

Briefing Document Blood Products Advisory Committee

Title: Briefing Document
Blood Products Advisory Committee Meeting
January 9, 2009

Product Name: ATryn® (Antithrombin III [Recombinant]) for Injection

Formulation: Lyophilized Powder

Indication: Hereditary Deficiency of Antithrombin

Sponsor: GTC Biotherapeutics, Inc.
175 Crossing Blvd, Suite 410
Framingham, MA 01702

Date: 03 December 2008

Advisory Committee Briefing Materials: Available for Public Release

1 EXECUTIVE SUMMARY

Background

GTC Biotherapeutics, Inc. has developed recombinant human antithrombin [rhAT, Antithrombin III (recombinant), antithrombin alfa, ATryn®], produced in the milk of transgenic goats, for use in patients with hereditary antithrombin deficiency (HD). The international nonproprietary name and the United States Adopted Name for this recombinant protein is antithrombin alfa and the product name for the formulated dosage form is ATryn. Antithrombin III (recombinant) is a highly purified, well characterized recombinant glycoprotein consisting of 432 amino acids, 3 disulphide bridges between cysteine residues and 4-N linked glycosylation sites, identical to human plasma-derived antithrombin.

In August 2006, ATryn was approved by the European Commission for the treatment of surgical patients with hereditary antithrombin deficiency to prevent venous thromboembolic events. Working closely and in collaboration with the Center for Biologics Evaluation and Research (CBER) and the Office of Orphan Products Development of the U.S. Food and Drug Administration (FDA), ATryn has been designated 1) an orphan drug for the treatment of hereditary antithrombin deficient patients and 2) as a drug for Fast Track development. The Biologic License Application (BLA) that is under review by CBER was submitted as a rolling application and has been granted Priority Review by CBER.

Transgenic Production of ATryn

Recombinant human antithrombin is produced in the milk of transgenic goats. For expression of rhAT, the DNA construct contained a goat beta casein promoter with the cDNA coding region for human antithrombin (hAT). The DNA construct was microinjected into fertilized one-cell goat embryos, which were transferred to female recipients. Kids born from the recipients were tested for presence of the transgene and expression of the human protein. A founder transgenic goat was selected from which subsequent offspring were generated by natural breeding to give rise to a production herd of hAT transgenic goats, which are milked to provide the source material for downstream purification of the rhAT. The rhAT is isolated from the goats' milk and conventionally purified using: tangential flow filtration, heparin affinity chromatography, viral removal filtration, anion exchange chromatography and hydrophobic interaction chromatography. A combination of validation of the impurity-removal capacity of the antithrombin alfa manufacturing process and the use of impurity-detecting assays is utilized to ensure purity of ATryn. The formulated drug substance is shipped for fill-finish where it is aseptically filled into 20 mL vials, lyophilized and subjected to terminal dry heat treatment for viral inactivation. The final product, ATryn®, is a sterile lyophilized powder for solution for infusion.

Overview of Adventitious Agents Risk Assessment for ATryn

A variety of general adventitious agent risk minimization measures have been implemented for initial selection of the GTC Farm site, sourcing of goats from the United States and New Zealand and maintenance of the biosecurity of the goat herd and their environment. The risk

from viral, prion (scrapie) and other adventitious agents was evaluated at the level of the goat (the bioreactor), the milk (the bioreactor harvest) and the removal/inactivation capacity of the manufacturing process. The hAT goats are part of a closed USDA certified scrapie-free, specific pathogen-free goat herd, are tested for a subset of viruses and are monitored for scrapie, the prion disease of goats. The source material is screened for adventitious viruses by *in vitro* cell line screening on four cell types. The two filtration steps and the three chromatographic steps in the purification process have been validated to remove scrapie and several viruses that model many of the common goat viruses. The column cleaning solutions, new and recycled TFF membranes and resins, and the heat inactivation step were also evaluated for their viral removal and inactivation capacities. Data supporting validated removal of potential adventitious viral contamination and prion/scrapie substantiate that ATryn is safe for human use.

Medical Condition to be Treated

Hereditary antithrombin deficiency is an autosomal dominant disorder characterized by a reduction in plasma antithrombin activity. It is an orphan medical condition in the US. The majority of affected individuals are heterozygotes. Antithrombin activity levels are usually between 40 and 50% of normal in these individuals. Hereditary AT deficiency causes a life-long increased risk of venous thromboembolism and up to 70% of cases do develop a venous thromboembolic event (VTE) during their lives. Failure to properly treat hereditary AT deficient patients, especially during high risk situations such as surgery or trauma or for pregnant women, during the peri-partum period, may result in VTE that may potentially lead to death due to life-threatening pulmonary emboli or may have other life-long sequelae. Deep venous thrombosis (DVT) of lower limbs and pulmonary embolism are the most common thromboembolic events reported in this population.

AT concentrate is used for the prophylaxis of the occurrence of venous thromboembolisms in hereditary AT deficient patients in high risk situations, e.g. surgery or delivery, known to be associated with the occurrence of such events. The target is to restore plasma AT activity levels in order to cover the period during which chronic anticoagulation often needs to be interrupted and ensure that the current thromboprophylaxis is effective. In addition, if a VTE does occur, AT concentrate can be used to support the anticoagulative therapy, which is dependent on sufficient AT activity levels.

ATryn Pivotal Trials

Per agreement with FDA, a comparative, historical control, international, multi-center study was performed in patients with HD who had undergone an elective procedure that placed them at high risk for the occurrence of a thromboembolic event, during which they were treated prophylactically with an intravenous administration of plasma AT (historical cohort in study GTC AT HD-R 013-04) or ATryn (GTC AT III 01002 and GTC AT HD 012-04). The incidence of thromboembolic events during treatment or up to 7 days post-treatment was compared. Patient populations in the historical study were matched as much as possible with those treated with ATryn.

Other Clinical Studies

ATryn also has been studied in clinical trials including patients with heparin resistance (HR). HR is defined as an inadequate response to heparin in patients undergoing cardiopulmonary bypass (CPB). Among risk factors for developing HR during CPB, decreased AT levels (acquired AT deficiency) present the greatest risk. However, HR is not being pursued as an indication in this BLA filing.

ATryn Dose

For treatment of HD, dosing with ATryn is initiated by a 15 minute loading infusion, immediately followed by a continuous infusion. Treatment is aimed at restoring AT activity to normal (with a range from 80 to 120%). Dosing for both prophylaxis and treatment are the same taking into account the type of patient (pregnant or non-pregnant surgical), baseline AT activity and body weight. Therapeutic drug monitoring is applied to maintain patients within the target range during the treatment period.

Efficacy: HD Trials

No plasma AT-treated patient experienced a thromboembolic event. Only 1 (3.2%) of the 31 patients in the ATryn treatment group experienced a confirmed thromboembolic event (acute DVT) and no patient experienced a confirmed thromboembolic event other than acute DVT. The non-inferiority of ATryn as compared to plasma AT treatment was therefore established and supports the efficacy of ATryn for the claimed indication.

The single patient from the GTC AT III 01002 study who had an acute DVT during treatment with ATryn was the only one who was treated accordingly by continuation of ATryn treatment together with low molecular weight heparin and Vitamin K antagonists. The patient remained asymptomatic and the event eventually resolved. It is likely that this patient who had a hip replacement procedure, which has a high risk of development of thrombosis, even without thrombophilia, represents the portion of thromboses normally seen in other studies with joint replacement surgery.

The analysis of 5 HD patients treated on 6 occasions in a compassionate use program with ATryn, further support the efficacy of ATryn. Although not treated by continuous infusion, multiple daily infusions were able to prevent the occurrence of any VTE.

Safety

The safety database for ATryn contains data on 235 subjects/patients that have been exposed to ATryn on at least one occasion. Additionally, data are available on 38 of these subjects/patients who have been exposed on two separate occasions to ATryn.

The type of adverse events reported in the clinical studies with ATryn reflects events often associated with surgery and delivery. Moreover, the combined use of AT and heparin (either unfractionated or low molecular weight heparin) during and just after surgery and delivery increases the risks of hemorrhagic complications. Among the reported events assessed related

to ATryn are also hemorrhagic events of different kinds. The rate seems in line with what can be expected in the populations studied.

Other minor events reported are infusion site reactions, and some other events generally seen in clinical trials, like headache, erythema, dizziness, etc. In general, it can be concluded that ATryn was well tolerated.

Assays have been developed and used for detecting any antibodies to antithrombin alfa or potentially contaminating goat milk proteins, including goat AT. No confirmed specific immune reaction to any of the components of ATryn has been detected during the clinical development.

Considering the infrequency with which patients may require treatment with ATryn on more than one occasion and given the relatively limited number of patients who have already been exposed to ATryn in clinical trials, the only way to gather additional clinical data assessing the immunogenic potential of ATryn, especially in those patients requiring re-exposure to ATryn, is in a post-marketing setting. The Applicant plans to conduct a post-marketing immunosurveillance study.

Unmet Medical Need

The need for a non-plasma derived alternative to the AT concentrates currently available is based upon patient preference as well as a concern, however small or large, about the potential risk of transmission of infectious agents, such as viruses and prions, from pools of human plasma. ATryn does not carry the risk of transmission of viruses derived from human plasma. The robust ATryn® manufacturing process has been extensively validated to demonstrate the removal/inactivation of potential viruses, as well as prions even though the herd of goats from which antithrombin alfa is derived, has been and remains a closed, certified scrapie-free and specific-pathogen-free herd, i.e. a strictly controlled source.

Finally, while it is presently claimed that Thrombate III® is available in the U.S. marketplace, this has not always been the case (as proven by the compassionate use program that existed for ATryn when Thrombate III® was not available) and there is no guarantee that this product will be readily available in the future. GTC Biotherapeutics, Inc. has established through the use of recombinant DNA technology a dedicated, steady and reliable source of recombinant human antithrombin, which may be made available unrestricted to physicians, pharmacists and distributors alike. Greater certainty around the continuous availability of product in the marketplace fulfills an unmet medical need.

Conclusion

On the basis of the development activities and data generated over the last 16 years, the Applicant maintains that ATryn has been shown to be safe and efficacious. As such, ATryn should be recommended for licensure for the treatment of a rare plasma protein disorder to prevent serious and potentially life-threatening venous thromboembolic events.

TABLE OF CONTENTS

1	EXECUTIVE SUMMARY	1
2	INTRODUCTION	12
2.1	Recombinant Human Antithrombin.....	12
3	THE APPLICANT	13
4	THE TRANSGENIC PRODUCTION PLATFORM	14
4.1	Introduction.....	14
4.2	History of Transgenic Production.....	14
4.3	Regulatory Oversight	14
4.4	Transgenic Goats for Production of Recombinant Human Antithrombin	15
4.4.1	Selection of the Species	15
4.4.2	Closed Specific Pathogen-free Herd.....	17
4.4.3	Housing of Goats	17
4.4.4	Vaccination of Goats.....	18
4.4.5	Animal Feeds	18
4.4.6	Viruses of the Goat	19
4.4.6.1	Eradication of Specific Viruses from the Herd.....	20
4.4.7	Site Directed Expression of Human Protein in Mammary Gland - Gene Construct.....	20
4.4.8	Production of Transgenic Founder Goats	21
4.4.9	Selection of Founder	22
4.4.10	Generation of the Production Herd.....	22
4.4.11	Characterization of Production Herd	23
4.4.12	Animal Health and Welfare	23
4.4.13	Management of a Transgenic Production Herd	24
4.4.14	Adventitious Agent Risk Minimization Measures for the Goat	24
5	RECOMBINANT HUMAN ANTITHROMBIN	25
5.1	Transgenic Goat Milk	25
5.1.1	Milking the Transgenic Goats.....	25
5.1.2	Adventitious Agent Risk Minimization Measures for the Milk	26
5.2	Purification of the rhAT from Milk	26
5.2.1	ATryn Manufacturing Process.....	26
5.2.2	Impurities (Including Viruses and Prions).....	27
5.2.2.1	Process Validation for Removal of Impurities.....	27
5.2.2.2	Analytical Determination of Impurities.....	28
5.2.2.3	Validated Removal of Viruses	30
5.2.2.4	Validated Removal of Prions/Scrapie.....	30

5.2.3	Characterization of Final Product and Comparability to hpAT	31
6	NON-CLINICAL SAFETY	33
6.1	Pharmacodynamic Studies	33
6.2	Pharmacokinetic Studies	33
6.3	Toxicology Studies	34
7	CLINICAL PROGRAM	34
7.1	Hereditary Antithrombin Deficiency	34
7.1.1	The Condition	34
7.1.2	Patient Population	35
7.1.3	Rarity of Disorder	36
7.1.4	Available Treatments	37
7.1.5	Clinical Development Program	37
7.1.5.1	Pharmacokinetics	40
7.1.5.2	Establishment of Dosing for Hereditary AT Deficient Patients	40
7.1.5.3	Efficacy Studies	43
7.2	Acquired Antithrombin Deficiency	57
7.2.1	Heparin Resistance in Cardiopulmonary Bypass Patients	57
7.3	Safety of ATryn	60
7.3.1	Hereditary AT deficient patients	64
7.3.1.1	Adverse Events in Treated Patients	64
7.3.1.2	Neonates	70
7.3.2	Acquired AT Deficient Patients	71
7.3.3	Healthy Volunteers	76
7.3.4	Immunogenicity	78
7.3.4.1	Anti-antithrombin alfa IgG and IgM Assays	78
7.3.4.2	Inhibition of Antithrombin Activity	81
7.3.4.3	Immunodot Blots	81
7.3.4.4	Overall Discussion and Conclusions Regarding PIR Results	81
7.3.5	Safety Conclusions	83
7.4	ATryn – Fulfilling an Unmet Medical Need	87
7.5	Benefit to Risk Assessment	87
8	REFERENCES	90

Table of Figures

Figure 1.	Considerations for Species Selection.....	16
Figure 2.	Human Antithrombin Transgene (7).....	21
Figure 3.	Making a hAT Transgenic Goat	22
Figure 4.	rhAT Purification Process.....	27

Table of Tables

Table 1.	Specific Pathogen-Free Herd	17
Table 2.	Expression of Major Proteins in Milk.....	21
Table 3.	Cumulative Log ₁₀ Clearance of Protein Contaminants.....	28
Table 4.	Methods to Detect Residual Goat Milk Proteins	29
Table 5.	Viral Clearance/Inactivation by the ATryn Manufacturing Process.....	30
Table 6.	Scrapie Clearance by the ATryn Manufacturing Process.....	31
Table 7.	Comparison of hpAT to Recombinant Antithrombin	32
Table 8.	Table of All Investigations.....	38
Table 9.	Population PK Parameters for Antithrombin alfa.....	40
Table 10.	Description of Clinical Efficacy Studies.....	44
Table 11.	Demographics and Baseline AT Activity (Per Protocol Population)	50
Table 12.	Patient Disposition Summary	51
Table 13.	Concomitant Anticoagulation Medications (Per Protocol Population)	52
Table 14.	Overall Incidence of Any Confirmed Thromboembolic Event (Per Protocol Population).....	54
Table 15.	Overall Incidence of Confirmed Acute DVT (Per Protocol Population).....	54
Table 16.	Overall Incidence of Confirmed Thromboembolic Events Other Than Acute DVT (Per Protocol Population).....	54
Table 17.	Procedure/Diagnosis Information (Safety Population).....	56
Table 18.	Results of Heparin Resistance Efficacy Studies	59
Table 19.	Numbers Subjects and Patients Exposed to ATryn, hpAT and Placebo.....	61
Table 20.	Demographic Characteristics (Safety Population).....	62
Table 21.	Total ATryn Dose (IU) (Safety Population)	63
Table 22.	Total ATryn Dose (IU/kg/day) (Safety Population)	64
Table 23.	Summary of Patients Experiencing Treatment-Emergent Adverse Events - Hereditary Deficient Patients (Safety Population)	64
Table 24.	Treatment-Emergent Adverse Events Reported by At Least 5% of Hereditary Deficient Patients (Safety Population).....	65
Table 25.	List of Severe Treatment-Emergent Adverse Events By System Organ Class - Hereditary Deficient Patients (Safety Population).....	66
Table 26.	List of Serious Treatment-Emergent Adverse Events By System Organ Class - Hereditary Deficient Patients (Safety Population).....	68
Table 27.	List of Related Treatment-Emergent Adverse Events By System Organ Class- Hereditary Deficient Patients (Safety Population).....	69
Table 28.	Summary of Neonates Experiencing Treatment-Emergent Adverse Events – Hereditary Deficient Pregnant Females (Safety Population).....	70

Table 29.	Treatment-Emergent Adverse Events Reported by Greater than One Neonate among Hereditary Deficient Patients (Safety Population)	71
Table 30.	Summary of Patients Experiencing Treatment-Emergent Adverse Events - Acquired Deficient Patients (Safety Population).....	71
Table 31.	Treatment-Emergent Adverse Events Reported by at Least 10% of Acquired Deficient Patients in Either Treatment Group (Safety Population)	72
Table 32.	Severe Treatment-Emergent Adverse Events Reported by at Least 2 ATryn Treated Acquired Deficient Patients (Safety Population).....	73
Table 33.	List of Related Serious Treatment-Emergent Adverse Events - Acquired Deficient Patients (Safety Population).....	74
Table 34.	Related Treatment-Emergent Adverse Events - Acquired Deficient Patients (Safety Population).....	75
Table 35.	Summary of Patients Experiencing Treatment-Emergent Adverse Events - Healthy Volunteers (Safety Population).....	76
Table 36.	Treatment-Emergent Adverse Events Reported by At Least 5% of Healthy Volunteers (Safety Population)	77
Table 37.	Related Treatment-Emergent Adverse Events Reported in 3 or More Subjects – Healthy Volunteers (Safety Population).....	77
Table 38.	Summary of PIR Assay Results for Antithrombin alfa Treated Subjects from the Clinical Trials.....	80
Table 39.	Related Treatment-Emergent Adverse Events – All Patients (Safety Population).....	84

List of Abbreviations

AAALAC Int.	Association for Assessment and Accreditation of Laboratory Animal Care International
ACT	activated clotting time
AE	adverse events
APHIS	Animal and Plant Health Inspection Service
aPTT	activated partial thromboplastin time
Arg	arginine
Asn	asparagine
AT	antithrombin or antithrombin III
AUC	area under the curve
BLA	Biologic License Application
BSE	bovine spongiform encephalopathy
BVD	bovine viral diarrhea
CABG	coronary artery bypass grafting
CAE	caprine arthritis encephalitis
CAEV	caprine arthritis encephalitis virus
CBER	Center for Biologics Evaluation and Research, FDA
CD	circular dichroism
CGMP	colloidal goat milk proteins
CHO	Chinese hamster ovary cells
CI	confidence interval
CJD	Creutzfeldt Jakob Disease
Cl	clearance
C _{max}	maximum concentration obtained
CPB	cardiopulmonary bypass
CT	computed tomography
CVM	Center for Veterinary Medicine, FDA
DNA	deoxyribonucleic acid
DVT	deep vein thrombosis
EDTA	ethylenediaminetetraacetic acid
ELISA	enzyme linked immunosorbent assay
EMA	European Medicines Agency
FDA	Food and Drug Administration
FFP	fresh frozen plasma
FPFV	first patient, first visit

gAT	goat antithrombin
GTC	GTC Biotherapeutics, Inc.
hAT	human antithrombin
HD	hereditary deficiency of antithrombin
HDR	heparin dose response
HIC	hydrophobic interaction column
HIT	heparin induced thrombocytopenia
HIV	human immunodeficiency virus
hpAT	human plasma derived antithrombin
HR	heparin resistance
HTST	high temperature short time
HUVEC	human umbilical vein endothelial cells
IDCP	immunological detection of contaminating proteins
INR	international normalized ratio
IU	international units
IV	intravenous
LMWH	low molecular weight heparin
LPLV	last patient, last visit
LPS	Lipopolysaccharide, endotoxin
MI	microinjection
MNC	mononuclear cells
MRT	mean residence time
n	number
NA or N/A	not applicable
No.	number
NOAEL	no observed adverse event level
NZ	New Zealand
PK	pharmacokinetics
Pts	patients
rhAT	recombinant human antithrombin, antithrombin III, recombinant, antithrombin alfa
SD	standard deviation
Ser	serine
SGMP	soluble goat milk proteins
SOP	Standard Operating Procedures
SPF	specific pathogen free

t _{1/2}	half-life
TB	tuberculosis
TDM	therapeutic drug monitoring
TFF	tangential flow filtration
UFH	unfractionated heparin
UK	United Kingdom
US	United States
USDA	United States Department of Agriculture
UV	ultraviolet
VSFCP	Voluntary Scrapie Flock Certification Program
VTE	venous thromboembolism
y	year(s)

Note! The approved INN and USAN name for the product is antithrombin alfa, the trade name is ATryn® and the FDA requested name is Antithrombin III (Recombinant). Therefore, in this document, the names interchangeably used for the active ingredient will be recombinant human antithrombin (rhAT), antithrombin III (recombinant) and antithrombin alfa.

