European Medicines Agency
Evaluation of Medicines for Human Use

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REFUSAL CHMP ASSESSMENT REPORT FOR RHUCIN

Common Name:
Recombinant human C1 inhibitor

Procedure No. EMEA/H/C/000769

Assessment Report as adopted by the CHMP with all information of a commercially confidential nature deleted.

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1. BACKGROUND INFORMATION ON THE PROCEDURE

1.1 Submission of the dossier

The applicant Pharming Group N.V. submitted on 20 July 2006 an application for Marketing Authorisation to the European Medicines Agency (EMEA) for Rhucin, through the centralised procedure falling within the Article 3(1) and point 1 of Annex of Regulation (EC) No 726/2004.

Rhucin was designated as an orphan medicinal product EU/3/01/036 on 11 May 2001. Rhucin was designated as an orphan medicinal product in the following indication: treatment of angioedema caused by C1 inhibitor deficiency. The calculated prevalence of this condition was 2.1 per 10,000 (EU population) at the time of granting the orphan status.

The applicant applied for the following indication: replacement treatment in acute attacks of angioedema in patients with congenital C1 inhibitor activity deficiency.

The legal basis for this application refers to:
Article 8.3 of Directive 2001/83/EC, as amended - complete and independent application

Protocol Assistance:
The applicant received Protocol Assistance from the CHMP on 20 November 2003. The Protocol Assistance pertained to quality, non-clinical and clinical aspects of the dossier.

Licensing status:
The product was not licensed in any country at the time of submission of the application.

The Rapporteur and Co-Rapporteur appointed by the CHMP and the evaluation teams were:
Rapporteur: **Ian Hudson** Co-Rapporteur: **Christian K. Schneider**

1.2 Steps taken for the assessment of the product

- The application was received by the EMEA on 20 July 2006.
- The procedure started on 16 August 2006.
- The Rapporteur's first Assessment Report was circulated to all CHMP members on 27 October 2006. The Co-Rapporteur's first Assessment Report was circulated to all CHMP members on 27 October 2006. In accordance with Article 6(3) of Regulation (EC) No 726/2004, the Rapporteur and Co-Rapporteur declared that they had completed their assessment report in less than 80 days.
- On 6 December 2006, the Biologics Working Party (BWP) adopted a recommendation to the CHMP for the list of questions related to quality aspects.
- During the meeting on 14 December 2006, the CHMP agreed on the consolidated List of Questions to be sent to the applicant. The final consolidated List of Questions was sent to the applicant on 15 December 2006.
- The applicant submitted the responses to the CHMP consolidated List of Questions on 12 July 2007.
- The outcome of the inspections carried out at the following sites Broekman Instituut B.V., Snijders Cryotheque B.V., NV Organon and Pharming Group N.V between 24 January and 7 March 2007 was issued on 6 July 2007.
- The Rapporteurs circulated the Joint Assessment Report on the applicant’s responses to the List of Questions to all CHMP members on 3 September 2007.
- On 12 September 2007, the BWP adopted a recommendation to the CHMP for the list of outstanding issues related to quality aspects.
- During the CHMP meeting on 20 September 2007, the CHMP agreed on a list of outstanding issues to be addressed in writing and in an oral explanation by the applicant.
- The applicant submitted the written responses to the list of outstanding issues on 2 November 2007. Additional written responses were provided after the deadline on 13 November 2007.

- The Rapporteurs circulated the Joint Assessment Report on the applicant's responses to the list of outstanding issues to all CHMP members on 23 November 2007.
- During the meeting on 3-5 December 2007, outstanding quality issues were addressed by the applicant during an oral clarification before the BWP on 3 December 2007 and the BWP adopted a recommendation to the CHMP with respect to quality aspects.
- During the CHMP meeting on 11 December 2007, outstanding issues were addressed by the applicant during an oral explanation before the CHMP.
- During the meeting on 10-13 December 2007, the CHMP, in the light of the overall data submitted and the scientific discussion within the Committee, issued a negative opinion by majority decision for granting a Marketing Authorisation to Rhucin on 13 December 2007.

1.3 Steps taken for the re-examination of the CHMP Opinion

The Rapporteur and Co-Rapporteur appointed by the CHMP and the evaluation teams were:
Rapporteur: **Bengt Ljungberg** Co-Rapporteur: **Pierre Demolis**

- On 8 February 2008, the applicant submitted the detailed grounds for the re-examination of the grounds for refusal listed above.
- During the plenary meeting on 18-21 February, the CHMP nominated the experts of the ad-hoc expert group on Rhucin to be held on 10 March 2008. Questions to be put to the expert group were adopted by written procedure after the meeting.
- The Rapporteur's first Assessment Report was circulated to all CHMP members on 27 February 2008. The Co-Rapporteur's first Assessment Report was circulated to all CHMP members on 28 February 2008.
- The Rapporteurs circulated the Joint Assessment Report on the applicant's grounds for reexamination to all CHMP members on 5 March 2008.
- On 10 March 2008 the ad-hoc expert group on Rhucin considered the questions from the CHMP and considered the oral clarifications provided by the applicant on the grounds for re-examination.
- During the meeting on 10-12 March 2008, the BWP adopted a recommendation to the CHMP with respect to quality grounds for re-examination.
- On 13 March 2008, the ad hoc expert group circulated their report.
- During the CHMP meeting on 17 March 2008, the grounds for re-examination were addressed by the applicant during an oral explanation before the CHMP.
- During the meeting on 17-19 March 2008, the CHMP, in the light of the overall data submitted and the scientific discussion within the Committee, issued a negative opinion by majority decision for granting a Marketing Authorisation to Rhucin on 19 March 2008.

2. SCIENTIFIC DISCUSSION

2.1 Introduction

The clinical syndrome of hereditary angioedema (HAE) ('swelling of the soft tissues') is related to the congenital deficiency of C1 esterase inhibitor (C1INH), which is a plasma protein. Two types of congenital functional C1INH deficiency (phenotypic variants) can be distinguished (HAE Type I and HAE Type II). Despite both types being autosomal dominant disorders, the levels of functional C1INH in plasma are much lower than 50 % of normal levels. This probably relates to a lack of control on C1 (auto) activation which results in a higher level of consumption of the inhibitor than in healthy people.

C1INH is primarily synthesized in the liver and its level in normal plasma is about 275 µg/ml (about 2.5 µM). C1INH belongs to the superfamily of serine proteinase inhibitors (serpins) in plasma. The members of this family, which includes amongst others alpha1-antitrypsin, $alpha_1$-antichymotrypsin,

alpha$_2$-antiplasmin and antithrombin III (ATIII), show structural homology and share a common inhibitory mechanism. The main function of C1 Inhibitor is inhibition of the complement system, which is a biochemical cascade of the immune system. Activation of this system leads to cytolysis, chemotaxis, opsonization, immune clearance, and inflammation, as well as the marking of pathogens for phagocytosis.

C1INH is the only known inhibitor of activated subcomponents C1s (C1 esterase) and C1r of complement component 1 (C1) of the classical pathway of the complement system. In addition, C1INH inhibits the Mannan Binding protein (MBP)-associated proteinases (MASPs) of Lectin pathway of complement activation. Furthermore, it is the major inhibitor of activated factor XII, factor XI and kallikrein of the contact system of intrinsic coagulation and fibrinolysis. The common final step of complement is the activation of C5 by C5 convertase that leads to the formation of the Membrane Attack Complex (MAC) C5b-9, which finally inserts into the target cell membranes and causes cell lysis. The peptides C3a, C4a, and C5a are known as anaphylatoxins and mediate several reactions in the inflammatory response, including smooth muscle cell contractions, changes in vascular permeability, histamine release from mast cells, neutrophil chemotaxis, and platelet activation and aggregation.

Most commonly, HAE provokes swelling of the face, mouth and/or airway but such swelling can occur in any part of the body. Attacks of oedema of the gastrointestinal tract are associated with severe pain similar to acute abdominal syndromes and may cause nausea, vomiting, diarrhoea, ascites and symptoms of hypovolemia. About 35% of patients suffering from abdominal attacks (stomach, intestines, bladder) undergo appendectomy or exploratory laporotomy due to misdiagnosis of HAE. Until recently, the lethality rates of attacks involving the upper airways leading to asphyxia were exceeding 25%.

Currently available treatments include androgens, antifibrinolytics and plasma-derived C1INH. Acute severe abdominal and laryngeal attacks can be successfully treated with intravenous infusion of plasma-derived C1INH. Plasma-derived C1INH is approved in only part of the European Union.

The applicant Pharming Group N.V. submitted a complete and independent application for Marketing Authorisation to the European Medicines Agency (EMEA) for Rhucin. Rhucin is a recombinant human C1 Inhibitor (rhC1INH), claimed as having the same serine protease inhibitory activity (i.e. C1s, Factors XIa and XIIa, and kallikrein) as human plasma C1 Inhibitor. The proposed indication of Rhucin is for use as replacement treatment in acute attacks of angioedema in patients with congenital C1 inhibitor activity deficiency.

Rhucin was designated as an orphan medicinal product in the following indication: treatment of angioedema caused by C1 inhibitor deficiency. The calculated prevalence of this condition was 2.1 per 10,000 EU population.

rhC1INH is produced using transgenic rabbits expressing the protein in milk. The transgenic rabbits, which are genetically modified organisms (GMO), are maintained in specific pathogen free enclosed housings in the Netherlands. The product, however, is not a GMO.

Abbreviations:
C1INH: C1 inhibitor:
rhC1INH: recombinant human C1 Inhibitor

2.2 Quality aspects

Introduction
C1INH is a single-chain plasma glycoprotein (Mr 76,000; 478 amino acids; cDNA 1.8 kb; gene 17 kb; heavily glycosylated: about 30 % w/w carbohydrate, 2 disulphide bonds) that belongs to the superfamily of serine proteinase inhibitors (serpins) in plasma. C1INH is a heavily glycosylated

protein but glycosylation is not required for its inhibitory activity. Recombinant human C1 Inhibitor (rhC1INH), the active substance of Rhucin, is produced in transgenic rabbits that express the protein in milk. The mRNA transcription product predicts that rhC1INH will have the same amino acid structure as C1INH.
Rhucin is presented as a powder for solution for injection to be reconstituted with water for injections before intravenous administration. Each vial contains 2100 units of rhC1INH (150 U/ml after reconstitution).

Active Substance

- Manufacture

The manufacture of the milk starting material, which includes breeding, maintenance and milking of transgenic rabbits, is performed by a manufacturer in the Netherlands in compliance with Good Manufacturing Practice (GMP). The transgenic rabbits are considered to be genetically modified organisms (GMO). The manufacturer has been authorised by the Dutch authorities to handle transgenic rabbits in a contained environment and the rabbit housing areas are classified as D-1 animal facilities in accordance with GMO regulations in the Netherlands.
The active substance is manufactured and routinely controlled by a second manufacturer in the Netherlands in compliance with GMP.

The manufacturing process for rhC1INH starts with the production of milk starting material which involves milking of rabbits, skimming of milk, filling in bags and freezing.
The downstream processing of the milk consists of thawing of milk, pooling of milk bags, fat removal by centrifugation and a succession of filtration and chromatography steps as well solvent/detergent treatment.

The process includes 2 virus removal/inactivation steps and formulation by ultra- and diafiltration.

The manufacturing process, including the handling of the transgenic rabbits, has been adequately described and validated. Three commercial scale validation batches, and 26 commercial batches in total have been manufactured and in-process control, batch release and extensive additional testing have shown that manufacture is well controlled overall.

Sufficient information was provided regarding derivation of genomic/promoter DNA and generation of the genetic founder animal in the genome of which the sequence coding for rhC1INH was inserted. The genetic testing on animals generated to date confirms that in a number of animals, a reduced copy number is present. Given the instability of the genetics of the production colony, methods of control are in place to assure the genetic acceptability of those animals accepted into the production colony. The number of generations between the Master Transgenic Bank (MTB) and the animals used for production is limited and fixed and the breeding males are screened to ensure they have the required number of copies of the transgene. This strategy limits the extent of the instability in the production colony. Genetic testing and specifications of breeding and production animals are such that mutation and copy number are satisfactorily controlled.

A combination of state-of-the-art techniques have been used to characterise rhC1INH. Protein sequencing has revealed large proportion of the amino acid structure, including all sites of N-linked glycosylation and sites of O-linked glycosylation. The applicant committed to improve the characterisation of the primary structure. An appropriate method has been used as a characterisation tool and has also been introduced as a batch release assay. Similarly, the N-linked and O-linked oligosaccharide profiles have also been characterised on the validation batches and will have to be incorporated in the batch release analysis. Monosaccharide composition has been characterised for a large number of batches and is a batch release assay. Product charge heterogeneity was not measured, due to the comparatively large amount of glycosylation of the product making development of a suitable assay difficult. Nevertheless, the applicant agreed to develop a quantitative method, characterise validation batches, and introduce this test as a batch release assay since this is an important characteristic of all glycoproteins, and especially from a transgenic source, where post translational modifications are not yet well understood. Molecular weight has been characterised by

several methods. The molecular weight is significantly less than human plasma derived C1INH due to differences in glycosylation. This may impact on the pharmacokinetics of rhC1INH and reduce its half life (see non-clinical and clinical aspects).

Product related impurities have been monitored in pilot scale batches, and some are analysed by peptide map for batch release. Although commercial scale validation batches have not been specifically analysed for these impurities, forced degradation studies reveal that the product is stable with regard to these impurities under mild process and storage conditions. Therefore it is considered not to be necessary to perform batch analysis for these impurities.

The relative activities of rhC1INH and human plasma derived C1INH have been compared by calculating second order rate constants of inhibition for 4 substrates of C1INH: C1s proteinase, Factors XIa and XIIa, and kallikrein. Results show that the rate constants obtained with rhC1INH and human plasma derived C1INH are similar. This indicates that the differences in glycosylation do not greatly influence activity of this protein. Still, the differences in glycosylation may impact on the pharmacokinetics (half life), stability and potential immunogenicity of the product (see non clinical and clinical aspects).

The applicant has provided robust data to support the consistency of the product regarding both characterisation of biochemical/structural properties, and product purity.

- The genetic stability of the rabbit colony is well controlled (see above)
- The raw material (milk) for each batch of active substance is analysed for each production run, including the gross milk protein composition of the pool by an adequate method. Characterisation of several batches of milk indicate that the composition regarding the major milk proteins is relatively consistent.
- The applicant has validated a consistent down stream purification process, and within the limits of the assays used (see discussion), have demonstrated that the process has additional capacity for the removal of milk proteins.
- Analysis of active substance demonstrate a consistent product. None of these methods detect significant quantities of Host Related Impurities (HRI) however there are concerns as to whether the assays used provide complete information on HRI (see discussion).
- Characterisation data, regarding primary structure, and post translational modifications (e.g. monosaccharide composition, N-glycan and O-glycan analysis and sialic acid content) has been provided for up to 26 batches of commercial product (see hereafter).

However, although product purity is likely to be consistent for the reasons detailed above, the actual purity of the product with respect to HRI (milk proteins) has not been adequately demonstrated (see discussion).

During the development cycle, and production of pre-clinical, clinical and commercial material, several changes have been made to the manufacturing process including use of different contract manufacturing organisations and scale up. The applicant has undertaken a comparability study which encompasses batch release data from development and commercial batches, in-process control data from development and commercial batches plus additional characterisation and a pharmacokinetics study performed in rats. Results using a number of methods, are very similar. Inhibitory kinetics of pilot and commercial scale material are essentially the same and overall comparability of active substance has been demonstrated.

- Specification

Critical parameters have been included in the active substance specifications to routinely confirm the quality of the active substance by a series of state-of-the-art methods which have been demonstrated to be suitable and have been adequately validated except for the method used to control Host Related Impurities (HRI) (see discussion) as well as a number of remaining concerns which would have to be addressed as part of follow-up measures undertaken by the company.

- Stability

The stability of active substance has been monitored in well controlled and justified stability studies which support the claimed shelf life (2 years at -20°C). Nevertheless, the stability studies for frozen skimmed milk (starting material) are not sufficiently satisfactory and the applicant should continue to monitor the protein profile of skimmed milk using a fully validated assay from 0 to at least 24 months when held under commercial storage condition.

Medicinal product

Rhucin is an aseptic lyophilised cake in a 25 ml Type I glass vial (Ph.Eur.), sealed with a siliconised 20 mm chlorobutyl "2-pin" lyophilisation stopper and capped with an aluminium overseal. Each vial contains 350 mg (2100 Units) of rhC1INH. The product is stored at 5°C ± 3°C. For administration of the product, the lyophilised cake is resuspended with 14.0 ml water for injections (supplied by the user) to prepare a solution at 25 mg/ml (150 U/ml) which is administered by intravenous injection.

- Pharmaceutical Development

The composition of the finished product is identical to the composition of the active substance. No additional excipients or formulation steps are involved in the manufacture of the product. The formulation does not contain any anti-microbial preservative and sterility testing is performed on each batch of product. Excipients are commonly used in freeze-dried pharmaceutical preparations and include a buffer system and cryoprotectant. Apart from the active substance, no materials of human or animal origin are included in the formulation. Development of the formulation was described and is considered acceptable.

- Manufacture of the Product

The finished product is manufactured and routinely controlled by a manufacturer in the Netherlands in compliance with GMP.

The product is manufactured by thawing one to four batches of formulated active substance, mixing, sterile filtration, aseptic filling followed by freeze-drying, labelling and packaging. The process has been adequately validated on the basis of 3 batches comprised of a single batch of active substance, and 3 batches comprised of four batches of active substance. In-process control and batch release data, along with extended testing demonstrate that this process is adequately controlled.

- Product Specification

Critical parameters have been included in the product specifications to routinely confirm the quality of the finished product by testing.

The activity-unit of rhC1INH is based on the C1 Inhibitor plasma unit definition: 1 unit (U) equals the inhibitory activity of 1 ml pooled human plasma towards the main natural target of the inhibitor, human C1s protease. An international standard is not available so the activity is calibrated on pooled human plasma. For this purpose, a citrated pool of 1000-1500 donors, obtained from a vendor was used. Several internal standards were established during development.

Batch release specifications are generally acceptable, although on the basis of batch release data obtained to date, some parameters should be tightened after manufacture of an appropriate number of batches.

- Stability of the Product

The stability of the product is monitored by well designed and controlled studies, which include product batches comprised of single and four–fold batches of rhC1INH. To date, 18 months real time data (single rhC1INH batches) and 6 months (4-fold rhC1INH batches) have been obtained. It can be concluded from these studies that Rhucin is stable for 18 months at 5°C ± 3°C and at 25°C ± 2°C / 60% RH ± 5% and for 6 months at 40°C ± 2°C/75% RH ± 5%. Overall, results support the claimed shelf life of 2 years at 25°C although the studies should be continued as detailed in the protocol.

- Adventitious Agents

The adventitious agents safety evaluation demonstrates a comprehensive series of controls to minimise the risk of contamination of finished product with non-viral and viral adventitious agents. A three fold approach has been adopted including control of the production colony by barrier technology and monitoring of production and sentinel animals, monitoring the raw material by in vitro testing on susceptible cell lines, and by validating the capacity of the process to remove or inactivate adventitious pathogens. The downstream process includes two specific effective virus reduction/inactivation procedures, a solvent/detergent incubation, and a nanofiltration procedure using small pore nanometre filters. Validation of a chromatographic step which has been indicated to contribute moderately towards virus reduction may be completed in an on-going procedure. Although it is not possible to monitor for every possible pathogen, the sampling regime and pathogens tested for largely conforms to guidance. In addition, the on-going monitoring of the health of the colony would be expected to quickly detect the presence of a pathogen in the colony. A risk assessment has been conducted of the capacity of the downstream process to remove pathogens which may be present at the limits of detection of the in vitro assay which is performed on every batch of raw material (milk), which confirms that the downstream process should be effective at removing undetected virus.

Discussion on chemical, pharmaceutical and biological aspects

rhC1INH is produced from milk obtained from transgenic rabbits. Milk is a complex mixture of proteins. The major proteins (aS1-casein, aS2a-casein, aS2b-casein, kappa casein, beta-casein, transferrin, whey acid protein, IgG, IgA, IgM, albumin, and a-lactalbumin) are mainly manufactured in the mammary gland and many are under the control of lactation specific promoters. However, plasma proteins can also diffuse from the bloodstream through the blood/mammary barrier and be present in low quantities in milk. The extent of leakage varies, and can be quite profound, potentially resulting in significant quantities of blood in the milk (sufficient to give it a noticeable red colour in worst cases, such as mastitis). Therefore, it is not sufficient to only be concerned about the classic milk proteins since plasma proteins also have to be considered Although generally plasma proteins are present in milk in low concentrations, co-partitioning with product during purification can result in immunologically significant quantities (i.e. that may cause antibody responses and related adverse events) being present in product. In this instance, other members of the serpin family in addition to C1INH should be specifically considered since members of the serpin family have a conserved structure and many structural similarities, resulting in a greater possibility of co-purification with rhC1INH.

At the oral clarification (new information), the applicant indicated that this has been addressed by nominating rabbit C1INH as a marker blood protein and demonstrating a relatively consistent concentration in pooled milk starting material. By proposing to introduce this as a batch release assay and specification for starting material, the applicant will be adequately controlling the starting material for variability in blood proteins.

Product purity has been probed by silver stained SDS-PAGE, immunoblotting and HRI-ELISA (host related impurities-ELISA), the latter two techniques using a polyclonal antibody mixture obtained by pooling antibodies obtained from animals immunised with milk or skimmed milk. Low levels of three purified standards (rabbit milk proteins) can be detected by silver stain if no rhC1INH is co-loaded onto the lane of the gel, but in order to obtain sufficient sensitivity to probe for impurities in product, very large amounts of product had to be loaded onto the gels. This resulted in product (and product related impurity) bands which covered approximately the 45 to 150 K.Daltons range of the gel. Therefore, many potential impurities which would be present in relatively low concentration, could be hidden by the product bands. The validation of the immunoblot to provide a limit of detection of milk proteins was not adequately controlled, and so the limits provided by the technique are not meaningful. In addition, some bands have been visualised by the anti-milk antibodies.

Consequently, the purity of the product has not been conclusively demonstrated and it should also be probed using a variety of separation techniques which use differing mechanisms, and sensitive, state of the art detection techniques.

