SCIENTIFIC DISCUSSION

1. Introduction

ATryn contains recombinant human antithrombin (rhAT), purified from the milk of transgenic goats.

Antithrombin (AT) is a serine protease inhibitor that is the principal inhibitor of the blood coagulation serine proteases thrombin and Factor Xa, and to a lesser extent, Factors IXa, XIa, XIIa, trypsin, plasmin, and kallikrein. AT neutralizes the activity of thrombin and other serine proteases by forming a complex between enzyme and inhibitor. This complex is rapidly cleared from the circulation by internalization into liver cells. In the absence of heparin, complex formation occurs at a relatively slow rate. When heparin or heparin sulfate on the vessel wall is present, however, it binds to lysyl residues on AT and dramatically accelerates the rate of complex formation. The ability of AT to inhibit thrombin can be enhanced by greater than 1000 fold when AT is bound to heparin. Antithrombin has also been referred to as a heparin cofactor. Localization of a fraction of the AT on the endothelial surface, where enzymes of the intrinsic coagulation cascade are commonly generated, enables AT to rapidly neutralize these activated clotting factors and protect natural surfaces against thrombus formation.

Normal human plasma AT concentrations range between 12.5 – 15 mg/dl. The AT activity of 1 ml of pooled normal human plasma in a thrombin or factor-Xa inhibition assay is defined as 1 IU/ml. AT activity levels are often reported as a percentage of the activity of normal human plasma and therefore set to 100%. Normal ranges for the assays mostly are between 80% and 120%.

Congenital AT deficiency is a heterozygous autosomal dominant disorder with a prevalence of about 1 in 3,000 to 5,000 in the general population, characterized by a decrease in antithrombin (type I) or the presence of a dysfunctional form (type II-a: reactive site defects: Type II-b: heparin binding defects; Type II-c: pleiotropic effects). In type I deficiency, AT activity and plasma AT concentration are concordantly reduced, indicating that no protein is produced by the mutant allele. In type II deficiency, the AT concentration is (near-) normal, but the AT activity is discordantly reduced, indicating a functional impairment of the molecule produced by the mutant allele. Distinction between the subtypes of antithrombin deficiency is of clinical relevance, since the incidence of thrombosis is higher in association with type I deficiency and type II-a deficiency, where the mutation affects the reactive site, as compared to type II-b deficiency where the mutation affects the heparin-binding site.
The genetic defect of the AT gene is located at chromosome 1q23-25. Type I deficiencies are produced by major gene deletions or point mutations. Single base substitutions within coding regions giving rise to variant antithrombins are the basis of all type II AT deficiencies identified to date.
Homozygotism (total deficiency of antithrombin) is very rare and thought to be not compatible with life. The majority of affected individuals are heterozygous. A partial (heterozygous) deficiency with a biological activity around 50% of normal levels may be associated with familial thrombophilia, predisposing up to 70% of affected family members to suffer from thrombosis, before they reach an age of 40 years. In patients with type I congenital AT deficiency, AT levels are usually between 40 and 50% of normal. The prevalence of congenital AT deficiency in the general population of asymptomatic subjects is unknown. Reported estimates of the prevalence of type I congenital AT deficiency in blood donors range from 0.02% to 0.14%. In unselected patients presenting with venous thrombosis, the prevalence of AT deficiency is around 1% but in patients with deep vein thrombosis who have a family history of venous thromboembolism the reported prevalence of AT deficiency is between 4% and 7%.

Thromboembolic events in congenital AT deficient patients are uncommon prior to puberty, but increase thereafter, particularly during periods of high risk, such as pregnancy, surgery, or bed rest. Regular prophylactic substitution of antithrombin in patients with congenital deficiency is not necessary. However, substitution with antithrombin in congenitally deficient individuals is considered for prophylaxis of thrombosis in certain risk situations such as the peri-partum period or major surgery, or for treatment of thrombosis:

- Surgical procedures are often accompanied by temporary immobility of the patient, which increases the risk of thromboembolism. Also, depending on the extent of tissue damage and hemorrhage, AT levels decline during surgery, which amplifies the deficiency in congenital AT deficient patients. Major surgery like orthopedic procedures, abdominal surgery or major vascular surgery is clearly associated with a high risk for thromboembolic events and consequently thromboprophylaxis is normally administered to subjects undergoing these procedures. This prophylaxis often consists of unfractionated (UF) or low molecular weight (LMW) heparin. The anticoagulant effect of either UF or LMW heparin, however, can only be established in the presence of sufficient levels of AT.
- Pregnancy is also a risk because it is typically a hypercoagulable state, due to a physiologic increase in coagulation factors and a decrease in fibrinolytic activity. Therefore, pregnant women with congenital AT deficiency are often given anticoagulant prophylaxis. Warfarin is usually avoided during pregnancy, particularly during the first 3 months due to teratogenic potential and during the last few weeks prior to delivery due to bleeding risk or the fetus. A heparin is often the anticoagulant of choice, as it does not cross the placenta. Rarely administration of AT concentrate in addition to a heparin may be necessary to achieve adequate anticoagulation. The use of antithrombin III concentrate during pregnancy in women can circumvent the problems associated with both warfarin therapy and prolonged high-dose heparin therapy.

It has been stated in literature that replacement therapy with AT concentrate (either alone or in combination with heparin therapy) would be beneficial when heparin therapy alone is not effective (i.e. heparin resistance), during acute thromboembolic episodes or when antithrombotic prophylaxis is needed in high-risk situations for both thrombotic and bleeding complications, such as delivery, surgery or trauma. The approved indications of AT concentrate in congenital AT deficiency are perioperative, post-surgical prophylaxis for deep vein thrombosis, acute thrombo-embolism, pregnancy (delivery and abortion) and neonates with congenital AT deficiency.

In the re-examination documentation the applicant discussed that eight EU countries (with a total populations of 23 million) do not have approved hpAT product, which limits the availability of AT concentrates to treat those patients

2. Part II: Chemical, pharmaceutical and biological aspects

Composition

ATryn contains recombinant human antithrombin (rhAT). The active moiety in ATryn is a recombinant form of the naturally occurring human antithrombin (AT) glycoprotein and is produced in the milk of transgenic goats.

ATryn is presented as a powder for intravenous infusion in a vial containing 250 mg antithrombin alfa. The 20 ml type I glass vial is closed with a siliconised bromo-butyl rubber stopper, sealed with an aluminium seal and a flip-off cap. ATryn is at a concentration of 175 IU/ml (25 mg/ml) after reconstitution with 10 ml water for injection.

The composition of the product is the following table:

Table 1

Name of Ingredient	Concentration	Composition per vial
Active Ingredient		
antithrombin alfa	175 IU/ml	250 mg (1750 UI)
Other Ingredients		
Glycine	133 mM	104.8 mg
Sodium Chloride	10 mM	82.8 mg
Sodium Citrate	135 mM	27.1 mg

Active substance

Manufacturing process
Antithrombin alfa is purified from transgenic goat milk (source material).

- *Transgenic herd and collection of the source material*
The transgenic goat was obtained after microinjection of the transgene in the pro-nucleus of a goat embryo. This transgene is composed of the gene of interest (human AT cDNA) and the regulatory regions of goat beta casein gene (CSN2) to direct tissue specific expression in goat mammary gland. The selection of the first transgenic goat F0 was based on its transgene status (mosaicism and germline transmission) and its capacity for producing large quantities of antithrombin alfa in its milk.

Goat breeding is then accomplished through a combination of natural breeding and artificial insemination. The herd is predominantly constituted of "Swiss breeds" dairy goats (namely Saanen, Alpine, Toggenburg breeds and mixtures thereof). Transgenic goat may be obtained from any combination of these breeds. A 2-tiered banking system was established: the Master Transgenic Bank (MTB) is comprised of semen from qualified F0 and F1 males, and the qualified P0 and F1 females, and the Working Transgenic Bank (WTB) is comprised of qualified male and female animals from qualified animals, and the semen from these qualified males. Considerable efforts have been made to identify the genotype of the goats, and to improve the "genetic consistency" of the herd.

The qualification of a production doe, and production group is mainly based on the transgenic animal capacity to produce antithrombin alfa in sufficient quantity, and on microbiological screening. Source material (milk) is then collected from qualified doe at GTC Biotherapeutics Inc., MA, USA,. Source material is then shipped to Cambrex, MA, USA for further processing.

In order to increase the consistency of the source material which shows variability in amounts of specific proteins, lactose, total protein and fat levels, the milk pooling strategy has been revised. Compositional analysis of the Cambrex batches (old pooling strategy) is compared with batches prepared with the new pooling strategy and demonstrates that greater consistency is achieved.

- *Active substance manufacturing process*

The purification process begins with the thawing, pooling and clarification of the source material. Purification is accomplished by a series of column chromatography steps and filtration through a virus filter. The formulated bulk of drug active is shipped to MedImmune BV, Nijmegen, The Netherlands.

Several modifications have been introduced in the active substance and finished product manufacturing process between 1991 and 2003.

The active substance is manufactured at Cambrex BioScience, USA; batch analyses of 3 consecutive batches and their in process controls analyses were provided to support the process consistency.

The validation of removal of goat milk impurities by the purification process has been adequately addressed by conducting further analysis on the raw materials and products from the first validation study and by performing a second validation study. More than 4 logs of removal are observed for all measured impurities, except for goat AT (2.9 logs). Suitable validated analytical methods were used assess removal of these impurities.

Characterisation
Antithrombin is a 58kDa single chain glycoprotein that contains 432 amino acids and has a carbohydrate content of about 15%. The protein has 3 disulfide bridges and 4 N-linked glycosylation sites (Asn 96, 135, 155 and 192).

The protein structure of antithrombin alfa is consistent with published data and hpAT. N-terminal variability (N-2 and N-3) is observed (≤10%); larger truncation has not been clearly detected, although

SDS-PAGE analysis (Coomassie reduced conditions) reveals faint presence of low molecular weight species. Four methionines have been identified as potential oxidation sites (Met314, 315, 17 and 20) .

The comparison of recombinant human AT (rhAT) to plasma-derived AT (hpAT) shows significant differences in glycosylation profiles and affinity to heparin. The glycosylation profile of antithrombin alfa differs from hpAT in terms of monosaccharide composition (fucosylated, less sialylated, high proportion of NGNA), oligosaccharide population, as well as glycoform species (different pI distribution). Differences are observed on heparin binding affinity analysis. Plasma derived hpAT is usually composed of ~90% alpha isoform (low binding affinity to heparin) and ~10% beta isoform (high binding affinity species). The antithrombin alfa demonstrates an hpAT "beta-like" affinity without any detectable impact on the in-vitro bioassay (assay performed in excess of heparin).

Active substance specifications
Satisfactory specifications and validation of analytical methodology have been provided for control of the active substance. Each batch of active substance is tested for identity, purity and potency. The specifications according to which the tests are performed are justified by batch data as well as data obtained for batches used in preclinical studies and clinical trials. All routine methods used as control or release tests of starting materials, process intermediate, drug product and stability samples were validated when appropriate. No compendial reference standard of antithrombin alfa is currently available . The company has developed its own reference standards for product testing purposes.

Milk contaminants are known allergens and may be present in trace amounts in the product. Therefore, they are monitored with a series of assays. A special warning in the SPC has been added to preclude patients with hypersensitivity to goat proteins or goat milk components.

Based on the stability data provided, a shelf life for the active substance of 80 days at 2-8 °C may be accepted.

Other ingredients

The other ingredients in the product are glycine, sodium chloride and sodium citrate. All of them are pharmacopoeial grade.

Product development and finished product

Pharmaceutical Development
The product was formulated at the active substance level, and no other excipient is added during the finished product manufacture.

Manufacture of the Product
The product is manufactured by MedImmune Pharma BV, Nijmegen, The Netherlands. Upon receipt from Cambrex Bio Science MA, the integrity of the drug substance vessel is inspected, and stored at 2 to 8°C. The formulated antithrombin alfa is filtered (0.22µm) and aseptically filled. The product is then lyophilized and subjected to heat inactivation.

Finished Product Specification
The controls performed on the finished products are similar to the one performed on the active substance. Biological activity is based on an antithrombin inhibition test using an excess of heparin. The Thrombin Inhibitory Activity assay is consistent with the Ph.Eur monograph on Assay of Human antithrombin III. The specification of release are that the calculated potency (IU) is not less that 80 % nor more than 120 % of the potency stated and the confidence interval (p=0.95) is not greater than 90 % to 110 % of the estimated potency. ATryn and hpAT behave differently in this thrombin inhibitory analysis (without heparin and with progressive amounts of heparin), even though the potency of both is expressed in international units (IU). Therefore, a statement is included in the SPC indicating that both products are not interchangeable.

Viral safety
The viral safety of this product mainly relies on:

1- The quality of starting materials (health status of animals, the quality of milk)
2- The capacity of the production process to remove or inactivate viruses.

The viral safety strategy of starting materials relies on a closed herd of selected and limited origins, together with a health monitoring program, and some viral screenings. During the process, specific viral removal and inactivation steps were introduced in the form of nanofiltration and dry heat treatment.