2 INTRODUCTION

If granted product licensure in the United States, antithrombin III (Recombinant), also known by the tradename ATryn®, will be the first recombinant DNA-derived product made available to the public in more than 20 years that is produced by an innovative and novel production process which utilizes transgenic animals for the site-directed expression of a human plasma protein. The production processes developed by GTC Biotherapeutics, Inc. enables it to economically produce recombinant human antithrombin (rhAT) in unlimited and unconstrained quantities to serve the needs of physicians and patients on a global basis. While others have attempted to produce recombinant human antithrombin by way of other expression systems, such as in Chinese hamster ovary cells, they have not been successful in developing an economically viable production process.

In August 2006, ATryn was approved by the European Commission for the treatment of surgical patients with hereditary antithrombin deficiency to prevent venous thromboembolic events. Working closely and in collaboration with the Center for Biologics Evaluation and Research (CBER) and the Office of Orphan Products Development of the U.S. Food and Drug Administration (FDA), ATryn has been designated 1) an orphan drug for the treatment of hereditary antithrombin deficient patients and 2) as a drug for Fast Track development. The Biologic License Application (BLA) that is under review by CBER was submitted as a rolling application and has been granted Priority Review by CBER.

GTC Biotherapeutics, Inc. is also collaborating with the FDA's Center for Veterinary Medicine (CVM) in response to their recently issued draft Guidance on Regulation of Genetically Engineered Animals. Personnel from GTC have met with CVM in Rockville and, at GTC's invitation, personnel from CVM have visited the GTC Farm to observe its operations.

2.1 Recombinant Human Antithrombin

Antithrombin (AT) is a serine protease inhibitor, which 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 and it also has anti-inflammatory properties. Antithrombin neutralizes the activity of thrombin, as well as, other serine proteases by forming a 1:1 stoichiometric complex between enzyme and inhibitor. Binding of heparin to AT results in a conformational change and a > 1,000 fold increase in thrombin inhibitory activity. Two isoforms of human plasma-derived AT (hpAT) have been identified, which differ in their affinity for heparin. The high affinity β -isoform (5-15% of total circulating AT) lacks glycosylation at Asn 135, while the low affinity α -isoform is glycosylated at this site.

Human plasma AT, which is synthesized in the liver, is present in serum at levels of 12.5 mg/dL. It has a molecular weight of approximately 58,000 daltons, contains 432 amino acids and has a carbohydrate content of about 15%. The protein has three disulphide bridges connecting Cys8-128, Cys21-95, and Cys247-430 and four carbohydrate side chains located at Asn96, Asn135, Asn155 and Asn192. The type of oligosaccharide found on the plasma

derived AT is consistent on all four sites and is comprised mainly of fully sialylated, non-fucosylated, biantennary complex oligosaccharides.

The only antithrombin that is currently commercially available in the US is derived from pooled human plasma (Thrombate III®, hpAT). To date, all attempts to produce AT using recombinant technologies in mammalian cell culture have been commercially unviable. Expression in other recombinant systems (e.g. pichia and insect cells) has produced product lacking glycosylation profiles essential for some of the normal pleiotropic properties of AT.

ATryn® is a nanofiltered sterile, terminally heat-treated, lyophilized product with recombinant human antithrombin III as active ingredient. Each vial of ATryn contains 1750 international units of recombinant human antithrombin (250 mg). The product is formulated with 26 mg sodium citrate, 79 mg sodium chloride and 100 mg glycine in each vial. Following reconstitution, the solution may be further diluted into 0.9 % sodium chloride for injection. ATryn is indicated for the prevention of peri-operative and peri-partum thromboembolic events, as well as the treatment of such events, in hereditary antithrombin deficient patients.

Deficiency of antithrombin leads to hypercoagulability, potentially resulting in thromboses in the veins of the extremities, as well as in the mesenteric, renal, hepatic and portal veins and vena cava (1). Hereditary AT deficiency (HD) is an orphan autosomal dominant disorder characterized by either a reduction in antithrombin (Type I) or the presence of a dysfunctional form (Type II) together with a functional form (except for some rare homozygous Type II patients). Hereditary AT deficiency causes a life-long increased risk of venous thromboembolism (VTE) and up to 70% of cases do develop a VTE during their lives (2-5). Often these are recurrent and may be life-threatening (6). The aim of treatment with ATryn is to restore and maintain peri-operative and peri-partum functional antithrombin activity levels in HD patients between 80 - 120% (0.8 – 1.2 IU/mL) of normal to reduce the risk of thromboembolic events, as well as, for treatment of such events.

3 THE APPLICANT

GTC Biotherapeutics, Inc. (GTC) is a US biotechnology business with corporate headquarters located in Framingham, MA. GTC is recognized as the world's leader in producing and developing recombinant proteins in transgenic animals including rabbits, goats, pigs and cows. GTC's near-term core focus is on the development of human plasma proteins for the treatment of rare plasma-protein disorders. In addition to ATryn, GTC is actively developing recombinant versions of human Factors VIIa, VIII and IX and alpha-I proteinase inhibitor.

GTC Biotherapeutics, Inc. has entered into an agreement with Ovation Pharmaceuticals, Inc. whereby Ovation will market and distribute ATryn in the United States. ATryn is already approved and commercially available in Europe.

4 THE TRANSGENIC PRODUCTION PLATFORM

4.1 Introduction

GTC Biotherapeutics, Inc. has been at the forefront of development of the transgenic animal production platform beginning its work in multiple species in the 1980s. Initial development work was performed using DNA constructs employing various promoters directing expression of the desired human protein in the mammary gland. In addition, initial development activities employed microinjection techniques to insert the DNA into the nucleus of a fertilized one-cell embryo, which was cultured and subsequently transferred into a recipient. For expression of recombinant human antithrombin, the DNA construct contained a goat beta casein promoter with the cDNA coding region for human antithrombin (hAT). The DNA construct was microinjected into fertilized one-cell goat embryos, which were transferred to female recipients. Kids born from the recipients were tested for presence of the transgene and expression of the human protein. A founder transgenic goat was selected from which subsequent offspring were generated by natural breeding to give rise to a production herd of hAT transgenic goats.

The following sections provide background information and more detailed information about the transgenic production platform developed and utilized by GTC Biotherapeutics, Inc. for the production of recombinant human antithrombin.

4.2 History of Transgenic Production

A transgenic animal is one whose genome has been modified by the incorporation of exogenous DNA, which has been stably integrated. In 1974, the first transgenic animals were mice produced by Rudolf Jaenisch, who successfully inserted foreign DNA into early-stage mouse embryos. Subsequent experiments, injecting leukemia genes into early mouse embryos using a retrovirus vector, proved that the exogenous DNA integrated not only to the mice themselves, but was also transmitted to their progeny. This was followed in 1980 with the production of the first transgenic mice by microinjection of a transgene into the pronucleus of a fertilized mouse egg by J.W.Gordon and colleagues. By the late 1980s, the first transgenic dairy and farm animals had been produced (sheep, goat, cow and pig). The 1990s brought the promise of human therapeutic proteins produced in the milk of transgenic animals into a commercial reality.

ATryn was the first recombinant human therapeutic protein produced from the milk of transgenic dairy animals to enter human clinical trials in 1996. However, it was not until 2006 that ATryn® became the first transgenically produced human therapeutic product approved by any regulatory authority (European Medicines Agency (EMA)) anywhere in the world.

4.3 Regulatory Oversight

The GTC Farm in Massachusetts is a United States Department of Agriculture (USDA)-registered research facility and the goatherd is inspected, monitored and certified scrapie-free

by Animal and Plant Health Inspection Service (APHIS) veterinary inspectors in the USDA Voluntary Scrapie Flock Certification Program (VSFCP). GTC Farm also has an Animal Welfare Assurance on file with the National Institutes of Health Office of Laboratory Animal Welfare and has been inspected and accredited since 1997 by the Association for Assessment and Accreditation of Laboratory Animal Care International (AAALAC Int.). As mandated under the Animal Welfare Act, all animal activities and related husbandry, facilities and veterinary care are overseen by GTC's Institutional Animal Care and Use Committee. Additionally, GTC Farm activities related to transgenic production have been inspected by representatives of the Center for Veterinary Medicine (CVM) of the FDA.

Activities related to transgenic production of recombinant human antithrombin have been inspected by representatives of the Center for Biologics Evaluation and Research, which has the ultimate regulatory oversight of activities leading to production of the medicinal product.

4.4 Transgenic Goats for Production of Recombinant Human Antithrombin

Recombinant human antithrombin is produced in the milk from qualified female dairy goats with the hAT transgene stably integrated into their genome. The transgene carries the genetic information directing the expression of recombinant human antithrombin in the milk secretory (epithelial) cells of the mammary gland. The milk secreting tissues are "turned on" during late pregnancy and milk secretion begins at parturition and continues as long as the milk (containing the rhAT) is removed daily from the mammary gland.

4.4.1 Selection of the Species

The selection of the production species for transgenic expression of a human therapeutic protein is largely driven by the expected quantity of the therapeutic protein required. Although GTC has produced transgenic mice, rabbits, goats and cows, the dairy goat was chosen as the species for expression of human antithrombin in its milk (Figure 1) because:

- the goat had a moderate time-frame from microinjection (MI) of the fertilized embryo to first milk from transgenic founders (16-18 months) due to its shorter gestation and maturation periods compared to the 10-fold higher milk-producing cow
- it produced a commercially reasonable amount of milk (600-800 L in approximately 300 days) to use as source material for recombinant protein isolation
- it usually produced multiple offspring (2-3) for more rapid herd expansion
- additionally, unlike the sheep, the dairy infrastructure and equipment were well established in the United States for the goat.







Animal Considerations					
Animal	Gestate (months)	Mature (months)	Offspring (number)	Yield (lactation)	Months from MI to Milk
	.75	1	10	1.5mL	3-6
	1	6	8	1-1.5L	7-8
	4	8	9	200-400L	15-16
	5	6	2	200-400L	16-18
	5	6	2	600-800L	16-18
	9	15	1	ca. 8000L	30-33

Figure 1. Considerations for Species Selection

The first transgenic goats produced by GTC between 1986 and 1994, including the male founder goat for the human antithrombin herd, were made using dairy goats sourced from New England and New York. However, the notoriety and concern generated by the mad cow disease (bovine spongiform encephalopathy-BSE) epidemic in England, led GTC to import dairy goats from New Zealand (NZ) to quell any perception of an increased scrapie risk in US domestic goats (although at that time only 5 US goats had ever been diagnosed with the transmissible spongiform encephalopathy of goats and sheep, scrapie). NZ is a country internationally recognized for its excellent animal health. In addition, in the 1950s NZ had eradicated scrapie from its sheep flocks and it was accepted by world-wide animal health authorities as being free of scrapie in goats and sheep and BSE in cattle. Therefore, after the appropriate quarantine in New Zealand and the US, GTC imported three shipments of health pre-screened NZ goats onto its dedicated farm site from 1994 to 1998. In 1998, the herd was closed to further importations from New Zealand to preserve the high health standards and specific pathogen-free status achieved in this closed herd.

At each importation, the goats from New Zealand were immediately entered into the USDA Voluntary Scrapie Flock Certification Program and due to their New Zealand origin were awarded certified scrapie-free status upon their entry into the US. In 1994, GTC also closed the domestic herd, which was located at a different farm site, and entered these goats into the VSFCP. In 1999, the closed domestic herd was awarded certified scrapie-free status after 5 years in the program and was then combined with the closed New Zealand herd in 2000. Thus, was formed the current closed certified scrapie-free and specific pathogen-free GTC goat herd, which includes the transgenic animals producing recombinant human antithrombin in their milk.

4.4.2 Closed Specific Pathogen-free Herd

GTC has developed a controlled and well-characterized herd of specific pathogen-free (SPF) goats at its farm site. This site was completely closed to any further live goat introductions in 2000 and since its establishment has been under a strict disease surveillance program. Testing is done on individual animals and/or the entire herd throughout the year dictated by pre-determined schedules. The specific disease pathogens for which the goats are routinely tested are listed in Table 1.

Table 1. Specific Pathogen-Free Herd

Disease	Causative Agent	Status of GTC Herd
Caprine Arthritis Encephalitis (C.A.E.)	Retrovirus	Herd is documented free of disease
Caprine Herpes	CapHV-1 Virus	Herd is documented free of disease
Contagious Ecthyma (Orf)	Parapox Virus	Herd is documented free of disease
Bovine Viral Diarrhea (BVD)	Pestivirus	Herd is documented free of disease
Johne's Disease (Paratuberculosis)	<i>Mycobacterium avium</i> , subsp <i>paratuberculosis</i>	Herd is documented free of disease
Brucellosis	<i>Brucella spp.</i>	Massachusetts is free of disease and herd is documented free of disease
Tuberculosis (TB)	<i>Mycobacterium tuberculosis</i>	Massachusetts is free of disease and herd is documented free of disease
Neospora	<i>Neospora caninum</i>	Herd is documented free of disease

4.4.3 Housing of Goats

Acquisition of the farm site was governed by a series of strict selection criteria:

- No occupation by bovine species for at least five years prior to purchase to reduce the risk from environmental pathogens.
- No evidence of occupation by sheep or goats to minimize the risk from scrapie or other species-specific pathogens
- Suitability of the terrain for agricultural operations
- No activities on abutting properties that would pose herd safety or health concerns
- No significant environmental risks on or close to the property
- Water that meets National Primary Drinking Water Standards

Once the site was selected and purchased by GTC, the construction of animal housing was designed to address animal comfort, efficiency of logistical operations, and to comply with

animal care and welfare regulations for animal spaces. Additionally, the potential impact of seasonal extremes and weather conditions on goat health and welfare were taken into consideration in the design of the buildings.

For the vast majority of animal housing located on site, a state of the art, dry lot design, center alley, large animal barn design, with internal penning and adjoining external paddock access for all goats was chosen. The surface materials are designed to withstand cleaning with detergents, disinfectants and high-pressure water. Passive ventilation is provided through screened ventilation curtains, and active ventilation in some buildings. Doors are located in the pens that permit goats to enter fenced outdoor paddocks that are surfaced with gravel and stone dust. Goats are allowed free access to these outside paddock area unless inclement weather dictates internal housing. No free-range pasturing is allowed for any goats. Lastly, there is a double fence system utilized at the site. Internal fencing maintains each group of animals within the adjoining paddocks attached to each barn, while the external fencing system encompasses the entire campus of buildings. Access to this site is highly restricted with both physical and electronic access restrictions in place.

4.4.4 Vaccination of Goats

All animals within the GTC herd are on a regular vaccination schedule based on known diseases to be found in the New England area. No live or modified-live vaccines are used within the goat herd. Rabies, tetanus, and *Clostridium* C and D vaccinations are administered semiannually or annually. A separate vaccination schedule is implemented for pregnant does and kids to provide additional protection for these specific groups of animals.

4.4.5 Animal Feeds

To ensure freedom from scrapie, only feeds that are specially formulated to be free of ruminant protein (1994) and ruminant fat (1995) are fed to the goats. The daily dietary ration for all adult goats is comprised of hay, pelleted concentrate, and trace mineral salt licks. Goats receive a nutritionally balanced ration with respect to the goat's age, size, and condition. All feeds are stored in facilities that protect them from the elements/weather and that inhibit access by potential pest species.

Hay is predominantly procured throughout the Northeastern USA and Southeastern Canada from contracted suppliers, including the GTC Farm. Representative samples are taken from each lot of hay to assess general quality, (e.g., presence of mold, dampness, off-color). All hay is tested for and approved based upon nutritional and mineral content. Testing for herbicides, pesticides, and aflatoxins is performed on a regular basis.

Mineral profile testing is performed to monitor that mineral levels are not significantly outside of the recommendations of the Subcommittee on Mineral Toxicity in Animals Committee on Animal Nutrition, Board of Agriculture's and Renewable Resources, Committee on Natural Resources, National Research Council. Based on the nutritional and mineral analyses, the hay is distributed to appropriate subpopulation groups within the farm.

All feed testing results are reviewed by a GTC Herd Veterinarian to ensure that it meets the nutritional requirements of the animals.

Testing is performed on hay and grain for pesticides and herbicides. Testing for aflatoxins is also performed on hay and grain. Lots that exceed action levels for pesticides, herbicides and aflatoxins in animal feed are rejected.

Bales of hay are stored in designated areas and facilities designed to restrict access by unauthorized personnel and inhibit access by pest species. Prior to feed out, hay bales are also visually inspected for gross contamination, such as mold or dampness. If any hay is found to be unacceptable at time of feeding, it is rejected and subsequently discarded.

At a minimum, two types of commercially prepared pelleted feed, referred to as concentrate and kid starter, are fed to the goats. Pelleted feeds are specially formulated to be free of animal fats and animal proteins. The concentrate is a pelleted formulation of corn, grains, and plant by-products commercially prepared by an approved feed manufacturer. Kid starter is a medicated concentrate containing decoquinate to prevent coccidiosis, an internal parasite known to negatively impact young goats. Concentrate is supplied to individual goats based on their dietary requirements.

All pelleted feed is analyzed for nutritional and mineral content. Pelleted feeds are routinely tested for residual pesticides, herbicides, and aflatoxins. Pelleted feed that exceeds the tolerances specified for pesticides and aflatoxin is rejected.

Commercial trace mineral salt blocks are also offered to all goats *ad libitum*. The raw ingredients used to make the blocks do not contain animal or animal-derived by-products.

Newborn kids are fed heat-treated colostrum during the first 24 hours of life. Colostrum is collected from non-transgenic GTC goats then heat-treated to reduce the microbiological load before feeding to the kids. After the first 24 hours, kids are fed pasteurized milk from GTC goats.

Goats have free access to water. The water is supplied from wells located on the property. The water supply is registered with the Massachusetts Department of Environmental Protection and is tested to ensure compliance with the National Primary Drinking Water Regulations and Massachusetts Primary Drinking Water Standards. Monitoring includes testing for coliforms, inorganic chemicals, sodium, copper and lead at point of use, synthetic organic chemicals, volatile organic compounds, radio nucleotides, and secondary contaminants.

4.4.6 Viruses of the Goat

During the 22 year development history of transgenic goats at GTC, consideration has been given to minimization of the risk of viral/other pathogen infection of the goatherd. A series of risk assessment, risk minimization, and expert consultations have been undertaken including:

- Expert small ruminant and virology panels
- Internal viral task force
- Consultations with outside experts on specific viruses and disease pathogens and their testing and eradication procedures
- Viral risk assessments updated periodically particularly for consideration of emerging viruses
- Comprehensive viral risk evaluation of the ATryn manufacturing process (from goat to final product) by an outside expert.

As a result of these assessments and subsequent actions, the specific pathogen free goatherd described above was established and the testing paradigms for goats and their milk were developed.

4.4.6.1 Eradication of Specific Viruses from the Herd

During the development and implementation of the current viral testing strategy, two specific viruses were detected in a small percentage of animals within the overall GTC herd of goats. However, neither of these viruses was found to be present in the hAT herd.

The two viruses that were found in the general goat herd were CAEV and CapHV-1. GTC worked with experts for these viruses to develop and implement an eradication program. These programs were remarkably successful and not only eliminated the respective virus from the entire herd but also assured continued freedom through repeated herd wide surveillance.