With the oral clarification, the applicant has provided new information to give further assurance about the purity of the product. The immunoblot method has now been adequately controlled, confirming that large concentrations of product do not affect the sensitivity of the method. In a complementary experiment, non-transgenic rabbit milk has been purified in a scale down version of the commercial process and no impurity bands are detected in product. Both provide additional assurance about product purity.

The HRI-ELISA, which has been used to characterise the purity of intermediates during characterisation studies, and for batch release of active substance has been designed and developed using the same principles as HRI-ELISAs to fermentation based products. Results from this assay indicate that the total HRI content of active substance is in the region of 10 ppm (~10 µg per 1000 mg adult dose). The standard curve is made from dilutions of skimmed milk and the applicant has been requested to ensure that titration curves of important milk proteins are similar to the titration curve of standard. The important proteins for which this is performed should be justified, either on the basis of being detected in active substance, or likely impurities from analysis of milk or intermediates. If the titration curves are too dissimilar from the standard, then the quantitative result from the ELISA would not be meaningful. In practice, the ELISA may only measure one or two of the most prevalent or immunogenic milk proteins and not effectively quantitate the more minor components. Thus, low concentrations of some milk proteins and proteins which co-purify with product may be un-detected by this assay. This level of validation is not always required of similar ELISAs for fermentation derived products, but it should be remembered that there tends to be a considerable amount of information available about the immunogenicity of HRI from conventional cell based products, and frequently the dose size is considerably smaller than in this case. The immunogenicity of milk proteins administered i.v. is not well understood, and, although the route of administration was different, it has been previously noted for product administered on a repeat basis to the lungs via a nebuliser that amounts of (sheep) individual milk proteins above 1.5 µg (~ 6 ppm in 250 mg of product) tend to cause antibody formation in some patients, and when the amount of individual sheep milk protein is approximately 15 µg (60 ppm in 250 mg of product), then essentially 100% of subjects developed antibodies to these individual proteins.

New quantitative data was provided at the oral clarification to show that the residual quantities of 3 marker milk proteins is very low or undetectable. In addition, the applicant has committed to explore and if necessary to improve the sensitivity of the HRI-ELISA as follows. They will continue to probe the purity of product for HRI using complementary techniques to those which have been employed. In addition, the specificity of antibodies which are raised by subjects after dosing with Rhucin will be examined using a combination of newly developed analytical tools. If it is found that patients are raising antibodies to specific HRI, then further analytical techniques will be developed and introduced in batch analysis to control for these.

In conclusion, except for remaining outstanding quality issues, the applicant has provided assurance regarding the quality of the product. Physicochemical and biological aspects relevant to the uniform clinical performance of the product have been investigated and are mostly controlled. Data have been presented to give reassurance on viral/TSE safety.

The remaining concerns relate to host related impurities (HRI), the inadequate control of HRI in the product and the use of a method (HRI-ELISA) which has not been adequately validated, and inadequate validation of the clinical assay for detection of human antibodies raised to rabbit milk proteins. In response to these concerns, the applicant has provided additional information in an oral clarification. The additional information was promising but still needs to be thoroughly evaluated before it can be concluded that the quality of this product is acceptable to support safe and effective use of Rhucin. In addition, there are a number of remaining concerns which would have to be addressed as part of follow-up measures undertaken by the company to ensure that host related impurities will be appropriately controlled and that the product will be consistent with material used in clinical trials.

2.3 Non-clinical aspects

Introduction

Two different pharmaceutical presentations were developed: a liquid formulation of 25 mg/ml in 20mM citrate buffer pH7.0 with 8% sucrose, and a freeze-dried presentation containing 6.5% sucrose serving as cryoprotectant. Liquid presentations were used in early non-clinical studies. Later studies were performed with the lyophilized presentations.

The non-clinical testing program was conducted to establish the safety of rhC1 INH for short term use (< 7 days) in this potentially life-threatening disease.
Studies presented covered *in vitro* pharmacology, *in vivo* safety pharmacology, pharmacokinetics, general toxicology with dosing of up to two weeks, teratology in rats and local tolerance.

Protocol Assistance from EMEA was obtained in November 2003 on quality, preclinical and clinical aspects.

The pivotal studies were performed under GLP.

Pharmacology

- Primary pharmacodynamics

Four *in vitro* studies assessing kinetics of binding with enzymes that are presumed to be inhibited by the product (C1s, FXIa, FXIIa, kallikrein) were reported as the primary pharmacodynamic characterisation of rhC1 INH. These studies report the second order rate constant of inhibition (k_{on}) of rhC1 INH, in comparison with that of human plasma-derived C1 INH, for batches used in preclinical and clinical studies. The results are presented in the table below.

Table 2.6.2-1 Second-Order Rate Constants for Inhibition of Target Proteases by rhC1INH (Batch 04I00013) and H-C1INH (Cetor)

	k_{on} ($M^{-1}.s^{-1}$)			
	C1s	**Factor XIa**	**Factor XIIa**	**Kallikrein**
rhC1INH[a]	$6.1 \pm 0.3 \times 10^4$	$9.8 \pm 0.5 \times 10^2$	$6.9 \pm 0.5 \times 10^3$	$9.1 \pm 0.1 \times 10^3$
H-C1INH[a]	$5.1 \pm 0.3 \times 10^4$	$9.0 \pm 0.2 \times 10^2$	$5.7 \pm 0.4 \times 10^3$	$7.6 \pm 0.3 \times 10^3$
H-C1INH[b]	6.3×10^4	-	5.7×10^3	8.2×10^3
H-C1INH[c]	$6.2 \pm 0.4 \times 10^4$	$3.9 \pm 0.3 \times 10^2$	$4.5 \pm 0.3 \times 10^3$	$7.8 \pm 0.4 \times 10^3$

(a) Data generated at Pharming Technologies B.V. The data are the mean ± sd of three experiments. The values for k_{off} were virtually zero.
(b) Data reported in literature[4]
(c) Data reported in literature [5]

These findings demonstrated that the inhibitory activity of rhC1INH towards the target proteinases can regarded to be comparable with plasma derived C1 inhibitor.

- Secondary Pharmacodynamics and Safety Pharmacology

Safety pharmacology was assessed in one *in vivo* study evaluating the effect of rhC1INH on cardiovascular and respiratory systems in anaesthetised dog.
Vehicle or 625 U/kg (corresponding to 104 mg/kg) rhC1INH was administered intravenously in a crossover design. Compared to the proposed clinical dose of 15 mg/kg, this choice of dose is acceptable
There were no marked changes in the QT_{CB} interval following treatment with vehicle or rhC1INH. No treatment-related effects were observed for the remaining monitored parameters.

- Pharmacodynamic drug interactions

No studies were performed.

Pharmacokinetics

Pharmacokinetics and toxicokinetics of rhC1INH were investigated in rats and dogs. The species used for analyses of pharmacokinetics were also used in the safety pharmacology and toxicology studies.
Single intravenous dose administration of several rhC1INH batches was studied in rats. Results of toxicokinetic blood sampling during toxicology studies in rats and dogs (single dose toxicity rats and escalating dose toxicity in dogs) were also presented.

Methods
Two methods for quantification of rhC1 INH in plasma were described. The first was a functional assay using the commercially available C1-inhibitor kit. This kit is used in clinical medicine for the determination of the functional activity of C1-inhibitor in plasma to diagnose states of reduced C1 inhibitor concentration in plasma and for monitoring substitution therapy in patients.
Validation of this method was shown for rat, dog and human plasma samples. Stability of plasma samples at room temperature was assured for at least 72 hours, and at -18ºC for at least 61 days, with stability over three freeze-thaw cycles.

The second method was an ELISA which was applied to rat and dog plasma samples and was validated for the range 0.0117 to 0.188 mU/ml.

Validation reports for the assessment of antibodies to rhC1 INH in rat plasma and their neutralising potential were provided and considered acceptable in spite of some deficiencies.
The ELISA used in toxicity studies met the validation criteria for precision and specificity at low, medium and high concentrations of anti-rhC1INH IgG

Absorption
Data on PK parameters following intravenous administration in rats and dogs were provided. As the product is intended for intravenous administration only, no studies in animals were done by other routes and no studies were conducted to assess bioavailability.

Distribution
No specific studies assessing distribution of rhC1INH were reported. However, from the single dose administration of rhC1INH in rats and escalating dose administration in dogs, the Cmax values were considered to be in accordance with the measured concentration of the injected dose.

Metabolism
The role of hepatic receptors in removing rhC1INH from the blood circulation was studied in a non-GLP single dose pharmacokinetic study. RhC1INH was administered in male Wistar rats treated with competitors for the asialoglycoprotein receptor on parenchymal liver cells and the mannose receptor on liver endothelial cells.
Results indicate that the exposure and half life were each greater and the elimination rate constant (ERC in the table below) was reduced when either, or both, inhibitors were injected just prior to injection of rhC1 INH. These results suggest that rhC1INH is mainly cleared from the circulation by the liver via receptor-mediated endocytosis.

Table 3: Pharmacokinetic data showing delayed clearance of Rhucin when blocking the asialoglycoprotein receptor, the mannose receptor, or both

Results

Mean Pharmacokinetic data of rhC1INH with GalNac and/or Mannan

rhC1INH Competitor	04I00013 -	04I00013 GalNAc	04I00013 Mannan	04I00013 GalNAc & Mannan
ERC	0.056 ± 0.009	0.013 ± 0.003	0.021 ± 0.006	0.004 ± 0.002
T1/2	12.6 ± 2.1	54.2 ± 12.7	35.5 ± 11.2	196 ± 101
AUC0-t	7380 ± 1430	11655 ± 1035	10788 ± 749	15783 ± 2825
AUC0-inf	7649 ± 1257	24272 ± 5123	16119 ± 4381	91257 ± 57785

Excretion

No specific excretion studies were reported as, according to the applicant, the pathway of amino acid degradation is generally understood. This was considered to be acceptable for this product.

Other Pharmacokinetic Studies

Pharmacokinetic comparison of batches of liquid formulation with batches of lyophilized formulation failed to demonstrate comparability between both formulations.

However, the analysis of pharmacokinetic parameters of 10 batches of lyophilised pilot scale product demonstrates consistent results within the experiment performed.

Results shown in the table below indicate that the half life of rhC1 INH was less than 20 minutes and the volume of distribution was close to the blood volume. No difference was seen between the batches.

Mean pharmacokinetic parameters of various rH-C1INH compounds after a single intravenous dose in male Wistar rats

		04I00011			04I00014		
Parameter	Unit	Mean		SD	Mean		SD
Body weight	Kg	248	±	7.1	249	±	11.0
Dose	U/kg	125	±	0.0	125	±	0.0
t_{last} (median value)	Min	30.0 (15-60)#			30.5 (30-32)#		
$AUC_{0\text{-}last}$	mU.min/ml	46066	±	12782	44556	±	4383
Dose normalized $AUC_{0\text{-}last}$	mU.min/ml	368.5	±	102.3	356.4	±	35.06
$AUC_{0\text{-}24h}$	mU.min/ml	46066	±	12782	44556	±	4383
Dose normalized $AUC_{0\text{-}24h}$	mU.min/ml	368.5	±	102.3	356.4	±	35.06
$AUC_{0\text{-}\infty}$	mU.min/ml	74353*	±	19073*	62798*	±	6960*
Dose normalized $AUC_{0\text{-}\infty}$	mU.min/ml	594.8*	±	152.6*	502.4*	±	55.68*
% extrapolated		34.15	±	23.91	28.69	±	6.788
β	1/min	0.04401*	±	0.02086*	0.04253*	±	0.008891*
$t_{1/2}$	min	18.71*	±	7.701*	16.89*	±	3.476*
Corr. coeff.	r^2	0.9849	±	0.01941	0.9779	±	0.02465
Cl	ml/min/kg	1.789	±	0.5113	2.012	±	0.2330
Vd_{area}	l/kg	0.04450	±	0.01075	0.04841	±	0.007221
Vd_{ss}	l/kg	0.04836	±	0.005962	0.04973	±	0.006128
MRT	min	29.15	±	9.527	25.08	±	4.380

* accurate determination not possible. When $t_{1/2}$ and β are approximations also the derived parameters $AUC_{0\text{-}\infty}$, Cl, Vd_{area}, Vd_{ss} and MRT are approximations.
Dose normalisation to 1 U/kg
median and range

An additional study comparing 2 pilot scale and 3 commercial scale batches of rhC1INH revealed differences for Cmax and AUC between groups. However no significant differences in pharmacokinetic parameters between pilot scale and commercial scale batches and between two different commercial scale batches were observed. Overall, bioanalytical comparability was considered to be demonstrated.

Toxicology

The toxicology program was designed to reflect the anticipated short-term use of rhC1INH in humans, and included single-dose studies in rats and dogs, repeat-dose studies in rats (up to 2 weeks) and dogs (up to 5 days) as well as a reproductive and developmental toxicology study in rats.
Toxicokinetic assessments were incorporated into the reproductive toxicity studies to determine exposure and corresponding safety exposure margins. Other toxicity studies included assessments of local tolerance in rabbits and antigenicity.

- Single dose toxicity

One single dose toxicity study was conducted in rats using intravenous administration. rhC1 INH was administered once at doses of 0, 25, 125, 625 and 1250 U/kg.

There were no deaths. Treatment-related clinical signs were mainly pilo-erection at the highest dose. In addition, the enlargement of spleen in 2/3 male rats may be evidence for immunological reactions.
The maximal exposure to functional rhC1 INH in this study was achieved in rats given the highest dose, 1250 U/kg. The mean maximal concentration was 18,562 mU/ml (3094 mcg/ml). In comparison, plasma concentration in patients after the proposed dose of 100 U/kg (approximating to the recommended dose of 15 mg/kg) was typically between 2,000 and 3,000 mU/ml. This concentration was achieved in some rats given 125 U/kg.

Overall, the study demonstrated tolerability at doses achieving about 7 to 9 fold excess the human plasma concentration.

There was no effect on coagulation and fibrinolytic parameters in this study. Considering the objective of the product is to interfere with coagulation, fibrinolysis, complement and kinin-releasing systems, the apparent absence of such effect even at large doses should be discussed by the applicant.

Repeat-dose toxicity

Toxicity after repeated dose was assessed in three studies, two, and one in the dog using repeated daily administration for 5 days.

In the rat, 2 studies were conducted, one using repeated daily administration for 4 days and one with continuous infusion over 14 days.

In the 4-day study rats were treated with rhC1 INH, from two batches, by slow intravenous infusion at doses of 0, 625 and 1250U/kg once per day and with 1250 U/kg twice per day, with a 7 hour interval between doses, for 4 consecutive days with toxicokinetic evaluation. In addition, 5 rats/sex were kept for a recovery period of 10 days. Anti-rhC1 INH antibody determination was undertaken on all rats, using samples taken just prior to termination on Days 4 and 14.
Almost all rhC1 INH-treated rats had a swollen muzzle and/or limbs after treatment. Swelling persisted beyond 7 hours for several animals but regressed within 24 hours. The incidence of swelling generally decreased as the study progressed.
There were no other signs of overt toxicity, no significant findings in laboratory investigations and no identified target organ of toxicity after histopathological examination.

Among 90 rats given rhC1 INH, 34 tested positive for IgG antibodies (38%). There was no correlation of antibody titre with dose, sex or whether rats were killed on Day 4 or 14.
This study indicates that rhC1 INH had little toxicity in rats when administered by daily intravenous injection for 4 days.
After two week recovery period product-specific antibody (IgG) titres were determined by ELISA. None or only relatively low rhC1INH-specific antibody titres were measured in all groups.

In the second repeated dose toxicity Dawley rats received a continuous intravenous infusion at doses of 25, 125 or 625 U/kg/day rhC1INH and 625 U/kg/day pdC1INH for 14 consecutive days, followed by a 14-day observation period, and including toxicokinetics. This dosing route design (i.e. continuous

infusion) was chosen to try to avoid immunogenicity associated with repeated bolus administration of Rhucin.

There were no effects of rhC1 INH detected on any parameters measured in the study. The NOEL was therefore determined to be 625 U/kg. In the comparator product (plasma derived C1INH) group, minimal changes in haematological and clinical biochemical parameters and in organ weights (increases to liver, kidney and spleen) were observed.

The exposure to rhC1 INH achieved in this was significantly less than that achieved in other studies and was less with Rhucin than with the comparator Esterasine.
The maximum reported plasma concentration was 14.3 mU/ml in this study, compared to rats treated with the same intravenous dose of 625 U/kg rhC1 INH once, where a plasma concentration in the range of 2351 - 3635 mU/ml was reported 2 hours after dosing.
The active comparator group had substantially greater exposure (concentrations range from 3179 to 6206 mU/ml in accordance with previous studies.
The low exposure was explained by faster clearance of rhC1INH compared to plasma derived C1INH through receptor-mediated endocytosis by the liver. Based on this limited exposure, the study was considered to be of limited toxicological relevance.

Investigation of antibody titres was performed for three groups (625 U/kg rhC1INH, 625 U/kg pdC1INH and vehicle) in order to determine the immunogenicity of the test substance in rats after prolonged exposure. After five days no significant differences was found between groups. However, after day 16 and day 29, differences in antibody titres between rhC1INH and vehicle became highly significant. In addition, the titres of the rats dosed with plasma-derived C1INH were not significantly increased as compared to the control group. Although these findings were regarded to be not fully predictive for the human use of rhC1INH, they might suggest differences in immunogenic properties of both either rhC1INH or pdC1INH. The immunogenic potency of plasma derived C1INH was not assessed in this study as immunogenicity was assessed using a validated test for rhC1INH.

In the dog, toxicity was investigated in a dose escalation study for 5 consecutive days administering doses of 25, 125, 625, or 1250 U/kg rhC1INH intravenously into 2 male and 2 female, including toxicokinetic sampling.
There were no mortality or clinical signs of overt toxicity in either stage of this study. Total white blood cell and platelet counts were decreased with reductions in the relative proportion of segmented neutrophils and increases in lymphocytes in both sexes. Neither APTT nor PT were altered by treatment with rhC1 INH. Histopathological examination of all tissues did not reveal treatment-related findings.

It was concluded that 5 daily treatments of 625 U/kg (104 mg/kg) did not results in toxic effects and that daily escalating doses that reached 1250 U/kg (208 mg/kg) were not associated with overt toxicity.

Toxicokinetic measurements demonstrated tolerability at doses achieving about 7 fold excess the human plasma concentration. (see toxicokinetic section).

Overall, the dose escalating study in dogs did not reveal treatment related adverse effects.

Genotoxicity

No genotoxicity studies were performed on the basis that the drug is unlikely to interact directly with DNA or other chromosomal material, in accordance with current guidelines (ICH S6)

Carcinogenicity

No carcinogenicity studies were performed, on the basis that such studies are generally inappropriate for biotechnology-derived pharmaceuticals. It is accepted that, based on the duration of clinical dosing and in view of its biological activity, rhC1 INH does not pose a carcinogenic risk. This is also in agreement with current guidelines (ICH S6).

Reproductive and developmental toxicity

An embryo-fetal development study in pregnant rats to assess the potential for teratogenicity was performed by intravenous injection at one dose level of 625 U/kg with a parallel control group.
Embryo-fetal toxicity evaluation in a single species was considered acceptable given the acute life-threatening disease and the short-term emergency use of the product.

During dosing, all drug-treated dams were observed to have swollen muzzles and limbs for up to 4 hours after dosing. However, there were no other anbormalities noted in the dams, including at necropsy. No adverse effects on the different parameters of pregnancy were observed. No external, visceral or skeletal abnormalities were noted in fetuses and there was no difference in the number or type of skeletal anomalies or variations.
Toxicokinetic measurements were of limited values as the sampling was performed 24h after dosing leading to values of endogenous concentration of C1INH. There was no evidence of development of IgG antibodies to rhC1 INH.

Studies on fertility, early embryonic and postnatal development were not performed. It could not be excluded that Rhucin will cross the placenta; fetal exposure and transfer in milk in lactating patients could not be excluded as there were no data to support this view. However, Rhucin was rapidly eliminated by receptor-mediated endocytosis. Given the acute life-threatening nature of the disease and the short-term emergency use, the absence of such data was considered to be acceptable. A corresponding statement should be mentioned in the SPC.

Local tolerance

Local tolerance was evaluated following administration of the liquid formulation in rabbits and. in the 4-day rat toxicity study using the lyophilised formulation. No concerns for local intolerance were observed across these studies.

- Toxicokinetic data

Toxicokinetic data from treated rats (4-day study) are shown in the table below.

– Toxicokinetic parameters from treated groups were as follows:

Occasion	Dose (U/kg/day)	Sex	C_{max} (mU/mL)	AUC_{0-2h} (mU.h/mL)
day 0	625	Male	15051	18870
		Female	15342	16523
	1250	Male	28679	33632
		Female	33855	35439
	2500*	Male	32026	41389
		Female	31464	40194
day 2	625	Male	16652	20272
		Female	15091	16424
	1250	Male	33161	41768
		Female	33108	35004
	2500*	Male	32312	42907
		Female	29390	37536

* given as 2 doses of 1250 U/kg with a 7 hour interval

The systemic exposure was similar after the first or the second daily administration and no accumulation was observed between days 0 and 2. AUC values increased proportionally between 625 and 1250 U/kg/day for both sexes. There were no sex differences in Cmax and AUC. The C_{max} data were approximately 10 fold the highest Cmax in human subjects at the recommended dose.

Toxicokinetic results in the dog (escalating dose study) are presented in the table below.

Parameters	**25 U/kg**		**100 U/kg**		**250 U/kg**		**625 U/kg**		**1250 U/kg**	
	M	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**

Half-life (min)	7.3	6.5	14.1	18.4	36.8	44.4	59.5	56.0	219	174
Clearance (ml/min/kg)	6.2	5.8	8.0	1.9	1.2	0.9	0.7	1.7	0.3	0.4
AUC $_{0\text{-inf}}$ (U.min/ml)	4.1	4.3	26.9	52.7	211	292	910	700	4437	3205
Dose norm. AUC $_{0\text{-inf}}$ (U.min/ml)*	0.2	0.2	0.3	0.5	0.8	1.2	1.5	1.1	3.6	2.6

* Dose normalization to 1 U/kg

- Other toxicity studies

Thrombogenicity

No specific studies were performed investigating the thrombogenic potential, and this remained a concern. (see "Discussion on non-clinical aspects" section).