Viral safety and TSE aspects are well addressed and documented. Several measures have been taken at the GTC farm to ensure health status and monitoring of transgenic animals (closed herd, controlled farm, good manufacturing practices, virological controls on animals). In case a confirmed infections occurs, the company commits to communicate the description of the case and the subsequent decisions to the competent authorities. Adventitious agents are searched on bulk milk. The only biological material used during the purification process is heparin of porcine origin. Global reduction factors were satisfactory regarding the virus removal/inactivation for enveloped viruses as well as for non-enveloped viruses.

Regarding TSE aspects, the goats were maintained in a closed herd in the USA. All GTC goats were certified scrapie-free by the USDA. Milk is considered as a product with no detectable infectivity. Moreover, some steps of the production process were subjected to scrapie removal/inactivation studies. The applicant committed to further investigate the use of a systematic scrapie search by ELISA or Western Blot on the CNS of each slaughtered animal in order to implement an acceptable health monitoring program. The CHMP will be kept updated on this investigation. As soon as a validated test is available, this test will be implemented.

Stability of the product

Stability studies were performed on 3 lots of product stored at 2-8°C. Results of the studies support an expiry period of 18 months.

A photodegradation study has been conducted: the product does not seem to be light sensitive. In-use stability study was conducted after dilution of the reconstituted product into infusion bags and infusion syringes stored at room temperature. Based upon the results of these studies, diluted solution prepared in infusion bags or syringes should be administered within 8 hours of preparation. In order to obtain lower particulates levels, the SPC mentions the need to bring the vial to room temperature prior to reconstitution and to limit the agitation.

3. Part III: Toxico-pharmacological aspects

The non-clinical program was performed according to the ICH S6 guideline "Preclinical Safety Evaluation of Biotechnology Derived Pharmaceuticals".

In order to ensure viral particle inactivation, a heat treatment step has been introduced. Nevertheless, the formulations used in preclinical studies with the exception of prenatal and postnatal toxicity studies were non-heat treated. Additional data submitted indicate there appear to be only minor biochemical differences between the non-heat treated, the heat treated and the nanofiltered heat treated antithrombin alfa products, which may not impact on the pharmacokinetics, distribution or activity of the antithrombin alfa in animals or humans. Definitive evidence of bioequivalence has been gained from the clinical PK study (GTC AT PK 011-04).

Pharmacodynamics

Pharmacological properties of antithrombin alfa were investigated in both *in vitro* and *in vivo* studies.

Primary pharmacodynamic studies

The anticoagulant activity of antithrombin alfa has been studied and compared to hpAT (human plasma-derived antithrombin)) in several *in vitro* studies: *in vitro* thrombin and factor Xa inhibition assay, effect on fibrinogen levels in blood obtained from Sprague-Dawley rats and humans, and *in vitro* coagulation in blood from cardiac surgical patients. These studies showed that antithrombin alfa and hpAT had equivalent activity. Heparin binding studies indicated that heparin affinity is 4 fold higher for antithrombin alfa than for hpAT.

Both anticoagulant and anti-inflammatory properties of antithrombin alfa have been studied in other *in vitro* studies; anti- inflammatory effect of antithrombin alfa on tissue factor and cytokines and regulation of neutrophil migration were shown.

In vivo studies have been carried out with several acquired AT deficiency models, including sepsis, disseminated intravascular coagulation (DIC) and organ transplantation. Some of these studies were reports from the published literature. In these studies, antithrombin alfa prevented sepsis and effects caused by septic shock. The results were similar between antithrombin alfa and hpAT.

Secondary pharmacodynamic studies

No secondary pharmacodynamics studies have been conducted. The applicant stated that due to the nature of antithrombin alfa and the lack of unexpected findings in the toxicity studies, these studies were not considered necessary, and this was agreed by CHMP. Anti-inflammatory properties of antithrombin alfa have been studied as part of the primary pharmacodynamic studies.

Safety pharmacology programme

No dedicated safety pharmacology studies have been conducted. However, effects on the CVS and respiration were monitored in a monkey study and a baboon toxicity study respectively. Also effects on respiration and on the CNS were monitored in a rat reprotoxicity study. From these studies there is no indication of any adverse effects on cardiac, respiratory or cerebrovascular system.

Pharmacodynamic drug interactions

A pharmacodynamic drug interaction study has been carried out with heparin and antithrombin alfa or hpAT. The presence of heparin did not modify anticoagulant activity. Moreover, no differences were noted between antithrombin alfa and hpAT regarding the profile of effect on coagulation factors.

Pharmacokinetics

Very few pharmacokinetic data were submitted. Protein binding, metabolism, metabolic pathway, excretion balance studies have not been performed. However, in view of the type of molecule, thd lack of these data is acceptable.

Five pharmacokinetic and three toxicokinetic (1 single, 2 repeated dose) studies were performed by intravenous route in mice, rats, dogs and monkeys.

Two types of assays have been carried out in the pharmacokinetic assessment, antithrombin assay and antithrombin alfa antibody assay.
Regarding antithrombin assay, AT ELISA method, antithrombin activity assay and radiometry were used. The lack of specificity (for antithrombin alfa) of the thrombin inhibition assay is a concern, however this is acceptable taking into account the aim of the studies in which this assay was used, ie measuring clearance rates of antithrombin alfa. For the thrombin inhibition assay, a posteriori validation has been performed, which is acceptable, given minor modification of the assay method.

Antithrombin alfa antibody assay, using an ELISA method was used in the repeat dose rat and monkey studies in order to investigate the production of antithrombin alfa antibodies.

Bioavailability - bioequivalence

A study has been performed in female mice in order to compare the pharmacokinetic profile of different lots of antithrombin alfa. Pharmacokinetic parameters have been determined in mice (6 animals per group) following a single bolus intravenous injection at 6 mg/kg with three batches. These three lots of antithrombin alfa have been individually purified from the milk of three different female goats. Data indicated similar results. Nevertheless, the results of this study could only be considered as supportive data and not as a proof of bioequivalence because of the lack of power (small number of animals and samples and the lack of validation for this assay).

Distribution

Distribution in the rat was generally similar for antithrombin alfa and hpAT however there was more drug related radioactivity associated with the gastrointestinal tract following ^{125}I-rhAT than ^{125}I-hpAT administration.

Toxicokinetics

In rat studies, AUC increased with dose in a non-linear manner. AUC were greater after 4 weeks of dosing compared with initial values.

The clearance was lower for hpAT comparing antithrombin alfa and hpAT dose for dose; this difference could be the result of glycosylation differences between antithrombin alfa and hpAT.

In cynomolgus monkey studies, AUC was generally about three times greater in this species than in the rat at all doses used. Some evidence of accumulation was noted at doses levels ≥ 300 mg/kg/day.

Toxicokinetics studies did not show gender differences. AUC were greater for hpAT than for antithrombin alfa, dose for dose.

Half-life increased and clearance decreased with dose across the 3 species. Depending on the dose, half-life ranged from approximately 0.75-2 h at 36-360 mg/kg dose in the rat, 2-8 h at 21-210 mg/kg dose in the dog and 2-6 h at 36-360 mg/kg dose in the primate. The clearance values ranged (with increasing dose) from 46,74 to 16,14 ml/hr/kg in male rats and from 34,5 to 16,86 in female rats; 23,3 to 4,9 ml/hr/kg in male dogs and from 19,6 to 4,6 ml/hr/kg in female dogs; 15,7 to 5,8 ml/hr/kg in male primate and from 17,5 to 6,1 in female primates.

Toxicology

Toxicology studies have been performed by the intravenous route in rats and dogs for single dose toxicity studies and in rats, dogs and cynomolgus monkeys for repeat dose toxicity studies. In two of the single dose studies and in the monkey repeated dose study, antithrombin alfa was administered by infusion.

Single dose

In the single dose toxicity studies in rats, doses up to 360 mg/kg were given (infusion/bolus). In the study in dogs, doses up to 210 mg/kg were given by infusion. The findings were limited to transient swellings observed in rats and dogs at the highest doses tested, and increased AST (with top-dose on day 2) in the dog study. The swellings resolved after 6 to 12 hours. AST was possibly associated with minor tissue damage.

Repeat dose toxicity (with toxicokinetics)

In repeat-dose toxicity study in rats, doses up to 360 mg/kg were given for 28 days (injection). Transient swellings have been observed at all doses tested.

In the 14 days cynomolgus monkey study, dosing was over 1 hour at 0, 36, 120 or 360mg/kg/day for 14 days followed by a 7 day treatment-free recovery period for the control and top-dose groups. The top dose was reduced to 300mg/kg/day on day 2 or 3 due to haemorrhage in one female (accidental piercing of the femoral artery during dosing/ sampling on day 1 in the presence of anticoagulant). Haematological disorders were noted in females at the highest dose tested. These effects included a decrease of red cells, haematocrit and haemoglobin and an increase in reticulocytes and

polymorphonucleocytes. These changes could be due to multiple blood samples collected during the first day of the study. On day 15 increased AST and ALT were observed in top-dose animals. The AST increase was most likely associated with muscle trauma; ALT returned to normal by day 22.

Antibody response was investigated in the repeated dose toxicity studies and toxicokinetic studies

There was an antibody response in both rat and monkey upon repeated dosing with antithrombin alfa but no consistent pattern of response in terms of dose level or gender in any study.

Genotoxicity

Genotoxicity studies are not needed according to ICH S6. The genotoxic potential of antithrombin alfa has been investigated in an *in vivo* mouse micronucleus test up to 360 mg/kg/day and in two *in vitro* studies: an Ames test in *Salmonella typhimurium and E. coli* strains and a chromosomal aberration assay in Chinese hamster ovary cells. The results of these studies were all negative.

Carcinogenicity

In view of the origin of the product and the duration of the treatment, no studies have been conducted in order to assess the carcinogenic potential of antithrombin alfa, which is acceptable and in agreement with ICH S6.

Reproduction Toxicity

A full package reproduction toxicity studies is not required for this therapeutic indication.

There were two pre- and post-natal studies performed in rats in order to support administration of antithrombin alfa to patients during pregnancy.

The first study investigated antithrombin alfa effects in dams and F_1 and F_2 offsprings. Pregnant rats (25/dose group) were given 0, 2.1, 21, 210 mg/kg/day by intravenous bolus from day 6 of gestation to day 20 of lactation (37 days). The NOAEL determined for maternal effects was 210 mg/kg/day. The NOAEL determined for F1 pup development is 21 mg/kg/day. Indeed, at the highest dose tested (210 mg/kg/day), a decrease of the pups viability index was observed in comparison with concurent and historical controls. In the second study, the investigation concerned dams and only F1 offsprings. Pregnant rats (30 animals/dose) were given 0, 52.5, 105, 210 mg/kg/day by intravenous bolus from day 20 of gestation to day 5 of lactation (8 days). The NOAEL for F_0 mothers and F_1 pups was 210 mg/kg/day. The maternal effects were a dose dependant incidence of swelling in limbs and snouts.

Local tolerance

No specific tests for local tolerance have been performed. Good local tolerance was observed during toxicology studies, and in clinical studies.

Ecotoxicity/environmental risk assessment

An environmental risk assessment has not been conducted. Since antithrombin alfa is a protein, the lack of an ERA is acceptable according to the draft ERA guideline.

4. Part IV: Clinical aspects

Initially, antithrombin alfa was developed for use in heparin resistant patients awaiting cardiac surgery requiring cardiopulmonary bypass (CPB). Following EMEA scientific advice in 1999 and 2000, further development focused on the use of antithrombin alfa in patients with hereditary AT deficiency in situations at high risk for thromboembolic events.

The clinical development consists mainly of the following studies (see table 2):

Pharmacokinetics

To fulfil the EMEA Note for Guidance (CPMP/BPWG/2220/99) request of a pharmacokinetic (PK) study in at least 12 congenital AT deficient patients not in a high risk situation to determine the PK characteristics of the antithrombin alfa product, a clinical PK study (GTC AT III- 009-00) has been performed in 15 patients with congenital AT deficiency (specific design features, timing of blood sampling and employment of three different lots of AT). In addition, two healthy volunteer PK studies were performed: GEN/G9601, to establish the PK characteristics of antithrombin alfa for the design of the acquired deficiency studies. Subsequently to the implementation of an active viral inactivation step in the production process of antithrombin alfa (heat treatment), a second healthy volunteer study (AT III-006-00) was then performed to evaluate whether the change in the production process of antithrombin alfa had impacted on the pharmacokinetic properties of antithrombin alfa.

In response to the LoQ, the Applicant performed a human PK study in healthy volunteers (GTC AT PK 011-04) in order to compare the heat-treated product with the to be commercialized heat-treated nanofiltered product in a standard cross-over design. Out of the 24 healthy volunteers (14 females and 10 males) included at the beginning of the study, a total of fourteen subjects (8 females and 6 males) completed the crossover part of the study.