4.4.7 Site Directed Expression of Human Protein in Mammary Gland - Gene Construct

Different milk proteins are expressed at different levels in milk (Table 2). The goat beta casein promoter was selected to drive transgene production in the milk of goats due to its strength of expression in the mammary gland. The beta casein is expressed in both a tissue specific and temporal fashion affording it the unique capability for expressing the gene product in the mammary gland only and specifically only during lactation.

Table 2. Expression of Major Proteins in Milk

Milk Protein	Expression in Milk
bovine alpha casein	10 mg/mL
bovine alpha lactalbumin	1 mg/mL
goat beta casein	16 mg/mL
sheep lactoglobulin	3 mg/mL
whey acid protein	2 mg/mL

The transgene incorporated into the founder hAT goats contains both goat and human DNA sequences (Figure 2). The transgene is comprised of the 5' and 3' genomic goat beta casein sequences devoid of the coding sequences for goat beta casein. In its place, the human AT cDNA coding sequence was inserted, thereby creating the transgene or construct.

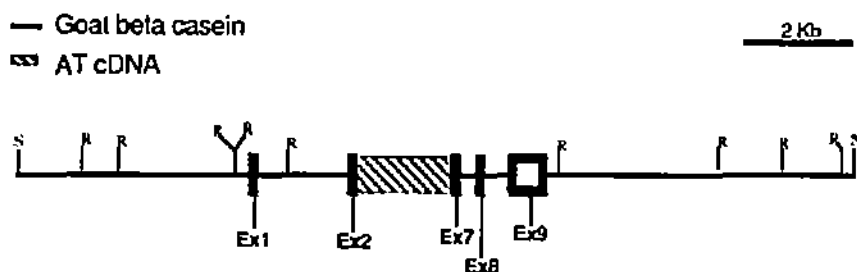


Figure 2. Human Antithrombin Transgene (7)

4.4.8 Production of Transgenic Founder Goats

The transgenic technology used for rhAT production utilized *in vitro* microinjection to introduce the hAT transgene into the genetic material of a fertilized goat egg or early stage embryo (Figure 3). After the exogenous DNA was introduced, the modified embryo was transferred to a recipient female. When the new genetic information (the transgene) successfully incorporated into the genetic material of the embryo, the goat, when mature, expressed human antithrombin in its milk. Once established in the founder transgenic animal, a male, the transgene was transmitted, as any other genetic trait, to subsequent generations through traditional breeding with either non-transgenic or other transgenic goats.

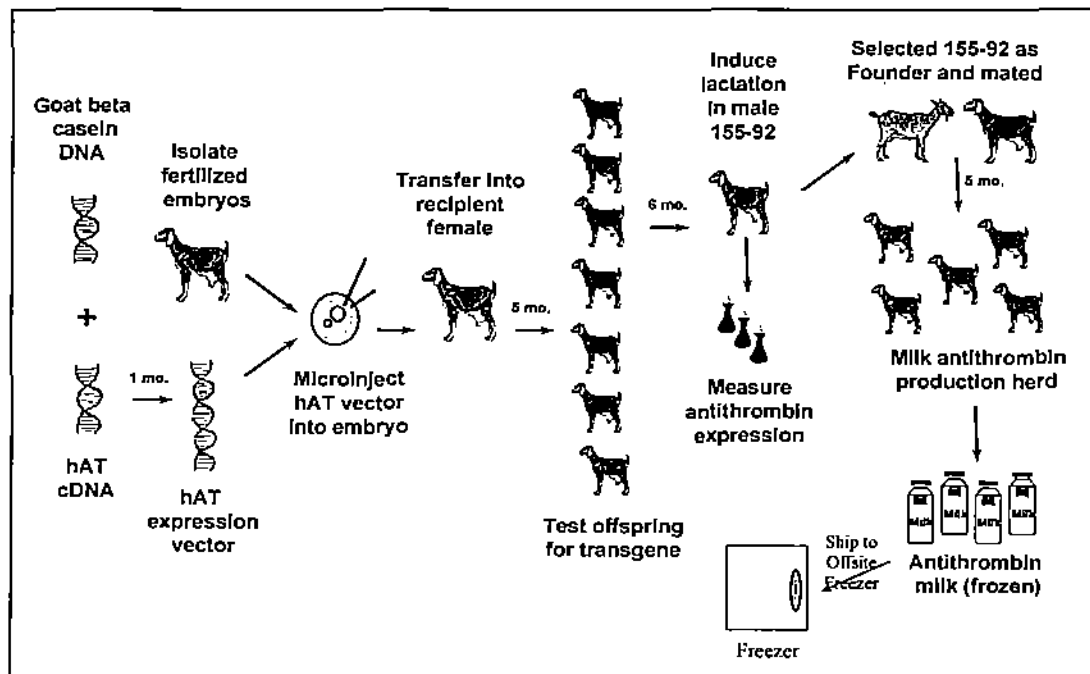


Figure 3. Making a hAT Transgenic Goat

4.4.9 Selection of Founder

The microinjection program generated 70 offspring of which only five were identified as potential founder transgenic goats. These five founder animals were subsequently thoroughly screened genetically to fully characterize the inserted transgene. This was done to document the integrity of the transgene sequence, to determine how many copies of the transgene were present, and on which chromosome(s).

Of the five potential founder goats produced, one was selected as the production founder line based on the integrity of the transgene, suitable copy number, acceptable lactational performance, and acceptable expression level of rhAT in the milk. The founder line selected produces rhAT at approximately 1 to 4 g/L in the milk with a lactation length of at least on average 150 days producing on average 2.0 liters per day.

4.4.10 Generation of the Production Herd

Once the founder transgenic hAT animal was identified, a production herd of goats was established by naturally mating of the founder to female goats. Mendelian inheritance of the transgene resulted in female transgenic offspring capable of producing rhAT in their milk and transgenic males who were also used for breeding purposes to increase the size of the herd. Herd size can be expanded as needed to fulfill production needs.

4.4.11 Characterization of Production Herd

To produce a recombinant human therapeutic from the milk of a transgenic animal, the production herd must be fully characterized for integrity of the transgene between animals and between generations. GTC employs a rigorous genetic testing of all its hAT production goats to genotypically qualify them for entry into the milking sub-group. All production goats must have an intact transgene with the correct copy number and with no sign of genetic rearrangement of the transgene.

In addition, phenotypic characterization is performed on the milk to confirm expression of the transgene (mRNA from the somatic cells in milk and rhAT levels).

Only after passing both the genotypic and phenotypic qualification, as well as having the appropriate health certification, is a hAT goat allowed to contribute to source material (milk) collection.

4.4.12 Animal Health and Welfare

GTC is committed to upholding the highest standards of animal health and welfare and to continuously improving the care of its animals. Farm procedures exceed those specified by regulatory agencies and industry groups for the humane use and care of animals. GTC strives to provide the best environment possible for all of the animals under its care.

GTC strictly adheres to the published guidelines regarding animal health and welfare, including those outlined in the following documents: the Animal Welfare Act, *The Guide for the Care and Use of Laboratory Animal*, including all amendments established by the National Institute of Health as combined in the *Public Health Service Policy on the Humane Care and Use of Laboratory Animals*, and also the *Guide For the Care and Use of Agricultural Animals in Agricultural Research and Teaching* published by the Federation of Animal Science Societies and accepted by the USDA-APHIS-AC and AAALAC Int. GTC also complies with all other federal, state and local requirements for the responsible use and care of animals.

Additionally, a regular review of morbidity and mortality (M&M) data is performed at least quarterly to evaluate the health status of the herd. The aims of this analysis are to lower M&M within the general herd and to improve overall animal health and welfare. Several full-time Herd Veterinarians provide 24/7 coverage year round. The veterinarians monitor the herd and individual animal health on a daily basis and can detect subtle changes that may be indicative of potential clinical or sub-clinical issues or possible adventitious agent concerns. Given the extensive care, monitoring and testing of the goatherd, it is a very healthy group of goats. As evidence, for example, the levels of clinical and sub-clinical mastitis in the herd are well below levels seen in conventional goat dairy operations world-wide.

4.4.13 Management of a Transgenic Production Herd

The limited numbers of goats required for production of recombinant human antithrombin to supply the commercial market allows for employment of rigorous quality and safety measures to manage the herd and prevent the potential ingress or transmission of pathogens and adventitious agents. GTC's comprehensive transgenic herd management program is based on a high level of control, good practices at the level of the farm and the animal, and appropriate and thorough documentation. Each goat in the herd has a unique identification number that is applied redundantly. A master list of all assigned numbers is maintained to facilitate traceability.

Control starts at the level of the farm with Good Agricultural Practices (GAP) employed for the majority of operations involved with maintaining the site and for caring of the animals. This control and these practices also apply to incoming materials which at a minimum includes all of GTC's hay, grain, and bedding materials. Lastly, this control encompasses monitoring, and restricting where necessary, flow of personnel/visitors and vehicular traffic.

GTC's QA documentation system utilizes a number of different categories of documents to encompass the activities that occur at the level of the farm and animal; standard farm practices, standard veterinary practices, and other standard operating procedures and good manufacturing practices that are aimed at defining best practice in an agricultural/pharmaceutical setting. These documents provide the exact procedure to be followed by trained personnel. Similar to conventional recombinant production systems, it is these practices and the documentation system that allows for a highly controlled, well characterized, and consistent product to be produced from the hAT goat herd.

4.4.14 Adventitious Agent Risk Minimization Measures for the Goat

A variety of general adventitious agent risk minimization measures have been implemented. Many of these measures are derived from generally accepted principles used to minimize risk of adventitious agent introduction into recombinant expression systems, downstream processing operations, and specific recommendations for animal derived products in regulatory guidance documents. The primary basis for such measures is the definition of an appropriate level of segregation and control at the level of the environment, the equipment, the raw materials and the manufacturing process. A similar approach has been taken for production of recombinant human antithrombin in transgenic goats.

The risk minimization started at the level of the farm with strict selection criteria for the farm site and the goats (domestic and NZ sourced). Following establishment of the base herd, closure of the herd reduced any potential for entry of adventitious agents into the herd via outside animal introduction resulting in the current certified scrapie-free and specific-pathogen free goatherd. A comprehensive biosecurity program was implemented which covers both internal and external aspects of farm operations and the overall animal care program.

From an external perspective, the program encompasses all personnel and visitors or service personnel/contractors. The program addresses known wildlife that exists in the surrounding environment and appropriate monitoring and controls to limit that population, where possible. The biosecurity program includes an Integrated Pest Management Program that monitors and controls incursions by birds, rodents and insects.

Internal aspects of the biosecurity program focuses on the goat herd itself and addresses herd closure, evaluation of raw materials provided to the goats (hay, grain, water, bedding, etc.), and the monitoring of overall clinical health as a tool for detecting potential disease entry.

5 RECOMBINANT HUMAN ANTITHROMBIN

GTC produces recombinant human antithrombin in the milk of transgenic goats that have been genetically engineered to express it. The rhAT is purified from the milk using conventional purification technology and then filled, lyophilized and finished similarly to other recombinant biopharmaceutical products. The transgenic goat can be considered a living production system/bioreactor for the target protein that then utilizes conventional downstream processing to purify the protein to an acceptable human dosage form. Thus, similar risk factor assessments are performed for the transgenic manufacturing process as for a bioreactor-based manufacturing process.

5.1 Transgenic Goat Milk

Milk is a nonsterile colloidal suspension of soluble whey proteins and insoluble casein micelles and milk fat globules. Protein makes up 3% to 4% of milk by weight or 30 to 40 g/L, of which 80% are caseins in colloidal suspension (formed into micelles) and 20% are the soluble whey proteins in colloidal solution. The whey proteins include those proteins specific to milk and to a much lesser degree those found in systemic circulatory system (at ~1/50-1/100 of their serum concentration). Recombinant human antithrombin, which is synthesized and secreted by the mammary epithelial cells, is found in the soluble milk whey fraction.

5.1.1 Milking the Transgenic Goats

Source material (milk) is collected from qualified hAT goats on a daily basis using conventional dairy practices, equipment, and sanitation technology. Several traditional dairy equipment units comprise the source material collection system. SOPs directed at the entire milking operation drive all the activities associated with rhAT source material collection.

To begin a milking session, goats from the hAT production milking herd are gathered together in a holding area. Individual animal identification is verified prior to their entry into the milking parlor. In the milking parlor, animal identification is verified again prior to milking. Milk from each animal is collected into a single-use bottle. The milk is weighed, sampled, labeled and frozen for long term storage until needed for downstream purification of rhAT. The information contained on the milk label allows the complete traceability backward to the individual animal and date of collection and forward to the final product.

Following the completion of a milking session, all milking equipment, the milk parlor, and milk room are cleaned appropriately to ensure that it is suitable for the next milking session.

Occasionally, a production goat is diagnosed with mastitis or another health problem that necessitates the administration of antibiotics to the animal. Use of drugs to treat clinical or subclinical mastitis is based on the severity of the clinical presentation and their use is recorded. Labeled withdrawal times are followed for drugs approved for use in lactating dairy goats. When using agents approved for other lactating ruminants, the milk withdrawal period followed is double that recommended by the manufacturer. If a production doe is treated with antibiotics, then that doe's source material must be tested to verify the absence of any antibiotic residue before source material collection resumes.

5.1.2 Adventitious Agent Risk Minimization Measures for the Milk

Prior to selection of the frozen bottles of milk for a manufacturing run, a composite that is representative of the ultimate manufacturing pool is created and tested for milk composition and adventitious agents including viruses. For the bottles of frozen milk to be released for manufacture of a lot of ATryn, the milk pool must meet the specification limits for milk composition and test negative for adventitious viruses.

The composite milk pool is screened for adventitious viruses using conventional, *in vitro* cell line screening methodology specifically validated for use with milk. Four cell lines with a wide range of viral sensitivities are used for *in vitro* screening of the milk pool. Additionally, specific tests for five zoonotic viruses (Powassan virus, West Nile virus, border disease virus, bunyavirus, and Borna disease virus) are performed utilizing an immunofluorescence assay. In all milk pools that have been tested, adventitious viruses have not been detected.

5.2 Purification of the rhAT from Milk

The rhAT is isolated from the goats' milk and conventionally purified using: tangential flow filtration (TFF), heparin affinity chromatography, viral removal filtration, anion exchange chromatography and hydrophobic interaction chromatography (refer to Figure 4). The purification process nominally produces 300 grams of purified rhAT per batch from no more than 375 liters of source material.

5.2.1 ATryn Manufacturing Process

The upstream purification process consists of shipping of the milk to the purification site for thawing, pooling and clarification of the milk and rhAT isolation. Thawed, pooled, milk is diluted with ethylenediaminetetraacetic acid (EDTA) buffer, and clarified by tangential flow filtration (TFF) with a hollow fiber membrane filter (Figure 4). Milk solids and colloidal materials are retained by the membrane. The soluble whey fraction permeate, containing the rhAT, is cycled through a closed loop linking the filtration system to a heparin column until > 90% of the rhAT is captured. The heparin column is then washed and decoupled from the closed loop. RhAT is eluted from the heparin column with a sodium chloride buffer and transferred to a downstream processing and formulation area.

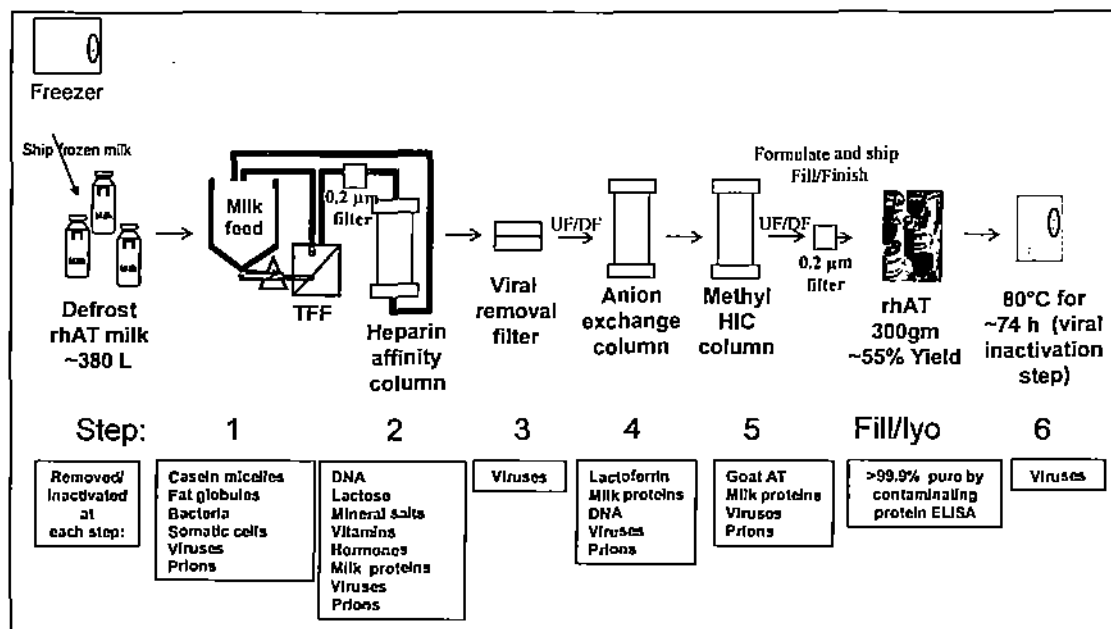


Figure 4. rhAT Purification Process

Downstream processing includes filtration using a nanofilter (a viral removal step) and subsequent purification by anion exchange chromatography and hydrophobic interaction chromatography (HIC) (Figure 4). Specifically, the collected heparin eluate is passed through a viral removal filter, concentrated and diafiltered by membrane filtration to adjust the ionic strength, and applied to the anion exchange column. After loading, the anion exchange column is washed and the rhAT eluted. The anion exchange-eluate is conditioned with citrate, applied to the methyl HIC column and the purified rhAT eluted from the media.

The HIC column eluate is concentrated and diafiltered into a citrate, glycine, sodium chloride buffer. The protein solution is adjusted to a final concentration of approximately 25 mg/mL and passed through a 0.2 µm filter into a pre-sterilized stainless steel shipping container. Upon release, formulated drug substance is shipped for fill-finish where it is aseptically filled into 20 mL vials, lyophilized and subjected to terminal dry heat treatment for viral inactivation. The final product, ATryn®, is a sterile lyophilized powder for solution for infusion.

5.2.2 Impurities (Including Viruses and Prions)

5.2.2.1 Process Validation for Removal of Impurities

Milk is a complex, nonsterile colloidal suspension of soluble whey proteins and insoluble casein micelles and milk fat globules. The rhAT manufacturing process has been designed to handle this complex mixture (see boxed text in Figure 4). Most of the insoluble solids are

removed during the first step in the purification process, dilution with EDTA and tangential flow filtration (TFF) through a 500 kD cut-off membrane. The insoluble materials include: milk fat globules, casein micelles, somatic cells, and microorganisms. The next step in the process is the heparin affinity chromatography, which binds a subset of the milk proteins and the recombinant antithrombin. This allows most of the nonbinding materials like vitamins, hormones, lactose, mineral salts, goat deoxyribonucleic acid (DNA), and some milk proteins to be washed away from those proteins that bind to the heparin column. The viral nanofilter placed between the heparin and anion exchange columns removes any particulate materials in addition to any potential viruses or prions. The anion exchange column is excellent for removal of lactoferrin, DNA, and some residual milk proteins. The final column, the methyl HIC column provides baseline separation of goat and human antithrombins and removes any last traces of potential contaminating milk proteins. The process has been validated at both lab and commercial scales to confirm the ability of each purification unit operation to remove these impurities, particularly the protein contaminants (Table 3).