Antigenicity

Investigation of antibody titres was performed in repeat-dose studies as described above. In the rat, differences in antibody titres between rhC1INH and vehicle became highly significant at the end of the investigation period whereas titres of the rats dosed with plasma-derived C1INH were not significantly increased as compared to the control group.

- Ecotoxicity/environmental risk assessment

According to the current guideline, the lack of environmental risk assessment studies is justified in view of the nature of the active substance (protein) and of the orphan medicinal product status. Therefore no risk to the environment is expected.

Discussion on Non-Clinical aspects

The *in vitro* target protease inhibition investigation demonstrated that the inhibitory activity of rhC1INH towards the target proteinases can be regarded to be comparable with plasma derived C1 inhibitor. The safety pharmacology study performed in dogs revealed no significant effects on vital functions such as cardiovascular or respiratory system.

Pharmacokinetic parameters have been characterised in the species used and bioanalytical comparability between batches was considered to be demonstrated. Clearance of the product is occurring through receptor-mediated endocytosis by the liver.

Single-dose and repeat dose toxicity of rhC1INH were assessed in rats and dogs. No significant treatment related adverse effects were observed at plasma concentrations corresponding to about 6 to 10 times the human exposure.
In the rat, differences in antibody titres between rhC1INH and vehicle became highly significant at the end of the investigation period whereas titres of the rats dosed with plasma-derived C1INH were not significantly increased as compared to the control group. These findings might suggest differences in immunogenic properties of both either rhC1INH or pdC1INH. The immunogenic potency of plasma derived C1INH was not assessed in this study as immunogenicity was assessed using a validated test for rhC1INH.

No genototoxic or carcinogenic potential is expected from this biotechnology-derived product, justifying the absence of such studies.

The reproductive toxicology did not indicate a teratogenic or fetotoxic effect of rhC1 INH in rats. Given the acute life-threatening nature of the disease and the short-term emergency use of the product, the lack of a teratology assessment in a non-rodent species in teratology assessment was considered acceptable. Potential effects on fertility and on peri- and postnatal development were not studied and

no data of transfer into milk are available. This should be mentioned in the SPC. No concerns for local tolerance were observed across all studies.
No risk for the environment is expected.

C1INH targets proteinases in the complement cascade and clotting pathway (factor XI, factor XII and kallikrein). Since C1 inhibitors can also inhibit serine proteinases like plasmin and tissue-type plasminogen activator of the fibrinolytic system, induction of thrombogenicity may be an issue which should be evaluated in non-clinical safety studies for this type of product. Due to its PK profile Rhucin has to be administered in relatively high doses when compared to plasma derived products. The biological effects of a sudden increase of C1INH activity in plasma from subnormal values in patients with HAE below 0.4 U/ml to a peak up to 5 U/ml were not considered. Since the number of patients treated with Rhucin was small, a potential thromboembolic risk can not be excluded. A non-clinical study investigating comparative thrombogenic potential of Rhucin with the human plasma-derived product in order to demonstrate whether the intended clinical use of Rhucin does pose a thrombogenic risk was not performed.

2.4 Clinical aspects

Introduction
Clinical data submitted for Rhucin were derived from the following clinical trials:

- Study C1 1101-01: A Phase I study in patients with asymptomatic HAE;
- Study C1 1202-01: A Phase II exploratory open-label study;
- Study C1 1203-01: A Phase II/III open-label study;
- Study C1 1106-02: A Phase I study investigating repeated intravenous doses of rhC1INH in healthy volunteers.

Studies C1 1202-01 and C1 1203-01 were the main efficacy studies, evaluating rhC1INH at the single dose of 100 U/kg in symptomatic HAE patients. The main pharmacodynamic results were derived from studies C1 1101-01, C1 1202-01 and C1 1203-01, whereas both pharmacokinetic and safety data were mostly derived from all four clinical studies.
A further two controlled clinical studies (C1 1205-01 and C1 1304-01) were in progress at the time of the evaluation. Study C1 1205-01 was a randomised, placebo-controlled, double-blind Phase II study on the safety and efficacy of rhC1INH at doses of 50 and 100U/kg in relieving eligible attacks of angioedema with involvement of submucosal tissues in patients with HAE. Results from this study were not available. Study C1 1304-01 was a randomised, placebo-controlled, double-blind, multi-centre study performed in order to demonstrate the efficacy of rhC1INH at 100 U/kg in patients with HAE with attacks of angioedema. Data from an interim analysis on Study C1 1304-01 were provided at day 121 of the procedure (July 2007), and were assessed and taken into account in the evaluation.

Table 1: Listing of all clinical studies

Type of Study	*Study Identifier*	*Loc. of Study Report*	*Objective(s) of the Study*	*Study Design and Type of Control*	*Test Product(s); Dosage Regimen; Route of Administration*	*No. Subjects*	*Healthy Subjects or Diagnosis of Patients*	*Duration of Treatment*	*Study Status, Type of Report*
Phase I	C1 1101-01	Module 5.3.3.1	Safety & Tolerability & PK/PD	Open-label	rhC1INH 6.25, 12.5, 25, 50, 100 U/kg 15 min. i.v. infusion 2 doses at least 3 week intervals	12	Asymptomatic HAE patients	2 Dosages	Completed GCP
Phase II	C1 1202-01	Module 5.3.5.2	Efficacy & Safety & Tolerability & PK/PD	Open-label	rhC1INH 100 U/kg 15 min. i.v. infusion	>5 - 15	Symptomatic HAE patients	(one dose per acute attack)	Ongoing
Phase II/III	C1 1203-01	Module 5.3.5.2	Efficacy & Safety & Tolerability & PK/	Open-label	rhC1INH 100 U/kg One dose per acute attack 15 min. i.v. infusion	>10 - 30	Symptomatic HAE patients	(one dose per acute attack)	Ongoing
Phase III	C1 1304-01	-	Efficacy & Safety & Tolerability	Randomised, placebo-controlled, double-blind	rhC1INH 100 U/kg Saline (vehicle) single dose 15 min. i.v. infusion	50	Symptomatic HAE patients	(single dose)	Ongoing
Phase II	C1 1205-01	-	Safety & Tolerability & Efficacy & PK/PD	Randomised, placebo-controlled, double-blind	rhC1INH 50 or 100 U/kg Saline (vehicle) single dose 15 min. i.v. infusion	39	Symptomatic HAE patients	(single dose)	Ongoing
Phase I	C1 1106-02	Module 5.3.3.2	Safety & Tolerability	Open-label	rhC1INH 100 U/kg i.v. infusion (6 mL/min) 5 doses at 3 week intervals	14	Healthy Volunteers	15 weeks	Completed GCP

Regulatory Guidance and Advice

The applicant justified the open-label trials for demonstrating efficacy during the oral hearing of a protocol assistance procedure at EMEA (November 2003). At that time results were available on the treatment with rhC1INH of only one attack of HAE.

The EMEA did not endorse the applicant's proposal for open-label trials and proposed an active-controlled study. The applicant initiated a randomised, placebo-controlled, double-blind clinical trial (C1 1304-01) to demonstrate rhC1INH efficacy in the treatment of acute attacks in patients with HAE**.**

GCP

All clinical trials were performed in accordance with GCP as claimed by the applicant.

The applicant has provided a statement to the effect that clinical trials conducted outside the community were carried out in accordance with the ethical standards of Directive 2001/20/EC.

Orphan Medicinal Products

According to the conclusion of the COMP (2001) the prevalence of the condition is 2.1 per 10000 individuals in the EU and although a satisfactory method of treatment was already authorised within the EU, the recombinant character of the product was supposed to be of significant benefit. Therefore orphan designation was granted (11th May 2001).

Biopharmaceutics

No biopharmaceutic studies were conducted as recombinant human C1 inhibitor is to be administered intravenously.

Pharmacokinetics

METHODS

PK measurements included functional and antigenic C1INH levels. Functional C1INH was determined by chromogenic assay. Antigen levels of C1INH were determined by a nepholemetric immunoassay.
The plasma levels of functional C1INH and antigenic C1INH were determined in a sequence of samples to evaluate their kinetics. The results for pharmacokinetic (PK) response parameters were derived from either model-independent (functional C1INH, C1INH antigen) or model-dependent (functional C1INH) analyses.

- In a Phase I study (C1 1101-01), 12 subjects with HAE but without clear symptoms of disease at the time of the trial were intravenously infused with escalating dosages (6.25 to 100 U/kg) of rhC1INH on two occasions.
  Treatment was rhC1INH at 6.25, 12.5, 25, 50, and 100 U/kg dosages, administered intravenously as a 15-min. infusion. Treatments were applied with a washout period of at least 3 weeks.

  Blood samplings were collected 30 days, 14 days and 1 day prior to the start of the trial, at day 0, and at different time-points (15 and 30 minutes, 1, 2, 4, 8, 12, 16, 24, 48, 96 and 144 hours) post-infusion.

- Pharmacokinetics of rhC1INH in Symptomatic Subjects with HAE were explored with phase II/III studies C1 1202-01 and C1 1203-01. Twelve subjects with 17 severe acute HAE attacks were treated with rhC1INH at 100 U/kg.
  Treatment was rhC1INH at a dose of 100 U/kg, administered intravenously as a 15-min. infusion.

- Pharmacokinetics of rhC1INH in Healthy Volunteers (C1 1106-02)**:** fourteen subjects received in total 59 administrations of rhC1INH in a dosage of 100 U/kg.
  Treatment was rhC1INH at 100 U/kg dosage, administered intravenously as a single infusion over a period of 15 min. For each subject five administrations were applied with a washout period between consecutive study drug infusions of approximately three weeks.

  Blood sampling for immunological analysis were collected at screening, pre-dose, at 7 and 21 days after each administration as well as at the close out visit at 90 days after the last study drug administration.

RESULTS

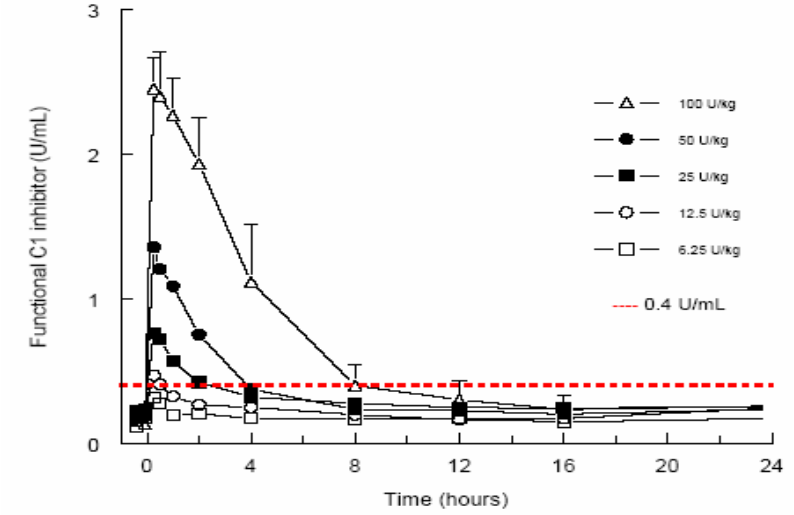
- In study (C1 1101-01), infusion of rhC1INH at escalated dosages resulted in dose-dependent increases of model-independent functional C1INH response parameters Cmax, Cmax above baseline, AUE above baseline, dose-normalized AUE, and of Time above 0.4 U/mL, whereas dose-normalized Cmax appeared constant (about 0.02 U/mL/U/kg). The profile of mean functional C1INH was similar to that of mean antigenic C1INH. The profiles of functional C1INH showed a full initial recovery and a dose-dependent clearance of rhC1INH indicating a saturable mechanism of elimination. That rates of clearance, half-life and endogenous infusion were dependent of dose which was confirmed with a standard one-compartment model of analysis. Application of the standard model after the infusion of rhC1INH at 100 U/kg revealed a clearance for functional C1INH of about 13 mL/min, a half-life of about 3 h, a volume of distribution of about 3 L and an endogenous infusion rate of about 2 U/min. After dosing of rhC1INH at 100 U/kg, the Cmax of functional C1INH was at least two-fold the normal level for about 2 hours and remained above 0.4 U/mL for about 9 hours.

- In Symptomatic Subjects with HAE after treatment, the levels of functional C1INH and C1INH antigen peaked at median times of about 20 min and thereafter in about 8 to 12 hour gradually declined to endogenous levels.

- In Healthy Volunteers (C1 1106-02) after administration of rhC1INH the levels of functional C1INH peaked at median times of about 20-30 minutes and thereafter declined to endogenous levels in about 8 to 12 hours. $T_{1/2}$, Clearance and Cmax were comparable to those estimated in the other studies.

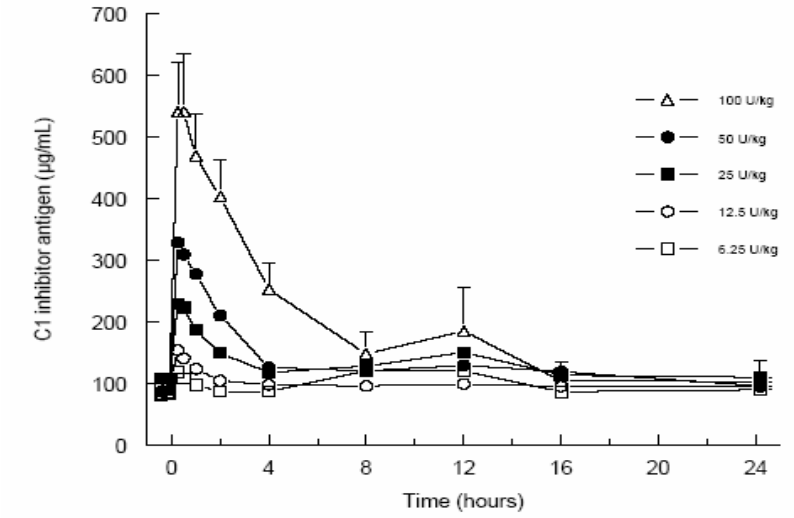
Model independent pharmacokinetics of functional C1 inhibitor (study C1 1101-01)
Time profiles of mean functional C1 inhibitor (U/mL) in the distinct dosage groups are shown in the figure below. The SD in the dose group 100 U/kg is represented by a bar.

Figure 1: Functional C1 inhibitor concentration (U/mL)



Model-independent pharmacokinetics of antigenic C1 inhibitor
The figure 2 below shows the time profiles of mean antigenic C1 inhibitor (µg/mL) in the distinct dosage groups. The SD in the highest dosage group (100 U/kg) is represented by a bar.

Figure 2: C1 inhibitor antigen concentration (µg/mL)



Time profiles of mean antigenic and functional C1 inhibitor appear similar apart from the second peak, the nature of which is unknown, and whose magnitude seemed to increase with dose.

Overall, following infusion, functional C1INH concentrations increased approximately 3- to 4-fold and declined to baseline concentrations over the subsequent 12 hours. Median baseline levels of functional C1INH appeared to be similar for each infusion and the dose-normalised C_{max} was similar for all subjects-visit combinations.

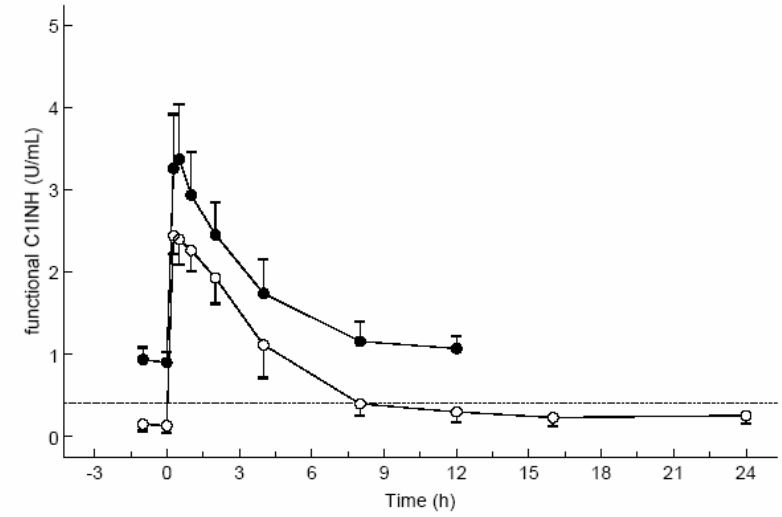
There appears to be dose-dependent increases of both Cmax (above baseline) and AUE above baseline of antigenic C1 inhibitor, whereas dose-normalised Cmax was constant (about 5 µg/mL/U/kg).

Pharmacokinetic comparability

Comparability data between the formulation used in clinical trials and the to-be-marketed formulation was presented.

The pilot scale finished product was used in study C1 1101-01, whereas the commercial scale product was used in study C1 1106-02. In both studies, the dose was 100 U/kg. For study C1 1101-01 six HAE patients were administered this dose. For study C1 1106-02 data were derived from the first administration in fourteen healthy volunteers. Results are shown in the graph and table below.

Average functional C1INH concentrations in healthy volunteers (C1 1106-02) and in asymptomatic HAE patients (C1 1101-01).



Pharmacokinetic parameters obtained in asymptomatic HAE patients (C1 1101-01) and healthy volunteers (C1 1106-02)

Parameter	Group	N	Mean	SD	Min	Median	Max
Baseline (U/mL)	HAE Phase I	6	0.14	0.08	0.06	0.14	0.25
	Healthy volunteers	14	0.92	0.12	0.68	0.93	1.15
Cmax (U/mL)	HAE Phase I	6	2.46	0.22	2.06	2.53	2.68
	Healthy volunteers	14	3.54	0.62	2.61	3.51	4.46
Cmax above baseline (U/ml)	HAE Phase I	6	2.32	0.20	1.97	2.32	2.54
	Healthy volunteers	14	2.62	0.65	1.70	2.56	3.58
Tmax (min)	HAE Phase I	6	18	6	16	16	30
	Healthy volunteers	14	25	12	16	23	60
AUE (U/ml)	HAE Phase I	6	1.28	0.28	0.79	1.29	1.62
	Healthy volunteers	13	1.96	0.30	1.42	1.94	2.42
AUE above baseline (U/mL)	HAE Phase I	6	1.14	0.24	0.70	1.19	1.42
	Healthy volunteers	13	1.04	0.30	0.51	1.00	1.61

In healthy volunteers (C1 1106-02; using commercial scale product) a higher C_{max} was observed than for the Phase I study carried out in asymptomatic HAE patients (C1 1101-01; using pilot-scale

product). According to the applicant, this difference was related to much higher C1INH baseline values in the healthy volunteers compared to HAE patients group.
The applicant considered the "pilot-scale" study drug and the "commercial-scale" product pharmacokinetically comparable.

Absorption
Rhucin is intended for intravenous administration.

Distribution and elimination
RhC1INH demonstrated a volume of distribution of approximately 2.8 L, a dose-dependent clearance of approximately 11.5ml/min and an elimination half-life of approximately 2 hours The plasma concentration vs. time profiles of C1INH showed a full initial recovery and a dose-dependent clearance of rhC1INH indicating a saturable mechanism of elimination.

Intra- and inter-variability
PK data obtained from study C1 1106-02 after intravenous infusion of rhC1INH in healthy volunteers revealed that the profiles obtained at three different visits were similar and that therefore intra-individual PK variability was low.

Special populations

Children
Studies in children were not performed and this was reflected in the SPC.

Special Studies
No additional special studies were conducted.

Drug interactions
No drug interaction studies were conducted (see section "Discussion on Clinical Safety").

Discussion on pharmacokinetics
Most of the PK data showing the anticipated range for this biological molecule but the recombinant product shows a rapid half-life/disappearance from the circulation of the patients which makes a higher dosing necessary, 100 U/Kg compared to usually administered 15-30 U/Kg with a plasma derived (pd) product (see figure in II.1.2) to cover a time period of 9 hours. This reduces the safety margin with respect to plasma derived products and could boost the risk for thrombembolic complications as already seen with plasma derived products, when they are given in high doses for off-label indications where the underlying mechanism is the C1 inhibitors involvement also in reducing fibrinolysis (See also discussion on safety).

Pharmacodynamics

Mechanism of action
C1INH inhibits targets in the complement cascade (C1r, C1s and MASPs) and clotting pathway (factor XI, factor XII and kallikrein). C1INH deficiency results in an inappropriate activation of these systems, in the release of vasoactive peptides (C2-kinin and bradykinin) and also in increased vascular permeability which causes uncontrolled, local oedema. Insufficient control of C1INH on the (auto)activation of the complement component 1 (C1) results in activation and consumption of complement component 4 (C4) through cleavage of native C4 by activated C1. As a result, the levels of C4 in plasma of asymptomatic HAE subjects are about 20 % of normal. In these patients C4b/c, a cleavage product of C4, increases with decreased levels of functional C1INH.
RhC1INH displayed biological activity in asymptomatic subjects through an increase in C4 antigen and inhibition of C4 cleavage.

Primary pharmacology and relationship between plasma concentration and effect

PD measurements included levels of C4 antigen, determined by a nephelometric assay. Based on the measurements of functional and antigenic C1INH levels and levels of C4 antigen, PK/PD parameters were calculated using compartmental and/or other applicable methodology.

METHODS

- In study C1 1101-01 12 asymptomatic subjects were treated and observed for C4 antigen and inhibition of C4 cleavage as well as for plasma levels of C4b/c, a cleavage product of C4. Pharmacodynamics endpoints were antigenic C4 plasma concentrations and magnitude and duration of plasma C4 responses compared to pre-infusion levels.

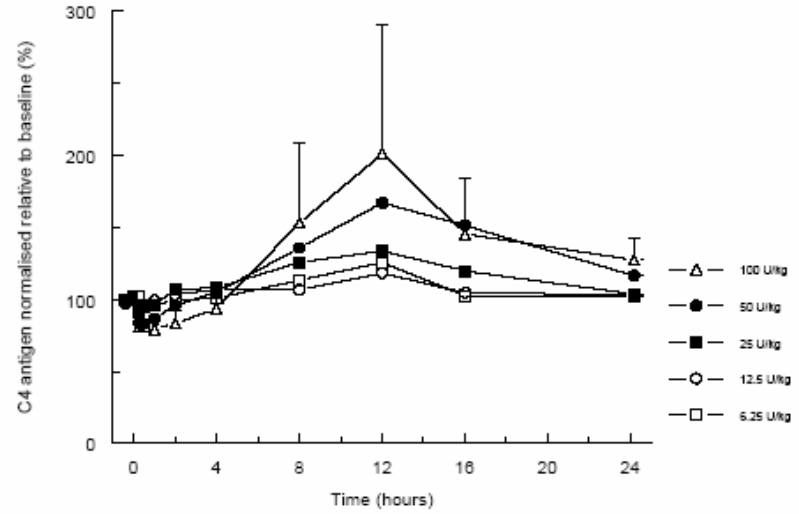
- In Studies C1 1202-01 and C1 1203-01 pharmacodynamic endpoints were concentrations of antigenic C4, C4b/c, prekallikrein, factor XII and PAP in plasma and magnitude and duration of plasma biomarker responses compared to pre-infusion levels.