Efficacy and Safety

As required by the EMEA Note for Guidance and the Scientific Advice, clinical efficacy data are presented from a formal clinical trial, which was set up to include 15 congenital AT deficient patients in high risk situations to be treated with antithrombin alfa in the peri-operative or peri-partum period (GTC AT III 01002). In the initial submission, a full evaluation of safety and efficacy of the first 10 patients enrolled (up to and including the Day 30 post treatment visit) has been provided. In the submission of the D121 responses to the CHMP LoQ, the Applicant has submitted the final clinical study. Fourteen hereditary AT deficient surgery (n=5) and delivery (n=9) patients, who were at high risk for the occurrence of a thromboembolic event, have been treated with antithrombin alfa replacement therapy.

Another study presented in the first submission, GTC AT III 011-003, provides retrospectively collected efficacy and safety data from 5 congenital AT deficient patients undergoing 6 surgical procedures treated under a compassionate use program in the US.

The applicant has also included in this application Phase II and III studies performed in acquired AT deficiency (although this development line was discontinued), mainly for purposes of the assessment of safety.

Justification has been provided for the absence of controlled large trials. The prevalence of clinically significant congenital AT deficiency is low (1 in 3000 to 1 in 5000). In addition, the need for AT prophylaxis to prevent the occurrence of thromboembolism in congenital AT deficient patients is limited to high-risk situations which is very difficult to predict and renders patient recruitment for clinical trials more difficult.

A tabular listing of all clinical studies is presented below in table 5.

The initially proposed indication of ATryn was for patients with congenital antithrombin deficiency for the prophylaxis of deep vein thrombosis and thromboembolism in clinical risk situations (especially during surgery or during the peri-partum period), in association with heparin if indicated. In the initial Marketing authorisation one dosing regimen was proposed for both types of patients (pregnant and non-pregnant). Thoughout the initial evaluation and re-examination the proposed

indication and posology changed several times based on difficulties with the dosing regimen in pregnant patients.

The final approved indication (section 4.1 of the SPC, Therapeutic Indications) is:

> *ATryn is indicated for the prophylaxis of venous thromboembolism in surgery of patients with congenital antithrombin deficiency. ATryn is normally given in association with heparin or low molecular weight heparin.*

Section 4.2 of the SPC, Posology, will match the dosing and monitoring as applied in the pivotal study:

> *The therapeutic goal of treatment with ATryn is to increase to, and maintain antithrombin activity between 80 – 120% (0.8 – 1.2 IU/ml) for the duration of treatment.*
> *Initial treatment starts with a loading dose of ATryn targeting an antithrombin activity level of 100%. This initial loading dose is based on body weight and on the pretreatment antithrombin activity level.*
> *In the applicant's posology proposal the required loading dose is determined using the following formula :*
>
> *Loading Dose (IU) = [(100 – patient's pre-treatment AT activity level in %) /2.28] x Body Weight in kg*
>
> *The usual loading dose in surgical patients (baseline AT activity 50%, bodyweight 75 kg) with congenital antithrombin deficiency in clinical risk situations is 20-25 IU/kg bodyweight. The loading dose should be given as a 15 minute infusion immediately followed by initiation of the maintenance infusion.*
>
> *The required maintenance dose for surgical patients is given as a continuous infusion and is determined using the following formula :*
>
> *Maintenance Dose (IU/hour) = [(100 – patient's pre-treatment AT activity level in %) /10.22] x Body Weight in kg*
>
> *The usual maintenance dose in surgical patients with congenital antithrombin deficiency in clinical risk situations is 4-5 IU/kg/h. During consumptive states (e.g. major surgery, concomitant use of heparin) the actual dose may be higher. See therapeutic monitoring and dose adjustment recommendations below.*

There is no paediatric development programme for this product.

The main trial was performed in compliance with Good Clinical Practice as claimed by the applicant.

A GCP inspection of trial GTC-AT PK 011-04 was performed due to unexpectedly low variability of the PK parameters calculated during the trial.
Overall, the observations of the inspection do not appear liable to jeopardise the acceptability of the trial data.

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.

Table 2: Tabular listing of all clinical studies (1)

Type of Study	Study Identifier	Objective(s) of the Study	Study Design and Type of Control	Test Product(s); Dosage Regimen; Route of Administration	Number of Subjects	Healthy Subjects or Diagnosis of Patients	Duration of Treatment	Study Status; Type of Report
Human Pharmacokinetics								
Comparative BA and BE	AT III-006-00	Compare PK of non-heat - treated with heat - treated rhAT	Randomized, cross-over study	rhAT; single dose 75 IU/kg; IV administration	26	Healthy Subjects	Two administrations, 28 days apart	Complete; Full
PK	GEN/G 9601	Define PK	Randomized, placebo controlled	rhAT; single dose, dose escalation (saline, 10, 50, 100, 150, 200 IU/kg); IV administration	20 (15 rhAT) (5 Placebo)	Healthy Subjects	Single Dose	Complete; Full
PK	AT PK 011-04	Compare PK of nanofiltered, heat - treated with non-nanofiltered, heat - treated rhAT	Randomized, two-period cross-over study	rhAT; single dose, 100 IU/kg; IV administration	24 (Period 1) 14 (Period 2)	Healthy Subjects	Two administrations	Complete

Type of Study	Study Identifier	Objective(s) of the Study	Study Design and Type of Control	Test Product(s); Dosage Regimen; Route of Administration	Number of Subjects	Healthy Subjects or Diagnosis of Patients	Duration of Treatment	Study Status; Type of Report
Congenital Antithrombin Deficient Subjects & Patients								
PK	AT III-009-00	Define PK	Randomized	Three different lots of rhAT; 50, 100 IU/kg; IV administration	15	Congenital AT Deficient Patient not in a high risk situation	Single Dose	Complete; Full
Efficacy Study	GTC AT III 01002	Efficacy of rhAT as prophylaxis in congenital AT deficient patients in high risk situations	Single Arm, Open Label with Blinded Evaluation	rhAT; individualized dosing targeting a plasma AT activity of 80-120%; IV administration	14	Patients in a high risk situation (delivery/ surgery)	3-14 days, Continuous Infusion	Complete; Full
Efficacy Study	GTC AT III 011-003	Efficacy of rhAT as prophylaxis in congenital AT patients in high risk situations	Compassionate Use Program; Retrospective Data Collection	rhAT; Individualized dosing targeting a plasma AT activity >80%; IV administration	6 treatment episodes	Patients in a high risk situation (delivery/ surgery)	2-16 days	Complete; Full

Type of Study	Study Identifier	Objective(s) of the Study	Study Design and Type of Control	Test Product(s); Dosage Regimen; Route of Administration	Number of Subjects	Healthy Subjects or Diagnosis of Patients	Duration of Treatment	Study Status; Type of Report
Acquired Antithrombin Deficient Patients								
Safety Study	GTC AT 96-0801	Safety; Dose Finding	Single Dose, Dose Escalation	Placebo or rhAT: 10, 25, 50, 75, 100, 125, 150, 175, 200 IU/kg; IV administration	36 (30 rhAT) (6 Placebo)	Acquired AT Deficient Patients (Heparin Resistance)	Single Dose	Complete; Full
Efficacy Study	GTC AT 97-0502	Efficacy for Restoration of Heparin Sensitivity	Single dose, placebo controlled, double blind	Placebo or rhAT: 75 U/kg	54 (27 rhAT) 27 (Placebo)	Acquired AT Deficient Patients (Heparin Resistance)	Single Dose	Complete; Full
Efficacy Study	GTC AT 97-0504	Efficacy for Restoration of Heparin Sensitivity	Single dose, placebo controlled, double blind	Placebo or rhAT: 75 U/kg	52 (28 rhAT) 24 (Placebo)	Acquired AT Deficient Patients (Heparin Resistance)	Single Dose	Complete; Full
Efficacy Study	GTC AT 97-0903	Compare dose response of rhAT with plasma derived AT (hpAT) in heparin resistant patients	Randomised, double blind, active control	rhAT 15, or 75 IU/kg, and hpAT 15 IU/kg	47 (33 rhAT) (14 hpAT)	Acquired AT Deficient Patients (Heparin Resistance)	Single Dose	Complete; Full

Clinical pharmacology

Pharmacodynamics

No pharmacodynamic studies were carried out either in healthy volunteers or in patients. This was accepted by CHMP, as the physiological action of antithrombin alfa is the inhibition of thrombin and factor Xa in the blood. AT levels in the PK studies reported are measured by activity assays that use thrombin or Factor Xa inhibition as a measure for AT activity, which is a surrogate for the final pharmacodynamic action of antithrombin alfa: prevention of clot formation.

Pharmacokinetics

Methods

No specific bioanalytical methods were developed for the determination of antithrombin alfa activity levels. Assays used for the determination of AT activity levels were commercially available antithrombin activity assays based on either thrombin or Factor Xa inhibition activity. The validation experiments, the comparison of the central lab and local lab values in the patients, and the comparative testing between plasma derived and recombinant AT in different test methods demonstrate that the commercially available assays seem to be accurate, reliable, and suitable for the measurement of AT activity in plasma of patients treated with antithrombin alfa.

Despite the several strategies managed by the Applicant, it appears that it has not been possible to develop a bioanalytical method to specifically measure antithrombin alfa protein levels and/or activity. Since antithrombin alfa cannot be distinguished from native AT, the PK studies are complicated by the presence of a baseline AT activity, with its own variability. This influences the total AT activity used to assess the PK parameters. Therefore PK parameters are both assessed using the total AT activity and the AT activity obtained after subtraction of the average baseline value for a specific patient. In response to CHMP concerns on this matter, the Applicant argued that, as the patients will be dosed to reach total plasma AT activity levels of 80-120% of normal, the proportion contributed by either antithrombin alfa or native AT is not clinically relevant. This was accepted by CHMP.

One of the major safety concerns of recombinant products is the potential for the induction of antibodies directed against the recombinant product. Although antithrombin alfa is very similar in structure to hpAT, it still has some differences in glycosylation. Therefore, throughout the whole clinical development of antithrombin alfa, both normal healthy volunteers and patients (congenital and acquired AT deficient), receiving one or more doses of antithrombin alfa, were tested for the occurrence of an immunological response to antithrombin alfa. An enzyme linked immunosorbent assay (ELISA) was developed to detect an IgG response to antithrombin alfa. Separate assays for both heat treated and non-heat treated antithrombin alfa were developed, since in theory, heat treatment could result in new epitopes, to which antibodies can be induced, compared to the non-heat treated antithrombin alfa. Both assays were validated in 100 samples of healthy blood donors, providing the normal range of optical density (OD). All patient samples were screened using the ELISA and samples with an OD above normal range were tested with a confirmative radioimmunoprecipitation (RIP) assay *(ITR-145-1200 and ITR-144-1200).*

Absorption, distribution and elimination

ATryn is intended for intravenous administration, hence absorption studies are not applicable.

Bioavailability

Bioavailability studies are not applicable for this application, because recombinant antithrombin alfa is administered intravenously and a 100% bioavailability can be assumed.

Bioequivalence

Study GTC AT III 006-00 (first submission), was performed in healthy volunteers to provide evidence that the addition of a heat treatment step in the production process of antithrombin alfa did not have a consequence for the PK characteristics of antithrombin alfa

Study participants were randomized to receive either 75 IU/kg heat treated antithrombin alfa and subsequently 28 days later 75 IU/kg non-heat treated antithrombin alfa or vice versa. Twenty-six subjects were enrolled, 3 subjects were excluded due to their withdrawal from the study after receiving only one treatment, 23 subjects completed both treatments and follow-up, including anti-antithrombin alfa antibody assessments.
Bioequivalence has been shown for AUC0-t, AUC0-inf, and Cmax only for the baseline uncorrected data. Half- life of the non-heat treated antithrombin alfa is approximately 4 to 6 hours. The studies with heat- treated antithrombin alfa showed half-lives ranging from 7.7 to 17.7 hours.

In response to CHMP concerns raised in the LoQ, the applicant has performed a human PK study in healthy volunteers (GTC AT PK 011-04), which was initiated in the United States in order to provide evidence of bioequivalence of the nanofiltered, finished commercial material and the material used in the pivotal clinical trial GTC AT III 01002 (heat-treated product). This study is an open-label, single-dose, randomized, two-period crossover trial, comparing the pharmacokinetics of intravenous administration of nanofiltered versus non-nanofiltered antithrombin alfa in normal healthy volunteers. For this study, 24 subjects received their first IV administration of study drug in mid September 2004. However, due to a regulatory delay before administration of the second dose to the subjects, results were presented as a parallel study initially. As a result, only PK and safety data obtained from the first administration of either antithrombin alfa drug product were available: primary (non-baseline corrected) and secondary (baseline corrected) endpoints have been met, the 90% confidence intervals of the ratio for Cmax and AUC0-24 being well within the bioequivalence range of 80% to 125%. In the response to LoI, the final pharmacokinetic results of study GTC AT PK 011-04 have become available. Out of the 24 healthy volunteers (14 females and 10 males) included at the beginning of the study, a total of fourteen subjects (8 females and 6 males) completed the crossover part of the study. Seven subjects received non-nanofiltered heat-treated antithrombin alfa (reference) first, and nanofiltered heat treated antithrombin alfa (test) second. The other 7 subjects were randomized to the opposite sequence. Both primary and secondary endpoints have been met, and bioequivalence has been shown, the 90% confidence intervals of the ratio for C_{max} and AUC_{0-24} being well within the bioequivalence range of 80% to 125%, for the non-baseline corrected and baseline corrected data. A GCP inspection concluded to the absence of concerns, making these results reliable.
Therefore, the applicant's conclusion that bioequivalence between the product used in the clinic and the to-be-commercialized product has been confirmed by the analysis of the completed crossover study is endorsed.