Table 3. Cumulative Log₁₀ Clearance of Protein Contaminants

	Commercial Validation Runs		
	Lot 1	Lot 2	Lot 3
Assay	Log ₁₀ Reduction		
Colloidal goat milk protein (CGMP)	>7.2	>7.1	>7.0
Soluble goat milk protein (SGMP)	>7.0	>6.9	>6.9
Process-specific contaminants (IDCP)	≥5.8	≥5.8	≥6.7
Goat IgG	>5.7	>5.7	>5.7
Goat antithrombin	>2.8	>3.1	>3.1

IDCP = immunological detection of contaminating protein
Ig = immunoglobulin

5.2.2.2 Analytical Determination of Impurities

The absolute purity of rhAT bulk drug substance is estimated by a combination of methods focused on the separation of rhAT from process- and product-related impurities.

- Potential process-related impurities include: residual host proteins (Table 4), DNA, leached materials from chromatographic media (e.g. heparin), adventitious agents and endotoxin.
- Potential product-related impurities include: molecular variants formed during manufacture and/or storage.

Table 4. Methods to Detect Residual Goat Milk Proteins

Assay	Detection
Soluble goat milk protein (SGMP) ELISA	Quantitative method for <i>whey proteins</i> α -lactalbumin, albumin, β -lactoglobulin
Colloidal goat milk protein (CGMP) ELISA	Quantitative method for casein: α S1-casein, α S2-casein, β -casein, κ -casein
Goat IgG ELISA	Quantitative method specific for goat IgG, with some cross-reactivity for IgA and IgM
Immunological detection of contaminating protein (IDCP) ELISA	Quantitative method for residual <i>lactoferrin</i> and <i>lactoperoxidase</i>
Goat antithrombin (gAT) Western blot	Limit test for <i>goat antithrombin</i>

Abbreviations: ELISA = enzyme-linked immunosorbent assay; Ig = immunoglobulin;

Suitable analytical methods have been developed and validated to estimate the levels of these impurities in bulk drug substance and/or drug product and the purification process was also validated for the removal of many of these possible impurities. Thus, ATryn is a highly purified product.

Like all processes for purification of human plasma-derived antithrombin (hpAT), the recombinant antithrombin process includes a heparin affinity chromatography step. Therefore, the possibility of contamination of the product by heparin that has leached off the column must be considered. Although some human AT concentrates have extremely low heparin levels (Thrombate III's specification is ≤ 0.004 U heparin/IU hpAT), there are others in Europe with measurable heparin content. The presence of measurable heparin in some of these products may be problematic for patients who have a heparin allergy. Heparin induced thrombotic thrombocytopenia syndrome is a highly prothrombotic immune reaction to heparin that may result in death, limb ischemia leading to amputation, graft occlusion, and other potentially severe thrombotic events ((8) and (9)). The drug product release specification for ATryn has been set as ≤ 0.001 IU heparin activity per IU recombinant antithrombin. The absence of significant levels of heparin in ATryn® was verified *in vitro* and *in vivo*. *In vitro*, ATryn®, in the absence of exogenously added heparin, produced no inhibition of thrombin or factor Xa. In the compassionate-use study, one of the ATryn®-treated HD patients, undergoing a hysterectomy, had a known severe allergy to heparin and was treated successfully with ATryn® with no allergic response (10).

Through a combination of validation of the impurity-removal capacity of the antithrombin alfa manufacturing process and the use of impurity-detecting assays, the purity of ATryn® is assured. A comprehensive validation of the impurity-removal capacity of the purification process has demonstrated the robustness of the process and of individual unit operations that have been engineered for removal of specific impurities. Additionally, through the use of a repertoire of assays available for quantification of both process- and product-related impurities, product purity is assessed on a lot-to-lot basis.

5.2.2.3 Validated Removal of Viruses

The results of the viral validation studies on the ATryn manufacturing process (Table 5) demonstrate that significant virus reductions of ≥ 8.5 to $\geq 25.3 \log_{10}$ were accomplished for 6 model viruses across the distinctly different modes of the recombinant antithrombin production process. The values used in the cumulative summary are the worst case reduction values for each step and each virus and thus represent the minimum viral reduction by the ATryn process.

Table 5. Viral Clearance/Inactivation by the ATryn Manufacturing Process

Process Step	Pseudorabies Virus	Xenotropic Murine Retrovirus	Human Adeno-virus	Porcine Parvo-virus	Polio Virus	Mouse Adeno-virus
VirA/Gard 500-kD	≥ 5.1	≥ 3.7	≥ 5.3	1.7	4.1	3.5
Heparin HyperD	1.6	1.2	N/A	1.4	4.0	2.3
Nanofiltration	≥ 2.9	≥ 3.8	≥ 6.3	3.7	ND	ND
ANX-Sepharose	3.6	1.0	≥ 7.1	N/A	2.4	N/A
Methyl HyperD	≥ 5.6	≥ 4.4	≥ 4.8	≥ 5.7	≥ 5.0	≥ 2.7
Heat Treatment	2.8	≥ 5.0	1.8	2.4	≥ 1.9	ND
Total Reduction	≥ 21.6	≥ 19.1	≥ 25.3	≥ 14.9	≥ 17.4	≥ 8.5

N/A = not applicable (since the reduction was below 1 \log_{10})

ND = Not Determined

Therefore, in addition to the control measures in place at the GTC Farm and the adventitious viral screen employed at the milk stage, GTC has shown robust viral removal capacity in the recombinant antithrombin manufacturing process. These data strongly support the conclusion that ATryn produced using these specific steps is safe for human use with respect to potential adventitious viral contamination.

5.2.2.4 Validated Removal of Prions/Scrapie

The cumulative scrapie removal capacity of the recombinant antithrombin purification process is $\geq 11.3 \log_{10}$ reduction (Table 6). Although the viral filter has not been validated for scrapie removal in this process, this filter has been reported to remove $\geq 2.8 \log_{10}$ of prions (11), which provides an additional presumptive level of safety. Therefore, the $\geq 11.3 \log_{10}$ reduction is the minimal reduction factor for the current manufacturing process. If one takes into account the reported reduction by the viral filter, then $\geq 14.1 \log_{10}$ is the minimal reduction factor for this process.

Table 6. Scrapie Clearance by the ATryn Manufacturing Process

Step*	Unit Operation	Log ₁₀ Reduction
1	Tangential flow filtration	2.0
2	Affinity chromatography	2.2
4	Anion exchange chromatography	≥ 3.3
5	Hydrophobic interaction chromatography	≥ 3.8
	Cumulative Reduction	≥ 11.3

*Refer to Figure 4 for the identity/location of the numbered steps.

Therefore, in addition to the scrapie-free certification of the goats producing the recombinant antithrombin and the verification that all materials in the downstream manufacturing process are free of ruminant derived ingredients, the recombinant antithrombin purification process has also been validated for its ability to remove prions/scrapie. This validation provides an even higher-level of safety assurance, in addition to the international acceptance of milk as safe with respect to TSE transmission (Category IV – no detectable infectivity; *Report of a WHO Consultation on Medicinal and other Products in Relation to Human and Animal Transmissible Spongiform Encephalopathies*).

5.2.3 Characterization of Final Product and Comparability to hpAT

Based on a detailed biochemical analysis, it was found that transgenically produced rhAT is comparable to clinical grade human plasma-derived AT with respect to: specific activity, purity, amount of aggregates, primary sequence, secondary and tertiary structure (Table 7). The major difference observed between hpAT and rhAT is in the glycosylation pattern.

As a result of the glycosylation differences, recombinant human AT has also been shown to have a fourfold higher affinity for heparin than the predominant α -isoform of hpAT, but similar to the less abundant hpAT β -isoform. Human plasma AT is glycosylated with biantennary complex structures which are predominantly disialylated at all four sites. Transgenic rhAT also contains biantennary complex structures at Asn 96, 135 and 192. However, these biantennary oligosaccharides are fucosylated and are a mix of mono and disialylated structures. Substitution of N-acetylgalactosamine for galactose is also observed on some of these complex glycans as is substitution of N-glycolyl neuraminic acid for N-acetyl neuraminic acid. Human plasma AT does not contain either N-glycolyl neuraminic acid or N-acetylgalactosamine on its oligosaccharide chains. Oligomannose and hybrid oligosaccharides are present at Asn 155 of the rhAT. These glycosylation differences are consistent with the species (goat vs. human) and mode of synthesis (mammary gland vs. liver). The differences in glycosylation account for the difference observed in heparin affinity between hpAT and rhAT with rhAT having a higher overall heparin affinity. The increased heparin affinity of rhAT does not affect the inhibition of thrombin in the presence of excess heparin. These glycosylation differences are also the primary determinants of the increased rate of clearance of the recombinant antithrombin from the circulatory compartment in both animals and humans.

Table 7. Comparison of hpAT to Recombinant Antithrombin

Parameter	hpAT	Recombinant Antithrombin
	Serine protease inhibitor (serpin)	Serine protease inhibitor (serpin)
Primary Structure	Single chain of 432 amino acids	Identical single chain of 432 amino acids
	Contains the reactive center Arg 393-Ser 394 which provides a cleavage site for proteinases such as thrombin	Contains the reactive center Arg 393-Ser 394 which provides a cleavage site for proteinases such as thrombin
	Low level of methionine oxidation (Thrombate & Kybermin P)	Level of methionine oxidation comparable to Thrombate & Kybermin P
Secondary Structure	6 cysteine residues forming 3 disulfide bonds (Cys 21-95, Cys 8-128, Cys 247-430)	Same 6 cysteine residues forming 3 disulfide bonds (Cys 21-95 Cys 8-28, Cys 247-430)
	Distinctive far and near UV CD profile	Same near and far CD profile
Glycosylation	4 N-linked glycosylation sites (Asn 96, 135, 155, 192)	4 N-linked glycosylation sites (Asn 96, 135, 155, 192)
	85% to 95% α isoform with four biantennary, mono and di-sialylated oligosaccharide chains	$\geq 80\%$ β -like isoform with biantennary, mono & di-sialylated oligosaccharide chains at three sites with hybrid & high mannose structures at Asn 155 and substitution of some N-acetyl neuraminic acid with N-glycolyl-neuraminic acid
	5% to 15% is the high heparin affinity β -isoform lacking glycosylation at Asn 135	Also contains the β -isoform lacking glycosylation at Asn 135 (< 20%)
	2-6 terminal sialic acid linkage	2-6 terminal sialic acid linkage
Purity	> 95% to 99% pure depending on manufacturer	> 99% pure
Inhibitor Activity	Inhibits thrombin and Factor Xa <i>in vitro</i> inhibition assay	Inhibits thrombin and Factor Xa <i>in vitro</i> inhibition assay
	Specific activity of US licensed Thrombate ~7 IU/mg	Specific activity of ATryn® ~ 7 IU/mg
Heparin Binding Affinity	Binds to heparin, which catalyzes a conformation change & an increase in activity	Binds to heparin, which catalyzes a conformation change & an increase in activity
	Contains 5% to 15% β -isoform with 3-fold to 10-fold higher heparin binding affinity	Contains mainly β -like heparin binding affinity (4-fold higher heparin binding than hpAT α -form) due to glycosylation differences
Antibody Reactivity	Recognized by polyclonal and monoclonal antibodies	Recognized by same polyclonal and monoclonal antibodies

Abbreviations: Asn = asparagine; Arg = arginine; CD = circular dichroism; Cys = cysteine; hpAT = human plasma-derived antithrombin; Ser = serine; US = United States; UV = ultraviolet.

6 NON-CLINICAL SAFETY

A range of *in vitro* and *in vivo* non-clinical pharmacology studies has been carried out with recombinant antithrombin. *In vitro* studies were performed to evaluate the activity and efficacy of recombinant antithrombin. It should be noted that there are no established animal models for AT deficiency. Thus, animals treated with rhAT in *in vivo* pharmacology and toxicology experiments were exposed to AT blood levels well in excess of those to be encountered in AT-deficient patients.

6.1 Pharmacodynamic Studies

Antithrombin is a naturally occurring complex glycoprotein with multiple pharmacologically important activities. It is the most critical modulator of coagulation and has potent anti-inflammatory properties independent of its effects on coagulation. Non-clinical studies have been performed to evaluate rhAT for efficacy as an anticoagulant and as an anti-inflammatory agent. In several *in vitro* and *in vivo* studies, hpAT was used as a direct comparator.

Pharmacodynamic studies were conducted *in vitro* by GTC and its collaborators to compare inhibition of thrombin by rhAT and hpAT (12) and to explore the effect of addition of rhAT on clotting times in blood from cardiac surgical patients. Additionally, the anti-inflammatory properties of rhAT were also investigated in *in vitro* model systems using: human neutrophils (13) (14), human umbilical vein endothelial cells (14), human eosinophils (15), human leukocytes (16) and human monocytes (17). RhAT was comparable to hpAT in both its expected anticoagulant and anti-inflammatory properties except for potential glycosylation related differences in heparin binding and tissue factor inhibition.

Since no established animal models are available for hereditary AT deficiency, *in vivo* animal models that demonstrate the efficacy of rhAT in several acquired AT deficiencies were utilized. The acquired AT deficiency animal models allowed the assessment of both the anticoagulant and anti-inflammatory properties of rhAT. Fifteen studies were conducted by GTC or its collaborators to assess the efficacy of rhAT in preventing disseminated intravascular coagulation (DIC) in rat (18) (19), neonatal pigs (20) and baboon (21) sepsis models, and to assess the efficacy of rhAT in sheep smoke inhalation (22-24) and pig-to-primate renal transplantation (25) (26) models. Pharmacodynamic data confirmed the activity of rhAT. In most of the studies, rhAT exerted protective impacts on parameters associated with bacterial or endotoxin induced sepsis, acute lung injury and organ transplantation.

6.2 Pharmacokinetic Studies

Thirteen pharmacokinetic studies were performed to determine if sex, dose and/or repeated administration affects the kinetic disposition of rhAT. The single dose pharmacokinetic studies were performed in Sprague Dawley rats, Beagle dogs, monkeys and Baboons. The repeat dose pharmacokinetic studies were performed in Sprague Dawley rats and monkeys. Pharmacokinetic studies indicated that the kinetic disposition of rhAT is similar between

sexes, remains constant with repeated exposure and appears to be nonlinear with administered dose. Clearance decreased as a function of increasing dose concurrent with an increase in half-life. In the repeat dose study, data indicates that rhAT accumulates at doses ≥ 300 mg/kg ($\approx 2,000$ IU/kg) in both sexes.

6.3 Toxicology Studies

Nine single dose and repeat dose toxicological studies were conducted to examine the safety of rhAT administered intravenously to rats, dogs and non-human primates at doses up to 10 to 20 times the highest anticipated dose in man. In addition, the safety of rhAT and heparin administered intravenously to rats was evaluated in a single dose toxicological study. In all safety studies, rhAT was well tolerated as evidenced by the absence of toxicologically relevant untoward clinical observations, appropriate body weight gain and, in general, unremarkable clinical pathology findings. Most clinical observations and/or adverse reactions were related to the pharmacological anticoagulant properties of the test article.

Since part of the intended HD population to be treated will be pregnant women, studies were performed to assess the reproductive toxicity of rhAT in rats during both long-term and short-term daily administration of ascending doses of rhAT. The NOAEL for rhAT in the rat reproductive toxicity study was 210 mg/kg/day.

Three studies were performed to evaluate the potential mutagenicity or genotoxicity of rhAT. The studies performed included an Ames assay that evaluated mutagenicity in five different *Salmonella typhimurium* strains and two *E. coli* strains, a mouse *in vivo* micronucleus assay that evaluated genotoxicity, and a CHO cell *in vitro* assay that evaluated chromosomal aberration. The results obtained did not show any potential mutagenicity or genotoxicity associated with rhAT.

7 CLINICAL PROGRAM

7.1 Hereditary Antithrombin Deficiency

7.1.1 The Condition

Antithrombin (AT) is the principle physiologic *in vivo* inhibitor of blood coagulation and therefore, plays a central role in the regulation of hemostasis *in vivo* (27). Deficiency of antithrombin leads to hypercoagulability, potentially resulting in thromboses in the veins of the extremities, as well as in the mesenteric, renal, hepatic and portal veins and vena cava (1). Patients with hereditary AT deficiency (HD) can experience spontaneous thromboembolic episodes during normal, every day events of living. These patients typically have an AT activity that is approximately 50% of normal (1). It is a heterogeneous disorder, with wide variations in the prevalence of its subtypes and in the incidence of thromboembolic events associated with the subtypes (28). Antithrombin deficiency may also be acquired secondary to other medical conditions, such as liver disease, major trauma, and burns resulting in active clot formation in these patients.

Hereditary AT deficiency is an autosomal dominant disorder characterized by either a reduction in antithrombin (Type I) or the presence of a dysfunctional form (Type II) together with a functional form (except for some rare homozygous patients). In Type I deficiency, AT activity and plasma AT concentration are concordantly reduced, indicating that the protein is not 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 some of the molecules (1;29). The majority of affected individuals are heterozygotes. Homozygosity is very rare (30) and is believed to be incompatible with life, with the exception of some Type II deficiencies. In patients with hereditary AT deficiency, AT activity levels are usually between 40 and 50% of normal.

7.1.2 Patient Population

Thromboembolic events in hereditary AT deficient patients are uncommon prior to puberty (1;28;31;32), but increase thereafter, particularly during periods of high risk, such as pregnancy, surgery, or bed rest (33;34). Asymptomatic hereditary AT deficient patients generally do not require anticoagulant prophylaxis, except in settings of increased risk (35). However, hereditary AT deficient individuals with a history of thrombosis may receive chronic oral prophylaxis, even outside of high risk settings.

Hereditary AT deficiency causes a life-long increased risk of venous thromboembolism and up to 70% of cases do develop a venous thromboembolic event (VTE) during their lives (36-38). Often VTEs are recurrent and may be life-threatening (39). Failure to properly treat hereditary AT deficient patients, especially during high risk situations such as surgery or trauma or for pregnant women, during the peri-partum period, may result in VTE. The risk of development of VTEs as compared to the normal population in these situations is increased by a factor 10 to 50 (40;41). VTEs potentially lead to death due to life-threatening pulmonary emboli or may have other life-long sequelae, e.g. post thrombotic syndrome. Recurrence of thromboembolic events is usually seen at a young age (<40 years). The recurrence of multiple thromboembolic events in AT deficient patients further increases the risk of morbidity and mortality. Deep venous thrombosis (DVT) of lower limbs and pulmonary embolism are the most common thromboembolic events reported in this population. In addition, mesenteric vein or cerebral vein thrombosis may occur (42) with associated mortality.

Surgical procedures are often accompanied by temporary immobility of the patient, which increases the risk of thromboembolism. Surgery alone, is also a risk because it typically causes a decrease in AT levels, with greater decreases occurring in procedures associated with more extensive tissue damage and hemorrhage (28). This decline in AT levels during surgery amplifies the deficiency in hereditary AT deficient patients. Major surgery like orthopedic procedures, abdominal surgery, or major vascular surgery is clearly associated with a high risk for thromboembolic events even in healthy normal individuals and consequently thromboprophylaxis is normally administered. In particular, hereditary AT deficient subjects undergoing these procedures will require anticoagulation. Chronic oral anticoagulation needs to be interrupted due to the risk of bleeding, so this prophylaxis often

consists of unfractionated (UFH) or low molecular weight heparin (LMWH). The anticoagulant effect of either UFH or LMWH, however, can only be established in the presence of sufficient levels of AT.

Pregnancy is a risk because it is typically a hypercoagulable state, due to a physiologic increase in coagulation factors and a decrease in fibrinolytic activity (43;44). Therefore, pregnant women with hereditary AT deficiency are often given anticoagulant prophylaxis (45). Warfarin is usually avoided during the first trimester of pregnancy due to its teratogenic potential, and during the last trimester of pregnancy due to bleeding risk for the fetus. Heparin, either UFH or LMWH, therefore, is often the anticoagulant of choice. Mainly during the peri-partum period, this may be interrupted and coverage with AT-concentrate is given to cover the period and make the heparin more effective just after delivery, when the risk for VTE is the highest.