RESULTS

- In study C1 1101-01 12 baseline C4 levels and C4 responses were highly variable between patients from the different dose groups. Individual C4 responses were expressed relative to individual C4 antigen values at baseline (normalised C4 antigen) in order to facilitate comparison of C4 responses both within and between dose groups. Therefore, the mean of individual baseline C4 levels was arbitrarily set at 100% and changes of C4 levels post-infusion were expressed as % change from baseline.

The figure below shows the time profiles of mean normalised C4 antigen (%) in the distinct dosage groups.

Fig 4: C4 antigen normalised (%)



A dose-dependent response in C4 antigen was seen to peak at approximately 12 hours post-infusion and thereafter to gradually decrease to baseline.
Immediate dose-dependent decreases of C4b/c plasma levels were observed; this can serve as an objective quantitative variable and can be assessed as a good therapeutic response although C4b/c levels in the normal range (i.e. below 8 nmol/L) were only observed when the mean functional C1 inhibitor concentration was at least normal (around 1 U/ml).
The magnitude of the decrease in C4bc as well as its duration appeared dependent on the dose of rhC1INH. The results indicated that cleavage of C4 starts occurring once functional C1 inhibitor drops below a level of about 70% of normal.

- In Studies C1 1202-01 and C1 1203-01 a single infusion of rhC1INH was followed by a sustained elevation of C4 antigen. The median C4 levels at screening and baseline are shown in the table below.

Table 13: Pharmacodynamic Parameters of C4 antigen after an IV infusion of rhC1INH 100 U/kg (ITT population)

Parameter	N	Mean	SD	Min	Median	Max
AUE (µg/mL)	17	307.0	62.85	189	319.6	444
AUE above baseline (µg/mL)	17	248.4	58.70	146	243.9	371
Baseline (µg/mL)	17	58.6	26.60	33	49.3	141
Cmax (µg/mL)	17	713.1	132.93	515	721.0	1023
Cmax above baseline (µg/mL)	17	654.5	134.64	467	683.1	951
Endogenous level (µg/mL)	17	83.1	38.31	37	76.5	184
Tmax (min)	17	20.3	11.79	15	15.0	60

Secondary pharmacology
No studies were performed.

Pharmacodynamic interactions with other medicinal products or substances
No studies were performed.

Genetic differences in PD response
No studies were performed.

Clinical efficacy

Clinical efficacy was mainly evaluated in two open-label studies C1 1202-01 (phase II, single centre, conducted in The Netherlands) and C1 1203-01 (phase II/III, multi-centre, conducted in Hungary, Poland, Spain and the UK). Data from both studies were pooled, analysed together and presented as an interim report in the initial application. The dose of rhC1INH of 100 U/kg was chosen based on PK results. A further two controlled clinical studies (study C1 1205-01 and study C1 1304-01) were in progress at the time of the evaluation.

The table below summarises the total exposures per study as described by the Applicant, taking to account that data from Study C1 1205-01 were not made available, whereas data from an interim analysis of Study C1 1304-01 were submitted at Day121 of the procedure and were evaluated.

Clinical trial exposure by type of Rhucin and study

CLINICAL TRIAL EXPOSURE BY TYPE OF RHUCIN and STUDY		
Pilot-scale Rhucin	**Total number of subjects**	**Total number of doses Rhucin administered**
Clinical Study		
C1 1101-01 (asymptomatic HAE)	12	24
C1 1202-01 and C1 1203-01(acute HAE)	14	21
C1 1304-01 (acute HAE, RCT)	**11**	**11**
C1 1205-01 (acute HAE, RCT)	7*	7*
Total	**43****	**63**
Up-scaled Rhucin	**Total number of subjects**	**Total number of doses Rhucin administered**
Clinical Study		
C1 1106-02 (healthy volunteers)	14	59
C1 1304-01 (acute HAE, RCT)	**3**	**3**
C1 1304-01 (acute HAE, Open-label)	**4**	**4**
C1 1205-01 (acute HAE, RCT)	19°	19°
C1 1205-01 (acute HAE, Open-label)	11°°	15°°
Total	**46°°°**	**100**

* *Estimate from 11 blinded administrations of Rhucin at 100U/kg, at 50 U/kg or placebo (1: 1: 1)*

** *The total number of subjects is one less than the addition of all subjects participating in each study, because one patient received 2 doses in study C1 1101-01, and one dose in study C1 1202-01*

° *Estimate from 28 blinded administrations of Rhucin at 100U/kg, at 50 U/kg or placebo (1: 1: 1)*

°° *15 open-label administrations in open-label extension study; 9 subjects received a single, one subject received 2 and another subject received 4 open Label Rhucin treatments, respectively;*

°°° *The total number of subjects is five less than the addition of all subjects participating in each study, because 2, 5 and 4 of the 11 subjects who received open-label treatment are estimated to have received pilot-scale Rhucin, up-scaled Rhucin or placebo as the first (randomized) treatment*

Dose response studies and main studies

The studies C1 1202-01 and C1 1203-01 explored the safety and tolerability, clinical effects, PK and PD of rhC1INH at 100 U/kg in acute attacks of angioedema. The two studies are by design almost identical, however, in study C1 1203-01 patients with laryngeal angioedema were also included.

METHODS

The evaluation of efficacy was performed on the basis of the Intention to Treat (ITT) set, which was composed of all subjects to whom study medication was administered and for whom any follow-up efficacy data were available.

Efficacy data were evaluated for:

- All eligible body sites, defined as body sites with an investigator score of 4 (severe symptoms) at 1h before start of study drug infusion.
- The most severe body site of each attack (defined as the site with the highest investigator severity score at 1h before start of study drug infusion).
- All body sites.

Patients were asked to score their perception of angioedema signs and symptoms by filling out two Visual Analogue Scales (VAS), one for treatment benefit and one for pain/swelling**.**

Analysis of efficacy was based on the recording of angioedema signs by the investigator (severity score) and by the patient (VAS scales). A series of efficacy evaluations was then calculated (time to the beginning of relief of an attack, time to minimal symptoms and relapses after the beginning of relief) and considered in an exploratory way.

Severity of angioedema signs were also rated by an investigator on a 6-point ordinal scale (depending

on whether the assessment occurred before or after infusion of the study drug).

Table 15: Investigator scores of angioedema signs and symptoms

Score	Before rhC1INH treatment	After start rhC1INH treatment (through 48 h)
0	No symptoms	Complete resolution
1	Almost no symptoms	Almost no symptoms without evidence for stabilization Almost complete resolution
2	Mild symptoms	Mild symptoms without evidence for stabilization Moderate but evidence for abatement
3	Moderate symptoms	Moderate symptoms without evidence for stabilization Severe symptoms but evidence for abatement
4	Severe symptoms	Severe symptoms without any evidence for stabilization
5	Life-threatening	Life-threatening

- **Outcomes/endpoints**

Primary endpoints:

Time to the beginning of relief

This was the primary efficacy variable in both studies.

Time to the beginning of relief at a site

The following definitions for time to the beginning of relief were used in this analysis.

- Based on the investigator severity score: The first time-point at which a decrease of at least 1 point was observed compared to time 0 (start of infusion), provided that the next assessment also showed a decrease of at least 1 point;
- Based on the treatment benefit VAS: The first time-point at which the treatment benefit VAS reached a value of at least 20 mm, provided that the next assessment still had a value of at least 20 mm;
- Based on the pain/swelling VAS: The first time-point at which the pain/swelling VAS decreased by at least 20 mm compared to time 0, provided that the decrease was still at least 20 mm at the next assessment.

Time to the beginning of relief of an attack

This was defined as the first-time relief observed for all sites (eligible or not) of the attack and was calculated based on the three definitions of time to the relief already described.

Response

Response was defined as time to the beginning of relief at a site within 4 hours. Evaluations were based upon the three definitions of time to the beginning of relief previously described. For the definition of response based on the investigator severity score and the definition based on the treatment benefit VAS, all patients (100%) were considered to be responders.

Relapse

Relapse was defined as follows:

- Based on investigator severity score: Reaching a value of at least that one at time 0 (start of infusion) at any time after the beginning of relief, but at 24 h-post-infusion at the latest;
- Based on treatment benefit VAS: Reaching a value below 20 mm at any time point after beginning of relief, but at 24 h-post-infusion at the latest;
- Based on pain/swelling VAS: Reaching at least the value at time 0 at any time-point after the beginning of relief, but at 24 h-post-infusion at the latest.

Secondary endpoints:

Time to Minimal Symptoms

Time to minimal symptoms was defined as follows.

- Based on investigator severity score: The first time-point at which a score of at most 1 is reached, provided the next assessment score is not higher than 1;
- Based on treatment benefit VAS: The first time-point at which a value of 80 mm or more is reached, provided that the value is still at least 80 mm at the next assessment
- Based on the pain/swelling VAS: The first time-point at which a value of 20 mm or less is reached, provided the value is still 20 mm or less at the next assessment.

Time to minimal symptoms of an attack

The time to minimal symptoms of an attack was defined as the first time-point at which minimal symptoms were observed for all sites (eligible or not) of the attack and was calculated based on the three definitions of time to minimal symptoms above.

Statistical and Analytical Plan

A first interim analysis was performed after 9 patients had been treated. A second first interim analysis was performed after 12 patients had been treated for at least one attack. Results from the second interim analysis are discussed below.

Study Populations

The following three study populations were used for analysis:

1. The ITT population included all patients who received at least one infusion of rhC1INH and had any post-treatment efficacy data. This population was evaluated for efficacy.
2. The safety population included all patients who received at least one infusion of rhC1INH and contributed post-treatment safety data. This population was evaluated for safety.
3. The total population included all patients screened for the study. This population was evaluated for the baseline characteristics.

RESULTS

- **Patient Disposition**

At the time of the second interim analysis, 14 patients were screened in Study C1 1202- 01, and 34 patients were screened in Study C1 1203-01.

12 patients were treated (4 from Study C1 1202-01, and 8 from Study C1 1203-01) and 5 patients presented for a second attack (2 from Study C1 1202-01 and 3 from C1 1203-01), so that a total set of 17 attacks was available for the second interim analysis.

For 3 of the treated attacks no data were collected for the Day 90 visit. For all 17 attacks, all patients were evaluated 24 h and 48 h after the start of the rhC1INH infusion and on Days 7 and 22.

- **Demographics**

The mean age for screened patients treated with rhC1INH in the study was 40 years and 34 years for those screened but who did not receive study medication.

The majority of subjects were female in both the screened and treated population and the screened but untreated group. For the screened and rhC1INH-treated population all subjects were Caucasian. 2 of the 36 patients screened but not treated with study medication were Asian.

- **Diagnostic Markers**

The summary statistics for diagnostic markers at screening are provided below.

Table 18: Diagnostic Markers at Screening – Treated Patients

Parameter	Units	N	Mean	SD	Min	Median	Max
Functional C1INH*	U/mL	12	-	-	<0.28	<0.28	59.0
Antigen C1INH	µg/mL	12	68.7	29.9	33.5	56.3	124.0
C1q	IE/mL	12	112.8	21.5	75.9	118.0	146.0
C4	µg/mL	12	100.2	56.8	39.9	77.2	207.0

* Seven out of 12 treated patients had a level <0.28

Table 19: Diagnostic Markers at Screening – Untreated Patients

Parameter	Units	N	Mean	SD	Min	Median	Max
Functional C1INH*	U/mL	36	-	-	<0.28	<0.28	124.0
Antigen C1INH	µg/mL	35	83.9	45.7	15.0	76.1	253.0
C1q	IE/mL	36	116.0	32.6	59.0	111.0	193.0
C4	µg/mL	35	99.3	51.1	29.7	93.1	253.0

* Nineteen of the 36 untreated patients had a level <0.28

- **Outcomes and estimation from the pooled analysis of studies C1 1202-01 and C1 1203-01**

Primary endpoints:

Time to the beginning of relief at a site

Table 20: Time to the beginning of relief (minutes) for all patients at a site

Set	Site	Minimum	Median	Maximum	N
Based on investigator severity score					
All eligible sites	Abdominal	15.0	22.5	60.0	8
	Non-abdominal	15.0	30.0	120.0	8
	All sites	15.0	30.0	120.0	16
Most severe sites	Abdominal	15.0	22.5	60.0	8
	Non-abdominal	15.0	30.0	120.0	9
	All sites	15.0	30.0	120.0	17
All sites	Abdominal	15.0	30.0	60.0	9
	Non-abdominal	15.0	30.0	120.0	13
	All sites	15.0	30.0	120.0	22
Base on treatment benefit VAS sensitivity analysis					
All eligible sites	Abdominal	15.0	15.0	60.0	3
	Non-abdominal	15.0	60.0	240.0	5
	All sites	15.0	45.0	240.0	8
Most severe sites	Abdominal	15.0	15.0	60.0	3
	Non-abdominal	15.0	60.0	240.0	5
	All sites	15.0	45.0	240.0	8
All sites	Abdominal	15.0	37.5	120.0	4
	Non-abdominal	15.0	90.0	240.0	6

	All sites	15.0	60.0	240.0	10
Based on pain/swelling VAS					
All eligible sites	Abdominal	15.0	45.0	60.0	8
	Non-abdominal	30.0	180.0	720.0	8
	All sites	15.0	60.0	720.0	16
Most severe sites	Abdominal	15.0	45.0	60.0	8
	Non-abdominal	30.0	120.0	720.0	9
	All sites	15.0	60.0	720.0	17
All sites	Abdominal	15.0	45.0	60.0	8
	Non-abdominal	30.0	180.0	720.0	12
	All sites	15.0	60.0	720.0	20

Time to the beginning of relief of an attack
In the table below the time to the beginning of relief is described for all attacks and for the first attack of each patient.

Table 21: Time to the beginning of relief (minutes) of an attack

	Minimum	**Median**	**Maximum**	**N**
Based on investigator severity score				
All attacks	15.0	30.0	120.0	17
First attack	15.0	30.0	120.0	12
Based on treatment benefit VAS sensitivity analysis				
All attacks	15.0	45.0	240.0	8
First attack	15.0	60.0	240.0	6
Based on pain/swelling VAS				
All attacks	15.0	60.0	720.0	17
First attack	15.0	60.0	720.0	12

Time to the beginning of relief at all sites
The mean and median values for time to the beginning of relief were longest for the assessment based on the pain/swelling VAS for both beginning of relief at sites and beginning of relief of attacks (for all sites or for any site). The median time to the beginning of relief of an attack for any site, calculated for all attacks, was 30 minutes based on the investigator severity score, 15 minutes based on the treatment benefit VAS, and 60 minutes based on the pain/swelling VAS.

Table 22: Time to the beginning of relief (ITT population: All sites)

	N	Time (min) to Beginning of Relief Mean ±SD	Min	Median	Max
Time to beginning of relief at sites					
Severity Score[1]	22	42.3 ± 30.6	15.0	30.0	120.0
VAS treatment benefit[2]	22	70.2 ± 79.7	15.0	22.5	240.0
VAS pain/swelling[2]	20[3]	134.3 ± 163.5	15.0	60.0	720.0
Time to beginning of relief of an attack for all sites					
Severity score[1]	17	43.2 ± 33.5	15.0	30.0	120.0
VAS treatment benefit[2]	17	67.9 ± 86.5	15.0	15.0	240.0
VAS pain swelling[2]	17	146.5 ± 174.2	15.0	60.0	720.0
Time to beginning of relief of an attack for any site					
Severity score[1]	17	35.3 ± 27.0	15.0	30.0	120.0
VAS treatment benefit[2]	17	54.7 ± 75.0	15.0	15.0	240.0
VAS pain swelling[2]	17	122.6 ± 173.7	15.0	60.0	720.0

[1] Investigator assessment
[2] Patient assessment
[3] For two body sites of subject 401 (study C1 1203-01), the time to the beginning of relief could not be determined because the pain/swelling VAS was already below 20 mm at the start of the infusion (time 0) and hence could not decrease for at least 20 mm at further time-points

Response
Based on the pain/swelling VAS, 1 patient (study C1 1203-01) was considered to be a non-responder, having a time to the beginning of relief of 12h.

Relapse
No patients were considered to have relapsed according to the definitions of relapse based on the investigator severity score and the pain/swelling VAS. 2 patients were considered to have relapsed after the beginning of relief (1 from each study) according to the definition based on the treatment benefit VAS. Based on changes in angioedema symptoms however, there were no clinical indications of relapse for either patient.

Secondary endpoints:

Time to minimal symptoms at a site
Based on the investigator severity score, the time to minimal symptoms was within 12 h for approximately 69%, 71% and 77% of all subjects, respectively for all eligible sites, the most severe site of each attack and for all body sites.
Based on the treatment benefit VAS and the pain/swelling VAS, the time to minimal symptoms was within 12 h for approximately 75%, 76% and 77% of all subjects, respectively for all eligible sites, the most severe site of each attack and for all body sites.

Time to minimal symptoms of an attack
The table below summarises the results for all attacks and for the first attack of each subject.
The median time to minimal symptoms of an attack, calculated for all attacks, was approximately 240 to 360 minutes based on the investigator severity score, 600 to 720 minutes based on the treatment benefit VAS, and 480 minutes based on the pain/swelling VAS.

Table 24: Time to minimal symptoms of an attack (minutes)

	Minimum	**Median**	**Maximum**	**N**
Based on investigator severity score				
All attacks	30	240	1440	17
First attack	30	360	1440	12

Based on treatment benefit VAS sensitivity analysis				
All attacks	30	600	>2880	8
First attack	120	720	>2880	6
Based on pain/swelling VAS				
All attacks	15	480	>2880	17
First attack	15	480	>2880	12

Time to minimal symptoms at all sites

The table below summarises results from the ITT population of each study separately and the results pooled together.

Table 25: Time to minimal symptoms at sites summarised by type of site (ITT population; all sites)

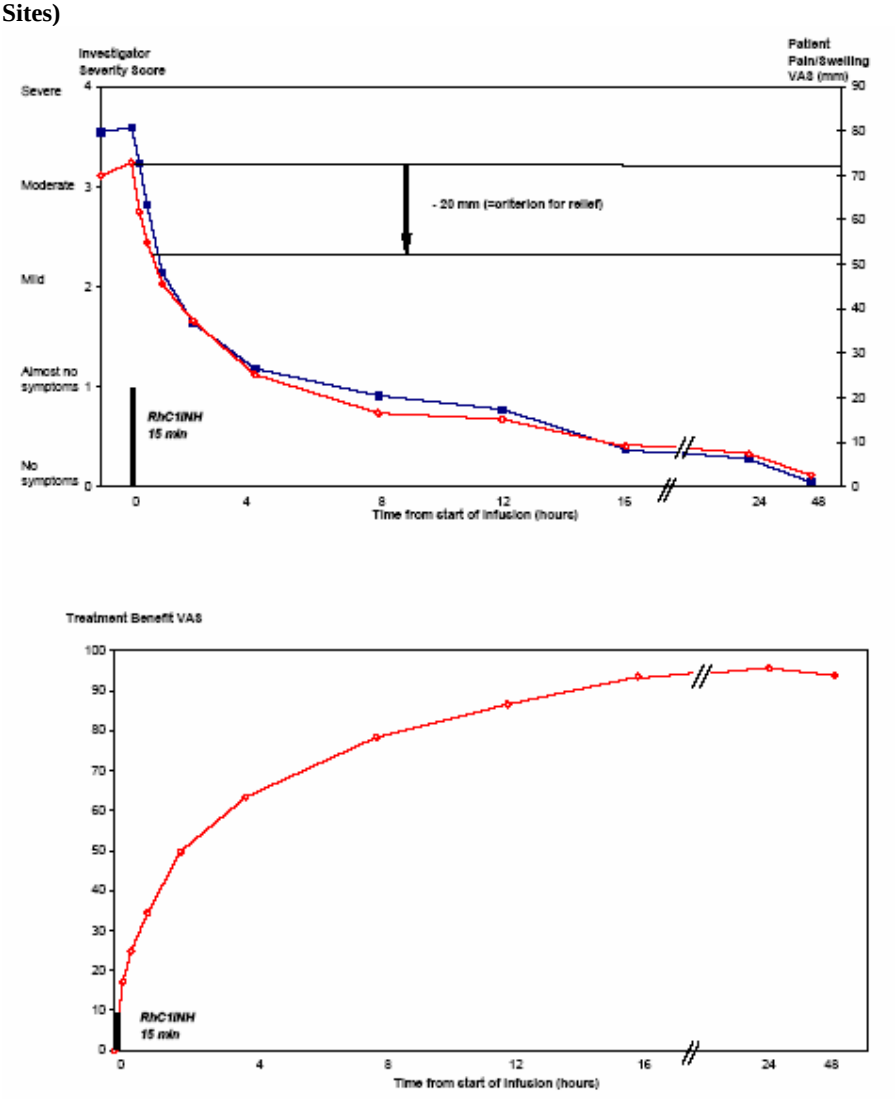
	Median Time (minutes) to Minimal Symptoms at Sites					
	Study C1 1202-01		Study C1 1203-01		All Patients	
	N	Median	N	Median	N	Median
Severity score[1]						
Abdominal	3	240	6	90	9	120
Non-abdominal	3	960	10	240	13	240
All sites	6	600	16	120	22	240
VAS treatment benefit[2]						
Abdominal	3	720	6	15	9	30
Non-abdominal	3	480	10	600	13	480
All sites	6	600	16	180	22	360
VAS pain/swelling[2]						
Abdominal	3	240	6	67.5	9	120
Non-abdominal	3	≥2880	10	480	13	720
All sites	6	360	16	240	22	240

[1] Investigator assessment
[2] Patient assessment

Investigator and Patient Assessments

Following infusion of rhC1INH, the mean severity score and the mean pain/swelling VAS promptly decreased from baseline over the first 4 h after infusion and continued to decrease over the 48 h observation time. The mean treatment benefit VAS rapidly increased over the first 4 h after the infusion and continued to gradually increase over the first 24 h after treatment.

