPT	0	0	0	0
ICT	15	58	12*	46
SCC	7	32	4	18
Total number of patients reporting ≥ 1 other (general) TEAE	9	35	2	8
SPT	0	0	0	0
ICT	6	27	1	4
SCC	4	18	1*	4

TEAE: Treatment-emergent adverse event. ICT: intracutaneous test. SCC: subcutaneous challenge. Local TEAEs of ICT included pain (15 patients; of which 12 related), and hematoma (1; unrelated).

Local TEAE of SCC included pain (3; of which 2 related), swelling (2), feeling of tight skin (2), and hematoma (2; unrelated).

General TEAEs included abdominal pain (1; unrelated), (feeling to) faint (3; unrelated), worsening of eczema (1; unrelated), rhinorrhea and eye pain (1; unrelated). Related general TEAEs consisted of nausea after ICT, and headache after SCC. One patient reported headache and nausea. *Moderate severity in 1 patient (mild in the others).

Screened: n=37

Excluded: n=11
- no allergy confirmed (n=11)

Included: n=26

SPT: n=26

ICT: n=26

Discontinued: n=4
- Positive ICT (n=2)
- Personal reasons (n=1)
- Lost to follow-up (n=1)

SC challenge: n=22
(completed study)

Table E1: Individual demographic and allergy data

Patient	Age (y)	Gender	History		SPT		Rabbit extract	Cow's milk extract	Specific IgE			Diagnosis	Other atopic diseases		
			Rabbit	Cow's milk	Rabbit	Cow's milk			Alfa-Lac (bos d 4)	Beta -Lac (bos d 5)	Caseine (bos d 8)		Allergic rhinitis	Asthma	Atopic eczema
1	49	f	+	-	+	-	5.0	0.33	0.02	0.07	0.45	RA	+	-	+
2	26	f	+	-	+	-	2.33	0.12	0.01	0.08	0.03	RA	+	+	+
7	37	m	+	-	+	-	2.59	0.04	0.02	0.03	0.05	RA	+	-	+
10	39	m	+	-	+	-	5.1	0.12	0.02	0.03	0.05	RA	+	-	+
11	20	f	+	-	+	-	1.50	0.11	0.04	0.03	0.04	RA	+	+	+
17	23	f	+	-	+	-	4.4	0.04	0.02	0.01	0.02	RA	+	+	+
18	45	f	+	-	+	-	0.19	0.02	0.01	0.02	0.01	RA	+	+	-
21	44	f	+	-	+	-	4.2	0.11	0.03	0.06	0.05	RA	+	+	+
25	31	f	+	-	+	-	0.16	0.04	0.02	0.04	0.03	RA	+	-	+
28	19	f	+	-	+	-	1.32	0.06	0.07	0.08	0.09	RA	+	-	-
30	30	f	+	-	+	-	3.8	0.11	0.01	0.11	0.03	RA	+	+	+
31	24	f	+	-	+	-	6.5	0.36	0.25	0.42	0.35	RA	+	+	+
34	25	f	+	-	+	-	1.51	0.02	0.01	0.02	0.01	RA	+	+	+
37	43	f	+	-	+	-	0.54	0.05	0.02	0.03	0.03	RA	+	-	-
38	46	m	+	-	+	-	26.3	0.06	0.03	0.04	0.02	RA	+	+	+
4	54	f	-	+	-	+	0.00	25.0	2.31	9.27	22.8	CMA	+	+	-
6	33	m	-	+	-	+	0.02	13.2	1.20	0.51	5.4	CMA	-	-	-
8	20	m	-	+	-	+	0.09	> 100	5.2	8.5	> 100	CMA	-	+	-
12	49	m	-	+	+	+	2.97	28.6	9.9	5.0	43.0	CMA	+	+	+
13	34	f	-	+	-	+	0.36	0.32	0.03	0.46	0.11	CMA	+	-	+
19	22	m	-	+	-	+	0.05	1.01	0.03	1.62	0.21	CMA	+	+	+
5	22	m	+	+	+	+	0.48	6.84	1.67	1.30	8.42	CMA+RA	+	+	-
14	24	m	+	+	+	+	12.7	59	10.9	1.33	70	CMA+RA	+	+	+
15	47	f	+	+	+	+	0.50	10.6	0.02	0.55	14.0	CMA+RA	+	+	+
23	23	f	+	+	+	-	4.2	0.36	0.33	0.15	0.11	CMA+RA*	+	+	-
32	48	f	+	+	+	+	8.6	67	39	29.7	43	CMA+RA	+	+	+

+: positive, -: negative, SPT: skin prick test, Alfa-Lac: alfa-lactalbumin, Beta-lac: beta-lactoglobulin, RA: rabbit allergy, CMA: cow's milk allergy, na: not applicable. For patient 12 the SPT was positive for rabbit dander but the patient is not allergic according to the medical history, and for patient 23 the SPT was negative for cow's milk but the subject is allergic according to the medical history and IgE value.