Patients with AT deficiency who do develop an acute VTE need to be treated, initially with heparin. Heparin will need sufficient AT to be active. Therefore, AT is historically used in these situations (33;46;47). A review of 70 cases of acute VTE in AT deficient patients showed that in approximately 9% of cases an insufficient rise in aPTT could be achieved, and in 11% of cases extension of the VTE occurred despite treatment with unfractionated heparin. The authors conclude that the use of AT concentrate should be considered when no sufficient aPTT can be achieved or progression of the VTE is seen despite apparent sufficient anticoagulation (48).

7.1.3 Rarity of Disorder

The prevalence of hereditary antithrombin deficiency has been estimated to be one per 2000 to 5000 (49) in the general population with the reported estimate in the United States being one per 2000 based upon clinical experience of one investigator in Boston, MA (50). Based upon a current estimate of 300 million US citizens and a prevalence estimate of one per 2000 would suggest a patient population size of 150,000 individuals who may have hereditary AT deficiency. However, the population who may benefit from treatment is significantly fewer than the above estimate considering that those patients who may require treatment are those hereditary AT deficient patients undergoing major surgical procedures and pregnant women during the peri-partum period. Therefore, the likelihood that patients will need treatment with AT concentrate several times in their lifetime is small.

In 1984, the FDA (Nov. 14, 1984 OPD Review of Request for Orphan Designation) estimated that there were approximately 12,000 – 24,000 US patients with inherited AT deficiency and only a portion of those would be expected to receive AT replacement therapy. GTC Biotherapeutics believes that the FDA's 1984 estimate of hereditary AT deficiency remains a good estimate of the prevalence today. Thus, hereditary AT deficiency is a rare condition for which antithrombin supplementation is indicated only during major surgical or obstetrical procedures and consequently the number of US patients requiring AT replacement therapy on an annual basis is a few thousand at best. ATryn has been designated an orphan drug.

7.1.4 Available Treatments

Patients with hereditary AT deficiency may need chronic treatment with an anticoagulant. This depends on their medical history, particularly whether there has been a prior venous thromboembolism(49). Antithrombin concentrates are not indicated for chronic treatment of hereditary AT deficient patients.

Prior to, during and following high risk situations (surgery, delivery, etc.), human plasma-derived antithrombin alone or in conjunction with heparin is used for the treatment of hereditary AT deficient patients (33;46;51;52). Treatment with hpAT has been effective in treating/preventing thrombotic episodes in hereditary AT deficient patients. The use of hpAT is well established in medical practice and has been licensed in the United States since 1989. It should be noted that since product licensure hpAT has been available in the US on an intermittent basis. In fact, during an interval of time when hpAT was not available in the US, GTC made ATryn available to physicians for the treatment of their patients on a compassionate use basis. Results of treatment of hereditary AT deficient patients with ATryn during that period have been published by Konkle *et al.* (53).

Although two human plasma-derived antithrombin products have been licensed in the US, only one product (Thrombate III) remains on the market. The package insert for Thrombate III carries specific WARNINGS about the potential risk of transmission of known and unknown infectious agents due to the fact that the product is derived from human plasma. The labeling further recommends that the physician discusses the risks and benefits of the product before prescribing or administering it to a patient. This type of class labeling is not necessary for ATryn as it does not contain any human plasma-derived components.

In summary, AT concentrate is used for the prophylaxis of the occurrence of venous thromboembolisms in hereditary AT deficient patients in high risk situations, e.g. surgery or delivery, known to be associated with the occurrence of such events. The target is to restore plasma AT activity levels in order to cover the period during which chronic anticoagulation often needs to be interrupted and ensure that the current thromboprophylaxis (UFH, LMWH) is effective. In addition, if a VTE does occur, AT concentrate can be used to support the anticoagulative therapy, which is dependent on sufficient AT activity levels.

7.1.5 Clinical Development Program

Table 8 provides an overview of all the clinical studies in the development of ATryn.

Table 8. Table of All Investigations

Protocol # Investigators Publications	Location	Population	Designs	Treatment Doses	Number Entered Each Treatment	Duration of Drug Treatment
AT III-006-00	USA	Healthy Volunteers	Randomized, cross-over study	Antithrombin alfa; 75 IU/kg; IV administration	26 (23 treated twice)	Single dose, cross-over
GTC AT PK 011-04	USA	Healthy Volunteers	Randomized, cross-over study	Antithrombin alfa; single dose 100 IU/kg; IV administration	24 (14 treated twice)	Single dose, cross-over
GEN/G 9601	UK	Healthy Volunteers	Randomized, placebo controlled	Antithrombin alfa; dose escalation (saline, 10, 50, 100, 150, 200 IU/kg); IV administration	5/3/3/3/3 (placebo/10/ 50/100/150/ 200 U/kg)	Single dose
AT III-009-00	Italy, UK	Hereditary AT deficient patients (not in high risk)	Randomization to one of three drug lots	Antithrombin alfa; 50, 100 IU/kg; IV administration	9 (50U/kg)/ 6 (100 U/kg)	Single dose
Leitner Study	Austria	Healthy Volunteers	Pilot study: randomized cross-over study Main study: Block- randomized, double blind, placebo-controlled study consisting of 3 parallel groups	Pilot: Antithrombin alfa 130 U/kg loading dose & 30 U/kg/h maintenance dose or saline placebo. Main: Antithrombin alfa 130 U/kg loading dose & 30 U/kg/h maintenance dose or 44 U/kg loading dose & 10 U/kg/h maintenance dose or saline placebo	Pilot: 4 Main: 10/10/10 (high/low/ placebo)	Pilot & main: 4 hour continuous infusion
GTC AT III 01002	USA, Europe	Hereditary AT deficient patients	Single arm, open label with blinded evaluation	Antithrombin alfa; individualized doses targeting a plasma AT activity of 80-120%; IV administration	14	3 -14 days of continuous infusion
GTC AT HD 012-04	USA, Canada, Europe, Australia	Hereditary AT deficient patients	Single arm, open label	Antithrombin alfa; individualized doses targeting a plasma AT activity of 80-120%; IV administration	18	3-14 days, continuous infusion

Protocol # Investigators Publications	Location	Population	Designs	Treatment Doses	Number Entered Each Treatment	Duration of Drug Treatment
GTC AT III 011-003	USA	Hereditary AT deficient patients	Compassionate Use Program; retrospective data collection	Antithrombin alfa; individualized doses targeting a plasma AT activity > 80%; IV administration	5 (6 treatment episodes)	2-16 days, multiple infusions
GTC AT 96- 0801	USA	Heparin resistant patients	Dose escalation	Placebo or antithrombin alfa: 10, 25, 50, 75, 100, 125, 150, 175, 200 IU/kg; IV administration	6/3/3/3/3/3/3/3/3 (Placebo/ antithrombin alfa 10/ 25/50/75/ 100/125/150/ 175/200 IU/kg)	Single dose
GTC AT 97- 0502	USA, Germany, Netherlands	Heparin resistant patients	Placebo controlled, double blind	Placebo or antithrombin alfa: 75 U/kg IV administration	27/27 (antithrombin alfa/ placebo)	Single dose
GTC AT 97- 0504	UK, Germany	Heparin resistant patients	Placebo controlled, double blind	Placebo or antithrombin alfa: 75 U/kg IV administration	28/24 (antithrombin alfa/ placebo)	Single dose
GTC AT 97- 0903	US, Germany, France, Netherlands	Heparin resistant patients	Randomized, double blind, active control	Antithrombin alfa 15, or 75 IU/kg, & hpAT 15 IU/kg IV administration	14/15/18 (hpAT/ antithrombin alfa 15 U/kg;/ antithrombin alfa 75 U/kg)	Single dose

7.1.5.1 Pharmacokinetics

Four pharmacokinetic (PK) studies were performed.

The first (GEN/G9601) was undertaken to establish the PK characteristics of antithrombin alfa in healthy volunteers. After implementation of an active viral inactivation step in the production process of antithrombin alfa (dry heat treatment), a second healthy volunteer study (AT III-006-00) was performed to evaluate whether the change in the production process had impacted the pharmacokinetic properties of antithrombin alfa. After implementation of a nanofiltration step early in the purification process of antithrombin alfa, a healthy volunteer study (GTC AT PK 011-04) was performed to evaluate whether the change in the production process of ATryn had impacted the pharmacokinetic properties of antithrombin alfa. The PK studies on assessing the potential effects of changes in the manufacturing process showed no significant impact on the pharmacokinetics, i.e. development was continued with the newly manufactured product.

For purposes of establishing a dosing regimen in hereditary AT deficient patients, a PK study in this patient population not in a high risk situation was performed (AT III-009-00). A population PK study was done on the data from this PK study. Population PK parameter estimates derived from this study are presented in Table 9.

Table 9. Population PK Parameters for Antithrombin alfa

Design	Treatment	Mean (SD) PK Parameters					
		Incr. Rec. %/IU/kg	C _{max} %	T _{1/2} h	AUC _{0-t} % x h	Cl l/h	MRT h
Population PK analysis of study AT III 009-00	recombinant human AT	2.07 (1.54)	132.73 (1.54)	10.16 (1.28)	587.88 (1.63)	0.665 (0.05)	8.57 (1.24)

Incr. Rec: Incremental recovery; C_{max}: maximum concentration obtained; T_{1/2}: half life; AUC_{0-t}: Area under the time concentration curve from start to end of sampling; Cl: Clearance; MRT: Mean residence time; SD: standard deviation; h: hour; IU: international units; kg: kilogram

The half-life of antithrombin alfa is significantly shorter as compared to the reported values for hpAT. The background for these differences in PK parameters is probably the distinct differences in glycosylation of antithrombin alfa compared to hpAT. Antithrombin alfa has the same amino acid sequence but differs in glycosylation profile as compared to hpAT. This may impact the clearance by a different affinity to certain receptors involved in the clearance, as well as by a difference in heparin affinity.

7.1.5.2 Establishment of Dosing for Hereditary AT Deficient Patients

Dosing of AT in general is aimed at restoring a normal plasma AT activity. This is true for plasma-derived AT, as well as ATryn.

Dosing of ATryn in the studies was determined by population pharmacokinetic (PK) modeling of data obtained from a pharmacokinetic study in hereditary AT deficient patients not in a high risk situation (AT III-009-00). Based on this modeling it was decided that the best way to maintain the AT activity within the target range of 80 to 120% of normal, ATryn was to be administered intravenously by a 15 minutes loading dose, immediately followed by a continuous infusion.

In the ATryn study GTC AT III 01002 all patients, pregnant and non-pregnant surgical patients, received ATryn according to a formula taking into account both baseline AT activity as well as the patient's weight.

The required ATryn loading dose (expressed in total IU) was determined using the following formula:

Loading dose (IU) = [(100 minus the patient's pretreatment antithrombin activity in %) divided by 2.28] times patient's weight in kg

For example, a loading dose in a patient with a baseline AT activity of 50% would be approximately 22 IU/kg bodyweight, which means a total loading dose of 1540 IU for a 70 kg patient. The loading dose was given as a 15 minute IV infusion immediately followed by initiation of the IV maintenance infusion. For easier programming of the infusion pump, multiplying the loading dose by 4 to obtain the IU/hour to be given over a period of 15 minutes was allowed.

The required ATryn maintenance dose was given as a continuous IV infusion and was determined using the following formula:

Maintenance dose (IU/hour) = [(100 minus the patient's pretreatment antithrombin activity in %) divided by 10.22] times the patient's weight in kg

For example, a maintenance dose in a patient with a baseline AT activity of 50% would be approximately 5 IU/kg/hour, which means an infusion rate of 350 IU/hour for a 70 kg patient.

In addition, in case a drop in AT activity below 80% occurred after the surgical procedure or delivery (due to e.g. blood loss), an extra 15 minute bolus dose could be given. This was determined by the following formula:

Loading dose (IU) = [(100 minus the patient's actual antithrombin activity in %) divided by 2.28] times patient's weight in kg

This formula is the same as the loading dose, with the exception that the actual, post-procedure plasma AT activity level is used (instead of baseline AT activity) to determine the number of IU to be administered.

Population PK analysis of this study revealed that pregnant HD patients had a different PK profile as compared to non-pregnant surgical patients. Both Volume of Distribution and Clearance are higher for pregnant patients as compared to non-pregnant surgical patients. Therefore, in study GTC AT HD 012-04 *non-pregnant surgical patients* were treated using the dosing formulae as stated above. For pregnant patients, the loading and initial maintenance dosing formulae were modified, as follows:

For *pregnant patients*, the required ATryn loading dose was determined using the following formula:

Loading dose (IU) = [(100 minus the patient's pretreatment antithrombin activity in %) divided by 1.25] times the patient's weight in kg

For example, a loading dose in a pregnant patient with a baseline AT activity of 50% would be approximately 41.5 IU/kg bodyweight, which means a total loading dose of 2905 IU for a 70 kg patient. The loading dose was given as a 15 minute IV infusion immediately followed by initiation of the IV maintenance infusion. For easier programming of the infusion pump, multiplying the loading dose by 4 to obtain the IU/hour to be given over a period of 15 minutes was allowed.

The required ATryn maintenance dose for *pregnant patients* was given as a continuous IV infusion and was determined using the following formula:

Maintenance dose (IU/hour) = [(100 minus the patient's pretreatment antithrombin activity in %) divided by 5.43] times the patient's weight in kg

For example, a maintenance dose in a pregnant patient with a baseline AT activity of 50% would be approximately 9 IU/kg/hour, which means an infusion rate of 630 IU/hour for a 70 kg patient.

The loading and initial maintenance dose are expected to bring the plasma AT activity of the patient in the desired range in the vast majority of patients. In order to make sure that patients indeed have an AT activity within the 80-120% of normal range, therapeutic drug monitoring (TDM) is applied. TDM consists of serial AT activity determinations and, if needed based on the AT activity results, infusion rate adjustments. If the AT activity is above 120%, the infusion rate is reduced, and if below 80% the infusion rate is increased. The timing of sampling for AT activity is important, as ideally a sample most indicative for the AT activity at steady state is used. On the other hand, the desired AT activity should be reached as soon as possible requiring an early sample. Analysis of study GTC AT III 01002 revealed that a sample taken 45 minutes after the initiation of the loading dose was too early to be representative for the steady state AT activity. Therefore, the first AT activity check in study GTC AT HD 012-04 was done 2 hours after initiation of the loading dose. Also the size of the dose adjustment was changed between studies. While Study GTC AT III 01002 used an increase of 50% when AT activity was below 80% of normal, this was changed to 30% for study GTC AT HD 012-04. The decrease of infusion rate when AT activity was found to be above 120% remained the same in both studies: 30%.

Both studies with ATryn utilized the same target AT activity, between 80 and 120%. How this was reached, i.e. the loading dose and initial maintenance infusion rate, however, was different for pregnant women in the first study (n=9) as compared to the latter study (n=12). In both studies the target range of AT activity was reached and maintained, and resulted in effective prophylaxis against venous thromboembolisms, in pregnant women and non-pregnant surgery patients. Study GTC AT HD 012-04 showed that the applied dosing formulae and TDM worked well, according to the low number of dose adjustments needed (median 1 dose adjustment vs. 4 in study GTC AT III 01002). The updated dosing formulae and TDM are to be used in the package insert.

7.1.5.3 Efficacy Studies

GTC Biotherapeutics is developing ATryn for the following proposed indication: ATryn is indicated for the prevention of peri-operative and peri-partum thromboembolic events, as well as the treatment of such events, in hereditary antithrombin deficient patients. Studies for the development of another indication (acquired AT deficiency) have been performed as well and will be described briefly in Section 7.2.

AGREEMENTS WITH CBER

Due to the rarity of the disorder, each year only a very small number of patients with hereditary AT deficiency require AT replacement for thromboprophylaxis in the defined high-risk situations. Large controlled trials are therefore not possible. Even major thrombosis centers usually have only a very few, if any, patients each year that need AT replacement and trials would require a huge number of sites and an extremely long recruitment period.

Based upon communication and agreement with the CBER, it was envisaged that a Biologic License Application may be submitted based upon an efficacy comparison of ATryn-treated hereditary AT deficient patients with that of a historical cohort of comparable patients treated with human plasma-derived antithrombin. The objective of the comparison is to confirm non-inferiority of efficacy of ATryn relative to hpAT-treated patients.

GTC has worked closely with CBER on the clinical development plan, which has resulted in study protocols and statistical analysis plans that have been reviewed and implemented based upon the received comments, suggestions and recommendations of CBER. Special Protocol Assessment was not pursued, since GTC did not feel it was necessary based upon the collaborative nature of the interactions with CBER.

Table 10 provides an overview of clinical efficacy studies supporting the proposed indication. GTC AT HD-R 013-04 is comparing data from a historical control group of patients treated with plasma derived AT, with data from 2 prospective studies with HD patients treated with ATryn (GTC AT III 01002 and GTC AT HD 012-04). For purposes of completeness, an outline of all three studies is provided in Table 10.

Table 10. Description of Clinical Efficacy Studies

Study ID	Number of Study Centers (Locations)	Study Start Enrollment Status, Date Total Enrollment/ Enrollment Goal	Design Control Type	Study and Control Drugs Dose, Route, Regimen	Study Objective	# Subjects by Arm Entered/ Completed	Duration ^c	Sex M/F Median Age (Range) ^d	Diagnosis Inclusion Criteria	Primary Endpoints
GTC AT HD-R 013-04	16 (USA, Europe, Australia) ^a	FPFV: 17 Jan 2007, LPLV: 05 Dec 2007 ^a	Comparison of historical control study with active treatment, non-inferiority design	HpAT vs. antithrombin alfa; historical data vs. prospective individualized dosing targeting a plasma AT activity of 80-120%; both IV administration	Efficacy of hpAT compared with antithrombin alfa as prophylactic treatment in HD patients in high risk situations (GTC AT III 01002 & GTC AT HD 012-04)	hpAT 50/35 vs. antithrombin alfa 37/31 ^b	Up to 7 days post-treatment	hpAT: 5/30, 41y (24 – 78) ATryn: 6/25, 35y (21 – 74)	Hereditary AT deficient patients in a high risk situation (delivery/surgery)	Antithrombin alfa is non-inferior to hpAT in preventing thrombo-embolic events
GTC AT III 01002	11 (USA, Europe)	FPFV: 02 Dec 2002, LPLV: 16 Feb 2004	Single arm, open label with blinded evaluation	Antithrombin alfa; individualized doses targeting a plasma AT activity of 80-120%; IV administration	Efficacy of antithrombin alfa as prophylaxis in HD patients in high risk situations	antithrombin alfa: 14/14 ^b	Up to 7 days post-treatment	2/12, 34y (21 – 74)	Hereditary AT deficient patients in a high risk situation (delivery/surgery)	Incidence of any thrombo-embolic event

Table 10. Description of Clinical Efficacy Studies

Study ID	Number of Study Centers (Locations)	Study Start Enrollment Status, Date Total Enrollment/ Enrollment Goal	Design Control Type	Study and Control Drugs Dose, Route, Regimen	Study Objective	# Subjects by Arm Entered/ Completed	Duration ^c	Sex M/F Median Age (Range) ^d	Diagnosis Inclusion Criteria	Primary Endpoints
GTC AT HD 012-04	17 ^e (USA, Europe, Canada, Australia)	FPFV: 22 Nov 2005, LPLV: 16 May 2008	Single arm, open label	Antithrombin alfa; individualized doses targeting a plasma AT activity of 80-120%; IV administration	Efficacy of antithrombin alfa as prophylaxis in HD patients in high risk situations	antithrombin alfa: 18/17 ^b	Up to 7 days post-treatment	4/13, 35y (21 – 62)	Hereditary AT deficient patients in a high risk situation (delivery/ surgery)	Incidence of any thrombo-embolic event

^a Historical part only

^b Intent to treat/Per protocol population

^c Efficacy endpoint

^d Per protocol population

^e No. of sites that treated patients

FPFV: First Patient First Visit; LPLV: Last Patient Last Visit; hpAT: human plasma derived AT

STUDY GTC AT III 01002

This was a multicenter, multinational open-label treatment study with independent blinded evaluation of efficacy data. The study population consisted of adult hereditary AT deficient patients (with a personal or family history of a thromboembolic event and with previously documented functional AT activity $\leq 60\%$ of normal) who were treated during high-risk situations for the occurrence of acute DVT. The population consisted of patients who were scheduled for surgery; pregnant patients scheduled for cesarean section or delivery induction; or were hospitalized pregnant patients in active labor.