Fig 15: Mean Scores for Investigator (*) and Patient Assessments (o) of Efficacy (ITT Population; All Sites)



- **Other Efficacy Analyses**

Investigator Severity Score

The mean investigator severity scores over time for all eligible body sites, for the most severe site of an attack, and for all body sites were presented. In general, the mean severity score decreased within the first 2 hours after study infusion and reached a score of 1 over 12 hours, and a level of 0 within 48 hours after study drug infusion.

VAS Assessing Treatment Benefit

A total of 7 subjects (2 from study C1 1202-01 and 5 from study C1 1203-01) reported treatment benefit at time points before the start or at the start of study drug infusion. The applicant considered this to be due to misinterpretation by the patients and disregarded these attacks from its analysis. Results showed an increase in the mean treatment benefit VAS after study drug infusion which stabilised at 16 hours following study drug infusion.

Intensity VAS Scales
The pain/swelling VAS combines the VAS scale assessing pain for the abdominal sites with the VAS scale assessing swelling for the other body sites. Results showed an increase in the mean pain/swelling VAS after study drug infusion which stabilised at 16 hours following study drug infusion.

Comparison of results in sub-population
No sub-populations were investigated in clinical studies.

Analysis of clinical information relevant to dosing recommendation
Based on the PK and PD of functional C1INH in the Phase I study in asymptomatic HAE subjects, a dose of 100 U/kg was used in the ongoing open-label studies as well as in the Phase III placebo-controlled studies in HAE subjects with acute attacks. No formal statistical meta-analysis was performed and the choice of dose was based on empirical considerations.

Persistence of Efficacy and/or Tolerance Effects
No patients were treated for long-term with rhC1INH, therefore there were no data on persistence of efficacy over time. Treatment of acute attacks with rhC1INH was considered to be replacement therapy.

A very limited number of patients underwent second or third administration.

Clinical studies in special populations
Clinical studies in special populations were not performed.

Analysis performed across trials (pooled analyses AND meta-analysis)
Results from the main studies C1 1202-01 and C1 1203-01 were in fact submitted as pooled data with pooled analyses (see above).

Additional clinical data provided during evaluation

Study C1 1304-01
Study C1 1304-01 was performed in order to demonstrate the efficacy of rhC1INH at 100 U/kg in patients with HAE with attacks of angioedema as compared to placebo. A set of data from this randomised, placebo-controlled, double-blind, multi-centre study was submitted at day 121 of the procedure as interim report.

METHODS
Twenty-five patients, 13 in the Rhucin group and 12 in the placebo group, were evaluated in this study.

The primary variable was the “time to the beginning of relief at eligible site(s) based on patients’ VAS scores”. The secondary variable analysed was “time to minimal symptoms based on patients’ VAS scores”.

Two different analyses were performed:
- An ITT (full analysis set (FAS): n=28) analysis including all the documented patients,
- A modified ITT (FAS: n=25) analysis excluding three patients for which approval was still pending.

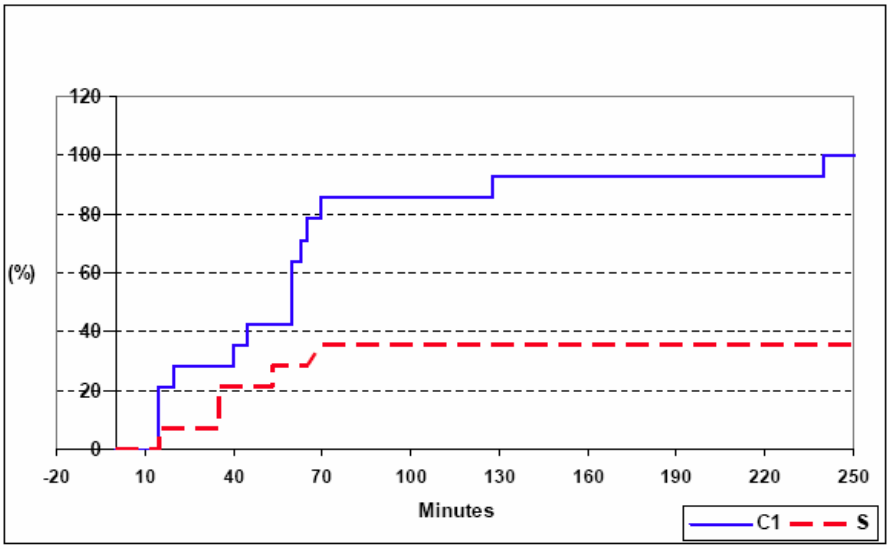
RESULTS
- **Outcomes and estimation**

The key primary, secondary and exploratory efficacy findings are summarised in the tables below (referring to the modified ITT on 25 patients).

Primary End Point

	Rhucin	Placebo	P-value (Log rank)
Median time to the beginning of relief (minutes) Primary Endpoint			
Overall VAS at any location decreased by ≥ 20 mm	**60.00**	**507.50**	**0.0009**
Exploratory variables/sensitivity analyses			
Overall VAS at all locations decreased by ≥ 20 mm	60.00	509.00	0.0001
Overall VAS at the most severe site decreased by ≥ 20 mm	60.00	509.00	0.0002
Overall VAS at any location decreased by ≥ 30 mm	121.50	720.00	0.0077
Overall VAS at any location decreased by ≥ 40 mm	133.50	960.00	0.0144
Investigator's Score at any location decreased by ≥ 1	30.00	300.00	0.0081
Investigator's Score at all locations decreased by ≥ 1	30.00	480.00	0.0028
Investigator's Score at the most severe site decreased by ≥ 1	30.00	480.00	0.0019

Proportion of patients with time to the beginning of the relief within 4 hours based on overall VAS (FAS)



Upper curve: rhC1INH (C1), lower curve: Placebo (Saline: S). FAS: full analysis set, VAS: visual analogue scale. Source: Section 14.2.of study report C1 1304-01

Proportion of patients with time to the beginning of the relief within 4 hours based on overall VAS and Investigator's Score

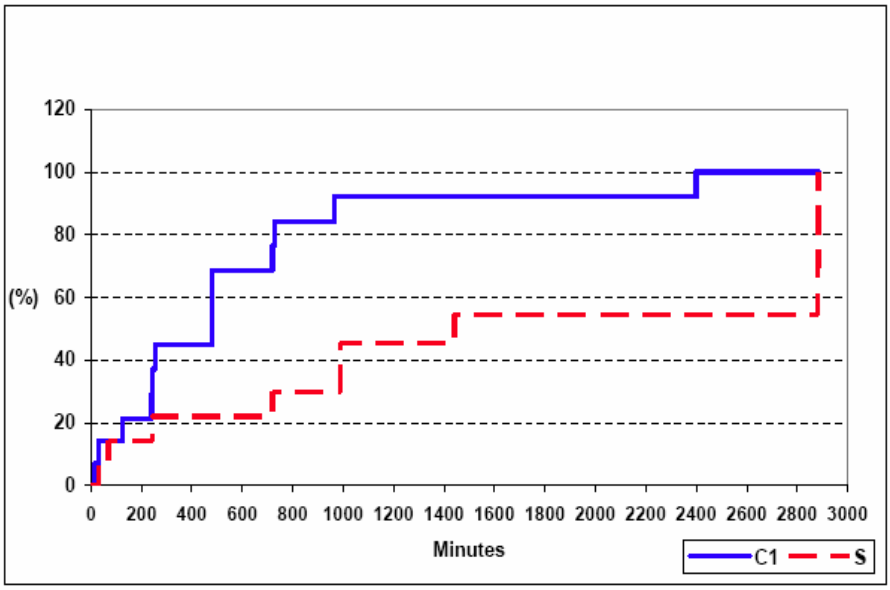
	Rhucin	Placebo	P-value (Fisher exact)
Response Rate (%)			
Based on overall VAS	100	35.7	0.0006
Based on Investigator's Score	92.9	50	0.0329

Secondary End Point

	Rhucin	Placebo	P-value (Log rank)
Median time to minimal symptoms (minutes) Secondary endpoint			
Overall VAS at all locations < 20 mm	**480.00**	**1440.00**	**0.0038**
Exploratory variables			
Overall VAS at any location < 20 mm	480.00	1440.00	0.0096
Overall VAS at the most severe site < 20 mm	480.00	1440.00	0.0069
Investigator's Score at any location ≤ 1	240.00	960.00	0.0442
Investigator's Score at all locations ≤ 1	240.00	1440.00	0.0269
Investigator's Score at the most severe site ≤ 1	240.00	1440.00	0.0297

The related Kaplan-Meier curve for the secondary endpoint is presented below with the proportions of patients with minimal symptoms and proportions of patients with minimal symptoms at 12 hours.

Proportion of patients with minimal symptoms by overall VAS



Left curve: rhC1INH (C1), right curve: Saline (S, placebo), VAS: visual analogue scale,

Discussion on clinical efficacy

At day 120 of the procedure the CHMP identified a number of major objections regarding the evidence of efficacy; the applicant was asked to address the following points:

- The two pivotal studies initially submitted (C1 1101-01 and C1 1202-01) were open-label and uncontrolled, despite EMEA advice to include a control arm. In line with the Protocol Assistance recommendation, the applicant was asked to perform a comparator-controlled, randomized and blinded study in at least 20 symptomatic patients prior to authorization.
- Patients' assessment of treatment effect was based on a VAS which on occasions had been misinterpreted; the applicant was asked to comment on the reliability of this data.
- The applicant was asked to provide information on how patients suffering from laryngeal oedema attacks (the most serious manifestation of the disease) would respond to treatment.
- The applicant was asked to address the issue of inadequate experience of re-dosing.
- The applicant was asked to comment on the paucity of data in support of the proposed posology. Specifically, the dosing recommendation, based on a Phase I study in asymptomatic HAE subjects, had not been adequately justified.

- The majority (75%) of treated patients were female. It was uncertain whether response in males would have been the same at the recommended dose.
- From the description of the used study drug within the reports it remained unclear to which extent the "pilot" study drug was clinically comparable to the future "commercial" drug. The applicant was asked to comment and provide a respective safety and efficacy comparison taking into account both analytical and pre-clinical data.

The applicant submitted new data at day 121 of the procedure, addressing some of the above-listed concerns.
The following major objections remained unresolved:

- The applicant initiated placebo-controlled, randomised trials; however, it would have been more informative to see comparator-controlled, randomised trials and to explore the question of superiority/non-inferiority and to compare the safety profiles with the acknowledged existing therapies for the claimed indication (i.e. plasma-derived C1 inhibitor (pdC1INH)).
  Thus, the lack of an active control arm was not adequately justified and the evidence of benefit over placebo (and a comparison of safety to placebo) was considered not sufficient to establish a favourable benefit/risk.

- There were insufficient data to support the proposed posology and the dosing recommendation, based on a Phase I study in asymptomatic HAE subjects, was not adequately justified.
  Because of the more rapid clearance compared to pdC1INH, Rhucin was evaluated at a higher dosage in order to ensure a duration of exposure above the minimum critical level of C1INH activity, 0.4 U/ml, for not less than 8 hours, and to minimize the potential for relapse and/or rescue treatment.
  A fixed dose of 100 U/kg of rhC1INH corresponds to 7000 U for a person of 70 kg bodyweight which is much higher than the usual doses administered for a plasma-derived product. Rhucin is differently glycosylated and has a shorter half-life and probably much higher Cmax values are reached with the proposed dose of 100 U/kg Rhucin than with pdC1INH.
  Data from a PK comparison between Rhucin and plasma-derived C1 inhibitor would have been useful.

- Laryngeal oedema is the most serious manifestation of the disease and there was not sufficient indication how such patients respond to the proposed treatment. Data were provided from the treatment of only two laryngeal angioedema attacks treated with Rhucin. Based on the successful outcome of these two treatments, according to the applicant there were no pathophysiological or pharmacological grounds to consider that laryngeal attacks would respond differently or require a modification of dosage schedule to C1INH replacement with Rhucin or plasma derived C1INH products.
  Moreover, in both cases laryngeal angioedema attacks were treated with a dose of rhC1Inhibitor equivalent to 50 U/Kg bodyweight, rather than the proposed dose of 100 U/kg body weight, and this raised further doubts on the proposed fixed dosage.
  Therefore, the company proposed to monitor in the Risk Management Plan the response from treatment with Rhucin in laryngeal attacks, and to submit documentation for review after each 100 treatments or yearly (whichever comes first) until 300 attacks will have been treated with Rhucin. Nevertheless, taking into account all the elements presently available, this remained a major concern.

- Overall and taking into account that hereditary angioedema is a chronic condition there was inadequate experience of re-dosing. The phase I Study C1 1101-01 and the two phase II studies C1 1202-01 and C1 1203-01 in asymptomatic and symptomatic patients evaluated 18 patients receiving 2 doses and 1 patient receiving 3 doses. In the open label extension to the placebo-controlled randomised clinical trial (C1 1205-01), 9 subjects received a single, 1 subject received 2 and another subject received 4 open label Rhucin treatments, respectively. Because of the double blind character of the study it was uncertain whether these patients

received Rhucin prior to participation in the open label extension. Finally, in a phase I study with healthy volunteers (C1 1106-02), 1 subject received 2 doses and 11 subjects received 5 doses of Rhucin.

These limited data were not considered sufficient to exclude the potential for an unsatisfactory clinical safety or efficacy response after re-exposure to Rhucin.

REPEAT EXPOSURES		
	N	Total number of doses
Open label, phase I and II studies (C1 1101-01, C1 1202-01 and C1 1203-01)		
Single dose	6	6
Two doses	**18**	**36**
Three doses (in 1101-01 and 1202-01)*	**1**	**3**
Randomised, placebo controlled trial (C1 1304-01)		
Single dose**	14 blind + 4 open label	18
Two doses	**0**	**0**
Randomised,placebo controlled trial (C1 1205-01)		
*** Single dose [Ongoing double blind study]	*** 39 [*** estimated, 26 Rhucin, 13 Placebo]	*** estimated 26 blinded Rhucin administrations as of 29 October, 2007
One or more Rhucin doses in open label extension phase of study	**11**	**15 Open-label administrations in open label extension study (9 subjects received a single , one subject received 2 and another subject received 4 Open-label Rhucin treatments, respectively)**
Healthy volunteers (C1 1106-02)		
Single dose	2	2
Two doses	**1**	**2**
Five doses	**11**	**55**

* One patient received 2 doses in study C1 1101-01 and 1 dose in study C1 1202-01

** Fourteen patients received 14 randomized treatments, 4 randomized saline treated patients 1 open label treatment.

*** Ongoing double blind placebo controlled randomized clinical trial with open label extension

- The majority (75%) of treated patients were female. It is uncertain whether response in males will be the same at the recommended dose.

  In the phase I clinical pharmacology study (C1 1101-01) in asymptomatic HAE patients, 4 (33%) of the participating 12 subjects were female. Although the numbers were small, according to the applicant the PK and PD findings were comparable between male and female patients and there was no evidence to suggest a gender difference in response to administration of Rhucin.

  A majority of female patients volunteered to participate in the open label treatment studies (C1 1202-01 and C1 1203-01). Of the volunteer patients enrolled but not treated in the open-label studies with Rhucin, 21 (62%) were female and 13 were male. This gender difference was also seen in the treated patients, 10 females (71%) and 4 men (29%). No gender differences were found with respect to efficacy or safety outcomes in the open label treatment studies. Additional data became available from Study C1 1304-01, showing no demographic differences between the patients that received Rhucin compared to those that received placebo. Since in study 1304-01 there were no differences in demographic characteristics, the majority of treated patients being female in the open label clinical studies C1 1202-01 and C1 1203-01 was likely due to coincidence.

  However, separate efficacy results for the groups of males or females, respectively, were not presented because of concerns over lack of statistical power in all studies. Therefore, differences in the treatment response in males and females could not be excluded. Furthermore, data on the concomitant use of androgens (which reduce the severity of acute

attacks when administered to HAE patients) were deemed necessary for assessment of efficacy results.

- During the CHMP meeting on 11 December 2007, outstanding issues were addressed by the applicant during an oral explanation before the CHMP.
  The CHMP acknowledged the additional clinical results and took them into consideration during the final discussion. The difficulty to conduct a study of non-inferiority against plasma-derived C1 inhibitor was also acknowledged. The proposed posology and dose recommendation was still not adequately justified. Major efficacy concerns remained unsolved: the size of the clinical database is small (with re-administration data particularly limited in the case of a life-long disease with repeated clinical attacks) and does not provide adequate reassurance in relation to efficacy in more severe forms of the disease, e.g. laryngeal oedema, and efficacy on re-administration.

Clinical safety

- **Introduction**

The initial safety data of Rhucin that were made available were derived from the four clinical studies C1 1101-01, C1 1106-02, C1 1202-01 and C1 1203-01. Results from these studies are described below.

- **Patient Exposure**

rhC1INH was administered 41 times to 24 patients: 24 administrations to 12 asymptomatic patients during the phase I study in asymptomatic patients and 17 administrations to 12 patients having an acute attack of angioedema in the open label studies. In addition 100 U/kg rhC1INH was administered to 14 healthy volunteers on 59 occasions.

- **Adverse events**

Only treatment emergent adverse events (TEAEs) were considered in the analysis, i.e. those AEs that occurred at or after study drug administration up to the last visit at day 90 post-infusion. A separate analysis was performed for AEs occurring within 72 hours of study drug administration. The same analyses were done for serious AEs (SAEs).

All of the adverse events reported during the course of Study C1 1101-01 were graded as “mild’’ or “moderate”, except for 1 SAE which was considered unrelated to study drug administration but related to pre-existent disease (HAE). Since a relationship with the study drug was not suspected this SAE was not reported to the Health Authorities.
The AEs occurred on single occasions or intermittently and none were persistent. Intermittent treatment-emergent AEs (n = 3) in most cases were considered to be related to pre-existent disorders (e.g back pain). All of the AEs occurring after study drug administration were classified as “possible”, “unlikely” or “definitely not” related to treatment. Of the 14 ‘possible’ related AEs, 9 presented as headache, 2 as abdominal pain, 1 as a vasovagal reaction and 2 as local haematoma or skin reaction. 13 out of the 14 ‘possible’ related AEs presented within 72 hour after study drug administration. None of the AEs were judged as being “definite” or “probable” related to study drug administration.

9 out of the 12 patients reported treatment emergent AEs, 1 from study C1 1202-01 and 8 patients from study C1 1203-01. The 1 subject from study C1 1202-01 at screening reported itching to occur as an early (prodromal) symptom before HAE attacks. This subject reported mild, possibly novel swelling behind the right ear after the 1st treatment and mild itching urticaria behind the right ear and on the occipital region of the head after the second treatment. These events occurred within 30 minutes after start of infusion and resolved within 1 to 2 hours. The swelling was possibly related and the itching definitely related. After the second treatment, the same subject reported mild headache after being awoken, which was considered definitely not related.
6 of the 8 patients with AEs in study C1 1203-01 reported events in the class “infections and infestations”. 2 further subjects reported events in “gastrointestinal disorders”. The patient with an SAE also experienced a partial muscle rupture at the right ankle. All AEs in study C1 1203-01 were

regarded as unlikely or definitely not related to study medication and had resolved by the time of the interim analysis.

Summary of TEAEs in asymptomatic HAE patients occurring within 72 hours

Dose (U/kg)	6.25	12.5	25	50	100
Body system	**(N=3)**	**(N=3)**	**(N=6)**	**(N=6)**	**(N=6)**
Preferred term	**n (%)**	**n (%)**	**n (%)**	**n (%)**	**n (%)**
Any adverse event	2 (67%)	2 (67%)	4 (67%)	3 (50%)	3 (50%)
Body as a whole – general disorders	0	0	0	2 (33%)	0
Back pain	0	0	0	1 (17%)	0
Syncope	0	0	0	1 (17%)	0
Centr & periph nervous system disorders	1 (33%)	2 (67%)	2 (33%)	1 (17%)	3 (50%)
Headache	0	2 (67%)	1 (17%)	1 (17%)	3 (50%)
Migraine	1 (33%)	0	1 (17%)	0	0
Gastro-intestinal system disorders	1 (33%)	0	1 (17%)	0	0
Nausea	1 (33%)	0	0	0	0
Abdominal pain	0	0	1 (17%)	0	0
Musculo-skeletal system disorders	0	0	1 (17%)	0	0
Arthropathy	0	0	1 (17%)	0	0
Platelet, bleeding & clotting disorders	0	0	1 (17%)	0	0
Haematoma	0	0	1 (17%)	0	0
Respiratory system disorders	0	0	0	0	1 (17%)
Asthma	0	0	0	0	1 (17%)
Skin and appendages disorders	0	1 (33%)	0	2 (33%)	1 (17%)
Skin reaction localized	0	1 (33%)	0	2 (33%)	1 (17%)

Summary of TEAEs in healthy volunteers occurring within 72 hours

Body system Preferred term	Period 1 (N=14) n (%)	Period 2 (N=12) n (%)	Period 3 (N=12) n (%)	Period 4 (N=11) n (%)	Period 5 (N=11) n (%)
Any adverse event	6 (43%)	4 (33%)	4 (33%)	2 (18%)	1 (9%)
Application site disorders	0	0	2 (17%)	0	0
Injection site reaction	0	0	2 (17%)	0	0
Body as whole – general disorders	2 (14%)	0	1 (8%)	0	1 (9%)
Syncope	1 (7%)	0	0	0	1 (9%)
Fever	0	0	1 (8%)	0	0
Allergic reaction	1 (7%)	0	0	0	0
Centr & periph nervous system disorders	2 (14%)	2 (17%)	1 (8%)	1 (9%)	0
Headache	2 (14%)	2 (17%)	1 (8%)	1 (9%)	0
Gastro-intestinal system disorders	0	1 (8%)	1 (8%)	0	0
Abdominal pain	0	0	1 (8%)	0	0
Gastroenteritis	0	1 (8%)	0	0	0
Platelet, bleeding & clotting disorders	0	0	0	1 (9%)	0
Haematoma	0	0	0	1 (9%)	0
Respiratory system disorders	0	0	0	0	1 (9%)
Upper resp tract infection	0	0	0	0	1 (9%)
Skin and appendages disorders	2 (14%)	0	0	0	0
Pruritus	2 (14%)	0	0	0	0
Special senses other, disorders	0	1 (8%)	0	0	0
Taste perversion	0	1 (8%)	0	0	0

- **Serious adverse event/deaths/other significant events**

No deaths were observed.
A female healthy volunteer was withdrawn from Study C1 1106-1 for an anaphylactic reaction, which was considered a related SAE and occurred during the first administration of rhC1INH at 100 U/kg.
1 SAE, 'unrelated' to drug treatment, was reported in Study C1 1101-01. Another SAE was reported in study C1 1203-01, which was also considered unlikely to be related to study drug. No AEs were reported that caused premature discontinuation of the study drug infusion.