Table 3 describes the non-baseline corrected and baseline corrected pharmacokinetic results for both the test and reference product in study GTC AT PK 011-04.

Table 3: Pharmacokinetic Results (Mean (SD))

	non-nanofiltered heat treated (n=14)		nanofiltered heat treated (n=14)	
	non-baseline corrected	baseline corrected	non-baseline corrected	baseline corrected
C_{max} (%)	353.6 (38.7)	256.6 (36.6)	343.3 (32.5)	247.2 (30.2)
AUC_{0-24} (%*h)	3545.9 (266.73)	1218.3 (158.04)	3507.3 (291.15)	1201.1 (149.23)
Incremental recovery (% per IU/kg)	NA	2.57 (0.366)	NA	2.47 (0.302)
$T_{1/2}$ (h)	NA	3.68 (1.01)	NA	3.97 (1.01.)
Kel (1/h)	NA	0.200 (0.0481)	NA	0.185 (0.0454)
Cl (L/hr/kg)	NA	0.00796 (0.00106)	NA	0.00798 (0.00093)

Study AT III-009-00, provided in the first submission, was an open-label, single dose pharmacokinetic study of 50 and 100 IU/Kg antithrombin alfa in 15 adult patients with confirmed hereditary AT III deficiency, currently not at high risk for or suffering from a thromboembolic event. The two primary pharmacokinetic parameters, calculated based on the observed (baseline corrected and uncorrected) AT activity, C_{max} and AUC_{0-t}, had higher values for the 100 IU/kg dose, as expected (baseline corrected data are presented in table 8 below). For baseline uncorrected data, AUC_{0-t} and C_{max} increased 1.11 and 1.48 fold respectively with a two-fold increase in dose.

To allow the determination of a dose recommendation for the efficacy study, a population PK analysis, has been performed in the first submission in order to calculate an optimal dosing regimen for antithrombin alfa to obtain plasma AT activity levels within the 80-120% range]
The population pharmacokinetic analysis was performed with pharmacokinetic data of antithrombin alfa and of hpAT:

- The pharmacokinetics of antithrombin alfa were obtained from the previous study, AT III-009-00, performed in 2001-2002 (see above), with 15 congenital antithrombin deficient patients, following a short intravenous infusion of 50 or 100 IU/kg.
- The pharmacokinetics of hpAT were obtained from a study performed in 1984 in the U.S.A. in 8 congenital AT deficient patients, following administration of a short intravenous infusion of 25 – 225 IU/kg hpAT marketed by Bayer Healthcare, Thrombate III.

The population PK analysis was performed with the Non-linear Mixed Effect Modeling language NONMEM. Based on the results of the population PK modelling, the plasma AT activity during and after intravenous infusion was simulated by means of a Monte Carlo simulation approach in a patient population of 100 subjects for both hpAT and antithrombin alfa. For hpAT, the dose recommendation of the Thrombate III package insert was followed. For antithrombin alfa, simulations were iteratively performed to calculate the optimal antithrombin alfa infusion regimen that results in plasma AT activity between 80-120 % of the mean population level of endogenous AT in the healthy population.
Pharmacokinetic analysis of the results of the two studies, on the basis of non-linear mixed effects modelling, yielded the following estimates of the population pharmacokinetic parameters for hpAT and antithrombin alfa. In addition, estimates of the inter-individual variability in these parameters were obtained (**Table 4**).

Table 4: Population PK parameters for rhAT and hpAT

Treatment	**# subjects M/F**	**Mean (SD) PK parameters**					
		Incr. Rec. %/IU/kg	**C_{max}** %	**$T_{1/2}$** h	**AUC_{0-t}** % x h	**Cl** l/h	**MRT** h
Recombinant human AT	2 M/ 13 F	2.07 (1.54)	132.73 (1.54)	10.16 (1.28)	587.88 (1.63)	0.665 (0.05)	8.57 (1.24)
Human plasma AT	8	1.71 (1.21)	113.53 (1.57)	91.21 (1.19)	6508 (1.63)	0.091 (0.00)	110.6 (1.19)

Incr. Rec: Incremental recovery - MRT: Mean residence time

Table 5: Results of Pharmacokinetic Studies with rhAT (Baseline Corrected Data)

Study Number	Design	# subjects M/F	HV/ P	Treatment (dose)	Mean (SD) PK parameters					
					Incr. Rec. %/IU/kg	C_{max} IU/ml	$T_{1/2}$ h	AUC_{0-t} IU/ml • h	Cl ml/h/kg	MRT h
GEN/G 9601	Randomized, placebo controlled, single dose, dose escalation; non-compartmental analysis	20 M	HV	10 IU/kg	2.7	NA***	NA***	NA***	NA***	NA***
				50 IU/kg		1.06	2.6	4.4*	10.3	4.1
				100 IU/kg		2.27	4.4	14.2*	6.2	6.7
				150 IU/kg		3.27	4.8	22.1*	5.9	7.0
				200 IU/kg		4.46	4.3	30.1*	5.7	6.3
AT III 006-00	Randomized, cross-over, heat treated vs. non-heat treated; non-compartmental analysis	13 M/ 13 F	HV	Heat treated; 75 IU/kg	1.601 (0.392)	1.256 (.334)	7.7 (5.1)	9.441 (3.043)	0.139 (0.094) h^{-1} ****	NA
				Non-heat treated; 75 U/kg	1.412 (0.272)	1.179 (.232)	5.7 (5.6)	8.503 (3.787)	0.204 (0.129) h^{-1} ****	NA
AT III 009-00	Two dose, single administration; non-compartmental analysis	2 M/ 13 F	P	50 IU/kg	2.24 (20.21)**	1.120 (20.21**)	11.6 (84.70)**	5.394 (38.47**)	9.6 (34.44)**	16.2 (74.90**)
				100 IU/kg	1.94 (14.81)**	1.938 (14.81**)	17.7 (60.91)**	12.898 (.1631**)	7.2 (15.29)**	20.5 (40.25**)
GTC AT PK 11-04	Randomized, cross-over, heat treated vs. nano-filtered heat treated rhAT, baseline corrected	6M / 8F	HV	100 IU/kg heat treated	2.60 (0.351)	2.573 (0.351)	3.68 (1.01)	12.11 (0.157)	0.00795 (0.00106)	NA
				100 IU/kg Nanofiltered heat treated	2.47 (0.302)	2.454 (0.302)	3.97 (1.01)	11.93 (0.157)	0.00798 (0.00093)	NA

NA: Not Available HV/P = Human volunteer (HV) or Patient (P) *: $AUC_{0-\infty}$; **: Coefficient of Variation (%); ***: Model independent parameters could not be calculated because all values were too close to the variation in baseline AT activity levels; ****: K_{el} given, no Clearance available

In order to determine the optimal infusion rate for antithrombin alfa to bring the AT activity in patients into the 80-120% range, simulations with various infusions rates of antithrombin alfa were performed iteratively. All PK parameters, except baseline AT activity, were fixed according to the population mean estimates derived from the PK model. A dosing schedule employing an initial 15 minutes IV loading infusion followed by a continuous infusion was calculated as being the best way to obtain and maintain the plasma AT activity levels within the target range. Loading dose and maintenance infusion rates were individually calculated using the patients pre-treatment AT activity and body weight. The dosing schedule with therapeutic drug monitoring as derived from the population PK analysis and simulations, has been tested in the clinical trial AT III 01002 and is the proposed dosing schedule in the Summary of Product Characteristics.

Dose proportionality and time dependencies
Study GEN/G9601, presented in the first submission, was a Phase I randomised, placebo-controlled study to evaluate the safety, tolerance, pharmacokinetic profile, and pharmacodynamic effect of single increasing doses of antithrombin alfa when administered intravenously over 30 minutes in 20 healthy subjects. Model independent parameters $T_{1/2}$, MRT and clearance indicate that there is an effect of dose on the clearance of AT III, which appears to saturate at doses >86 IU/kg. At doses >80 IU /kg, T1/2 is 4-5 hours, the MRT is 6-7 hours and the clearance is approximately 6 ml/hr/kg. Maximum plasma concentrations of AT III increased 0.27 IU/ml for each 10 IU/kg of AT III infused.
The results of this study are presented in **Table 5**.

Special populations
No studies were done in special populations.

Clinical efficacy

A table of studies and enrolled patients has been presented above (**Table 2**).

Dose-response studies and main clinical studies

Dose response studies
Data derived from the PK study performed in the target population (AT hereditary deficiency) were in addition to the classical PK calculations evaluated by population PK modelling, in order to establish the most optimal dose to be used in the clinical study to evaluate the efficacy of antithrombin alfa as a prophylactic treatment during high risk periods in congenital AT deficient patients (see Pharmacokinetic part of this assessment).

Main studies

A Phase 3 Study to Assess the Incidence of Deep Vein Thrombosis (DVT) Following Prophylactic Intravenous Administration of Recombinant Human Antithrombin (rhAT) to Hereditary AT Deficient Patients in High-risk Situations (GTC AT III 01-002)

Methods

- Study Participants
  - This study was a multi-center, multinational (Italy, United-Kingdom, France, Germany and USA) open-label treatment with independent blinded evaluation of efficacy data.
  - 15 hereditary AT deficient patients were planned to be enrolled in the study with the expectation that 12 of the patients would be evaluable.

Inclusion criteria required that study patients are :

- congenital AT deficient patients with a personal or family history of venous thrombotic events ;
- with a history of congenital AT deficiency that includes 2 or more plasma AT activity levels ≤ 60% of normal ;
- who are scheduled to have an elective procedure known to be associated with high risk for occurrence of acute DVT. This includes surgical patients or pregnant patients scheduled for cesarean section or delivery induction. In addition, hospitalized pregnant patients in active labor were to be allowed entry into the study ;
- and who are at least 18 years of age, not exceeding 70 years of age.

Exclusion criteria:

- patients who had a diagnosis of another hereditary APC resistance/Factor V Leiden, Protein S or C deficiency, prothrombin gene mutation (G20210A), or acquired (lupus anticoagulant) thrombophilic disorder ;
- patients who were scheduled for a neurosurgical procedure or open-heart surgery ;
- patients who had an underlying medical condition, which in the opinion of the investigator could complicate the interpretation of the primary efficacy endpoint ;
- patients who had a known allergy to goats or goat products ;
- patients who had participated in a study employing an investigational drug within 30 days of the start of their participation in the current trial ;
- patients using fondaparinux sodium or were expected to be treated with fondaparinux sodium during the study period.

- Treatments

Treatment administration

- All patients were administered an initial intravenous (IV) loading dose of antithrombin alfa designed to increase AT activity to a level expected to result in a therapeutic range between 80% and 120% of normal. AT activity levels measured prior to the initial dose and patient weight were the basis for the initial intravenous loading dosing. Continuous intravenous infusion of antithrombin alfa, intended to maintain AT activity levels between 80% and 120% of normal, was initiated immediately following administration of the loading dose.
- Treatment was intended to start approximately 24 hours prior to initiation of their scheduled procedure and continue for a minimum of 3 days and a maximum of 14 days. AT activity levels were used to monitor and adjust dosing. End of treatment was determined when the investigator judged that the patient was no longer at high risk for the occurrence of a thromboembolic complication.
- In pregnancy patients not scheduled for caesarean section or induction of delivery, treatment was initiated only when the patient was hospitalized and in active labor.

Investigational product

- This trial was a single arm open-label treatment, blinded evaluation study.
- The product administered was heat treated antithrombin alfa (batch numbers T8011 and T8015).
- The vials of lyophilized antithrombin alfa were reconstituted with 10 ml of sterile water for injection (WFI), to provide a 25mg/ml solution.

Selection and timing of dose for each patient

For a patient with a pretreatment baseline AT activity of X%, the dose required to increase and maintain the AT activity level at 100% would be as follows :

Loading Dose (IU) = [(100 – X)/2.28] x Patient Weight

The loading dose was to be given as a 15 minutes infusion immediately followed by maintenance dosing.

Maintenance Dose (IU/day) = [(100 – X)/0.426] x Patient Weight

Therapeutic Monitoring and Dose Adjustment

After the **start of the maintenance dose infusion**, blood for AT activity levels had to be drawn at 0.5 hour (i.e. this is 45 minutes after the start of the loading dose infusion).
Based on the result of this AT activity level, the infusion rate (and consequently the dose) had to be adjusted using the following guideline :
1. If the AT activity level was between 80% and 120%, no dose adjustment was needed. An AT activity level 4 hours calculated from the time of the previous AT activity blood draw had to be taken.
2. If the AT activity level was less than 80%, the maintenance infusion rate had to be increased by 50% and a blood AT activity level to be taken 0.5 hour after the infusion rate adjustment.
3. If the AT activity level was greater than 120%, the infusion rate had to be decreased by 30% and a blood AT activity level taken 0.5 hour after the infusion rate adjustment.