Surgery patients and pregnant patients scheduled for a cesarean section or a vaginal delivery were administered ATryn intravenously to normalize and maintain functional AT activity $\geq 80\%$ and $\leq 120\%$ of normal. Treatment was intended to start the day 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. Pregnancy patients not scheduled for cesarean section or delivery induction began ATryn administration as soon as they had been admitted to the hospital and active labor had begun. End of treatment was determined when the investigator felt confident that the patient was no longer at high risk for the occurrence of a thromboembolic complication. This generally occurred at the time when the investigator established effective chronic anticoagulation therapy (if applicable) and the patient was mobilized and ready for hospital discharge.

Duplex ultrasounds were performed and interpreted by qualified specialists within the same hospital/institution on a real-time basis for the timely and appropriate clinical care of the patient. Contrast venography and other scanning techniques could be used, when clinically indicated, if the study investigator felt that duplex ultrasound results were inconclusive or additional tests were appropriate. For purposes of this clinical trial and the primary efficacy endpoint, duplex ultrasound scans were videotaped for a subsequent standardized blinded interpretation by a qualified, independent laboratory to provide an unbiased evaluation of the incidence of acute DVT. The independent laboratory was provided with videotapes of all scans and no other information about the patient's history or course of treatment.

In the event of the occurrence of a thromboembolic event other than acute DVT, the event was assessed using those validated diagnostic procedures routinely used at the investigational site (e.g., for suspected pulmonary embolism, ventilation/perfusion lung scan, spiral computerized tomography, and/or pulmonary angiography). All diagnostic procedures and any other test results used to evaluate such events were collected. All imaging procedures in addition to duplex ultrasound examinations that were performed to confirm or exclude a thromboembolic event were submitted for blinded review by an independent laboratory to provide an unbiased evaluation. A variety of standard safety evaluations were performed.

Fourteen (14) hereditary AT deficient surgery ($n = 5$) and pregnant ($n = 9$) patients were treated with ATryn replacement therapy (mean 7.05, median 4.55 and range of 3.0-18.6 days).

Results of this study are provided by both central reviewers based solely on images provided to them, as well as the local interpretation of the clinical and imaging performed on a real time basis. The central review of recorded ultrasound examinations, although robust in terms of standardization, is subject to cautious interpretation. Not sitting at the bedside performing the ultrasound themselves limits the central reviewer's ability to adequately assess the ultrasounds. This is underscored by the discordance between local and central reviews. Results are presented for central and local review separately.

Central review: Thirteen (13) of the 14 patients were evaluable since one patient was thought to have an acute DVT at baseline, prior to administration of ATryn and was therefore excluded from the per protocol population for this purpose. One patient was diagnosed by the central reviewers as having an acute DVT at the intended last day of dosing of ATryn and 1 patient with an acute DVT that occurred 7 days after discontinuation of treatment with ATryn.

Local review: All 14 patients were evaluable according to local review, as the alleged acute DVT by central review was assessed as chronic changes by local review, and patient was treated according to protocol. Local review also assessed one patient as having an acute DVT at the intended last day of dosing of ATryn. Although asymptomatic, the patient was treated for acute DVT. The local interpretation of the ultrasound images of the one acute DVT at 7 days post-dosing was presence of chronic changes as opposed to the blinded central review diagnosis of acute DVT. This patient showed no clinical signs or symptoms of DVT during or after rhAT treatment and was not treated for acute DVT. Two unscheduled ultrasounds in the period after 7 days post treatment revealed no acute DVT by both central and local review. One diagnosis of an 'other thromboembolic event' was made locally by standard ultrasound imaging at day 7 after discontinuation of rhAT treatment. A crural vein thrombosis, not involving the deep venous system, was seen and assessed as not relevant, i.e. no treatment was initiated after this finding. This thrombosis was not observed during central review of the ultrasound examination.

In addition to the prophylactic use of ATryn, it was also used as part of the treatment regimen for the one patient diagnosed locally with an acute DVT. ATryn was continued after diagnosis until a therapeutic level of INR was reached. Total duration of treatment therefore exceeded 14 days (total duration was almost 19 days). The patient remained asymptomatic and the acute DVT resolved.

AT activity levels were maintained close to or within the normal AT activity range. However, pregnant patients had more fluctuations in AT activity levels and consequently required more frequent dose adjustments in order to reach and maintain the AT activity levels within the target range.

In summary, according to the central reviewers 2 out of 13 patients had a thromboembolic event during treatment with ATryn or up to 7 days after cessation of ATryn. Local assessment regarded 1 out of 14 patients as having a thromboembolic event that was treated accordingly.

STUDY GTC AT HD 012-04

This study was similar to GTC AT III 01002 as described above. Differences in design were:

1. Number of patients treated: 18
2. All patients had to have a personal history of a thromboembolic event
3. Distinct dosing algorithm for pregnant patients and non-pregnant surgical patients (see section 7.1.5.2)
4. Protocol mandated ultrasound only at baseline, and after that only when clinically indicated
5. No central review of diagnostic evaluations, only local real time assessment
6. Primary endpoint was any thromboembolic event defined as a combination of signs and/or symptoms for such event and concurrent diagnostic evaluation providing confirmation of the suspected event.

A total of 18 patients received ATryn treatment (mean 5.1, median 3.2 and range of 0.9-14.0 days). One patient was, in violation of the study, treated after delivery and did not receive the full minimum dosing of 3 days; she was excluded from the per protocol analysis. None of the treated patients developed an acute DVT or thromboembolic event other than acute DVT during the treatment with ATryn or within 7 days after cessation of ATryn treatment.

AT activity was reached and maintained more easily as compared to the previous study as indicated by the need of only 1 dose adjustment (median) per treatment. This indicates that the updated dosing algorithm for pregnant patients together with the revised therapeutic drug monitoring were effective.

GTC AT HD-R 013-04

The objective of the study was to estimate the incidence of thromboembolic events (acute DVT and/or thromboembolic events other than acute DVT) in hereditary AT deficient (HD) patients who received prophylactic treatment with plasma-derived AT during elective procedures having a high risk for the occurrence of a thromboembolic event. Additionally, the study was designed to assess the non-inferiority of ATryn compared to plasma-derived AT for prevention of thromboembolic events.

This was a historical cohort study. Study centers that had a comprehensive way of identifying eligible patients for this study were selected and data from all eligible patients from that site were collected by site personnel on a case report form. Patients that participated in the current historical cohort study were ages 18 to 80 years, had HD documented by 2 or more plasma AT activity values $\leq 60\%$ of normal, and had a personal history of venous thromboembolic events. In addition, patients had an elective procedure performed since 01 January 1997 that was associated with a high risk for the occurrence of a thromboembolic event (i.e., non-pregnant surgical patients or pregnant patients undergoing cesarean section, delivery induction, or natural child birth). At the time of their high risk procedure, all study patients were treated prophylactically with intravenously administered plasma AT for a minimum of 2 calendar days.

Standard statistical methods were used to estimate the incidence of thromboembolic events (acute DVT and/or thromboembolic events other than acute DVT) for patients treated with plasma AT. Subsequently, the incidence of thromboembolic events (acute DVT, and/or thromboembolic events other than acute DVT) that were associated with plasma AT administration was compared with the incidence for patients treated with ATryn as determined in studies GTC AT III 01002, and GTC AT HD 012-04 in order to assess the non-inferiority of ATryn.

A total of 50 patients treated with plasma derived AT were enrolled into the historical part of the study. Of the 50, 15 did not meet all of the inclusion/exclusion criteria and/or did not receive treatment with plasma-derived AT for a minimum period of 2 days. Thus, 35 patients were evaluable as the per protocol population (treatment duration mean 9.2, median 5.0 and range of 2.0-45 days). In none of the patients was an acute DVT or other thromboembolic event recorded during the treatment with plasma AT or the 7 day post-treatment.

PATIENT DEMOGRAPHICS

Plasma AT and ATryn treated patients were of a similar age, had a similar division of male and females. Both groups were for the vast majority Caucasian and had median plasma AT levels at baseline as can be expected for HD patients, i.e. $\leq 60\%$. Although some baseline plasma AT activity may be above 60%, all patients had historical AT activity levels of $\leq 60\%$.

Table 11 provides the overall demographic profile of patients in controlled trial GTC AT HD-R 013-04 (hpAT vs. ATryn) and provides evidence that the two treatment groups were balanced in terms of age, sex and race, as well as numbers of surgical and pregnant patients in each group.

Table 11. Demographics and Baseline AT Activity (Per Protocol Population)

Parameter	Statistic	Plasma AT			ATryn		
		Overall	Non-Pregnant Surgery Patients	Pregnant Patients	Overall	Non-Pregnant Surgery Patients	Pregnant Patients
Age (years)	n	35	16	19	31	11	20
	Mean	44.0	55.1	34.6	37.1	49.9	30.1
	Median	41.00	53.00	36.00	35.00	49.00	31.50
	Std. Dev.	14.34	13.40	6.00	12.80	12.18	5.82
	Minimum	24.0	33.0	24.0	21.0	35.0	21.0
	Maximum	78.0	78.0	43.0	74.0	74.0	40.0
	p-value ²	0.323					
Sex ¹	p-value ³	0.022					
	Male	n (%)	5 (14.3)	5 (31.3)	0 (0.0)	6 (19.4)	6 (54.5)
	Female	n (%)	30 (85.7)	11 (68.8)	19 (100.0)	25 (80.6)	5 (45.5)
	p-value ²	0.264					
Race ¹	p-value ³	NA					
	Caucasian	n (%)	34 (97.1)	16 (100.0)	18 (94.7)	29 (93.5)	11 (100.0)
	Black	n (%)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
	Hispanic	n (%)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
	Asian	n (%)	1 (2.9)	0 (0.0)	1 (5.3)	0 (0.0)	0 (0.0)
	Other	n (%)	0 (0.0)	0 (0.0)	0 (0.0)	2 (6.5)	0 (0.0)
	p-value ²	NA					
Baseline AT Activity Level (%)	p-value ³	0.487					
	n	25	11	14	30	11	19
	Mean	65.7	61.9	68.6	50.0	56.1	46.5
	Median	59.00	59.00	58.00	49.00	53.00	45.00
	Std. Dev.	36.35	32.69	39.94	17.89	23.80	12.86
	Minimum	22.0	22.0	22.0	25.0	25.0	28.0
	Maximum	153.0	148.0	153.0	118.0	118.0	79.0

¹ Percentages are based on number of patients in the Per Protocol population for each treatment group.

² P-values comparing plasma AT versus ATryn among non-pregnant surgery patients from Fisher's Exact test for sex and race, and Wilcoxon Rank-Sum test for age, weight, and height

³ P-value comparing plasma AT versus ATryn among pregnant patients from Fisher's Exact test for sex and race, and Wilcoxon Rank-Sum test for age.

AT=Antithrombin; Std. Dev.=Standard Deviation; NA=Not Applicable

PATIENT DISPOSITION

Table 12 provides a summary of the patient disposition by treatment group.

Table 12. Patient Disposition Summary

Parameter	Number (%) of Patients ²					
	Plasma AT			ATryn		
	Overall	Non-Pregnant Surgery Patients	Pregnant Patients	Overall	Non-Pregnant Surgery Patients	Pregnant Patients
All Enrolled Pts. ¹		22 (44.0)	27 (54.0)		12 (32.4)	25 (67.6)
Per Protocol Population	35 (70.0)	16 (32.0)	19 (38.0)	31 (83.8)	11 (29.7)	20 (54.1)
Efficacy-Excluded Patients ¹	15 (30.0)	6 (12.0)	8 (16.0)	6 (16.2)	1 (2.7)	5 (13.5)

¹ Surgery and pregnant patient counts for the plasma AT group do not sum to the overall count as Patient 09-01-05 had insufficient data to determine whether a surgery or pregnant patient.

² Percentages are based on all enrolled patients for each treatment group.

AT=Antithrombin; Pts.=Patients

Exclusion from efficacy evaluation in the plasma AT treated group was mainly due to non-compliance with inclusion and/or exclusion criteria or treatment duration less than 2 calendar days. None of the patients in the prospective ATryn studies withdrew after initiation of treatment. One patient in the ATryn treated group was excluded from the per-protocol evaluation since treatment was initiated only after the delivery had begun and treatment duration was <3 days. Five other enrolled (consented only) patients were not treated with ATryn. The various reasons for not being treated included 1) ineligible due to DVT at baseline, 2) spontaneous labor prior to treatment, 3) spontaneous amniorrhexis before treatment, 4) fetal abnormalities detected prior to treatment, and 5) withdrawal of consent.

AT replacement is targeted to restore normal AT activity, i.e. bringing the risk for thromboembolic complications to a level similar to a patient in the same situation without AT deficiency. This means that in most cases, additional anticoagulant medication is needed to sufficiently protect the patient against thromboembolic complications. Vice versa, heparin and to a lesser extent low molecular weight heparin, need a certain AT activity level to be able to have a pharmacodynamic effect.

This interrelationship is reflected in the fact that almost all patients treated with ATryn also received anticoagulant therapy during as well as after cessation of treatment with ATryn. Table 13 shows the concomitant anticoagulant use during each period. All but one patient in each patient group used a concomitant anticoagulant.

Table 13. Concomitant Anticoagulation Medications (Per Protocol Population)

Time Period	Therapeutic Category	Statistic ¹	Plasma AT (N=35)	ATryn (N=31)	P-value ²
During or After (+7 Days) Treatment	Any Anticoagulant	n (%)	34 (97.1)	30 (96.8)	1.000
	Heparin Group	n (%)	33 (94.3)	30 (96.8)	
	Vitamin K Antagonists	n (%)	21 (60.0)	20 (64.5)	
	Platelet Aggregation Inhibitors Excluding Heparin	n (%)	0 (0.0)	1 (3.2)	
During Treatment	Any Anticoagulant	n (%)	33 (94.3)	29 (93.5)	1.000
	Heparin Group	n (%)	32 (91.4)	29 (93.5)	
	Vitamin K Antagonists	n (%)	19 (54.3)	15 (48.4)	
	Platelet Aggregation Inhibitors Excluding Heparin	n (%)	0 (0.0)	1 (3.2)	
After Treatment (+7 Days)	Any Anticoagulant	n (%)	32 (91.4)	30 (96.8)	0.616
	Heparin Group	n (%)	28 (80.0)	27 (87.1)	
	Vitamin K Antagonists	n (%)	19 (54.3)	20 (64.5)	
	Platelet Aggregation Inhibitors Excluding Heparin	n (%)	0 (0.0)	1 (3.2)	

¹ Percentages are based on total number of patients. Patients using multiple medications under the same therapeutic category are counted only once for that therapeutic category.

² P-value comparing percentages of patients having any anticoagulation medication between plasma AT versus ATryn from Fisher's Exact test.

AT=Antithrombin

DEFINITION OF ENDPOINTS FOR COMPARISON OF ATRYN AND PLASMA AT TREATMENT

To account for the differences in collecting outcome data in the studies, the following definition of the endpoint was applied to compare ATryn treated HD patients with plasma AT treated HD patients.

The first outcome assessment was a clinical diagnosis of the occurrence of acute DVT. An acute DVT was considered to have occurred if: a) during treatment with or within 7 days following discontinuation of treatment with plasma AT or b) during treatment or within 7 days following discontinuation of treatment with ATryn:

Clinical symptoms consistent with the occurrence of an acute DVT were present. Clinical symptoms of acute DVT included calf pain, swelling/edema, redness, venous distention, or pain on dorsiflexion.

AND

Confirmation of acute DVT was obtained by diagnostic imaging [e.g., ultrasound, venography, or computed tomography (CT)].

In addition, for study GTC ATIII 01002 where protocol mandated ultrasounds were required (i.e., without clinical symptoms of acute DVT), an acute DVT was considered to have

occurred if local diagnostic imaging indicated the presence of an acute DVT and the patient was treated for an acute DVT.

The second assessment was the clinical diagnosis of any thromboembolic event other than acute DVT (e.g., pulmonary embolism). All analyses used only data from local assessments. A thromboembolic event other than DVT was considered to have occurred if: a) during treatment or within 7 days following discontinuation of treatment with plasma AT or b) during treatment or within 7 days following discontinuation of treatment with ATryn:

Clinical symptoms consistent with the occurrence of a thromboembolic event other than acute DVT were present. Clinical symptoms of thromboembolic events differ with the location, but shortness of breath, chest pain, headache, severe abdominal pain may all be indications that such an event occurred.

AND

Confirmation of thromboembolic event other than acute DVT was obtained by diagnostic imaging (e.g., ultrasound, angiography, CT, ventilation/perfusion scan).

In addition, for study GTC ATIII 01002 where protocol mandated ultrasounds were required (i.e., without clinical symptoms of thromboembolic events other than acute DVT), a thromboembolic event other than acute DVT was considered to have occurred if local diagnostic imaging indicated the presence of a thromboembolic event other than acute DVT and the patient was treated for a thromboembolic event other than acute DVT.

The two clinical outcome assessments (acute DVT and thromboembolic events other than acute DVT) combined, determined the primary endpoint for the comparison, which is the incidence of the occurrence of any thromboembolic event.

EFFICACY OUTCOME

The integrated efficacy data from two studies of ATryn provided a per protocol population of 31 evaluable patients. In the ATryn treated group there was one confirmed diagnosis of an acute DVT. The incidence of any thromboembolic event from the start of treatment to 7 days after last dosing is summarized by treatment group in Table 14 as are the Clopper-Pearson exact 95% CI for the proportion of patients with a thromboembolic event and the exact 95% lower confidence bound for the difference between treatments.

Table 14. Overall Incidence of Any Confirmed Thromboembolic Event (Per Protocol Population)

Plasma AT				ATryn				Lower 95% Confidence Bound of Difference
No. of Pts. Assessed	No. of Pts. With Events	% of Pts. with Events	95% CI ¹	No. of Pts. Assessed	No. of Pts. With Events	% of Pts. with Events	95% CI	
35	0	0.0	0.00, 10.00	31	1	3.2	0.08, 16.70	-0.1722

¹ The 95% confidence intervals were calculated using Clopper-Pearson methodology.

AT=Antithrombin; No.=Number; Pts.=Patients; CI=Confidence Interval

The lower 95% confidence bound of difference between treatment groups was -0.1722, a value that is greater than the protocol-specified lower confidence bound of -0.20. This demonstrates that ATryn was non-inferior to plasma AT in terms of the prevention of peri-operative or peri-partum thromboembolic events.

Table 15 and Table 16 provide the incidence of confirmed acute DVT and of confirmed thromboembolic events other than acute DVT respectively.

Table 15. Overall Incidence of Confirmed Acute DVT (Per Protocol Population)

Plasma AT				ATryn				Lower 95% Confidence Bound of Difference
No. of Pts. Assessed	No. of Pts. With Events	% of Pts. with Events	95% CI ¹	No. of Pts. Assessed	No. of Pts. With Events	% of Pts. with Events	95% CI	
35	0	0.0	0.00, 10.00	31	1	3.2	0.08, 16.70	-0.1722

¹ The 95% confidence intervals were calculated using Clopper-Pearson methodology.

AT=Antithrombin; No.=Number; Pts.=Patients; CI=Confidence Interval

Table 16. Overall Incidence of Confirmed Thromboembolic Events Other Than Acute DVT (Per Protocol Population)

Plasma AT				ATryn				Lower 95% Confidence Bound of Difference
No. of Pts. Assessed	No. of Pts. With Events	% of Pts. with Events	95% CI ¹	No. of Pts. Assessed	No. of Pts. With Events	% of Pts. with Events	95% CI	
35	0	0.0	0.00, 10.00	31	0	0.0	0.00, 11.22	-0.1157

¹ The 95% confidence intervals were calculated using Clopper-Pearson methodology.

AT=Antithrombin; No.=Number; Pts.=Patients; CI=Confidence Interval

Non-inferiority of ATryn as compared to plasma AT, the current standard prophylactic treatment of HD patients in a high risk situation was established for each type of event, as confirmed by the lower 95% confidence bound of the difference (plasma-ATryn) which is ≥ -0.20 .