- **Laboratory findings**

For studies C1 1101-01 and C1 1106-02 none of the abnormal values for laboratory safety parameters were considered related to the administration of study medication.

Post-infusion, **haematology** values outside the normal range were found occasionally for ESR, haemoglobin, haematocrit, RBC, MCV, MCHC, WBC, monocytes, eosinophils and basophils.
For 1 patient a relative high frequency of abnormal neutrophile values was measured on Day 22 post- the LLN and almost concomitant with the low values for neutrophils, several abnormal values were detected for lymphocytes on Day 22 post-infusion.
None of the abnormalities observed for haematology parameters after treatment were considered clinically significant.

Biochemistry parameters were measured at screening, 20 minutes before and 24 hours after infusion and on Day 7 and 22 after infusion. Overall, values outside the normal range at this time points were found once or twice for sodium, potassium, AST/SGOT, ALT/SGPT, alkaline phosphatase, albumin, total protein, LDH, uric acid and triglycerides. None of these values outside the normal range, which were measured in the post-infusion period, was considered to be clinically significant.

Urinanalysis
Urine samples were measured at screening, 20 minutes before and 24 hours, 7 days and 22 days after infusion. In all studies no clinically significant abnormalities were reported.

Coagulation assays
APTT and PT were assessed at screening, 20 minutes before and 30 minutes, 1 hours, 2, 8, 12, and 24 hours and 7 and 22 days after infusion. In all studies no clinically significant abnormalities were reported.

Viral serology
Tests for hepatitis BsAg, hepatitis antibodies, and HIV 1 and 2 antibodies were performed at screening and on Day 7 and 22 after infusion. All tests were negative.

Immunogenicity

As for any recombinant product, antibodies may be raised against recombinant C1 inhibitor. These antibodies may cross-react with plasma-derived C1 inhibitor which would render treatment with plasma-derived product ineffective. Also, Rhucin is derived from milk of transgenic rabbits and the removal and control of host related impurities (i.e. from the milk) is critical since very large doses of this product will be given (typically over 1000 mg for an adult) and that several milk proteins are known to be immunogenic.

Anti-C1INH antibody subclasses IgG, IgA, and IgM (tested against immobilized plasma derived C1INH and rhC1INH) and antibodies against rabbit milk protein (anti-Host Related Impurities) were evaluated. The analyses were performed at screening, 20 minutes before start of study drug infusion (baseline), at Day 7 and 22 post-infusion and at the Day 90 visit. Antibodies were determined by an ELISA validated for precision and specificity.

In Studies C1 1101-01, C1 1202-01 and C1 1203-01 no immune reactions against study medication were reported. 1 subject tested positive for anti-C1INH antibodies subclass IgM at screening. At baseline and at the post-infusion time points, none of the subjects had positive results in any of the anti-C1INH assays or the anti-HRI test.

In Study C1 1106-02 an increase in the plasma level of anti-HRI (Host Related Impurities) antibodies was observed in 7 subjects following the fourth administration of rhC1INH. All assays for antiC1INH antibodies of IgM, IgG and IgA isotype remained negative. There were no clinical symptoms of an immunological reaction observed in any of the subjects.

Nevertheless, during early clinical trials (37 patients and healthy volunteers) at least one suspected immunogenic reaction has been noted. In a study in healthy volunteers (C1 1106-02), antibody responses were seen in 8 out of 10 subjects where the antibody levels rose above the cut off value of 120% to levels of responsiveness between 128 and 564% following the fourth administration of Rhucin. Also, given that the validation of the assay designed to detect patient antibodies to rabbit milk proteins is also inadequate (see Quality aspects), this results in a lack of analytical assurance that patients are not raising antibodies to host related impurities. Since repeat doses are expected throughout a patient’s life, the available clinical safety database with respect to repeat administration in patients is insufficient to assess the immunogenicity of Rhucin at the present point in time.

- **Vital signs, physical finding and other observations related to safety**

Vital signs
In Studies C1 1101-01 and C1 1106-02 no clinically significant changes in any of the vital signs or physical findings were observed.
In Studies C1 1202-01 and C1 1203-01 systolic blood pressure and heart rate at post-baseline visits were in general lower than at the baseline visit. The baseline visit occurred 20 minutes before the start of study drug infusion, i.e. while the subject was in an HAE attack.

ECG
ECGs were recorded at screening, 20 minutes before start of study drug infusion, 24 hours and 7 days after infusion. Abnormal ECG results were reported for two subjects at screening and/or baseline and on Day 7. None of them was considered to be clinically significant. At 24 hours post-infusion the ECGs were normal.

- **Safety in special populations**

Not performed.

- **Safety related to drug-drug interactions and other interactions**

Drug interactions were not considered (see "Discussion on clinical safety" section).

- **Safety in Special Groups and Situations**

Intrinsic Factors
There was no reported bias of HAE incidence among different ethnic groups, and symptoms did not appear to correlate necessarily with the type of genetic defect responsible for the C1INH deficiency in HAE (in instances of the inherited condition).
No separate analysis was conducted for special populations. Patients younger than 16 or 18 years were excluded from enrolment in the three trials presented.

Extrinsic Factors
Effect of extrinsic factors was not investigated.

Use in Pregnancy and Lactation
No investigations specific to use of rhC1INH in pregnant or breastfeeding women were conducted and these individuals were excluded from all three studies performed. Animal studies did not indicate direct or indirect harmful effects on embryonal / foetal development.

Overdose
Doses above the intended therapeutic dose of 100 U/kg bw were not investigated. No case of overdose was reported. C1INH after therapeutic administration in patients with HAE were similar to the levels of functional C1INH that were reported to be safe in the clinical studies exploring the potential of C1INH in various pathological conditions.

Drug Abuse
Drug abuse was not investigated.

Withdrawal and Rebound
Rebound and/or withdrawal following rhC1INH treatment was not investigated.

Effects on Ability to Drive or Operate Machinery or Impairment of Mental Function
Treatment with rhC1INH did not have any effects on the ability to drive or to operate machinery and did not impair mental function in patients.

Additional clinical data provided during evaluation

A set of data from C1 1304-01 study was submitted at day 121 of the procedure as interim report.

The table below summarises adverse reactions reported over all studies.

Adverse reactions reported in clinical studies with Rhucin

TYPE OF RHC1INH and ADVERSE REACTIONS	
	Adverse reactions < 72 hours
Pilot-scale rhC1INH 63 administrations in 43 patients	
Clinical Study	
C1 1101-01 (asymptomatic HAE)	9 Headache (6 pts) 1 Abdominal pain 1 Haematoma 1 Syncope 1 Injection site reaction
C1 1202-01 and C1 1203-01(acute HAE)	1 Swelling 1 Urticaria*
C1 1304-01 (acute HAE, RCT)	0
C1 1205-01 (acute HAE, RCT)	No reports of allergic reactions
Up-scaled rhC1INH: 100 administrations in 46 subjects	
Clinical Study	
C1 1106-02 (healthy volunteers)	2 Pruritus 1 Anaphylactic reaction** 1 Headache 1 Dysgeusia
C1 1304-01 (acute HAE, RCT)	0
C1 1304-01 (acute HAE, Open-label)	No reports of allergic reactions
C1 1205-01 (acute HAE, RCT)	No reports of allergic reactions
C1 1205-01 (acute HAE, Open-label)	No reports of allergic reactions

*All adverse reactions were of possible relationship, except for * (Definite) and ** (Probable)*

In the additional Study C1 1304-01 submitted during evaluation, none of the patients (treated with Rhucin or placebo) showed increased (above cut-off) pre-exposure values for anti-rabbit milk protein antibodies or for anti-C1INH antibodies (against either plasma-derived or Rhucin) of IgM or IgA isotype. No increases of anti-rabbit milk antibodies or anti-C1INH antibodies of IgM or IgA isotype were observed after treatment. According to the applicant these results provided no evidence for (primary) immune responses against host-related impurities or C1INH after treatment with Rhucin.
Prior to treatment with Rhucin, two patients showed isolated slightly increased values (16%) for anti-pdC1INH antibodies of IgG isotype only. After treatment with Rhucin, increased values for anti-C1INH antibodies (against either plasma-derived or Rhucin) of IgG isotype were not observed.
Following treatment with Rhucin, two patients showed isolated slightly increased values (17-20%) of anti-Rhucin antibodies of IgG isotype only, which were not considered to represent anti-C1INH antibody responses.
Still, given that the validation of the assay designed to detect patient antibodies to rabbit milk proteins was not deemed adequate (see above), the clinical evidence provided was not considered sufficiently reassuring and the concern with respect to immunogenicity of Rhucin remained.

- **Discontinuation due to adverse events**

One healthy volunteer due to a SAE.

- **Post marketing experience**

No post-marketing experience data were available, as the product had not yet been placed on the market in the European Union or in any other countries.
The applicant proposed to initiate a surveillance program after market approval, whereby patients were to be monitored for immunosafety at least twice every year (or once per year in case the frequency treatment is lower) at each treatment occasion.

- **Discussion on clinical safety**

At day 120 of the procedure the CHMP came to the conclusion that evidence of safety for Rhucin was insufficient. Specifically, the following major objections were identified:

- In the absence of a control arm, the clinical relevance of the adverse events observed was difficult to interpret. The applicant was asked to discuss this issue.
- Only a very small number of patients had been studied with limited re-exposure whereas the product is intended for a hereditary disease with repeated clinical attacks during their life. The Applicant was asked to discuss and provide justification for this deficiency.
- TEAEs had been reported in 75% of patients of whom 67% were of hereditary angioedema symptoms. One anaphylactic reaction had been reported in one healthy volunteer and an increase of anti-HRI antibodies in 7 subjects. The applicant was asked to better define the frequency of sensitisation and the seriousness of the potential reactions to this recombinant product or to rabbit impurities especially since these patients will receive repeated injections.
- Compared with published data of plasma-derived C1INH, the recombinant product showed a rapid half-life/disappearance from the circulation of the patients which makes higher dosing necessary, recommended as 100 U/Kg. This could boost the risk for thromboembolic complications which were already reported with very high doses of plasma-derived C1INH. Therefore, the applicant was asked to explore sufficiently thrombogenicity of the proposed treatment prior to marketing authorisation.

The applicant submitted new data at day 121 of the procedure, addressing some of the above-listed concerns.
The following major objections remained unresolved:

The risk of thromboembolic complications was not adequately addressed.

The applicant considered that there was no evidence to support a concern about thromboembolic risk arising from the proposed use of Rhucin in the treatment of acute angioedema attacks in HAE patients. In particular, there were significant differences between:

- the patient population from which the risk was reported, severely ill neonates treated with pdC1INH, and the proposed “healthy” HAE population that will be treated with Rhucin
- the administered dosage, 100 U/kg Rhucin in HAE versus 500-1050 U/kg plasma derived C1INH in neonates
- the plasma clearance of Rhucin versus pdC1INH.

The CHMP objected that since very high doses of C1INH were administered with Rhucin the biological effects of a sudden increase of C1 activity in plasma from subnormal values in HAE patients below 0.4 U/ml to peaks of up to 5 U/ml (data from study C1 1202-01/1203-01) are unknown. Consequently, resulting effects on the complex system of coagulation and complement activation could not be reliably anticipated. Data showing similarly high C_{max} after infusion of normally administered doses of pdC1INH were requested in order to resolve the concern of a potential thrombogenic risk through suspected high C1INH activities after administration of Rhucin but such data were not provided. Therefore, the thrombogenic risk would have to be addressed as part of the risk management plan.

In addition, the following concerns remained unresolved:

- The potential risk of immunogenicity had still not been adequately addressed in view of the potential for raising antibodies against C1 inhibitor and against host related impurities (see above).

- The interaction potential of Rhucin with concomitant treatments had not been adequately discussed.

Following the Oral explanation, the CHMP considered that overall, the safety database submitted remains limited and it was insufficient to recommend a marketing authorisation for Rhucin.
The answers from the Applicant were only partially reassuring as far as the single dose of 100U/kg body weight was concerned. It remained unclear whether the safety profile would remain the same if higher or repeated doses of Rhucin are used.
Specifically the potential of immunogenicity during repeated administration has been insufficiently explored.
The risk of thrombogenicity remained a concern, and this was requested to be included in any case in the RMP as an important potential risk.

Interactions with other concomitant treatments could not completely be ruled out.

2.5 Pharmacovigilance

Detailed description of the Pharmacovigilance system

The CHMP considered that the Pharmacovigilance system as described by the applicant fulfils the legislative requirements.

Risk Management Plan

The MAA submitted a risk management plan

The CHMP, having considered the data submitted in the application, was of the opinion that the proposed pharmacovigilance and risk minimisation activities were not sufficient to characterise or to reduce the risks to an acceptable level.

2.6 Overall conclusions, risk/benefit assessment and recommendation

Quality

Although, the data submitted provide substantial reassurance with respect to the quality of Rhucin, there are remaining issues related to the quality aspects of the product and their potential impact on efficacy and safety. Rhucin is derived from milk of transgenic rabbits and the importance of the accuracy of measurement of host related impurities (i.e. from the milk) in final product is emphasised because at the moment, there is not sufficient reassurance with respect to the concentration and range of proteins in the raw material, and thus the variability of the protein burden to be removed by the down stream process. Following review of the data submitted, there are concerns regarding the characterisation of the active substance with respect to host related impurities (HRI), the inadequate control of HRI in the product and the use of a method (HRI-ELISA) which has not been adequately validated. Furthermore, the validation of an assay designed to detect patient antibodies to rabbit milk proteins is also inadequate.

In response to these concerns, the applicant has provided additional information in an oral clarification. The additional information was promising but still requires thorough evaluation before it can be concluded that the quality of this product is acceptable to support safe and effective use of Rhucin. In addition, there are a number of remaining concerns which would have to be addressed as part of post-authorisation commitments undertaken by the company.

As a consequence, the quality of the product is not controlled in a satisfactory way and the evidence provided was not sufficient to conclude that the manufacturing process and the methods of control will guarantee the uniform clinical performance of the product.

Non-clinical pharmacology and toxicology

C1INH targets proteinases in the complement cascade and clotting pathway (factor XI, factor XII and kallikrein). Since C1 inhibitors can also inhibit serine proteinases like plasmin and tissue-type plasminogen activator of the fibrinolytic system, induction of thrombogenicity may be an issue which should be evaluated in non-clinical safety studies for this type of product. Due to its PK profile Rhucin has to be administered in relatively high doses when compared to plasma derived products. The biological effects of a sudden increase of C1INH activity in plasma from subnormal values in patients with HAE below 0.4 U/ml to a peak up to 5 U/ml were not considered. Since the number of patients treated with Rhucin was small, a potential thromboembolic risk can not be excluded. A non-clinical study investigating comparative thrombogenic potential of Rhucin with the human plasma-derived product in order to investigate whether the intended clinical use of Rhucin does pose a thrombogenic risk was not performed.

Efficacy

Two pivotal phase II open-label uncontrolled studies (C1 1202-01 and C1 1203-01) were performed in order to explore the safety and tolerability, clinical effects, pharmacokinetics and pharmacodynamics of rhC1INH at 100 U/kg in symptomatic patients suffering from hereditary angioneurotic oedema (HAE). Overall, in the absence of a control arm, the clinical relevance of the effects observed was difficult to interpret.

To address this point the company submitted on day 121 of the procedure an interim report of a confirmatory placebo controlled clinical trial (C1 1304-01). This was a randomised, placebo-controlled, double-blind study of recombinant C1 inhibitor for the treatment of acute attacks in patients with hereditary angiodema. Apart from some residual doubts on the validity of the Visual Analogue Scales (VAS) to assess efficacy due to their structure, there are no major methodological concerns with this study and there is evidence in support of efficacy relative to placebo. Nevertheless, the clinical database is small and does not provide adequate reassurance in relation to efficacy in more severe forms of the disease, e.g. laryngeal oedema. Laryngeal oedema is the most serious manifestation of the disease and therefore it is these patients that will greatly benefit from an appropriate treatment. According to the company's consideration, there are no pathophysiological or pharmacological grounds to consider that laryngeal attacks respond differently or require a modification of dosage schedule to C1INH replacement therapy. So far, only two successful treatments of laryngeal angioedema attacks with Rhucin have been reported. It is considered that this is too limited indication on how such patients might respond to the proposed treatment. Furthermore, the clinical data available does not provide adequate reassurance in relation to safety and efficacy on readministration, since the database for repeat administration is very limited. This is of particular concern since the treatment of HAE and of angioedema attacks requires more than one administration and therefore further evidence is required to support repeated administration of Rhucin.

It is also considered that the data to support the proposed posology is limited. Compared with published data on plasma-derived C1 inhibitor, the recombinant C1 inhibitor (Rhucin) shows a rapid half-life/disappearance from the circulation of the patients which makes higher dosing necessary, with a proposed fixed dose of 100 U/kg of Rhucin. No intraindividual pharmacokinetic comparison between Rhucin and a plasma-derived product has been performed. Rhucin is differently glycosylated, which explains a shorter half-life, but it has to be assumed, that much higher Cmax values are reached with the proposed dose of 100 U/kg Rhucin than with plasma-derived C1 inhibitor. Data from a pharmacokinetic comparison, even of a small exploratory study, would have been useful. Furthermore, it was noted that two patients with laryngeal attacks were successfully treated with a dose of Rhucin equivalent to 50 U/Kg bodyweight, rather than the proposed dose of 100 U/kg body weight. Overall it is considered that the optimum dose of Rhucin has not been sufficiently explored.

Safety

Very high doses of C1 inhibitor are administered with Rhucin (see above). The biological effects of a sudden increase of C1 activity in plasma from subnormal values in HAE patients below 0.4 U/ml to

peaks of up to 5 U/ml (data from study C1 1202-01/1203-01) are unknown. Consequently, resulting effects on the complex system of coagulation and complement activation can not be reliably anticipated. Therefore, the risk for thromboembolic complications which were already reported with very high doses of plasma-derived C1 inhibitor can not be excluded. Although there is currently no clinical evidence to suggest that there is thrombogenic risk with Rhucin, given the small clinical safety database available, the thrombogenic risk would have to be addressed as part of the risk management plan.

The major safety concern with Rhucin, however, is the potential for immunogenicity. First, as for any recombinant product, antibodies may be raised against recombinant C1 inhibitor. These antibodies may cross-react with plasma-derived C1 inhibitor which would render treatment with plasma-derived product ineffective. In a repeat-dose study in healthy volunteers (C1 1106-02), increases of IgM antibodies against native C1 inhibitor and against recombinant C1 inhibitor were measured starting from the third administration of Rhucin and increasing with each further application. Although still remaining below the assay's cut-off level, this is a signal for an immunological reaction. Second, Rhucin is derived from milk of transgenic rabbits and the removal and control of host related impurities (i.e. from the milk) is critical since very large doses of this product will be given (typically over 1000 mg for an adult) and that several milk proteins are known to be immunogenic. During early clinical trials (37 patients and healthy volunteers) at least one immunogenic reaction has been noted. In a study in healthy volunteers (C1 1106-02), anti-Host Related Impurities (anti-HRI) antibody responses were seen in 8 out of 10 subjects where the antibody levels rose above the cut off value of 120% to levels of responsiveness between 128 and 564% following the fourth administration of Rhucin. Also, given that the validation of the assay designed to detect patient antibodies to rabbit milk proteins is also inadequate (see Quality), this results in a lack of analytical assurance that patients are not raising antibodies to host related impurities. Since repeat doses are expected throughout a patient's life, the available clinical safety database with respect to repeat administration in patients is insufficient to assess the immunogenicity of Rhucin at the present point in time.

Risk-benefit assessment

In conclusion, the CHMP considered that, following review of the data provided, there are concerns with respect to the risk-benefit of Rhucin for use in the treatment of acute attacks of oedema in patients with hereditary angioedema for the following grounds:

- The size of the clinical database is small and does not provide adequate reassurance in relation to
  i. efficacy in more severe forms of the disease, e.g. laryngeal oedema
  ii. safety and efficacy on readministration

- There is insufficient reassurance on immunogenicity with repeat administration in patients, including its impact on safety and efficacy

- The choice of dose has not been sufficiently justified

- Sufficient reassurance on quality aspects has not been provided. The following issues are outstanding:
  i. It has not been adequately justified that the characterisation of active substance or product is sufficient to detect and identify Host Related Impurities (HRI) which could be present in immunologically significant quantities
  ii. The validation of the HRI-ELISA has not been justified and it has not been justified that this test is capable of detecting quantitatively any HRI in the active substance which is present in immunologically significant quantities (i.e. that may cause antibody responses and related adverse events)
  iii. It has not been adequately justified that the quantity of host related impurities in the active substance from the validation batches, as detected by the HRI-ELISA, is quantitatively accurate and within acceptable specifications

iv. It has not been adequately justified that the ELISA to detect antibodies to HRI raised by patients is adequately validated to ensure detection of antibodies raised to relevant proteins
v. Satisfactory post-authorisation commitments to address other outstanding quality issues would need to be agreed

– A satisfactory summary of product characteristics and risk management plan would need to be agreed.

Two CHMP members did not agree with the conclusions of the CHMP with the following divergent position:

– Although the clinical database is small, Rhucin has been used in the most severe form of hereditary angioedema, laryngeal oedema, and there is no evidence of lack of efficacy.

– With regard to antigenicity, the antigenicity of protein medicinal compounds, and the clinical consequences thereof, are never known at the time of licensing; consequently this is not a reason to refuse the granting of the Marketing Authorisation for Rhucin.

Recommendation

Based on the CHMP review of data on quality, safety and efficacy, the CHMP considered by a majority of 22 out of 24 votes that the risk-benefit balance of Rhucin in the treatment of acute attacks of oedema in patients with hereditary angioedema was unfavourable and therefore did not recommend the granting of the marketing authorisation.