When the **next AT activity level** was available, based on these results the dose was adjusted again using the following guideline:

1. If the AT activity level was between 80% and 120%, no dose adjustment was needed.

- In case this was the second consecutive AT activity level that was within the target range, the next blood sample had to be taken at least every 24 hours later (calculated from the start of treatment) for the duration of treatment with antithrombin alfa.
- In case this was the first AT activity level that was within the target range, an AT activity level had to be taken 4 hours calculated from the time of the previous AT activity blood draw.

2. If the AT activity level was less than 80%, the maintenance infusion rate had to be increased by 50% and a blood AT activity level taken 0.5 hour after the infusion rate adjustment.

3. If the AT activity level was greater than 120%, the infusion rate had to be decreased by 30% and a blood AT activity level taken 0.5 hour after the infusion rate adjustment.
   This cycle of AT activity checking was repeated until there were 2 consecutive samples that showed an activity in the target range of >80% and <120%, and at least every 24 hours afterwards (calculated from the start of treatment) for the duration of treatment with antithrombin alfa.

It was possible that the procedure or delivery would influence AT activity levels.
Therefore, an additional check of the AT activity level was to be done approximately one hour after the surgery or delivery. In case the activity level was below 80%, a 15 minutes bolus infusion of AT could be given to quickly restore the AT activity level.
The dose was calculated with the formula :
***Bolus Dose** (IU) = [(100 – Y)/2.28] × Patient Weight*
where 'Y' is the patient's post-surgery or delivery AT activity level.
In order to check the effect of this, an AT activity level blood sample was recommended 0.5 hour after the bolus dose administration was stopped.

All administrations of antithrombin alfa were carefully documented in the patient's CRF.
Maintenance dosing was to continue for all patients until the study investigator determined that the patient was no longer at high-risk for the occurrence of a thromboembolic complication up to a maximum of 14 days. It was expected that this would generally occur at the time when the investigator established effective chronic anticoagulation therapy and the patient was mobilized and ready for hospital discharge

- Objectives

The objectives of this study were:

- to assess the incidence of deep venous thrombosis (DVT) following prophylactic intravenous administration of antithrombin alfa to congenital AT deficient patients during high risk situations for the occurrence of DVT ;
- to perform also clinical and diagnostic monitoring for thromboembolic events other than DVT;
- to assess the safety of antithrombin alfa.

- Outcomes/endpoints

Primary efficacy endpoint :

- The incidence of acute DVT at any time point after start of study drug treatment as assessed by a blinded, independent central review of duplex ultrasonography and/or venography testing, if the study investigator felt that duplex ultrasound were inconclusive. The incidence of acute DVT is defined as the percentage of patients with acute DVT among the total number of evaluable patients.
- The incidence of acute DVT at the last day of dosing, 7 (± 1) days post dosing, and 30 (± 2) days post dosing is also summarized.
- The central evaluation of the presence or absence of acute DVT was based on the interpretation by two independent reviewers of all scheduled and unscheduled duplex ultrasounds and, when clinically indicated, venograms. Ultrasound evaluations included each leg and 5 different vein segments in each leg. Venography evaluations included each leg and 7 different vein segments in each leg. Potential outcomes for each section by both methodologies included normal, acute DVT, indeterminate, chronic changes, and not done. If the two reviewers agreed on their initial venogram assessments, the conclusion was final. If the two reviewers disagreed on their initial venogram interpretations for any examination time point, a third reviewer reviewed all venograms for that patient in a blinded fashion.

Secondary efficacy endpoints :

- the combined incidence of acute DVT and other thromboembolic events after start of study drug treatment as assessed by the independent central review ;
- the incidence of acute DVT after start of study drug treatment as assessed by local (study site) review
- the incidence of thromboembolic events other than acute DVT after start of study drug treatment as assessed by local (study site) review ;
- the combined incidence of acute DVT and other thromboembolic events after start of study drug treatment as assessed by the local (study site) review.

- Sample size

- The applicant determined sample size for this study based on the "Note for Guidance on the Clinical Investigation of Plasma Derived Antithrombin Products (CPMP/BPWG/2220/99)" distributed by CPMP and additional Scientific Advice from the EMEA.
- 15 hereditary AT deficient patients were planned to be enrolled in the study with the expectation that 12 of the patients would be evaluable.
    - An interim clinical study report submitted with the initial MAA presented detailed safety and efficacy data from the first 10 patients treated in the study. The patient enrollment cut-off date for this report was 4 August 2003.

- Randomisation

No randomisation of patients was performed. All study patients received antithrombin alfa.

- Blinding (masking)

All patients were to receive antithrombin alfa. However, an independent review of the serial imaging scans was performed in a manner such that the independent evaluators were blinded to the treating physician`s assessment of treatment or treatment outcome.

- Statistical methods

A detailed statistical plan was developed prior to the completion of patient enrollment. Prior to locking the database, decisions were made regarding the evaluability of all patient data for inclusion in the statistical analysis. The rationale for excluding any data from the analysis was prospectively defined, and classification of all or part of a patient's data as non-evaluable was completed and documented before the statistical analysis began. All data collected in this study was documented using summary tables and patient data listings. Demographic, efficacy, and safety data are summarized overall and by subgroup. The subgroups were non-pregnant surgery patients and pregnant patients. For categorical

variables, frequencies and percentages are presented. For continuous variables, descriptive statistics (n, mean, median, standard deviation, minimum, and maximum) are presented. The statistical analysis of the data derived from this study was to be performed using SAS Version 8.0 or higher.

Results

- Participant flow

Fourteen (14) patients having documented hereditary AT deficiency (HD) were enrolled based on the inclusion/exclusion criteria outlined in the study protocol and completed their 30-day post dosing visit.

- Recruitment

The first patient entered study GTC ATIII 01002 on December 2, 2002, the last patient entered on November 3, 2003 and completed on February 16, 2004.

- Conduct of the study

- Amendment 1 was to correct the calculations for the loading dose and continuous infusion rate. No patients were enrolled before the implementation of Amendment 1.
- Amendment 2 was implemented to clarify which data were to be obtained regarding the outcome of the pregnancy (including the status of the newborn) and surgical procedure and correct some inconsistencies in the protocol. For all patients included in the study, the additional data that were requested in Amendment 2 were collected.
- Amendment 3 was implemented to include an additional safety assessment intended to identify potential bleeding events and include an extension of the follow-up period to 90 days after last study drug administration (instead of 30 days).

- Baseline data / AT Activity levels

Of the 14 patients enrolled, 5 were surgery patients and 9 were delivery patients. Patients` ages ranged from 21 to 74 years, with a mean of 36.7 years. As expected the mean age of delivery patients (29.7) was lower than that of surgery patients (49.4). Two patients were male and 12 patients were female.
Four of the surgical patients were scheduled for prosthesis implants involving the hip; the fifth was scheduled for a bilateral breast reduction.

Table 6 provides the data necessary for calculating the initial bolus dose, the initial dose that was administered, in parentheses the dose that was to be given based on the dosing formula, and the procedure for which each of the patients had been scheduled.

Table 6: Patient Weight and Local Laboratory Baseline AT Activity Levels (Safety Population)

Patient Number	Patient Weight (kg)	Local Laboratory Baseline AT Activity Level (%)	Initial Bolus Dose IU Given (Calculated)
Surgery Patients (N = 5)			
Patient 1601	84	37*	2500 (2321)
Patient 3001	85	42	2000 (2162)
Patient 3002	57	53	1425 (1174)
Patient 3003	57	62	1000 (950)
Patient 3501	82	57	1726 (1546)
Mean	73	50	1730 (1631)
Median	82	53	1726 (1546)
Delivery Patients (N = 9)			
Patient 0401	75	33	442 + 2400 (2203)
Patient 1501	105	52	2210 (2210)
Patient 2201	67	44	1800 (1647)
Patient 2401	61	38	1659 (1659)
Patient 3401	66	55	1303 (1303)
Patient 4001	84	45	1989 (2026)
Patient 8101	97	33**	3900 (2888)
Patient 8102	79	58***	2000 (1455)
Patient 8301	77	53	1564 (1587)
Mean	79	46	2141 (1664)
Median	77	45	1989 (1659)

* A baseline value (local laboratory) taken approximately 45 minutes later was 118% but was not used by the Study Investigator for calculation of the initial bolus dose.
** Screening value taken approximately 3 days prior to initiation of rhAT administration.
*** Screening value taken approximately 1 day prior to initiation of rhAT administration.

Patient weight and AT activity level measured at Baseline were used to calculate the initial bolus dose for each patient according to the following formula :
Initial Bolus Dose (IU) = [(100 – Baseline AT Activity)/2.28] x Patient Weight

Patients received antithrombin alfa treatment for periods of time ranging from 3.0 to 18.6 days and received total doses from 37,884 IU up to 227,817 IU. Infusion rates ranged from 3360 IU per 24 hours to 64800 IU per 24 hours for varying periods of time.
In most of the cases, the doses actually given differ from the calculated doses, particularly for delivery patients. Also, the individual response to the proposed therapeutic dose regimen for antithrombin alfa varies extremely. One to fifteen dose adjustments have been needed among the delivery patients and 1 to 8 among the surgery patients. The starting dose ranges from 20 to 81 IU/kg and the further daily injections between 79 and 451 IU/kg. For three patients in the delivery group, despite increased repeated doses of antithrombin alfa, the target of a 80 % mimimal AT III activity has been reached with difficulties.

The therapeutic schedule used for the pivotal study, based upon the population pharmacokinetic analysis performed from PK data in patients not in high risk situations, did not take into account the realities of the intended use. Therefore, further to the CHMP LOQ the Applicant has performed

additional pharmacokinetic analyses from study GTC AT III 01002. These revealed that the initial pharmacokinetic model overestimated the expected plasma AT activity levels in pregnant women. This was due to a higher Clearance and Volume of Distribution as compared to non-pregnant patients. Therefore the dosing regimen applied in the study GTC AT III 01002 was not appropriate for delivery patients. For surgical patients the model predicted the AT activity very well.

- Numbers analysed

Per Protocol (PP) population was used for the evaluation of antithrombin alfa efficacy and includes all patients who satisfy the following criteria (efficacy-evaluable):

- Have congenital AT deficiency with a personal or family history of venous thrombotic events (Protocol Inclusion Criteria 1).
- Have a history of congenital AT deficiency that includes 2 or more plasma AT activity levels ≤ 60% of normal (Protocol Inclusion Criteria 2).
- Have at least one evaluable post baseline ultrasound in the period up to 7 days post dosing after last study drug administration.
- Do not have a diagnosis of another hereditary APC resistance/Factor V Leiden, Protein S or C deficiency, prothrombin gene mutation (G20210A), or acquired (lupus anticoagulant) thrombophilic disorder.
- Do not have an acute DVT diagnosed at baseline.

Of the 14 patients who received antithrombin alfa, one patient was excluded from the efficacy evaluation since an acute DVT was detected by central evaluation at baseline ultrasound.
One patient (Patient 1601) had a protocol deviation based on age (74-years-of-age). However, as this is not considered a protocol violation, this patient was not excluded from the evaluation of efficacy.
Patients received antithrombin alfa treatment for periods of time ranging from 3.0 to 18.6 days and received total doses from 37,884 IU up to 227,817 IU. Infusion rates ranged from 3360 IU per 24 hours to 64800 IU per 24 hours for varying periods of time.

- Outcomes and estimation

Primary efficacy endpoint
The incidence in the PP population of acute DVT diagnosed by central evaluation is summarized overall and by subgroup in Table 10. The incidence of acute DVT on the last day of dosing, 7 (± 1) days post dosing, and 30 (± 2) days post dosing are also summarized in **Table 7**.

Table 7: Incidence of Acute DVT Based on Independent Central Review (PP Population)

Time Point	Evaluable Patients [1] N	Patients Having Acute DVT N (%)
Any Time Point		
Overall	13	2 (15.4)
Surgery Patients	5	1 (20.0)
Delivery Patients	8	1 (12.5)
Last Day of Dosing		
Overall	12	1 (8.3)
Surgery Patients	4	1 (25.0)[2]
Delivery Patients	8	0 (0.0)
7 Days Post Dosing		
Overall	13	2 (15.4)
Surgery Patients	5	1 (20.0)[2]
Delivery Patients	8	1 (12.5)[3]
30 Days Post Dosing *		
Overall	2	0 (0.0)
Delivery Patients	2	0 (0.0)

* Unscheduled (not per protocol) ultrasound between 7-30 days post dosing
[1] Patients who were not evaluable by independent central review (Patient 1601 on last day of dosing) or who had an acute DVT at Baseline (Patient 2201) are excluded.
[2] Patient 3003 (left total hip replacement)
[3] Patient 8301 (DVT detected 7 days after discontinuation of rhAT treatment)

Among the 13 evaluable patients, 2 patients (15.4%) had an acute DVT diagnosed by central evaluation. A pregnant patient (Patient 8301; caesarean section) experienced acute DVT 7 days after discontinuation of antithrombin alfa (in this patient, the assessment differed between the central reviewers and local assessor). A non-pregnant patient (Patient 3003) had an acute DVT diagnosed during antithrombin alfa dosing by central and local review.