One patient, suffered a DVT 10 days post-treatment, another a pulmonary embolism 14 days after treatment. These were not regarded related to the study drug, as due to the short half life of recombinant human antithrombin, it will have completely cleared from the circulation approximately 2 days post-treatment and as such these thrombotic events may have been a failure of follow-up anticoagulation rather than a failure of antithrombin replacement.

SUPPORTIVE CLINICAL EFFICACY DATA

One additional study on the assessment of the incidence of DVT following prophylactic administration of ATryn to hereditary AT deficient patients in high-risk situations was done (GTC AT III 011-003). This was a retrospective collection of data from patients treated in a compassionate use program for ATryn. The objective of this compassionate use treatment series was to provide AT replacement therapy with ATryn during the supply shortage of human plasma derived AT in the United States, specifically, to hereditary antithrombin deficient patients with an acute need for AT replacement therapy in high-risk situations for the development of thromboembolic complications.

Patients that qualified for compassionate use treatment were administered ATryn by bolus (slow intravenous push) injection. The goal of ATryn treatment was individualized to maintain plasma AT activity levels within normal range (80-120% activity). Implicit with the goal of maintaining AT activity levels within normal range was the prevention of new thromboembolism. ATryn was administered by periodic intravenous bolus infusion.

The following formula was to be employed for each single or periodic dose of ATryn (in IU) that was administered when the baseline AT level was known:

$$\frac{\text{Desired AT Level (\%)} - \text{Baseline or Current AT Level (\%)}}{1.5 (\% \text{ increase per unit rhAT})} \times \text{Patient's Weight (kgs)}$$

Five patients, with 1 patient having 2 separate treatments were enrolled in the program and data on their clinical course collected.

Five hereditary AT deficient surgical patients, who were at high risk for the occurrence of a thromboembolic event, were treated with ATryn replacement therapy. All had a personal history of thromboembolic events. One of the 5 patients was treated a second time with ATryn after approximately a 4-week interval. See Table 17 for a description of the procedures.

Table 17. Procedure/Diagnosis Information (Safety Population)

Patient Number	Patient Type	Procedure	Baseline AT Activity Level (%)
(b)(6)	Surgical	Two-vessel CABG	28
	Surgical	Laparoscopic Splenectomy	80
	Surgical	Bilateral Hip Replacement	56
	Pregnancy/Surgical	Cesarean Section; Bilateral Tubal Ligation	58
	Surgical	Supracervical Hysterectomy	53**
	Surgical	Left Total Knee Replacement	38***

CABG = Coronary Artery Bypass Graft

* Same patient, different surgeries

** Historical level, no baseline level available

*** Post treatment value, no baseline level available

All patients were observed for clinical signs and symptoms of thromboembolic events and 5 of the patients were specifically evaluated for DVT by duplex ultrasound. Neither DVT nor any other thromboembolic event was diagnosed based on clinical symptoms or by duplex ultrasound. In addition, dosing with ATryn resulted in an increase in AT activity level close to and within the normal AT activity range. This study has also been published by Konkle et al. (54). The data from this study provide additional evidence of the efficacy of ATryn for the prevention of peri-operative and peri-partum thromboembolic events in hereditary antithrombin deficient patients.

TREATMENT OF THROMBOEMBOLIC EVENTS

No ATryn-treated patient from the GTC AT HD 012-04 study experienced a thromboembolic event during or up to 7 days post-treatment. The single patient from the GTC AT III 01002 study who had an acute DVT during treatment with ATryn was the only one who was treated accordingly by continuation of ATryn treatment together with LMWH and vitamin K antagonists. This acute DVT was not associated with any clinical signs and symptoms indicating such event. Whether this would have become a clinically overt DVT if no protocol mandated ultrasound was performed and therefore the acute DVT only would have been detected when it became symptomatic, is uncertain. Treatment for acute DVT started before symptoms were present and afterward the patient remained asymptomatic for acute DVT. This patient was successfully maintained within the target range of AT activity for most of the time during the ATryn treatment period. Moreover, to support the treatment of the acute DVT, ATryn treatment was continued for an additional 6 days. The event eventually

resolved. It is likely that this patient who had a hip replacement procedure, which has a high risk of development of thrombosis, even without thrombophilia, represents the portion of thromboses normally seen in other studies with joint replacement surgery.

The use of antithrombin for the *treatment* of thromboembolic complications is a rational use. Reports on HD patients with thrombosis that are treated with AT show a favorable outcome (55). The indication for use of Thrombate III includes the treatment of thrombotic events. The package insert of Thrombate III reports on the treatment of 8 patients with thrombosis of which 7 were treated successfully. ATryn has been shown to have the same pharmacodynamic actions as compared to plasma derived AT, together with the experience in the one patient in the clinical study, supports the inclusion of the treatment of acute thrombosis in hereditary AT deficient patients.

7.2 Acquired Antithrombin Deficiency

7.2.1 Heparin Resistance in Cardiopulmonary Bypass Patients

Cardiopulmonary bypass (CPB) is associated with substantial activation of the haemostatic system. This is caused, amongst others, by the interface between blood and the large, non-endothelial extracorporeal circuit. Elevated haemostatic activity associated with CPB may induce a systemic inflammatory reaction and lead to clinical complications such as hemorrhage, postoperative thrombosis, and organ dysfunction (56). Adequate anticoagulation is a critical component of successful management of haemostatic and inflammatory responses associated with CPB. Heparin is commonly administered during CPB to achieve systemic anticoagulation. Heparin alone has no direct anticoagulant effect, but it potentiates the activity of AT. Heparin enhances AT mediated inhibition of coagulant enzymes more than 1,000-fold. An inadequate response to heparin, known as heparin resistance (HR), has been reported in up to 22% of patients undergoing CPB (57). Among risk factors for developing HR during CPB, decreased AT levels present the greatest risk (58). ATryn has been studied in clinical trials including patients with HR. However, HR is not being pursued as an indication in this BLA filing.

Protocol AT 96-0801 was a phase I – II, single dose, open label, dose escalation study of the safety of antithrombin alfa in patients scheduled for primary cardiac surgery requiring cardiopulmonary bypass. The study was designed to test the safety of administering antithrombin alfa to patients who had been on heparin therapy for ≥ 12 hours prior to their scheduled cardiac surgery. A total of 36 patients participated in the study where 30 patients received antithrombin alfa while 6 patients were managed with heparin alone to serve as the comparative control group. One control patient was included in each of the first three dosing cohorts, and three control patients were added (non-randomized) at the end of the study. All patients received a pre-bypass, standard heparin-loading dose of 300 IU/kg. For patients in the first dosing cohorts (10, 25, 50, 75, 100, 125, and 150 IU/kg) and the placebo dosing cohort, additional heparin was administered as needed to maintain the heparin concentrations at approximately 3.4 U/mL. For patients enrolled in subsequent dosing cohorts 150, 175, and 200 IU/kg, additional heparin was administered as needed to maintain the heparin

concentrations between 1.5-2 IU/mL in order to maintain an activated clotting time (ACT) ≥ 480 seconds. Following the induction of anesthesia prior to the administration of heparin and initiation of CPB, intravenous antithrombin alfa was infused continuously over 30 minutes. Safety follow-up was done up to 1-2 months after treatment.

Protocol AT 97-0502 was a phase III, double blind, randomized, placebo controlled, multi-center study of the safety and efficacy of antithrombin alfa in heparin resistant patients scheduled for cardiac surgery requiring cardiopulmonary bypass. For identification of heparin resistant patients, the investigators used the Hepcon Hemostasis Management System with the Heparin Dose Response (HDR) cartridge as a screening device. Randomization assignment was given only to those patients who's ACT remained below 480 seconds following IV administration of a total of 400 IU/kg of heparin. Fifty-four patients were randomly assigned to either antithrombin alfa (75 IU/kg) or normal saline placebo. Subjects were followed for 4 weeks following surgery.

Protocol AT 97-0504 was a phase III, double blind, randomized, placebo controlled, multi-center study of the safety and efficacy of antithrombin alfa in heparin resistant patients scheduled for cardiac surgery requiring cardiopulmonary bypass. For identification of heparin resistant patients, the investigators used the Hepcon Hemostasis Management System with the HDR cartridge as a screening device. Randomization assignment was given only to those patients who's ACT remained below 480 seconds following IV administration of a total of 400 IU/kg of heparin. Fifty-two patients were randomly assigned to either antithrombin alfa (75 IU/kg) or normal saline placebo. The study was conducted at 6 study centers. Subjects were followed for 4 weeks following surgery.

Protocol AT 97-0903 was a phase III, double blind, randomized, multi-center study comparing recombinant human antithrombin and human plasma antithrombin (hpAT) in heparin resistant patients undergoing elective cardiac surgery requiring cardiopulmonary bypass. Patients who had been on IV heparin for 6 or more hours prior to surgery were eligible to participate. Randomization assignment was given only to those patients whose ACT remained below 480 seconds after receiving an initial loading dose of 300 IU/kg heparin IV. The study was conducted at 14 U.S. and European study centers. Approximately 270 patients were to be enrolled in three dosing cohorts (90 per treatment arm); 15 IU/kg antithrombin alfa, 75 IU/kg antithrombin alfa, and 15 IU/kg hp AT. Subjects were followed for 4 weeks following surgery. Forty-seven patients received treatment before the study was closed. Reasons for closure of the study were twofold. The medical practice of treatment with intravenous heparin prior to undergoing elective cardiac surgery, a requirement for study entry, had decreased significantly, thereby reducing the number of eligible study patients and therefore, the feasibility of completing the trial. Additionally, discussions with several health regulatory authorities resulted in the realization that the clinical trial as designed did not fulfill the regulatory requirements for licensure of the product.

Table 18 provides a summary of the results of ATryn efficacy studies in the heparin resistance indication.

Table 18. Results of Heparin Resistance Efficacy Studies

Study	Treatment Arm	# Enrolled/ Completed	Primary Endpoint	Statistical test/ <i>P</i> value	Secondary Endpoint	Other Comments
			# Patients Who Required FFP/ No. Patients ^d (%)			
GTC AT 97-0502	75 IU/kg ATryn vs. Placebo	Placebo: 24/23 ^b ATryn: 28/28	Placebo: 22/27 (81) ATryn: 5/26 (19)	< 0.001	AT levels significantly higher in ATryn treated subjects; no differences in D-Dimer or fibrin monomer	Achieved primary endpoint
GTC AT 97-0504	75 IU/kg ATryn vs. Placebo	Placebo: 27/27 ATryn: 27/24 ^c	Placebo: 22/24 (92) ATryn: 6/28 (21)	< 0.001	AT levels significantly higher in ATryn treated subjects; no differences in D-Dimer or fibrin monomer	Achieved primary endpoint
GTC AT 97-0903	75 IU/kg ATryn vs. 15 IU/kg ATryn or 15 IU/kg hpAT ^a	ATryn 75 U/kg: 18/18 ATryn 15 U/kg: 15/15 hpAT 15 U/kg: 14/13 ^e	N/A ^e	N/A ^e	AT levels significantly higher in 75 IU/kg ATryn treated subjects	Study ended prematurely ^f

a) hpAT = human plasma derived antithrombin

b) One patient died during surgery

c) One ATryn patient received a partial dose (1 mL) of study medication and two ATryn patients died post-operatively (2 & 18 days) due to complications associated with their underlying disease.

d) Intent to treat population

e) N/A = not applicable

f) Discussions with several health regulatory authorities resulted in the realization that the clinical trial as designed did not fulfill the regulatory requirements for licensure of the product.

In the GTC AT 97-0502 Intent-To-Treat population, 22 of 27 placebo treated patients required the infusion of FFP, while only 5 of 26 ATryn treated patients required FFP prior to initiation of CPB. In the GTC AT 97-0504 Intent-To-Treat population, only 6 of 28 ATryn treated patients required FFP prior to initiation of CPB while 22 of 24 placebo-treated patients required the infusion of FFP. The proportion of patients requiring the administration of two units of FFP prior to proceeding to CPB was significantly smaller ($p < 0.001$) in the ATryn treatment group compared to the proportion among placebo treated patients in both studies. The same level of statistical significance was observed in each study when the analysis was performed using the Per Protocol population.

Study GTC AT 97-0903 lacked the statistical power to allow for a complete and meaningful analysis of efficacy.

The study results in acquired AT deficient patients provide additional support for the pharmacodynamic action of ATryn in hereditary AT deficient patients. Patients with heparin resistance were given ATryn to restore the response to heparin, providing sufficient anticoagulation to initiate CPB. ATryn was very effective in restoring heparin resistance, which provides additional evidence for the pharmacodynamic properties. Similar to acquired AT deficient patients, hereditary AT deficient patients may be heparin resistant when they are treated for an acute DVT or other acute VTE. Some patients do not reach an adequate aPTT, or some who do still show progression of the clot. Plasma AT is used in such situations as well.

7.3 Safety of ATryn

Provided in Table 19 is a tabulation of the number of individual subjects and patients who were enrolled and treated in clinical trials of ATryn. Included in the table are the number of subjects/patients treated with ATryn, human plasma-derived antithrombin and placebo by subject/patient population (i.e., HD patients, acquired AT deficient patients and normal healthy volunteers). Thus, extent of exposure to ATryn in the HD patient population is 52 patients, 118 patients with acquired AT deficiency and 65 normal healthy volunteers for a total exposure to ATryn of 235 subjects/patients. Some of the subjects and patients were exposed to ATryn on two separate occasions as shown in Table 19 for a total of 273 unique exposures to ATryn of which 38 were from re-treatment. The duration between treatments with ATryn ranged from 28 days to months. No subject or HD patient was exposed to ATryn on more than two occasions consistent with the rarity of the patient population. Likewise, the patients with acquired AT deficiency were only treated once during cardiac surgery requiring cardiopulmonary bypass.

Table 19. Numbers Subjects and Patients Exposed to ATryn, hpAT and Placebo

Patient Type/Protocol	Number of Patients				
	ATryn	hpAT	Placebo	Total	ATryn Treatment Twice
All Patients	235	14	62	311	38
Hereditary Deficient Patients	52	0	0	52	1
GTC AT III-009-00	15	0	0	15	0
GTC AT III 01002	14	0	0	14	0
GTC AT III 011-003	5	0	0	5	1
GTC AT HD 012-04	18	0	0	18	0
Acquired Deficient Patients	118	14	57	189	0
AT 96-0801	30	0	6	36	0
AT 97-0502	27	0	27	54	0
AT 97-0504	28	0	24	52	0
AT 97-0903	33	14	0	47	0
Healthy Volunteers	65	0	5	70	0
GEN/G-9601	15	0	5	20	0
AT III-006-00	26	0	0	26	23
PK 011-04	24	0	0	24	14

The demographic characteristics data are summarized for the safety population in Table 20.

Table 20. Demographic Characteristics (Safety Population)

Parameter/ Statistics	Hereditary Deficient	Acquired Deficient			Healthy Volunteers	
	ATryn	ATryn	hpAT	Placebo	ATryn	Placebo
Age (year)						
n	53	118	14	57	102	5
Mean	40.0	63.5	64.0	64.6	26.8	25.0
Median	36	64	67	65	23	25
Std. Dev.	13.8	9.6	10.1	10.1	7.6	2.7
Minimum	21	35	47	38	17	22
Maximum	74	81	82	86	44	29
Sex						
Male	12 (22.6%)	90 (76.3%)	11 (78.6%)	42 (73.7%)	56 (54.9%)	5 (100%)
Female	41 (77.4%)	28 (23.7%)	3 (21.4%)	15 (26.3%)	46 (45.1%)	0 (0.0%)
Pregnant	22 (41.5%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
Non-pregnant	19 (35.8%)	28 (23.7%)	3 (21.4%)	15 (26.3%)	46 (45.1%)	0 (0.0%)
Race						
Caucasian	51 (96.2%)	115 (97.5%)	14 (100%)	56 (98.2%)	71 (69.6%)	5 (100%)
African-American	0 (0.0%)	3 (2.5%)	0 (0.0%)	0 (0.0%)	27 (26.5%)	0 (0.0%)
Hispanic	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (1.0%)	0 (0.0%)
Asian	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (1.8%)	2 (2.0%)	0 (0.0%)
Other	2 (3.8%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
Missing	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (1.0%)	0 (0.0%)
Weight (kg)						
n	53	118	14	57	102	5
Mean	79.0	78.5	82.1	76.5	71.7	70.3
Median	78	77	85	78	71	66
Std. Dev.	19.1	14.6	10.4	13.6	11.0	10.1
Minimum	45	52	64	48	48	60
Maximum	140	125	105	104	96	84

Subjects/patients aged 17 to 86 have been included in studies with ATryn. HD deficient patients were mainly female while acquired deficient patients were mainly male. This is probably due to the fact that HD patients were mostly pregnant women and acquired deficient patients were mostly elderly male patients undergoing cardiac surgery. Healthy volunteers were, as expected, relatively young and fairly balanced with regard to gender. The vast majority of patients were Caucasian. Weight was comparable among the types of subjects/patients.

Almost all hereditary and acquired AT deficient patients received some type of concomitant medication. This is expected since they were treated around a surgical procedure or delivery.

For hereditary deficient patients, 52 patients (98%) had used concomitant medications. Concomitant anticoagulant medication was used by the majority of HD patients. Vitamin K antagonists were used in 79% of patients and heparin group medication in 68% of patients. All but 1 of the HD patients treated in the phase III studies GTC AT III 01002 and GTC AT HD 012-04 used an anticoagulant during or within 7 days after stop of treatment with ATryn.

All 118 acquired deficient patients who received ATryn had used concomitant medications. Heparin group medication was used in 87% of patients treated with ATryn. This was comparable in the hpAT and placebo treated group.

Among the healthy volunteers who received ATryn, 30 (29%) had used concomitant medications, with no concomitant medication (therapeutic category) used by more than 10% of subjects. None of the 5 healthy volunteers who received placebo used any concomitant medications.

All 52 hereditary deficient patients were treated with ATryn. Among them, 9 patients received an intravenous infusion of 50 IU/kg, 6 patients received an IV infusion of 100 IU/kg, 32 patients received treatment by continuous infusion, and 5 patients received multiple ATryn infusions one of which was treated on two separate occasions.

Among the 118 acquired deficient patients who received ATryn, 15 patients received an intravenous infusion of 15 IU/kg; 76 patients received 75 IU/kg; 6 patients received 150 IU/kg; and the 10, 25, 50, 100, 125, 175 and 200 IU/kg dose groups had 3 patients each.

Among the 65 healthy volunteers who received ATryn, there were 49 exposures to 75 IU/kg, 41 to 100 IU/kg; and the 10, 50, 150, and 200 IU/kg dose groups had 3 subjects each.

Table 21 provides the total ATryn dose (IU) for each patient type. Since ATryn is dosed per kg body weight, dose per kg body weight (IU/kg) is provided in Table 22. The vast majority (72%) of HD patients were treated with a dose of more than 10,000 IU, and more than half (57%) of the HD patients was dosed with >150 IU/kg/day.

Table 21. Total ATryn Dose (IU) (Safety Population)

Patient Type	Number (%) of Patients					Total
	<3000 IU	3000- <6000 IU	6000- <10000 IU	10000- <50000 IU	≥50000 IU	
All Patients	27 (9.9%)	100 (36.6%)	92 (33.7%)	27 (9.9%)	27 (9.9%)	273 (100%)
Hereditary Deficient Patients	3 (5.7%)	6 (11.3%)	6 (11.3%)	11 (20.8%)	27 (50.9%)	53 (100%)
Acquired Deficient Patients	21 (17.8%)	49 (41.5%)	37 (31.4%)	11 (9.3%)	0 (0.0%)	118 (100%)
Healthy Volunteers	3 (2.9%)	45 (44.1%)	49 (48.0%)	5 (4.9%)	0 (0.0%)	102 (100%)

Table 22. Total ATryn Dose (IU/kg/day) (Safety Population)

Patient Type	Number (%) of Patients					Total
	10-50 IU/kg/day	>50-100 IU/kg/day	>100-150 IU/kg/day	>150-200 IU/kg/day	>200 IU/kg/day	
All Patients	39 (14.3%)	176 (64.5%)	19 (7.0%)	21 (7.7%)	18 (6.6%)	273 (100%)
Hereditary Deficient Patients	9 (17.0%)	7 (13.2%)	7 (13.2%)	12 (22.6%)	18 (34.0%)	53 (100%)
Acquired Deficient Patients	24 (20.3%)	79 (66.9%)	9 (7.6%)	6 (5.1%)	0 (0.0%)	118 (100%)
Healthy Volunteers	6 (5.9%)	90 (88.2%)	3 (2.9%)	3 (2.9%)	0 (0.0%)	102 (100%)

Patients who were treated with human plasma AT were administered a single intravenous dose of 15 IU/kg.