3. RE-EXAMINATION OF THE CHMP OPINION

At the December 2008 CHMP meeting, the CHMP concluded that the risk-benefit balance of Rhucin in the treatment of acute attacks of oedema in patients with hereditary angioedema was unfavourable and therefore did not recommend the granting of the marketing authorisation.

On 8 February 2008, the applicant submitted the detailed grounds for the re-examination of the grounds for refusal listed above.

Ground for refusal 1:

The size of the clinical database is small and does not provide adequate reassurance in relation to
i. efficacy in more severe forms of the disease, e.g. laryngeal oedema
ii. safety and efficacy on readministration

Applicant's position:

Pharming considers that the extent of the clinical database is reasonable taking into consideration the low prevalence of the orphan disease hereditary angioedema (HAE) as well as the low frequency of attacks that require replacement treatment with C1INH. HAE is extremely rare (1:100,000), and does not need chronic daily or even regular replacement treatment with C1INH. Only acute angioedema attacks that are severe enough to affect the patient's daily activities qualify for the substitution of the deficient C1 esterase inhibitor (C1INH). The typical number of acute attacks that require replacement C1INH treatment is in the order of less than 1 attack per year per patient. Two plasma derived C1INH products are licensed in a limited number of countries (Berinert® P: Germany, Hungary, Austria and Cetor®: the Netherlands). Rhucin is a recombinant form of C1INH with comparable pharmacokinetic and pharmacodynamic properties.
Several clinical studies have been performed with Rhucin, and all have indicated a consistent and satisfactory clinical effect. Efficacy is clearly shown in findings from open label uncontrolled studies (C1 1202-01 and C1 1203-01) when compared to what has been reported in literature about the natural course of acute angioedema attacks. Compelling and reassuring evidence is derived from the blinded placebo controlled randomized study (C1 1304-01), where a highly statistically significant and clinically relevant response to treatment of acute angioedema attacks with Rhucin was found. Positive

treatment effects were consistently reported both by the patient and the investigator. More information from additional treatments of acute attacks with Rhucin have been and are continued to be collected from a placebo controlled study (C1 1205-01) and from open label extensions of the two placebo controlled randomized studies (C1 1205-01 and C1 1304-01). To date, clinical efficacy, safety and laboratory data are available to support the benefit to risk evaluation from 189 administrations of Rhucin to 96 healthy subjects or patients. At the time of the CHMP's negative opinion (13 December 2007), the clinical database contained 163 administrations of Rhucin to 89 healthy subjects or patients. In light of the orphan nature of the disease, the fact that replacement of C1INH to treat acute angioedema attacks is approved in a number of EU-member states and the evidence of efficacy observed in clinical studies, the company considers the size of the clinical database for Rhucin to be sufficient.

The clinical studies also provide evidence for the efficacy of Rhucin in more severe forms of HAE, e.g. laryngeal oedema. HAE is characterised by the occurrence of recurrent acute episodes of peripheral subcutaneous and submucosal swellings ('angioedema attacks') of any part of the skin, and respiratory and gastro-intestinal (GI) tracts. From a clinical point of view, abdominal (GI) and respiratory (laryngeal) submucosal attacks are considered more severe than peripheral attacks as they are associated in the case of abdominal attacks with severe pain similar to acute abdominal syndromes and may cause nausea, vomiting, diarrhea, ascites and symptoms of hypovolemia, and in the case of laryngeal attacks may lead to asphyxia and death. Laryngeal attacks occur in less than 1:125 angioedema attacks. In clinical studies with Rhucin sixty-two patients with submucosal angioedema attacks have been treated (abdominal 58; laryngeal 4).

HAE is a genetic disorder characterized by an inherited deficiency of C1INH activity. The pathophysiological mechanism for angioedema attacks is the same for the different peripheral or submucosal attack localizations. This is confirmed by an analysis comparing the response to Rhucin treatment in non-abdominal (cutaneous peripheral) attacks with abdominal (submucosal) attacks. The evidence for efficacy was highly comparable for the treatment with Rhucin of attacks at these different localizations.

Therefore, there is adequate and reassuring evidence to support the extrapolation of the efficacy findings to laryngeal attacks from the open label and blinded clinical studies of Rhucin in the treatment of other manifestations of both subcutaneous and submucosal attacks.

In addition, information on the successful treatment with Rhucin of 4 acute laryngeal attacks is presented in this re-examination submission. All 4 cases showed a rapid and consistent relief of symptoms as reported by both patients and investigators.

The number of angioedema attacks that require C1INH replacement therapy is low. Therefore, the probability of patients having more than one attack to be treated in the course of a clinical study is small. Despite this low attack frequency, the company recognizes that data on re-administration of Rhucin are needed. During the clinical development program information has been obtained on repeated administration of Rhucin to healthy volunteer subjects and HAE patients. In total 22 patients and 1 healthy volunteer received two administrations of Rhucin, 2 patients received 3 administrations, 11 healthy volunteers received 5 administrations and one patient received 6 administrations.

No safety concerns have been reported from studies with repeated dosing in asymptomatic HAE patients, healthy volunteer subjects and symptomatic HAE patients. In addition, efficacy findings after the first, second, and subsequent treatments have been compared, and no differences were found in patients' responses to Rhucin treatment.

To date, there is no evidence, from the currently available clinical study and laboratory testing database, to indicate that there is any immunogenicity concern that has impacted on efficacy and safet in HAE patients exposed to single or repeat doses of Rhucin. In particular, the available immunogenicity data after single and repeat administration of Rhucin in patients and healthy volunteer subjects support a positive benefit to risk evaluation for the use of Rhucin in the treatment of acute angioedema attacks.

Findings from the clinical studies with Rhucin provide evidence for a clinically relevant benefit of treatment of acute angioedema attacks with Rhucin. In addition, Rhucin treatment appeared to be safe and well tolerated.

To provide more reassurance about the adequacy of the clinical database in particular with respect to severe submucosal attacks, and the number of repeat administrations it is proposed to provide post marketing safety and treatment outcome reports to the CHMP, first, after 6 months post-market introduction, and than every 100 treated patients or yearly whichever is earlier.

Therefore the company requests the CHMP for a scientific consideration for a positive opinion for Rhucin under the provisions of Article 11 of Commission Regulation (EC) No. 507/2006 for a Conditional Marketing Authorisation (CMA) for medicinal products for human use falling within the scope of Regulation (EC) No 726/2004 with commitments as laid out in the Risk Management Plan.

CHMP position:

The number of patients with HAE, treated with Rhucin for acute attacks is very limited. In total 163 Rhucin administrations in healthy volunteers and in HAE patients have been given.
In the limited number of patients treated in the placebo-controlled study, a better efficacy was found with single administrations of Rhucin (14 attacks) compared with placebo (14 attacks).
In total for the development of Rhucin, 13 patients had more than one administration of Rhucin, whereof one received 3 and one subject received 6 administrations. Efficacy data is available for 12 patients. From these limited data it is not possible to perform an evaluation of efficacy over time.
Within the performed studies there was a restriction for administration of Rhucin, with at least 21 days between administrations.
No experience exists with the recommended dose, from repeated treatment within the same attack.
Laryngeal symptoms were treated in only 4 individuals (2 until the oral hearing), whereof 3 received half the recommended dose, 50 U/kg, with sufficient effect.

A few patients have been described as potentially insufficient responders with both the recommended dose (100U/kg) and half the dose (50U/kg). In the ongoing open label studies there is an option to repeat the dose of 50U/kg within the same attack but thereafter not until 22 days later. In addition, in the open label phase of study C1 1304-01, 2100 U and a second dose of 2100 U or 4200 U can be used.
The safety database provided, does not allow any sufficient assessment of the risk for hypersensitivity/ allergic reactions or for reduced efficacy with repeated administrations although there are safety signals of concern emanating from administrations to healthy volunteers in the early development of the product (see Grounds for re-examination 2).
One case report of an anaphylactic reaction in a healthy volunteer is of concern. This reaction occurred 2 minutes after the first administration of Rhucin. From the case narrative of this female, hypersensitivity against rabbit was revealed after the first dose. An increase in IgE was found in pre-dose blood sample. This reaction strengthens the already included contraindication for rabbit hypersensitivity, but also shows the potential risk of allergic reactions in patients with unknown hypersensitivity towards rabbit.

i. The size of the clinical database is small and does not provide adequate reassurance in relation to efficacy in more severe forms of the disease, e.g., laryngeal attacks

The following is based on data presented during the original assessment procedure. Experience from treatment of HAE attacks is limited. The included patients presented with severe symptoms in accordance with inclusion criteria. Laryngeal symptoms were treated in only 2 patients, who received half the recommended dose, apparently with sufficient efficacy.
To extrapolate data on efficacy in laryngeal oedema from other submucosal manifestations of HAE might, as the applicant argues, be of additive value, but experience from other manifestations is too limited for Rhucin.
As the pharmacokinetic profile indicates a short half life, a high initial dose has been chosen during the development of Rhucin. However, there is not sufficient clinical experience from severe attacks with the recommended dose to exclude a need for repeated dose within the same life-threatening severe attack.

ii. The size of the clinical database is small and does not provide adequate reassurance in relation to safety and efficacy on re-administration

Experience from re-administration of attacks is very limited. There is no experience from re-administration within the same attack. In total, only two patients have received more than 2 repeated

administrations. Due to restrictions in the inclusion criteria, there is no experience from re-administration within 21 days from a treated attack.
Sufficient data are lacking to prove that the sought dose (14 times the recommended dose for plasma derived C1INH) studied and proposed 100 U/Kg (one dose per attack), is appropriate in patients with a need for life-long repeated administrations of C1INH.
There are insufficient safety and efficacy data to reassure that the signal from the healthy volunteer study is of no concern. These findings indicate an increased antibody development with increasing number of administrations.

Conclusion
Thus, safety and efficacy in re-administration have not been sufficiently shown.

Ground for refusal 2:
There is insufficient reassurance on immunogenicity with repeat administration in patients, including its impact on safety and efficacy

Applicant's position:
Insufficient reassurance on immunogenicity with repeat administrations in patients, including its impact on safety and efficacy, is one of the grounds for refusal of Rhucin by the CHMP. Rhucin, as any therapeutic protein, potentially can elicit an immune response. This response can be directed to the protein itself (anti-rhC1INH antibodies) and/or to host related impurities (anti-HRI antibodies, i.e. anti-rabbit milk proteins).

Impact of antibodies against C1INH: These antibodies potentially impact on the efficacy of Rhucin. To address impact of antibodies against C1INH on the efficacy of Rhucin, Pharming has undertaken the following studies: analysis of pharmacokinetics (PK) of C1INH activity upon repeated administrations of Rhucin; evaluation of the formation of antibodies against C1INH following single and repeated Rhucin administrations; and analysis of clinical responses after repeated administrations of Rhucin.

PK of repeated Rhucin administrations have been analyzed in 11 healthy volunteer subjects who received 5 doses of Rhucin at 100U/kg at 3 weekly intervals. No differences in PK profiles between the 1st, 3rd and 5th administration were found. PK profiles were also determined in 7 HAE patients who received repeated administrations of Rhucin for subsequent acute angioedema attacks. In these patients there was no difference between the PK profiles after the 1st and 2nd administration of Rhucin.

Immunogenicity of Rhucin was tested in 82 HAE patients and 14 healthy volunteer subjects participating in the clinical development program of Rhucin. Blood samples were collected pre- and post-administration of Rhucin for up to 90 days. Validated tests for the detection of antibodies (IgG, IgM and IgA) to recombinant and plasma derived C1INH have been developed. The cut-off levels for each of these tests were determined by testing blood plasma samples from normal healthy volunteers who never previously received Rhucin. Due to the nature of HAE, being a heterozygous disorder with patients still expressing normal C1INH, patients are not naïve and immuno-tolerant C1INH. This is different from deficiencies like haemophilia A. Patients with haemophilia have no FVIII, are naive for FVIII administered, and are at risk to develop inhibitors to FVIII. In contrast patients with HAE are immuno-tolerant to C1INH similar to healthy volunteers. Testing a large number of plasma samples from healthy volunteers or HAE patients that had received Rhucin, for the presence of antibodies to C1INH, indicated that above cut-off levels of anti-C1INH occurred sporadically. Similar above cut-off level findings occurred in plasma samples of patients prior to the first administration of Rhucin and in post-treatment plasma samples from placebo treated subjects. Therefore, the isolated above cut-off anti-C1INH antibody findings in subjects after the administration of Rhucin, often returning to below cut-off levels at later time-points, are regarded not to be clinically relevant.

Clinical efficacy responses were similar in 12 HAE patients who were treated with repeat administrations of Rhucin for subsequent attacks.

Impact of antibodies against HRI: These antibodies potentially impact on the safety of Rhucin. Pharming has evaluated the development of anti-HRI antibodies in all subjects participating in the clinical development program for Rhucin.

In 11 healthy volunteer subjects, that were administered Rhucin on 5 subsequent occasions, above cut-off levels of anti-HRI antibodies were found in 8 subjects after the fourth Rhucin administration. The relevance of these findings is doubtful since 2 subjects had above cut-off levels prior to the first administration of Rhucin. In addition, no clinical safety concerns including allergic reactions occurred in these healthy volunteer subjects.

One female healthy volunteer subject, who had developed an anaphylactic reaction during the first administration of Rhucin, retrospectively appeared to have a history of multiple clinically relevant allergies including rabbit allergy, and was excluded from further participation. Rabbit allergy has been included in the SmPC as a contra-indication to receive Rhucin.

In HAE patients, there were no anti-HRI antibody findings above the arbitrary cut off level before or after a single dose administration of Rhucin.

Findings from the anti-HRI ELISA's did not show any evidence for the development of anti-HRI antibodies after repeat administration of Rhucin, except for one finding in one patient. In this particular patient, after the 5th administration of Rhucin, an isolated above cut-off value was found, without any report of adverse reactions.

The company has proposed and confirmed in a draft risk management plan to establish a prospective post-approval registry of the first 300 administrations of Rhucin. The company understands that this registry is accepted by the CHMP as a satisfactory means to monitor for the possible development of anti-C1INH and anti-HRI antibodies as well as the possible clinical consequences for efficacy and safety, particularly, in their conclusions of the review of the company's responses to Day 180 List of Outstanding Issues questions 1D, 7, 12 and 16. As part of the risk management post-marketing surveillance program/ post-approval registry, blood sampling will be undertaken to test for the development of antibodies to HRI and C1INH. Above cut-off findings will be reviewed by an independent data monitoring committee. For anti-HRI antibodies, there will be further laboratory testing using confirmatory displacement assays for specificity with rabbit milk and a range of immunologically relevant individual milk proteins. For anti-C1INH antibodies there will be further laboratory testing to confirm the potential of these antibodies to adversely effect the activity of C1INH ("neutralizing antibodies").

CHMP position:
In healthy volunteers receiving 5 administrations of Rhucin 100 U/kg, an IgM response was seen at 7 days after the 3rd, 4th and 5th administration. There was no increase in IgM antibody level in predose samples before these administrations. Thus, as the IgM response would have taken some time to develop after administration of Rhucin while rhC1INH is relatively rapidly cleared from plasma ($t_{1/2}$ 2-3 hours), the fact that there was no increase in clearance of rhC1INH after the 3rd, 4th and 5th administration cannot be regarded as proof that the antibodies formed are not neutralising or could not affect elimination in the long-tem.

Although the incidence was high, and eight out of eleven healthy volunteers developed antibodies upon repeated administration of Rhucin, it is agreed that the clinical consequences most likely are limited as the reaction was mild, leading only to a moderate increase in anti-rhC1IN IgM levels and no persistent IgG response. However, data currently available are too limited to confirm a similar immunogenic profile in HAE patients and support that C1 inhibitor deficiency by either lower inhibitor levels or the expression of a dysfunctional protein will not confer an increased risk for development of an IgG response. In the study on healthy volunteers, the increase in anti-rhC1IN IgM levels was seen starting from the third administration, and so far, data is solely available on two HAE-patients receiving the third dose.

As the anti- rhC1IN antibodies appear to be cross-reactive, they may act to reduce the activity of the endogenous protein. Therefore it can not be excluded that an immune reaction could increase the frequency of more severe attacks.

Ground for refusal 3:
The choice of dose has not been sufficiently justified

Applicant's position:

The clinical development justification to evaluate the efficacy and safety of Rhucin administered at a dose of 100 U/kg to treat acute angioedema attacks in HAE patients was based on the following considerations.

Clinical pharmacology
The normal physiological state in healthy subjects supports the pharmacodynamic assumption that acute angioedema attacks cannot occur if endogenous C1INH activity is maintained above the lower limit of the normal range (0.7-1.3 U/ml plasma).
Pharmacology findings submitted in the original submission indicate that Rhucin has a different pharmacokinetic profile from that published and provided in the summary of product characteristics of plasma derived C1INH products. In particular, Rhucin has a substantially shorter half life. This is readily explained by the well understood differences found in glycosylation of recombinant DNA proteins compared to their endogenous counter parts.
As would be expected from the known differences in half life, the duration of these pharmacological effects of Rhucin are shorter. The observed duration of Rhucin's favourable effect on C4 consumption is dose dependent. The pharmacodynamic effect of Rhucin at 100 U/kg persists for at least four hours whereas that of 50 U/kg persists less than 2 hours. Most importantly, it is clear from the PK and PD data that to restore C4 homeostasis in HAE patients a C1INH activity level greater than 0.7 U/ml is required, that is, the lower limit of the normal range. Rhucin administered at doses of 50 and 100 U/kg was found from these data to achieve an activity level greater than 0.7 U/ml for more than 2 hours.
Finally, the Applicant selected 100 U/kg for the Rhucin clinical development program and 50 U/kg was rejected because this dose was not expected to maintain C1INH activity above 0.7 U/ml for more than 4 hours.

Rhucin dose response
The Company considers that the absence of a thorough dose response evaluation to determine the optimal effective dose of Rhucin for use in the treatment of acute angioedema attacks needs to be placed in the context of:
- the many examples of medicinal products with regulatory approval where the optimal dose and dosage schedule has not been established
- the prevalence of Hereditary Angioedema (HAE)
- the low frequency of acute angioedema attacks that require replacement therapy with C1INH are critical and justifiable mitigating factors that need to be taken into consideration to determine the acceptability of the extent of the Rhucin clinical database currently available to provide adequate reassurance about efficacy and safety.

Because of the more rapid clearance, Rhucin was clinically evaluated at a higher dosage compared to current and historical clinical practice with pdC1INH. The company considers that the 100 U/kg dose has proven to be appropriate because there has been a favorable therapeutic response in all cases of replacement treatment with Rhucin in the reported open label and blinded studies (C1 1201-01, C1 1203-01 and C1 1304-01) and there have been no reported relapses or need for any other supportive care or rescue treatment.
It should be noted that there are no adverse event data to suggest that the differences found in the PK profile between Rhucin and pdC1INH raise any clinical safety or efficacy concern.

Recently results of placebo controlled double blind clinical trials on the efficacy of Cinryze™ and Berinert® P have been presented.

- In the clinical study for Berinert® P, IMPACT, two doses of were investigated, 10 U/kg and 20 U/kg. The Applicant concludes that it can be assumed that a dosage of 20 U/kg of Berinert® P is the minimal effective dose for the treatment of acute HAE attacks.
- The dose used for Cinryze™ in the CHANGE study was 1000 U followed by another administration of 1000 U (1000 U + 1000 U). The results of the CHANGE trial (Relief within 4 hours: Cinryze™ 60%, placebo 42%) suggest that Cinryze™ at a dose of 1000 U + 1000 U (equal to 30 U/kg) may be an inadequate dose in the treatment of HAE attacks.
From the preliminary reports of these studies it has become apparent that there is no "well established" agreement about the appropriate "normal dose" of pdC1INH.

Efficacy in support of Rhucin at a dose of 100 u/kg
The efficacy of Rhucin at 100 U/kg was evaluated in three clinical studies: C1 1202-01, C1 1203-01 and C1 1304-01. In addition, in the United States of America and Canada the efficacy of Rhucin is being evaluated in another placebo controlled randomized clinical trial with an open label treatment extension (study C1 1205-01). This is a study in which Rhucin at 50 U/kg and 100 U/kg is compared to placebo. The Applicant considers that these preliminary data indicate that the clinical outcomes were less satisfactory compared to those obtained from studies with Rhucin 100 U/kg. In particular, there are several concerns,
- Not all 45 acute angioedema attacks in 34 patients treated at lower dosages in the open label extensions of studies C1 1205-01 and C1 1304-01 responded to treatment.
- In study C1 1205-01, where the protocol specified and permitted (provisional) second dose of Rhucin, 50 U/kg, was administered to three patients at the discretion of the investigator, these patients appeared to achieve minimal symptoms later compared to the patients who responded after a single dose of Rhucin.
- Four patients, in study C1 1205-01, did not respond to treatment, after Rhucin, 50 U/kg; two did not respond after a single dose of Rhucin, 50 U/Kg, and one did not respond after Rhucin, 50 U/kg followed by the provisional second dose of Rhucin. For one patient, who reportedly did not respond to treatment, VAS data are presently unavailable.
- In study C1 1304-01, one patient received a provisional second and third dose of Rhucin (Total dose 6300 U), at the discretion of the investigator. Also this patient appeared to achieve minimal symptoms later (24 hours) compared to the patients who responded after a single dose of Rhucin.
The company concludes that taken overall, these clinical pharmacology, efficacy and safety data findings suggest that the most optimal and preferred dose of Rhucin for the treatment of acute severe angioedema attacks is 100 U/kg.