Secondary efficacy endpoint

✓ Central Review: Combined Incidence of Acute DVT and Other Thromboembolic Events

As previously discussed there was only one delivery patient (Patient 8301) and one surgery patient (Patient 3003) who had an acute DVT or other thromboembolic event based on the central independent review.

✓ Local Review (Study Site): Individual and Combined Incidence of Acute DVT and Other Thromboembolic Events

Local evaluation for thromboembolic events included clinical and diagnostic imaging assessments. Of the 13 evaluable patients, 1 patient (patient 3003) had an acute DVT diagnosed on day 13 of treatment and 1 patient (patient 3501) had crural vein thrombosis diagnosed by the ultrasound performed at day 7 after stop of antithrombin alfa treatment at local review, but not at central review. Both patients had had hip replacement surgery.

In only one of the 3 cases described above an asymptomatic DVT was diagnosed and treated by antithrombotic therapy in order to prevent clinical symptoms of DVT (patient 3003). In one case (patient 3501) the finding was regarded not relevant, and the other (patient 8301) was diagnosed only at central review, a week after the administration of study treatment.

Data illustrating antithrombin alfa dosing and AT activitly levels for each patient taken prior to, during and post-treatment with antithrombin alfa are given in the study report.

Clinical studies in special populations

No studies were performed.

Supportive studies

An Assessment of the Incidence of DVT Following Prophylactic Administration of rhAT to Hereditary AT Deficient Patients in High-risk Situations; A Compassionate Use Program (GTC AT III 011-003)

This study describes the clinical course of 5 congenital AT deficient patients undergoing 6 surgical procedures treated under a compassionate use program in the US. Safety and efficacy data on these patients have been obtained retrospectively, using a standardised data collection protocol. Patients were all prophylactically treated with antithrombin alfa during and just after a high risk period for the occurrence of thromboembolic events. Efficacy was assessed clinically and in 5 of the 6 procedures through ultrasonography. Safety follow-up up to approximately 30 days post treatment included the occurrence of adverse events and the assessment of the immunogenicity of antithrombin alfa by testing for the presence of anti-antithrombin alfa antibodies.
Neither DVT nor any other thromboembolic event was diagnosed based on clinical symptoms or by duplex ultrasound. In addition, dosing with antithrombin alfa resulted in an increase in AT activity level close to and within the normal AT activity range.

Studies in acquired AT deficiency were performed in patients with heparin resistance scheduled for coronary artery bypass surgery (CABG) with the use of cardio-pulmonary bypass (CPB) machines.
Patients treated with heparin prior to the CABG procedure often show a diminished response to the large heparin dose administered to prevent clotting in the extracorporeal circulation. This diminished response is due to the consumption of AT during prolonged heparin use prior to surgery. In these studies the effect of antithrombin alfa on the activated clotting time (ACT), a marker of the anti-coagulative effects of heparin, was investigated. The general design of these studies was as follows : Patients received a standard dose of heparin, and when the ACT did not reach the required value, patients received a standard dose of antithrombin alfa or placebo. Efficacy was assessed by the response of the ACT value to the administration of antithrombin alfa (percentage of patients reaching the required ACT value).

A total of 189 patients were enrolled in 4 studies :

- GTC AT 96-0801: A Phase I-II Open, Dose Escalation Study of the Safety of Recombinant Human (transgenic) Antithrombin III in Patients Scheduled for Primary Cardiac Surgery Requiring Cardiopulmonary Bypass (36 acquired AT deficient patients ; 10, 25, 50, 75, 100, 125, 150, 175, 200 IU/kg or placebo)
- GTC AT 97-0502: A Phase III, Double Blind, Placebo-Controlled, Multi-Center Study of the Safety and Efficacy of rh AT III in Heparin Resistant Patients Scheduled for Cardiac Surgery Requiring Cardiopulmonary Bypass (54 acquired AT deficient patients; either 75 IU/kg antithrombin alfa or placebo)
- GTC AT 97-0504: A Phase III, Randomized, Double-Blind, Placebo-Controlled Study of the Safety and Efficacy of rh AT III in Heparin Resistant Patients Scheduled for Cardiac Surgery Requiring Cardiopulmonary Bypass (52 acquired AT deficient patients; either 75 IU/kg antithrombin alfa or placebo)
- GTC AT 97-0903: A Phase III, Double Blind, Randomized Study Comparing Transgenic Antithrombin III and Plasma Antithrombin III in Patients Undergoing Elective Cardiac Surgery Requiring Cardiopulmonary Bypass (47 acquired AT deficient patients; compared a single

administration of two different dosages of antithrombin alfa (75 IU/kg and 15 IU/kg) with a single administration of 15 IU/kg human plasma derived AT).

These four studies are conducted in a patient population different from the intended clinical population claimed in the SPC. These patients display an acquired, and not hereditary AT III deficiency. Furthermore, most of these patients have a median age > 63 years. Therefore these studies could only be taken into account for the safety evaluation, but not for the efficacy evaluation.

Discussion on clinical efficacy

In total, 19 hereditary AT deficient patients (14 patients from study GTC AT III 01002 and 5 patients in the compassionate-use treatment GTC AT III 011003) were treated during and after 20 high risk situations:

- the Compassionate Use Study (GTC AT III 011-003), retrospective collection data performed on 5 patients (6 treatments) with non-heat treated antithrombin alfa administered by multiple daily infusions (due to a shortage of hpAT in the US),
- the Efficacy Study (GTC AT III 01002), prospective study performed on 14 hereditary AT deficient surgery (n=5) and delivery (n=9) patients with heat treated antithrombin alfa administered by continuous infusion and central independent evaluation of standardised ultrasound examinations for detection of DVT. The incidence of DVT was assessed both locally and centrally by an independent group of reviewers. One patient in the Efficacy Study was excluded since an acute DVT was detected by central evaluation at baseline ultrasound. Patients received antithrombin alfa treatment for periods of time ranging from 3.0 to 18.6 days and received total doses from 37,884 IU up to 227,817 IU. Infusion rates ranged from 3360 IU per 24 hours to 64800 IU per 24 hours for varying periods of time.

According to the study GTC AT III 01002 protocol, the primary efficacy endpoint was defined as "incidence of acute DVT at any time point after start of study drug treatment as assessed by a blinded, independent central review of duplex ultrasonography and/or venography testing". In this context, 3 out of 13 efficacy-evaluable patients thromboses were diagnosed by ultrasonography (2 asymptomatic DVT, confirmed by central review, and 1 superficial vein thrombosis). Nevertheless, in only one of these 3 cases, an asymptomatic DVT (patient no. 3003) was diagnosed and treated by antithrombotic therapy in order to prevent clinical symptoms of DVT. In one case the finding was regarded not relevant, and the other was diagnosed only at central review, weeks after the administration of study treatment.
The discrepancies observed between local and central review questioned the relevance of central examination, as no DVT occurred in the follow-up of patients with acute DVT reported by central review. In addition, two of the three ultrasound findings were made at seven days after the stop of treatment with antithrombin alfa (with a half-life of approximately 10 hours).
In the meta-analysis of low molecular weight heparin and unfractionated heparin in thrombosis prophylaxis published by Koch et al. in 2001 provided by the Applicant, an incidence of DVT of about 27% especially in orthopaedic surgery was reported in the large comparative studies in non-congenital AT deficient patients treated with currently approved effective anticoagulants (LMWH and heparin).
Therefore, in the applicant's opinion efficacy of antithrombin alfa has been established in patients with congenital antithrombin deficiency for the prophylaxis of deep vein thrombosis and thromboembolism in clinical risk situations.

A major issue was the large variability in dose requirements during treatment of pregnant patients with ATryn and the difficulties to maintain therapeutic AT levels in some of these patients. In order to address this, the applicant proposed in the submission of the responses to the Consolidated List of Questions (day 121) a new dosing regimen and therapeutic monitoring schedule for the pregnant patients). As no prospective clinical data had been provided regarding the newly proposed dosing schedule, the Applicant agreed with the CHMP that since this has not been tested in a controlled

environment of a clinical study, this can not be supported yet for use in the marketed situation. Improvements in the dosing regimen for pregnant women and the therapeutic drug monitoring need to be confirmed in a prospective manner before being applied in clinical practice. The applicant therefore agreed to limit the indication for use of antithrombin alfa to non-pregnant surgical patients with the dosing and therapeutic drug monitoring (TDM) schedule as applied in the pivotal study where it has shown to maintain the AT-activity well in the desired therapeutic range.

From the initial evaluation several points were highlighted:

- In the context of limiting the indication for use of antithrombin alfa to surgical patients, the assessment of the clinical efficacy of ATryn took into account the data resulting from the high-risk surgical situation in the pivotal study for non-pregnant patients only, i.e. only 5 surgical patients among 14 patients in the pivotal study GTC AT III 01002. Therefore the initial evaluation concluded that, for the proposed indication (i) efficacy has been investigated in only 5 surgical patients, a very small number that makes efficacy assessment impossible, (ii) this is far from the level of 12 patients recommended by the EMEA scientific advice and, (iii) not in accordance with the Note for Guidance CPMP/BPWG/2220/99. The results from the five patients treated in the compassionate-use program must be considered as a supportive data set, as in this retrospective collection of data no well defined dosing instruction, nor the desired targeted AT level could be given.
- Two out of the three thromboses diagnosed by ultrasonography were in surgical patients and the only case needing an antithrombotic therapy, in order to prevent clinical symptoms of DVT, was administered in a hip replacement surgical patient.
- While the Applicant stated, that the original TDM schedule, applied in the pivotal study, should be improved in order to provide a simpler and more practical approach, the original TDM (with very close and heavy monitoring) as applied in the pivotal study, would need to be followed, as long as no prospective clinical data have been provided with the "improved" TDM in surgical patients (proposed by the Applicant in the response to the CHMP List of Questions - 2nd submission-).
- Despite the fact that bioequivalence between the product used in the clinic and the to-be-commercialized product has been confirmed in healthy volunteers (see pharmacokinetics part), the proposed finished commercial product - nanofiltered antithrombin alfa - was not yet used in the patient population.

In order to provide appropriate data, the CHMP considered that the post-marketing safety monitoring study proposed by the Applicant should be performed before any marketing authorization of the product. The aim of this study was to provide sufficient prospective clinical efficacy and safety data in surgical and pregnant patients population using the finished intended to market product, nanofiltered antithrombin alfa. This study should include patients with confirmed hereditary AT deficiency in a high risk situation, prospectively investigating a the new dosing regimen for pregnant women, and updated therapeutic drug monitoring for all treated patients. The inclusion of patients who have previously been treated with antithrombin alfa, would be encouraged, enabling to obtain safety data on repeat administration of antithrombin alfa in patients. According to the Note for Guidance on the clinical investigation of plasma derived antithrombin products (CPMP/BPWG/2220/99), which also applies for recombinant products with regard to efficacy, the occurrence of deep vein thrombosis should be actively assessed by objective measures, such as doppler sonography. Diagnostic imaging should therefore be performed in each patient at baseline, at the last day of dosing and 7 days post dosing, and, if needed, additionally at 42 days in order to follow up any signs and symptoms indicative of a thromboembolic event.

At the February 2006 CHMP meeting the CHMP issued a negative opinion for ATryn in surgical patients with congenital antithrombin deficiency for the prophylaxis of deep vein thrombosis and thromboembolism in clinical risk situations, i.e. during the peri-surgical period.

One ground for refusal, related to the evaluation of efficacy, was that "efficacy data within the claimed indication are limited to only 5 patients (5 surgical, non-pregnant patients out of 14 patients treated

overall with antithrombin alfa in the single pivotal study submitted) making an assessment of the efficacy impossible and also being below the number recommended by the EMEA scientific advice (12 patients). The CHMP felt that the data obtained in patients treated in the compassionate use programme and at childbirth could not support the results obtained in the 5 surgical patients. Furthermore, the CHMP considered that ATryn could not be granted a marketing authorisation under exceptional circumstances, as proposed by the applicant during the oral explanation, due to the absence of objective and verifiable reasons".

In the re-examination documentation, the applicant argumented that all patients, pregnant and non-pregnant, from the pivotal trial and the Compassionate Use programme (19 in total) should be considered for the evaluation of efficacy. With regard to the dosing recommendations, the applicant refers to the well established use of antithrombin treatment in patients with congenital antithrombin deficiency, and states that in the development of Atryn, a similar approach was taken as for human plasma derived AT concentrates, ie initial dose recommendation followed by individualised dose titration based on actual plasma AT levels. While it may be possible to improve the dosing paradigm (and hence dosing convenience for the treatment of pregnant women), safety and efficacy were demonstrated when patients were treated using the dosing instructions specified in the pivotal trial. The difference in dosing convenience between surgical and pregnant patients is attributable to a difference in volume of distribution and clearance.
The applicant believes that the indication should be congenital deficiency as a whole, and that there are no grounds to regard pregnant congenital AT deficient patients as a separate subtype, in which safety and efficacy has not been established. The indication proposed by the applicant together with the grounds for the re-examination (SPC section 4.1. Therapeutic indication) is : "ATryn is indicated in patients with congenital antithrombin deficiency for the prophylaxis of deep vein thrombosis and thromboembolism in clinical risk situations (especially during surgery or during the peri-partum period), in association with heparin if indicated." Text to indicate that there may be differences between surgical and pregnant patients, requiring more frequent monitoring in pregnant women treated with Atryn, was proposed for SPC section 4.6 (Pregnancy and lactation).