<u>CHMP position:</u>
Based on the literature, a duration of at least four hours of C1INH activity above the lower limit of the normal range is necessary to obtain a satisfactory treatment result, e.g. time to the onset of relief within four hours in 100% of cases without relapse of the initial angioedema attack.
The results of the study C1 1205-01 are of great relevance for posology, since doses of 50 and 100 UI/kg are investigated for safety and efficacy in HAE patients.
In the study C1 1205-01, 31 open-label treatments with Rhucin for acute attacks of angioedema have been given to a total number of 20 patients. For 29 attacks in 19 patients data were available. For 26 attacks, patients responded to treatment with Rhucin for an acute attack of angioedema. In 23 of those 26 attacks, patients responded to treatment after a single dose of Rhucin, 50 U/kg.
Clinical observation data are available from four acute laryngeal attacks successfully treated with Rhucin in the open label phase of North American study protocol C1 1205-01. For three laryngeal attacks in four patients treated with Rhucin, 50U/kg, time to the beginning of relief occurred within 4 hours. One patient who had a laryngeal attack (patient 33-001), received an initial dose of Rhucin at 50 U/kg, and although the attack had responded (time to the beginning of relief after 60 min), the patient was administered the provisional second dose of Rhucin, 50 U/kg, 3 hours after the first dose, because at that time the patient still was not free of attack symptoms (total dose 100 U/kg). Time to minimal symptoms occurred after 214 minutes after the first administration of Rhucin.
The applicant concludes that these findings add more reassurance, at present, that the optimal dose of Rhucin for the treatment of acute severe angioedema attacks is 100 U/kg.
Furthermore, in study C1 1304-0, the posology informations are only expressed as a total administered dose, without any indications of patients weight. Noteworthy, 12 out of 14 patients received a single

dose of 2100 U Rhucin, which appears to be efficacious and safe. If the SPC recommendation of a dosing of 100 U/kg has been followed, the 12 patients' weight would be around 21 kg, which seems unlikely for adults.
Moreover the higher doses required for Rhucin, compared to the doses of plasma-derived C1INH (10-20 U/kg), could boost the risk for thromboembolic complications which were already reported in very high doses of plasma-derived C1INH. This risk has not been adequately addressed by the applicant.

Based on the data available to date, the CHMP considers that the choice of the dose recommended in the SPC is not fully justified.

Ground for refusal 4:
Sufficient reassurance on quality aspects has not been provided. The following issues are outstanding:

i. It has not been adequately justified that the characterisation of active substance or product is sufficient to detect and identify Host Related Impurities (HRI) which could be present in immunologically significant quantities
ii. The validation of the HRI-ELISA has not been justified and it has not been justified that this test is capable of detecting quantitatively any HRI in the active substance which is present in immunologically significant quantities (i.e. that may cause antibody responses and related adverse events)
iii. It has not been adequately justified that the quantity of host related impurities in the active substance from the validation batches, as detected by the HRI-ELISA, is quantitatively accurate and within acceptable specifications
iv. It has not been adequately justified that the ELISA to detect antibodies to HRI raised by patients is adequately validated to ensure detection of antibodies raised to relevant proteins
v. Satisfactory post-authorisation commitments to address other outstanding quality issues would need to be agreed

Applicant's position:
i. In order to provide re-assurance with respect to the level of HRI in active substance, the Company has conducted further studies. Three analytical approaches have been applied to address the concerns of the CHMP:

- First, a lab-scale purification run with non-transgenic rabbit milk was performed in order to assess the concern of co-migrating proteins in the region of rhC1INH on SDS-PAGE.
- Second, the limit of detection of the immmunoblotting method has been confirmed in the presence of large amounts of rhC1INH.
- Finally, in order to provide re-assurance with respect to the 'true' level of HRI in active substance, the Company has developed a strategy to assess the removal of 'model' rabbit proteins by the down stream process.

ii. With respect to the proposed HRI-ELISA and the lack of justification that this test is capable of detecting quantitatively any HRI in the active substance which is present in immunologically significant quantities (i.e. that may cause antibody responses and related adverse events), the applicant has argued that as with all recombinant DNA platforms, the impurities do not consist of a finite number of proteins that can be quantified but rather a theoretical range of contaminants that can only be present at very low concentrations. The strategy applied by the Company in controlling total HRI(s) is in accordance with Ph.Eur. and the HRI-ELISA is considered appropriate for the monitoring and consistent removal of HRI by the purification process. The Company acknowledges that the ELISA is not intended and suitable for the quantification and characterisation of individual HRI(s) in active substance. The Company will address the immunological significance of individual HRI(s) by implementation of confirmatory testing using competitive displacement with diluted milk and individual milk proteins. In the circumstances where the confirmatory displacement assays identify specific immunogens, the Company will assess the responsiveness of the protein in the HRI-ELISA. If required, a specific ELISA will be developed and implemented at the level of active substance.

iii. With respect to the inadequate justification for the quantity of host related impurities in the active substance based on results from the validation batches, as detected by the HRI-ELISA, the Company indicated that this concern can only be resolved prospectively by the proposed specific studies to

extend identification of possible individual impurities in active substance using approaches such as immuno-affinity chromatography.

iv. With respect to the inadequate justification for the use of the ELISA test to detect antibodies to HRI raised by patients, the Company clarified that the current anti-HRI testing strategy to detect possible antibodies raised by patients or healthy volunteers relies on a two-step approach: an anti-HRI ELISA and a confirmation assay intended to discriminate between specific and non-specific responses in the anti-HRI ELISA. The confirmatory assay has been validated and has been implemented into the standard testing approach for the detection of anti-HRI antibodies. Retrospectively, subject samples that have shown responses above the cut-off level of the anti-HRI ELISA are presently being subjected to the confirmatory displacement assay.

v. The applicant have provided an updated list of post-authorisation actions to address outstanding quality issues and made the commitment to undertake these post-authorisation commitments to fulfil the conditions for approval of Rhucin on Quality grounds.

CHMP position:
With their grounds for re-examination, the Company provided in writing data they presented at an oral clarification meeting held on 3 December 2007.

Regarding the presence of HRI in active substance, the Company has applied three analytical approaches:

- First, a lab-scale purification run with non-transgenic rabbit milk was performed in order to assess the concern of co-migrating proteins in the region of rhC1INH on SDS-PAGE.
- Second, the limit of detection of the immunoblotting method has been confirmed in the presence of large amounts of rhC1INH.
- Finally, in order to provide re-assurance with respect to the 'true' level of HRI in active substance, the Company has developed a strategy to assess the removal of 'model' rabbit proteins by the down stream process.

This additional characterisation data presented together with the post-approval commitments are considered satisfactory to assure that Host Related Protein (HRI) is limited to acceptably low levels.

To demonstrate that the proposed HRI-ELISA is capable of detecting quantitatively any HRI in the active substance which is present in immunologically significant quantities, the company has provided data to demonstrate that three model proteins can be determined by individual specific ELISAs. The new data provided show that the residual quantities of the three model proteins are very low or undetectable. This is evidence that titration curves of important milk proteins are similar to the titration curve of the standard (skimmed milk).

With respect to the inadequate justification for the use of the ELISA test to detect antibodies to HRI raised by patients, the company proposed a two-step strategy to assess specific immune response to individual milk proteins in treated patients: an anti-HRI ELISA and a confirmation assay intended to discriminate between specific and non-specific responses in the anti-HRI ELISA. This approach is adequate and the applicant's commitment to develop further analytical assays to control identified immunogen and to include them as active substance batch release assays is acceptable.

In conclusion the quality related grounds for refusal are not maintained.

Grounds for refusal 5:

A satisfactory summary of product characteristics and risk management plan would need to be agreed.

Applicant's position:
An updated Summary of Product Characteristics was provided. In the updated version, appropriate changes have been made based on the comments made during the initial evaluation. QRD comments have been implemented except for one QRD comment which was not considered relevant for a parenteral product according to the relevant guideline on excipients in the labelling.

The Risk Management Plan (RMP) has been adapted in accordance with the outcome of the initial evaluation of Rhucin. This revised RMP takes into account all previous CHMP comments and includes the applicant's proposals for post authorisation commitments. In addition the RMP has been updated based on the clinical database available at 1 February 2008.

CHMP position:
The submitted risk management plan is in accordance with the EU guideline on risk management systems for medicinal products.
In the RMP, the safety specification adequately reflects the available safety data based on the very limited clinical experience and highlights the need for a post-authorization safety study, in the case of a MA approval. However, the applicant considers thrombo-embolic complications as a potential risk in case of off- label use at very high dosages of rhC1INH. Given the biological plausibility, this potential risk should also be considered for indicated dosages and should be followed through the ongoing studies and post-authorization safety study.

Given the limited extent of the safety database, there is a need to further assess the nature, frequency of identified and potential risks of the products as well as to identify risk factors.
In this respect, the results of the two ongoing randomised placebo-controlled clinical trials will be of particular interest.

Overall, the safety specification adequately reflects the safety information obtained from the very limited clinical safety database and highlights the need for post-authorisation safety study. Such a study needs to be set up to further assess the nature, frequency, risks factors and preventability of allergic reactions and infusions reactions as well as important potential risks including development of anti-HRI antibodies and anti-rhC1INH-antibodies (IgM, Ig G and IgA isotypes) as well as thrombo-embolic complications.
The applicant proposes to set up a post-marketing registry with the aim to follow the first 300 infusions of Rhucin. A detailed synopsis for this registry was lacking. No details of objectives, inclusion and exclusion criteria, sample size, duration of follow-up have been provided.

As a consequence the proposed post-approval measure is not considered sufficiently documented and the RMP is not considered satisfactory.

Views of the ad hoc expert group on Rhucin

For the evaluation of the grounds for re-examination, the CHMP decided to consult an ad hoc expert group for Rhucin consisting of:
- experts with expertise in hereditary angioedema (HAE) including physicians with personal experience in the treatment of patients with HAE and
- experts with specific experience about immunological consequences of the administration of recombinant DNA proteins to patients.

The ad hoc expert group met on 10 March 2008.

The expert commented that currently available therapies consist of plasma-derived products, which, in clinical practice are given for treatment of serious, life-threatening attacks or when swelling is very painful and can affect blood flow. These severe attacks are not observed frequently.
Patients are reluctant in receiving this sort of treatments due to their concerns about possible transmission of pathogens (e.g. viruses and prions). Delayed treatment may be fatal in case of laryngeal oedema. C1 INH could also be useful in the lesser forms of attacks.
Patients expect the best treatment they can tolerate. For some patients, tranexamic acid is used for years or danazol for male subjects. Short-term therapy using e.g. Rhucin would allow better control of attacks since the patient would worry less about taking the product (e.g. because of fear of infection with plasma-derived products).
Besides the indications given above, preventive treatment is recommended before surgery and before dental extractions. During pregnancy, the frequency and severity of attacks is often increased while the use of tranexamic acid or attenuated androgens is contraindicated; therefore one relies more easily on

C1 INH infusions. Prophylaxis using C1 INH concentrates is essential for patients who are resistant to tranexamic acid and with contra-indications for danazol (androgen).

With respect to the grounds for re-examination the experts had the following views:

1. The size of the clinical database is small and does not provide adequate reassurance in relation to
i. efficacy in more severe forms of the disease, e.g. laryngeal oedema
ii. safety and efficacy on readministration

It was acknowledged that the size of the clinical database is in fact limited; of special concern is the very limited number of patients treated for laryngeal attacks. Nevertheless, the experts agreed that the data provided demonstrated efficacy in less severe forms of attacks and could be extrapolated to the more severe forms of the condition (e.g. laryngeal oedema).

Extrapolation of efficacy from the experience of treatment of less severe forms to the laryngeal oedema attacks was considered acceptable based on the following argumentation. The pathophysiology of HAE attacks is considered to be the same regardless of the attack location. Laryngeal mucosa is expected to be the same as the other kinin targets, the difference being in terms of diffusion through blood vessels from the injection site. The data given at the expert meeting are in agreement with this postulate. The severity of the attacks depends on their location: submucosal attacks (laryngeal and abdominal) are the most severe forms, although the extent of oedema in these forms is smaller. The extent of oedema plays a major role in the response to treatment; thus, one can assume that the smaller the extent of the oedema, the faster the response to treatment. Generally severe attacks involving laryngeal oedema respond faster to the therapy with plasma-derived products than attacks involving the gastrointestinal system or the skin.

Clinical experience with respect to re-administration with Rhucin is very limited so far. Nevertheless, the experts considered that there was no indication of loss of efficacy over time on re-administration. However, only 2 patients received more than 2 administrations. Furthermore, there was no evidence of an increase in side effects, and in this respect safety data are reassuring. However this would need to be confirmed. An appropriate follow-up protocol for patients treated with Rhucin would include measuring C1 INH function, C4 antigenic level, and the titration of anti-C1INH antibodies each year (see also below).

2. There is insufficient reassurance on immunogenicity with repeat administration in patients, including its impact on safety and efficacy

The experts acknowledged that anti C1 INH antibodies development can also occur after treatment with plasma-derived C1 INH, in particular when treatment is frequent.

The experts reported a case with antibodies raised against plasma-derived C1 INH. The patient had an increased frequency of attacks and need for treatment and had urticaria. This was an exceptional occurrence. Although there was no strong evidence for the development of neutralising antibodies to C1-inhibitor with repeat administration of Rhucin, it was recognised that the available clinical data were limited. Furthermore, the ELISA method used by the applicant may lead to underestimation of the level of antibodies since antibodies are captured by the high excess of the administered target antigen, i.e. C1 INH.

It was considered that immunogenicity should be further investigated. In this respect, the tests on kinetics of the C1 INH activity in deficient patients which are treated repeatedly are very important and useful.

On the other hand, the possibility of anaphylactic and severe allergic reactions was viewed as a serious concern. During the clinical development of Rhucin one anaphylactic reaction was observed in one healthy volunteer; no such episodes were seen in any of the patients. Such a reaction in a patient with an angioedema attack could be life-threatening. Although it is highly likely that the anaphylactic reaction in the healthy volunteer, who was a polysensitised atopic individual, was against rabbit milk

protein and due to her rabbit dander allergy (positive RAST), the exact nature of the allergen present in Rhucin that had triggered the reaction has not been further investigated whereas tests could have been performed using the serum. In this respect, it was noted that the risk of IgE response in the treated population had not been adequately addressed by the applicant. Risk to patients with allergic reactions to animals and to milk proteins has to be considered since the trigger had not been identified and cross-reactivity is possible (in particular cross reactivity to rabbit, cat and rat allergens). The experts highlighted that about 8-15% of the general population, and probably of the HAE population, was atopic with a risk of developing IgE following repeated injections of any foreign protein.

The experts considered that it was unfortunate that the applicant did not take the opportunity of obtaining a serum sample from the normal subject who had an anaphylactic reaction, most likely to a component of Rhucin, to elucidate the allergenicity of impurities. This could easily be done with IgE immunoblots on Rhucin. Not only the serum of this patient could be used, but also that of other rabbit and cow's milk allergic patients, first to identify the responsible allergens, secondly to further elucidate the risk of anaphylaxis. Such studies should be undertaken as soon as possible. Also, all participants for further studies, and all patients who might be treated with Rhucin, should be screened on RAST for IgE antibodies against rabbit dander, rabbit meat and cow's milk proteins. Alternatively, a RAST analogue could be developed to detect IgE against host derived impurities in Rhucin.

In conclusion, the experts considered that further investigation is required including screening of patients for possible allergies with monitoring of IgE responses before and after treatment.

3. The choice of dose has not been sufficiently justified

The experts observed that interpretation of the data was more difficult given that in some cases the dose was not expressed as U/Kg.
The experts agreed that the proposed dose (i.e. 100 U/Kg) was acceptable although it had been poorly justified (since it had been calculated based on badly documented weights of patients) and that a lower dose of 50 U/Kg may be equally efficacious although this has not been demonstrated on the basis of available data. The experts agreed that proper dose response studies in a condition as rare as HAE would be difficult to perform and therefore the strategy followed by the applicant to study the higher dose of 100 U/Kg first and subsequently investigating the efficacy of lower doses was thus considered an acceptable approach.

Additional comment from the experts

The applicant confirmed that Rhucin is not intended for prophylactic use. The experts considered that the wording in the SPC "replacement treatment" could be misleading. There should be no suggestion that Rhucin was indicated for prophylactic use and the indication should rather state only "treatment". It should be specified that it was for use in patients with HAE type 1 and type 2. It is not for treatment of type 3.
Therefore, the correct clinical diagnosis is important in this respect and although genetic tests are not easily available, low C4 and low C1 INH activity in-between attacks is sufficient proof to confirm the diagnosis.

Overall conclusions on grounds for re-examination

Quality issues
With their grounds for re-examination, the Company provided in writing data they presented at an oral clarification meeting held on 3 December 2007.
The grounds for refusal relating to product purity, validation of a method (HRI-ELISA assay) for batch release purposes and validation of the clinical assay for detection of human antibodies raised to rabbit milk proteins have been satisfactorily addressed by the Company.

Except for a number of points, which will have to be addressed as part of post-authorisation commitments, the quality of this product is considered to be acceptable when used in accordance with the conditions defined in the SPC. Physicochemical and biological aspects relevant to the uniform

clinical performance of the product have been investigated and are sufficiently controlled. Viral safety or freedom from other adventitious agents (including TSE) has been adequately demonstrated. Batch to batch consistency has been documented and the relevant tests will be performed according to the agreed specifications.

Clinical efficacy and safety issues

The CHMP agreed with the ad hoc expert group that there is insufficient reassurance on immunogenicity with repeat administration in patients, and that this should be further investigated. In the clinical studies performed, only 2 patients received more than 2 repeated administrations. No individual received Rhucin within 3 weeks from the previous administration. Overall, the efficacy data made available from re-administrations were too limited and therefore the evaluation of efficacy over time appeared not possible. Moreover, it was noted that Rhucin, as any therapeutic recombinant protein, can potentially elicit an immune response, which can have an impact on both anti C1 INH and anti-HRI antibodies development. In the repeat-dose study in healthy volunteers (Study C1 1106-02), there was a trend to increasing anti C1INH IgM levels with increasing number of administrations, starting from the third administration of Rhucin, although levels remained below the assay cut-off. According to the last submitted listings of laboratory data, one patient in the open label extension of study C1 1205-01, who was the first to receive more than three doses of Rhucin, showed an increased anti-rhC1INH IgM level of 56% (cut-off level 50%) and an increased anti-rhC1INH IgG of 14% (cut-off level 15%) on one occasion. No conclusions can be drawn from these currently available laboratory data. Since repeated doses are expected throughout a patient's life, the available clinical safety database with respect to repeat administration in patients was deemed to be insufficient to assess the immunogenicity of the product.

In addition, the safety database provided did not permit a sufficient assessment of the risk for hypersensitivity/allergic reactions. In fact, during the product's early development there were safety signals of concern emanating from Rhucin administration to healthy volunteers. One anaphylactic reaction occurred in a healthy volunteer 2 minutes after Rhucin administration. IgE RAST and medical history indicated rabbit allergy. In this respect, it was considered that the risk of IgE response in the treated population had not been adequately addressed by the applicant. The CHMP agreed with the ad-hoc expert group that the possibility of anaphylactic and severe allergic reactions was a serious concern.

Overall, the clinical dataset was judged to be too limited to sufficiently show safety and efficacy of Rhucin in re-administration, and this remained a major objection. The CHMP concluded that further studies in patients were required to explore an immunogenic potential with repeat administration including the possibility of anaphylactic and severe allergic reactions elicited by treatment with Rhucin.

The CHMP took into account the ad hoc expert group's position that, based on the clinical experience with the plasma-derived C1INH, the efficacy data from the less severe forms of the disease could be extrapolated to the laryngeal oedema attacks.

With respect to the choice of the dose, the data provided indicated that a lower dose of 50 U/Kg may be equally efficacious, but this remained to be demonstrated. The CHMP agreed that extensive dose-finding studies in a condition as rare as HAE would be difficult to perform and agreed with the ad hoc expert group's conclusion on the acceptability of the dose recommended (i.e. 100 U/Kg), even if it was poorly justified.

Finally, the CHMP agreed with the ad hoc expert group that appropriate risk management activities (e.g. the need for a post-authorization safety study) would need to be put in place.

The safety specification included in the revised risk management plan reflected the available safety data which were based on the very limited clinical experience. The potential risk for thrombo-embolic complications was also considered in the revised RMP, both in case of off- label use at very high dosages of C1INH and for the indicated dosages. This potential risk for thrombo-embolic complications was proposed to be followed through the ongoing studies and the proposed post-authorisation safety observational study.

Following the applicant's initial proposal to follow up the 300 first infusions of Rhucin in a post-marketing observational study and to set up a post-marketing registry, a synopsis for the observational study C1 1407-01 was submitted within the revised RMP but without detailed information on the proposed registry and a detailed protocol. Importantly, a rationale for the proposed sample size (evaluation of data on 300 treatments in enrolling 600 patients) was not provided nor was there any information about the expected number of patient with repeated treatments. Although, during the oral explanation the applicant additionally informed the CHMP about the possibility to use an already existing EU-registry of HAE patients currently covering 11 EU Member States, the CHMP did not consider that enough information was available at this time to evaluate the adequacy of the proposed measures to collect missing data regarding the risk of immunogenicity with repeated treatment and of anaphylactic or severe allergic reactions.
In addition it was considered that the applicant did not investigate enough the anaphylactic reaction that occurred in the healthy volunteer in order to identify with certainty the responsible allergen. As a consequence the exact risk factors for potential anaphylactic or severe allergic reaction have not been fully characterised and, with the data currently available, the adequacy of the risk management proposed cannot be evaluated.

The CHMP considered that because the safety profile has not been sufficiently elucidated, it is not possible at this time to evaluate the adequacy of the risk management plan.

Overall conclusions on grounds for re-examination

In conclusion, the CHMP considered that, following re-examination of the data provided, major concerns remained with respect to the safety and efficacy of Rhucin and that the risk-benefit balance of Rhucin was unfavourable.

Three CHMP members did not agree with the conclusions of the CHMP with the following divergent position:

– The antigenicity and allerginicity of protein medicinal compounds, and the clinical consequences thereof, are never known at the time of licensing; consequently this is not a reason to refuse the granting of the Marketing Authorisation for Rhucin.

With the re-examination, the CHMP considered whether the application for Rhucin met the requirements for a conditional marketing authorisation as proposed by the applicant. The CHMP concluded that Rhucin did not meet the requirements for a conditional marketing authorisation since the risk-benefit balance of the product was not positive.

GROUNDS FOR REFUSAL

Whereas

– The size of the clinical database is considered too small to provide adequate reassurance on efficacy and safety, including immunogenicity with repeat administration in patients (and its impact), and serious concerns with respect to the occurrence of anaphylactic and severe allergic reactions with Rhucin.

the risk-benefit balance of Rhucin in the treatment of acute attacks of oedema in patients with hereditary angioedema cannot be considered positive, and therefore the CHMP has recommended the refusal of the granting of the Marketing Authorisation for Rhucin.

Recommendation

Based on the CHMP review of data on quality, safety and efficacy, the CHMP considered by a majority of 27 out of 30 votes that the risk-benefit balance of Rhucin in the treatment of acute attacks of oedema in patients with hereditary angioedema was unfavourable and therefore did not recommend the granting of the marketing authorisation.