The CHMP considered that the indication proposed by the applicant together with the grounds for the re-examination differs from that which was subject of the negative CHMP opinion of February, but is in line with the originally proposed wording at the time of submission of the Marketing Authorisation Application. The limited experience in the target population (non-pregnant surgery patients on one hand, and patients in the peri-partum period on the other hand), along with the large variability in dose requirements during treatment of pregnant patients with ATryn and the difficulties to maintain therapeutic AT levels in some of those patients remained a concern to CHMP.

The CHMP consulted an ad hoc expert group. The view expressed by the consulted experts during the ad-hoc expert meeting was that the surgical subpopulation (5 patients from the single pivotal trial), considered separately, was insufficient to establish the efficacy of ATryn in the surgical setting. Likewise, the 8 peri-partum patients considered separately were insufficient to establish efficacy in that clinical setting. However, in the opinion of the experts, for the evaluation of efficacy the important issue is the thrombotic risk, and not the specific situation determining such a risk (surgery or delivery). As the level of thrombotic risk for both clinical settings is high, and in both situations the target AT level is the same, in the experts view the entire population from the pivotal trial can be considered as a whole for the evaluation of efficacy.

Therefore the experts agreed that the available data would allow for proper dosing recommendations in non-pregnant surgical patients, and this view was accepted by CHMP. Whether the available data would be sufficient for use in pregnant patients was questioned by some experts and by the CHMP members, in view of the uncertainties with the dose recommendations in this group.

Another ground for refusal (related to both efficacy and safety) was that "the proposed finished commercial product, nanofiltered antithrombin alfa, was not the one used in the patient population".
The applicant's response to this point was that although nanofiltered ATryn was not used in the pivotal clinical trial, no material differences were observed in the physico-chemical and activity properties between the nanofiltered and non-nanofiltered product (used in the pivotal trial). No

material differences were shown in a nonclinical pharmacokinetic and biodistribution study in rats. Furthermore, a two-period cross-over human pharmacokinetic study was performed which demonstrated comparable safety and bioequivalence between the products.

In conclusion, in the opinion of CHMP only 14 subjects have received the nanofiltered antithrombin alfa (pharmacokinetic study), none of them repeated doses. Data obtained in this clinical trial can support that the pharmacokinetic profile of both products is similar but do not allow to conclude that the clinical safety, particularly in terms of immunogenicity, or clinical efficacy is the same. Changes in the manufacturing process of biotechnology-derived proteins may have an influence on the occurrence of an immunogenic response. CHMP therefore requests that safety and efficacy data of the nanofiltered antithrombin alfa should be provided; these should be obtained post-authorisation from the ongoing clinical trial and from a post-authorisation surveillance program.

Clinical safety

Patient exposure

All patients who took at least one dose of the product are included in the safety analysis. A total of 217 subjects were included, exposed to the recombinant product, of which 35 had congenital AT deficiency and 118 had acquired deficiency (relating to supportive studies and are not relevant to this application). The remainder included healthy volunteers. All 35 congenital AT III deficient patients received antithrombin alfa. Among them, 9 patients received 50 IU/kg, 6 patients received 100 IU/kg, 14 patients received continuous antithrombin alfa infusion, and 6 patients received multiple antithrombin alfa infusions over multiple days.

Adverse events and serious adverse events/deaths

Congenital AT deficient population

In the congenital AT deficient population, the most frequently reported adverse events were anaemia (17% of patients), hypotension (14% of patients), nausea (14% of patients), and abdominal pain /post operative pain/ fever (each 11% of patients). Severity of adverse events was generally mild to moderate (45.5% and 27.3% of the patients with at least one adverse event respectively). Adverse events were severe in 9.1%, and unknown in 18.2% of the patients with at least one adverse event. No dose relationship was seen for any adverse event in the congenital AT deficient patients. One patient experienced bronchospasm and rash erythematous at the second administration. One patient in a PK study reported one related adverse event coded to Preferred Term "Pruritis". This event was considered not serious with mild severity. Following review of the case this was considered to be an "Injection Site Reaction".

Acquired AT deficiency population

In the acquired AT deficiency population, the most frequently reported adverse events in antithrombin alfa treated patients were post operative pain (43% of patients), hypotension (24% of patients), haemorrhage, Not Otherwise Specified (NOS, 24% of patients), fever (20% of patients), fibrillation atrial (14% of patients), nausea (13% of patients), pleural effusion (12% of patients), hypertension (11% of patients), anaemia (11% of patients), hyperglycaemia (10% of patients).

Healthy volunteers

In the initially submitted healthy volunteer studies, the most commonly reported adverse events were headache (13% of patients), dizziness (11% of patients), and injection site reaction (11% of patients). Related adverse events reported in the healthy volunteers were injection site reaction (3.1% of patients), injection site pain (4.7% of patients), dizziness (4.7% of patients), headache (7.8% of patients), and nausea (6.3% of patients). Chest pain, hot flushes, pallor, and gastrointestinal reflux were all reported only once.

In the human PK bioequivalence study (GTC AT PK 011-04 1) performed in healthy volunteers in order to compare the heat-treated product with the heat-treated nanofiltered product, the adverse event profile for the two treatments are very similar, and no apparent safety concerns have arisen with any of

the two test drugs. Also, in the serum samples taken to determine an immune response to antithrombin alfa, no IgG or IgM antibodies directed against antithrombin alfa were found.

Immunogenicity

In response to concerns expressed by the CHMP regarding the immunological potential of antithrombin alfa (including the limited duration of follow-up, lack of data on repeated exposure and antibody testing methodology), the applicant showed that the follow-up of subjects post-administration varies from 23 days to 90 days in patients and from 14 to 90 days after a break of 5 months (cross-over PK study) in healthy volunteers. For the repeated administration concern, in the human volunteer PK cross-over study, comparing the to-be-commercialized nanofiltered, heat-treated, product with the heat-treated, non-nanofiltered product (GTC AT PK 011-04), the follow-up for anti-antithrombin alfa antibodies and AT-activity levels was until 90 days after the second administration (5 months after the first part of the study). All the retained samples, previously tested only for the presence of IgG antibodies, were tested again in the newly developed IgG antibody test, as well as with the new IgM assay, both fully validated. No IgG, nor IgM antibodies against antithrombin alfa were detected in these retained samples.

Therefore, on the basis that among the different studies performed in healthy volunteers and patients with antithrombin alfa, no inhibiting antibodies to antithrombin alfa have been detected, nor events that might indicate an immunological reaction, the applicant requested the repeated administration of antithrombin alfa to be allowed. In order to warn treating physicians to be cautious in case of repeated administration of antithrombin alfa, since the experience is limited, the following statement was proposed in section 4.8 of the SmPC: "No antibodies have been detected up to 90 days following treatment with ATryn. However, the experience with repeated doses is limited."

Despite reassuring data from the bioequivalence PK study, regarding the repeat-use of antithrombin alfa, the CHMP considers that the available experience concerns 38 subjects of which 37 are healthy volunteers and only one hereditary AT deficient patient included in the compassionate use protocol. From an immunological point of view, it appears very difficult to extrapolate limited safety data from use of the nanofiltered antithrombin alfa in healthy volunteers (14) to congenital AT deficiency patients, where their deficiency in AT could play a role in the susceptibility to develop inhibiting anti-antithrombin alfa antibodies. In haemophilia A and B, it is well known that mutations resulting in the absence or severe truncation of the factor VIII / factor IX proteins are associated with highest risk for inhibitor formation, indicating that a major driving force in inhibitor development is the presentation of a novel antigen to the patient's immune system. During the oral explanation held on 21 February 2006, the applicant `s argumentation that the total absence of normal factor VIII / factor IX proteins presents a different situation from hereditary (heterozygous) antithrombin deficiency, where AT levels are usually between 40%- 50% of normal was accepted.

Another aspect regarding immunogenicity came from the Quality Assessment Report, which clearly stated that "the amount of goat AT, which may potentially cause cross-reactive immune response against human AT, cannot be quantified as the level is below the limit of detection of 2.5ng/mg. Based on process performance, gAT may be estimated to be near 0.3µg/250mg dose, which is below the limit of detection of the method (LOD correspond to 0.625µg / 250 mg dose). The presence of residual goat AT in the product is drawn to the attention of the clinical assessors."

The clinical assessors referred to a Letter to the Editor published by L.T. Spencer et al. in NEJM of the May 12, 2005. In this article, the authors describe that they observed systemic antibody responses to non-human protein that was present in very low concentrations (<100 ppm) in a sheep-derived transgenic human alpha-1 antitrypsin formulation: 24% of patients with a positive response to sheep alpha-1 antitrypsin, and 78% of patients with a positive response to sheep alpha-1 antichymotrypsin. Among subjects who withdrew from the study (4), there was a possible relationship between drug-related adverse events (dyspnea and a decline in lung function) and a high-titer antibody response. The clinical symptoms (exertional dyspnea and hypoxemia) and secondary antibody responses that occurred in the one subject who participated in both studies suggest that re-exposure could result in intolerance of non human proteins for therapeutic use.

These findings, reported by L.T. Spencer, raised concerns for ATryn application, as the manufacture concept appears similar and the amount of contaminant non-human proteins observed compatible with those expected in the Quality Assessment report for ATryn, i.e. near 0.3µg/250mg dose, which is below the limit of detection of the method. Furthermore, as stated in the Letter to the Editor, these

findings underline the concern of re-exposure to contaminant non-human proteins and the possible resulting intolerance.
Until the time of the Day 210 rapporteurs`s assessment report in January 2006, no tests had been developed by the Applicant in order to detect potential immunogenicity to contaminant goat proteins, and notably goat AT, in subjects receiving antithrombin alfa.
In the oral explanation held on 21 February 2006, the applicant presented summary of results from a new immunodot blot assay for antibodies to goat AT and goat milk proteins. The documentation submitted with the grounds for re-examination included the detailed results and a report on the validation of the assay.

Serious adverse event/deaths/other significant events
Five treatment emergent serious adverse events were reported in 3 (12% of patients) congenital AT deficient patients, all from study GTC AT III 01002. Of these three patients, 1 was reported in a pregnant female and 2 were reported in male or non-pregnant females. Fever in a pregnant female patient was considered not related. Hypotension and haemorrhage NOS in a non-pregnant female, and convulsions Grand Mal and fracture trauma in a male, were all considered as being remote/unlikely.
Two additional SAEs were reported in neonates (congenital malformations, unrelated). No deaths were reported from this study.

Serious adverse events were reported in 29 acquired AT deficient patients (24.6%) treated with antithrombin alfa, in 3 patients (21.4% of patients) treated with hpAT and in 11 patients (19.3% of patients) in the placebo group. For patients who received antithrombin alfa, there were seven patients (6%) who reported ten related serious adverse events. These included 5 events of Haemorrhage NOS, of which 4 were considered as being possibly and 1 probably related and 1 case of concomitant anaemia, considered as possibly related. Two cases of myocardial infarction were considered to be possibly related, as were 2 events of hallucination and psychosis in 1 patient. All patients recovered from these events. For patients who received hpAT, there was one patient (7%) who reported two related serious adverse events but recovered. No placebo patients reported any related serious adverse events. With regard to SAE myocardial infarction, discrepancies in causality assessments, between the integrated summary of safety and the text in the study report, were confirmed by the applicant in responses to LOI, and were attributed to differences between the initial SAE report, discharge letter ad the CRF.

Three patients died in the antithrombin alfa treatment group, one in placebo and one in hp-AT treatment group. Death was considered not related to study drug in the 5 cases (cardiogenic failure, arterial occlusion, cerebral infarct, disseminated intravascular coagulopathy, cardiogenic shock).

Laboratory findings
No clinically significant laboratory changes were identified.

Safety in special populations

No difference in the adverse event profile was found between the pregnant females and the male/non-pregnant females. None of the neonates delivered from women participating in the Efficacy Study had any adverse event that could be attributed to maternal antithrombin alfa treatment.

Discussion on clinical safety

Safety data are very limited: only 14 patients with congenital deficient AT were included in the pivotal trial and 5 in the compassionate use group.
The two safety populations (congenital and acquired deficient AT) were not comparable at baseline as the demographic parameters (age, sex) were different. Congenital deficient AT is mainly represented by young pregnant females while the acquired deficient concerned old male patients scheduled for cardiac surgery with a history of cardiovascular underlying diseases and concomitant treatments.

Haemorrhage was the main AE in acquired deficient AT patients. This is understandable as those patients underwent surgery and had a concomitant anticoagulant therapy (mainly heparin). The bleeding events observed in acquired deficient AT patients, but also to a lesser extent in congenital deficient AT patients (three bleeding events in two patients: one vaginal haematoma in a pregnant patient, and a wound haemorrhage and haematoma in a patient with a hip replacement surgery), raise the safety issue of haemorrhage occurrence, which could be increased by the difficulties encountered in some patients with the posology adjustment of antithrombin alfa in order to achieve AT activity between 80 and 120%. Headache, hypotension, nausea and fever were also observed in this study population.

At the time of the initial CHMP Opinion (February 2006) the safety concern of potential immunogenicity of antithrombin alfa had not been resolved by the additional information provided during the assessment. Indeed neither IgG, nor IgM antibodies against antithrombin alfa were detected in the patient studies nor in the human volunteer bioequivalence PK finalized study. However it is difficult to extrapolate these limited safety data, particularly on the repeated administration of the product and namely the nanofiltered antithrombin alfa, only tested in healthy volunteers (14), to the situation of congenital AT deficiency patients. Furthermore, there was uncertainty about the validation of the new immunodot blot assay for antibodies to goat AT and goat milk proteins. Thus the CHMP concern of re-exposure to contaminant non-human proteins and the possible resulting intolerance could not be resolved, leading to the following ground for refusal (in addition to the 2 grounds discussed in the section on Efficacy): the safety database for patients with congenital antithrombin deficiency is felt insufficient considering the observed safety signals and the potential problem with immunogenicity.
The Applicant responded that "the observed safety signals" for ATryn (bleeding events, hypotension, fever) are the same as those described in the package inserts for hpAT products marketed in Europe. These alleged signals are related to the pharmacological class of antithrombin, to concomitant medications (e.g., induction of anesthesia) and to the procedures patients undergo while being treated with AT (ATryn or hpAT). The Applicant has assessed patients for IgM and IgG (all subtypes) antibodies to Atryn, as well as for antibodies to potential residual goat milk proteins (including goat AT) using validated assays. To date, the Applicant has not observed any clinical or laboratory evidence for an immune response in over 200 patients treated on one or more occasions with ATryn. Furthermore, AT activity levels following treatment returned to baseline indicating a lack of neutralizing antibodies.

The CHMP sought input from an ad-hoc expert group on the issue of limited safety data, especially with regard to concerns for possible immunogenicity of antithrombin alfa and of remaining goat proteins in the product. The vast majority of the (over 200) individuals tested for IgG and IgM antibodies to ATryn were given a single Atryn dose. Immunodot Blot Assay (for remaining goat proteins) has only been carried out in 24 subjects. To the experts, the concerns raised related to possible immunogenicity of ATryn would not be an issue precluding clinical use, provided appropriate surveillance would be in place. The lack of clinical experience in the patient population with the nanofiltered product was identified as an element of uncertainty (please refer to the discussion on Efficacy).
CHMP therefore considers the potential problem with immunogenicity does not preclude granting a marketing authorisation, provided that the applicant addresses these points as part of post-authorisation activities. This also includes questions raised during the expert meeting regarding test methodology (the positive controls used, and IgE testing).

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 CHMP, having considered the data submitted in the application, is of the opinion that no additional risk minimisation activities are required beyond those included in the product information.

Summary of the risk management plan for ATryn

Safety concern	Proposed pharmacovigilance activities	Proposed risk minimisation activities
Bleeding	• Routine Pharmacovigilance • Additional information from ongoing trials • Additional information from post-marketing surveillance programme	• Warning in SmPC Section 4.4 "Special warnings and special precautions for use" • Warning in SmPC Section 4.5 "Interaction with other medicinal products and other forms of interaction" • Haemorrhage listed in the SmPC Section 4.8 "Undesirable effects".
Immunogenicity	• Routine Pharmacovigilance • Additional information from ongoing trials • Additional information from post-marketing surveillance programme • Immunosurveillance program	• Report results of immunosurveillance program to treating physicians • Contraindication in section 4.3 for patients with hypersensitivity to goat proteins or goat milk components • SmPC includes following text in section 4.4 "Special warnings and special precautions for use": Patients treated with ATryn should be monitored for possible clinical immunological reactions. Antibody status should be monitored and reported.
Hypotension, fever	• Routine Pharmacovigilance • Additional information from ongoing trials • Additional information from post-marketing surveillance programme	
Potential medication error	• Routine Pharmacovigilance	• The SmPC, section 4.2 *Posology and method of administration*, contains a statement about the differences between plasma and recombinant AT. • The need for a correct dosing, will be clearly mentioned in all communications to health care professionals. All marketing material will have the dosing regimen listed, and where appropriate the distinction with plasma derived AT will be mentioned.

6. Overall conclusions and benefit/risk assessment

Quality

ATryn contains recombinant human antithrombin (AT) produced in transgenic goats.
Considerable efforts have been made by the applicant to improve the genetic consistency of the transgenic herd and the consistency of the goat milk (source material).
Testing of the drug substance include elaborate monitoring of goat milk contaminants and glycoprotein consistency. Trace amounts of goat AT (up to 0.3 µg/dose) and other goat proteins in the product raises concern regarding (i) the potential cross-reactive immune response of residual goat AT against endogenous human AT and (ii) hypersensitivity reactions against other goat proteins and milk components. The latter concern is addressed adequately in the SPC. The specifications for the drug substance and drug product will be reviewed when an appropriate number of commercial scale batches have been produced.
It can be concluded that the applicant has provided evidence that ATryn is manufactured in a consistent way and is tested satisfactorily. All outstanding quality issues can be resolved on an ongoing basis.
Appropriate measures are in place to assure the viral and TSE safety of ATryn.

Non-clinical pharmacology and toxicology

The non-clinical studies were limited. Non-clinical data demonstrated the anticoagulant activity of antithrombin alfa and revealed no special hazard for humans based on studies of single dose toxicity, repeated dose toxicity, genotoxicity and reproductive toxicity.

In non-clinical studies, exposure to antithrombin alfa was similar or higher (up to 2-fold) than in the clinical situation. The formulations used in most non-clinical studies were non-heat treated. Minor biochemical differences with the heat treated, and finally nanofiltered heat treated antithrombin alfa products are not thought to impact on the pharmacokinetics, distribution, or activity of the antithrombin alfa in animals and humans.

Efficacy

The proposed clinical use of ATryn is for the prophylaxis of venous thromboembolism in surgery of patients with congenital antithrombin deficiency. ATryn is normally given in association with heparin or low molecular weight heparin.

In total, 19 hereditary AT deficient patients were treated during and after 20 high risk situations: Five (5) patients /6 treatments with non-heat treated antithrombin alfa in the Compassionate Use Study (GTC AT III 01, 03), and 14 hereditary AT deficient patients in the context of surgery (n=5) and delivery (n=9) with heat treated antithrombin alfa in the pivotal Efficacy Study (GTC AT III 01002).

The initial CHMP opinion was negative on the following grounds:

- Efficacy data within the claimed indication are limited to only 5 patients (5 surgical, non-pregnant patients out of 14 patients treated overall with antithrombin alfa in the single pivotal study submitted) making an assessment of the efficacy impossible and also being below the number recommended by the EMEA scientific advice (12 patients). The CHMP felt that the data obtained in patients treated in the compassionate use programme and at childbirth could not support the results obtained in the 5 surgical patients. Furthermore, the CHMP considered that ATryn could not be granted a marketing authorisation under exceptional circumstances, as proposed by the applicant during the oral explanation, due to the absence of objective and verifiable reasons;
- The proposed finished commercial product, nanofiltered antithrombin alfa, was not the one used in the patient population

Further to the applicant`s request for re-examination, during the ad-hoc expert meeting held within the re-examination procedure, the experts considered that the entire population could be used for the evaluation of the efficacy of ATryn. The expert also agreed that the available data would allow for proper dosing recommendations in surgical patients. Whether the available data would be sufficient for use in delivery patients was questioned by some experts, in view of the uncertainties with the dose recommendation. The lack of experience with the nanofiltered product in the patient population is an element of uncertainty.

Taking into account the recommendations from the ad-hoc expert meeting, CHMP agreed that the efficacy data from 13 evaluable patients (pregnant and non-pregnant) in the pivotal trial can be considered to support the claimed indication. Further data on efficacy of the nano-filtered product from the ongoing study and from post-authorisation surveillance are required, these can be provided post-authorisation.

Safety

The safety database for patients with congenital antithrombin deficiency is limited considering the observed safety signals and the potential problem with immunogenicity. The other patient population studied, patients with acquired AT deficiency, has different demographic parameters.
Bleeding events observed in patients with acquired AT deficiency, and to a lesser extent in patients with congenital deficiency, raise the safety issue of haemorrhage occurrence, which could be increased by the difficulties encountered with antithrombin alfa posology adjustment needed to achieve antithrombin activity between 80 and 120 %.
Headache, hypotension, nausea and fever were also observed in the study population.
There is a potential risk of an immune response to the antithrombin alfa or residual goat proteins, including goat AT. Such an immune response could potentially cross-react with human AT. Neither IgG, nor IgM antibodies against antithrombin alfa were detected even after repeated exposure. Nevertheless, it appears very difficult to extrapolate the limited safety data, particularly on the repeated administration of the product and namely the nanofiltered antithrombin alfa (only tested in 14 healthy volunteers), to the situation of congenital AT deficiency patients.
In the oral explanation the applicant presented a new immuno dot blot assay for antibodies to goat AT and goat milk proteins. In the initial opinion CHMP concluded that the safety database for patients with congenital antithrombin deficiency is felt insufficient considering the observed safety signals and the potential problem with immunogenicity.

In the re-examination documentation the applicant provided further details on the immuno-assay methodology.
The ad-hoc experts concluded that the concerns raised related to possible immunogenicity of ATryn would not be an issue precluding clinical use, provided appropriate follow-up would be in place. The lack of clinical experience in the patient population with the nanofiltered product was identified as an element of uncertainty.
CHMP therefore considers the potential problem with immunogenicity does not preclude granting a marketing authorisation, provided that the applicant addresses these points as part of post-authorisation activities. This also includes questions raised during the expert meeting regarding test methodology (the positive controls used, and IgE testing).

Close monitoring for haemorrhage and signs of immunogenicity and systematic serum sample collection to study immunogenicity should be performed

User consultation

The company provided a justification why no user consultation was performed:

- The targeted patient population group (hereditary antithrombin deficient patients undergoing surgery) is very limited;
- The product will be administered intravenously and in a hospital setting. The package leaflet will only be seen by the patient upon their personal request;

- As there is no patients association for people with hereditary antithrombin deficiency, it is very difficult to find relevant people to conduct a user consultation.

The justification provided by the company is acceptable.

Benefit/risk assessment

Considering the available data submitted in the Marketing Authorisation Application, the Marketing Authorisation Holder`s answers to the grounds for refusal, the recommendations expressed at the ad-hoc expert group meeting, and the oral explanations provided by the applicant, the CHMP conclusions at the end of the re-examination were that:

- The applicant has provided evidence that ATryn is manufactured in a consistent way and is tested satisfactorily. Appropriate commitments have been taken by the applicant to resolve all outstanding quality issues on an ongoing basis.
- Adequate measures are in place to assure the viral and TSE safety of ATryn.
- Due to the rarity of this clinical condition, the efficacy in terms of prevention of thromboembolic events cannot be fully established
- Some EU countries do not have approved hpAT product, which limits the availability of AT concentrates to treat those patients
- From the data provided it can be reasonably concluded that the proposed dosing schedule for Atryn will allow to bring antithrombin levels in the target range (80 – 120%), which in the view of the experts would be the immediate treatment target.
- Further data are necessary to draw conclusions on the most suitable dosing schedule in pregnant women with congenital AT deficiency.
- Although the safety database is limited and there are remaining concerns regarding the potential immunogenicity of Atryn, the CHMP feels that this should not preclude the clinical use of Atryn. Surveillance of immunogenicity (to Atryn or residualt goat proteins), together with other relevant safety signals (bleeding, hypotension, fever, hypersensitivity reactions) should be addressed by the marketing authorisation holder as specific obligations.

Therefore the CHMP agrees that based on the quality data submitted, the efficacy data from 13 evaluable patients (pregnant and non-pregnant) in the pivotal trial, and based on the overall safety database of Atryn, a restricted indication (limited to non-pregnant surgery patients) can be granted under exceptional circumstances.

A risk management plan was submitted. The CHMP, having considered the data submitted, was of the opinion that:

- pharmacovigilance activities in addition to the use of routine pharmacovigilance were needed to investigate further some of the safety concerns
- no additional risk minimisation activities were required beyond those included in the product information.

ATryn is indicated for the prophylaxis of venous thromboembolism in surgery of patients with congenital antithrombin deficiency. ATryn is normally given in association with heparin or low molecular weight heparin.

Recommendation

Based on the CHMP review of data on quality, safety and efficacy, the CHMP considered by consensus that the risk-benefit balance of ATryn for the prophylaxis of venous thromboembolism in surgery of patients with congenital antithrombin deficiency, was favourable and therefore recommended the granting of the marketing authorisation under exceptional circumstances.