7.3.1 Hereditary AT deficient patients

7.3.1.1 Adverse Events in Treated Patients

Table 23 summarizes the number of HD patients experiencing any treatment emergent adverse events.

Table 23. Summary of Patients Experiencing Treatment-Emergent Adverse Events - Hereditary Deficient Patients (Safety Population)

Category	Overall (N=53) ^d	Pregnant Female (N=22)	Male and Non- Pregnant Female (N=31)
Any Serious Adverse Event ^a	9 (19%)	4 (19%)	5 (19%)
Any Adverse Event	40 (76%)	17 (77%)	23 (74%)
Death	0 (0%)	0 (0%)	0 (0%)
Any Severe Adverse Event	7 (13%)	5 (23%)	2 (6%)
Any Related Adverse Event ^{a,b}	7 (15%)	3 (14%)	4 (15%)
Any Severe Related Adverse Event ^{a,b}	1 (14%)	1 (5%)	0 (0%)
Any Serious Related Adverse Event ^{a,b}	2 (2%)	1 (5%)	1 (3%)
Any Adverse Event Leading to Withdrawal ^c	0 (0%)	0 (0%)	0 (0%)

^a Protocol GTC AT III 011-003 was excluded because seriousness and relationship information were not available, so denominators are 47, 21, and 26 for overall, pregnant female, and male and non-pregnant female, respectively.

^b Related includes possibly, probably, and definitely related.

^c Protocol GTC AT III 011-003 was excluded because an indication of events leading to withdrawal was not available.

^d One patient treated on 2 occasions is counted twice

In hereditary AT deficient patients, the rate of adverse events reported (76%) was expected considering the fact that these patients were for the most part treated around the time of a surgical procedure or delivery. The rate of serious and/or severe events though was low (19 and 13%, respectively). Only 7 of 47 patients (15%) reported an adverse event that was thought to be related to ATryn, but never lead to withdrawal from the study. No control arms were used in the studies in HD patients and therefore, the adverse event rates can not be compared with any control treatment.

Table 24 shows all treatment emergent adverse events that occurred in at least 5% of patients.

Table 24. Treatment-Emergent Adverse Events Reported by At Least 5% of Hereditary Deficient Patients (Safety Population)

System Organ Class/Preferred Term	Statistic	ATryn	
		All (N=53)	Related (N=47) ^a
Blood and Lymphatic System Disorders			
Anemia	n (%)	7 (13.2)	0 (0)
Gastrointestinal Disorders			
Abdominal pain	n (%)	3 (5.7)	0 (0)
Nausea		4 (7.5)	0 (0)
Vomiting		6 (11.3)	0 (0)
General Disorders and Administration Site Conditions			
Edema peripheral	n (%)	4 (7.5)	0 (0)
Pyrexia		3 (5.7)	0 (0)
Injury Poisoning and Procedural Complications			
Incision site complications	n (%)	5 (9.4)	0 (0)
Procedural pain		3 (5.7)	0 (0)
Musculoskeletal and Connective Tissue Disease			
Pain in extremity	n (%)	3 (5.7)	0 (0)
Nervous System Disorders			
Headache	n (%)	6 (11.3)	0 (0)
Reproductive System and Breast Disorders			
Vaginal laceration	n (%)	3 (5.7)	0 (0)
Respiratory, Thoracic and Mediastinal Disorders			
Epistaxis	n (%)	3 (5.7)	0 (0)
Vascular Disorders			
Hematoma	n (%)	4 (7.5)	1 (2.1)
Hypotension		5 (9.4)	0 (0)

^a Study GTC AT III 011-003 has no relationship assessments available

The highest incidences of adverse events reported were in the body systems of gastrointestinal disorders; general disorders and administration site conditions; injury, poisoning and procedural complications; and vascular disorders, with events in these body systems being reported in 30%, 26%, 26%, and 21%, respectively, of patient exposures. The most commonly reported adverse events were anemia (13%), vomiting (11%), and headache (11%). Only one of the more frequently reported adverse events was assessed as probably related to ATryn.

The reported adverse events reflect the fact that the patients (except those in the PK study) were treated in the peri-operative and peri-partum period. The most frequently reported events are common during those periods, with or without treatment with AT.

Table 25 provides a list of all severe adverse events reported, with their relationship and whether they were serious.

Table 25. List of Severe Treatment-Emergent Adverse Events By System Organ Class - Hereditary Deficient Patients (Safety Population)

<i>System Organ Class</i>					
Protocol / Patient Number	Preferred Term	Reported Term	Serious	Relation	Outcome
<i>Blood and Lymphatic System Disorders</i>					
AT 01204/ (b)(6)	Anemia	Unstable HCT/anemia	No	Not Related	Recovered
<i>Gastrointestinal Disorders</i>					
AT 01204/ (b)(6)	Intra-abdominal Hemorrhage	Intra-abdominal Bleeding	Yes	Probable/Definite	Recovered
<i>Infections and Infestations</i>					
AT 01204/ (b)(6)	Enterobacter Sepsis	Septicemia Enterobacter Cloacae	Yes	Not Related	Recovered
<i>Injury, Poisoning and Procedural Complications</i>					
AT 01002/ (b)(6)	Femur Fracture	Traumatic Fracture of Left Femur	Yes	Remote/Unlikely	Recovered
<i>Musculoskeletal and Connective Tissue disorders</i>					
AT 01204/ (b)(6)	Muscle Spasms	Muscle Spasm at RT Shoulder	No	Not Related	Recovered
<i>Nervous System Disorders</i>					
AT 01002/ (b)(6)	Grand Mal Convulsions	Grand Mal Seizures	Yes	Remote/Unlikely	Recovered
AT 01204/ (b)(6)	Headache	Headache	No	Not Related	Recovered
<i>Pregnancy, Puerperium and Perinatal Conditions</i>					
AT 01002/ (b)(6)	Umbilical Malformation	Cord Prolapse	No	Not Related	Recovered
<i>Reproductive System and Breast Disorders</i>					
AT 01204/ (b)(6)	Vaginal Laceration	Vaginal Tear	No	Not Related	Recovered
<i>Respiratory, Thoracic and Mediastinal Disorders</i>					
AT 01204/ (b)(6)	Pulmonary Embolism	Bilateral Pulmonary Embolism	Yes	Not Related	Recovered with Sequelae

^a pregnant female, ^b surgical female, ^c male

There were seven patients (13%) who reported ten severe adverse events, of which only one occurrence of intra-abdominal hemorrhage was considered related to treatment; the other events were considered either not related or remote/unlikely to be related to treatment. For fourteen patients (26%), the highest AE severity rating was moderate, fifteen patients (28%) reported only mild adverse events and four patients (8%) reported all adverse events with unknown severity. Among pregnant females, five patients (23%) reported seven severe

adverse events. Among the males and non-pregnant females, two patients (6%) reported three severe adverse events.

The serious treatment-emergent adverse events for hereditary deficient patients are listed in Table 26. There were nine patients (19%) who reported a total of 13 serious adverse events. Two events were assessed as potentially related to treatment (i.e., severe intra-abdominal haemorrhage and moderate hemarthrosis). All others were considered either not related or remote/unlikely related to treatment. Among pregnant females, four patients (19%) reported six serious adverse events. Among the males and non-pregnant females, five patients (19%) reported a total of seven serious adverse events.

Table 26. List of Serious Treatment-Emergent Adverse Events By System Organ Class - Hereditary Deficient Patients (Safety Population)

<i>System Organ Class</i>					
Protocol / Patient Number	Preferred Term	Reported Term	Severity	Relation	Outcome
<i>Gastrointestinal Disorders</i>					
AT 01204 (b)(6)	Intra-abdominal Hemorrhage	Intra-abdominal Bleeding	Severe	Definite/ Probable	Recovered
<i>General Disorders and Administration Site Conditions</i>					
AT 01002 (b)(6)	Pyrexia	Fever Unknown Origin	Moderate	Not Related	Recovered
<i>Injury, Poisoning and Procedural Complications</i>					
AT 01002 (b)(6)	Femur Fracture	Traumatic Fracture of Left Femur	Severe	Remote/ Unlikely	Recovered
<i>Infections and Infestations</i>					
AT 1204 (b)(6)	Enterobacter Sepsis	Septicemia Enterobacter Cloacae	Severe	Not Related	Recovered
<i>Investigations</i>					
AT 1204 (b)(6)	Hemoglobin Decreased	Low Hemoglobin Level	Moderate	Not Related	Recovered
<i>Musculoskeletal and Connective Tissue disorders</i>					
AT 1204 (b)(6)	Hemarthrosis	Clinical Hemarthrosis R Knee	Moderate	Possible	Recovered
<i>Nervous System Disorders</i>					
AT 01002 (b)(6)	Grand Mal Convulsions	Grand Mal Seizures	Severe	Remote/ Unlikely	Recovered
<i>Respiratory, Thoracic and Mediastinal Disorders</i>					
AT 1204 (b)(6)	Pulmonary Embolism	Bilateral Pulmonary Embolism	Severe	Not Related	Recovered with Sequelae
<i>Vascular Disorders</i>					
AT 01002 (b)(6)	Hypotension	Hypotension	Moderate	Remote/ Unlikely	Recovered
AT 01002 (b)(6)	Wound Hemorrhage	Wound Hemorrhage	Moderate	Remote/ Unlikely	Recovered
AT 01002 (b)(6)	Deep Vein Thrombosis	Muscular Vein Thrombosis Left Upper Leg	Mild	Remote/ Unlikely	Recovered
AT 01204 (b)(6)	Deep Vein Thrombosis	Popliteal Non-occlusive Thrombus in RLE	Mild	Not Related	Recovered
AT 1204 (b)(6)	Hematoma	Hematoma	Moderate	Remote/ Unlikely	Recovered

^a pregnant female, ^b non-pregnant female, ^c male

The related treatment-emergent adverse events for hereditary deficient patients are listed in Table 27. There were seven patients (15%) who reported eight related adverse events. Only severe intra-abdominal bleeding among a pregnant female was considered serious.

Table 27. List of Related Treatment-Emergent Adverse Events By System Organ Class- Hereditary Deficient Patients (Safety Population)

System Organ Class						
Protocol / Patient Number	Preferred Term	Reported Term	Serious	Severity	Relation	Outcome
Gastrointestinal disorders						
AT01204 (b)(6)	Intra-abdominal Hemorrhage	Intra-abdominal Bleeding	Yes	Severe	Probable/Definite	Recovered
General disorders and Administration Site Disorders						
AT 009 (b)(6)	Application site pruritus	2x2cm Red Itchy Area At Left Antecubital Fossa	No	Mild	Probable/Definite	Recovered
AT01204 (b)(6)	Feeling Hot	Feeling Hot	No	Mild	Possible	Recovered
AT1204 (b)(6)	Non-cardiac Chest Pain	Non-cardiac Chest Pain	No	Moderate	Possible	Recovered
Investigations						
AT1204 (b)(6)	Hepatic Enzyme Abnormal	Abnormal Liver Enzyme Tests AST 52 ALT 77	No	Mild	Possible	Recovered
Musculoskeletal and Connective Tissue Disorders						
AT1204 (b)(6)	Hemarthrosis	Clinical Hemarthrosis R Knee	Yes	Moderate	Possible	Recovered
Renal and Urinary Disorders						
AT01204 (b)(6)	Hematuria	Microscopic Hematuria	No	Mild	Possible	Recovered
Vascular Disorders						
AT01204 (b)(6)	Hematoma	Anterior Rectus Hematoma	No	Mild	Probable/Definite	Recovered

^a pregnant female, ^b non-pregnant female, ^c male

Note: Related includes possibly, probably, and definitely related.

ATryn has not been studied in a prospective, comparative study with either placebo or an active control arm (except for efficacy, where a historical control study with patients treated with plasma derived AT served as the active control arm, Study GTC AT HD-R 013-04). A comparison of the adverse event rates, seriousness and severity is therefore not possible. The adverse events that have been reported, though, are what one would expect in a population of patients that are treated during and just after a surgical procedure, or during and just after delivery (either vaginal or through cesarean section). Frequently reported events as listed in Table 24 may well be due to anesthesia (e.g. nausea, vomiting, hypotension), the surgery

(e.g. abdominal pain), or delivery (vaginal laceration). Consistent with this, events have rarely been assessed as related to the ATryn treatment by the investigators. Likewise, severe and serious adverse events have occurred, but again, are as can be expected in patients having surgery or delivery.

Events that have been assessed as potentially having a relationship with ATryn treatment as presented in Table 27 do not indicate a particular safety concern. Each preferred term is only listed once. However, a few of the events have as a common theme that these are different types of bleeding (intra-abdominal hemorrhage, hemarthrosis, hematuria and hematoma, all reported once as a related adverse event). Since ATryn is an anticoagulant, especially when given concomitantly with unfractionated heparin (UFH) or low molecular weight heparin (LMWH), this would be consistent with the pharmacodynamic action of the drug. In the phase 3 studies, ATryn was administered in almost every patient concomitantly with another anticoagulant (see Table 13). Next to the surgical procedure or the delivery, these bleeding events may therefore either be related to the concomitant anticoagulant, or due to the combination of ATryn and the anticoagulant.

7.3.1.2 Neonates

Of the 22 neonates born from HD patients treated with ATryn, 10 were reported to have had an adverse event, of which 2 were assessed as serious although none of the adverse events were assessed as related to treatment with ATryn. Table 28 summarizes the data.

Table 28. Summary of Neonates Experiencing Treatment-Emergent Adverse Events – Hereditary Deficient Pregnant Females (Safety Population)

Category	ATryn (N=22)
Any Serious Adverse Event	2 (9.1%)
Any Adverse Event	10 (45.5%)
Death	0 (0.0%)
Any Severe Adverse Event	2 (9.1%)
Any Related Adverse Event ^a	0 (0.0%)

^a Related includes possibly, probably, and definitely related.

The treatment-emergent adverse events reported by more than 1 neonate are summarized for the safety population in Table 29. None of the reported adverse events in neonates was assessed as related to ATryn treatment.

Table 29. Treatment-Emergent Adverse Events Reported by Greater than One Neonate among Hereditary Deficient Patients (Safety Population)

System Organ Class/Preferred Term	Statistic	ATryn (N=22)
Cardiac Disorders		
Cyanosis	n (%)	2 (9.1%)
Hepatobiliary Disorders		
Jaundice	n (%)	2 (9.1%)

Adverse events reported for the neonates born from the pregnant HD patients treated in the ATryn studies were all assessed as not related to the drug. Only single events have been reported for most of them, except for cyanosis and jaundice which were reported twice each. These are common events just after birth, and hence none of these, nor any of the other events were assessed as related to ATryn.

7.3.2 Acquired AT Deficient Patients

Table 30 summarizes the number of acquired AT deficient patients experiencing a treatment emergent adverse event by treatment.

Table 30. Summary of Patients Experiencing Treatment-Emergent Adverse Events - Acquired Deficient Patients (Safety Population)

Category	ATryn (N=118)	hpAT (N=14)	Placebo (N=57)
Any Serious Adverse Event	29 (24.6%)	3 (21.4%)	11 (19.3%)
Any Adverse Event	111 (94.1%)	12 (85.7%)	53 (93.0%)
Death	3 (2.5%)	1 (7.1%)	1 (1.8%)
Any Severe Adverse Event	25 (21.2%)	3 (21.4%)	7 (12.3%)
Any Related Adverse Event ^a	20 (16.9%)	2 (14.3%)	6 (10.5%)
Any Severe Related Adverse Event ^a	3 (2.5%)	1 (7.1%)	0 (0.0%)
Any Serious Related Adverse Event ^a	5 (4.2%)	1 (7.1%)	0 (0.0%)
Any Adverse Event Leading to Withdrawal	1 (0.8%)	1 (7.1%)	0 (0.0%)

^a Related includes possibly, probably, and definitely related.

Almost all acquired AT deficient patients suffered an adverse event which is not unexpected in CABG surgery patients. This is confirmed by the incidence of the occurrence of adverse events in the hpAT and placebo groups. Three deaths occurred in the ATryn treated group, all assessed as not related to ATryn. In the hpAT and placebo treated groups 1 death occurred in each. The incidence of related adverse events was a little higher in the ATryn treated group compared to the placebo group, but not the hpAT group. The incidence of related serious adverse events was low and not very different from the other groups.

Table 31 shows all treatment emergent adverse events that occurred in at least 10% of patients in either treatment group.

The most commonly reported adverse events were procedural pain (39% of patients), hypotension (21% of patients), pyrexia (19% of patients), atrial fibrillation (14% of patients), nausea (12% of patients), pleural effusion (11% of patients), and post procedural hemorrhage (10% of patients).

Table 31. Treatment-Emergent Adverse Events Reported by at Least 10% of Acquired Deficient Patients in Either Treatment Group (Safety Population)

System Organ Class/Preferred Term	Statistic	ATryn (N=118)	hpAT (N=14)	Placebo (N=57)
Cardiac Disorders				
Atrial Fibrillation	n (%)	17 (14.4)	3 (21.4)	11 (19.3)
Tachycardia		6 (5.1)	0 (0.0)	6 (10.5)
Gastrointestinal Disorders				
Nausea	n (%)	14 (11.9)	0 (0.0)	13 (22.8)
General Disorders and Administration Site Conditions				
Pyrexia	n (%)	22 (18.6)	1 (7.1)	14 (24.6)
Pain		6 (5.1)	2 (14.3)	1 (1.8)
Injury, Poisoning and Procedural Complications				
Post Procedural Hemorrhage	n (%)	12 (10.2)	0 (0.0)	2 (3.5)
Procedural Pain		46 (39.0)	0 (0.0)	22 (38.6)
Investigations				
Blood glucose increased	n (%)	6 (5.1)	0 (0)	6 (10.5)
Metabolism and Nutrition disorders				
Hyperglycemia	n (%)	6 (5.1)	3 (21.4)	4 (7.0)
Hypokalemia		8 (6.8)	3 (21.4)	2 (3.5)
Psychiatric Disorders				
Confusional state	n (%)	4 (3.4)	0 (0)	6 (10.5)
Respiratory, Thoracic and Mediastinal Disorders				
Pleural Effusion	n (%)	13 (11.0)	0 (0.0)	5 (8.8)
Vascular Disorders				
Hypotension	n (%)	25 (21.2)	5 (35.7)	4 (7.0)
Hypertension		10 (8.5)	2 (14.3)	9 (15.8)

Table 32 provides a list of severe adverse events reported by at least 2 acquired deficient patients treated with ATryn, with their relationship and whether they were serious. For patients who received ATryn, no body system reported an incidence of severe adverse events of 10% or greater. The most commonly reported adverse events were post procedural hemorrhage (3% of patients), hemorrhage (vascular body system, 3% of patients), arrhythmia, myocardial infarction, cerebral infarction,, and pleural effusion (each term reported by 2% of patients).

Table 32. Severe Treatment-Emergent Adverse Events Reported by at Least 2 ATryn Treated Acquired Deficient Patients (Safety Population)

System Organ Class/Preferred Term	Statistic	ATryn (N=118)	hpAT (N=14)	Placebo (N=57)
Cardiac Disorders				
Arrhythmia	n (%)	2 (1.7%)	0 (0.0%)	0 (0.0%)
Myocardial Infarction	n (%)	2 (1.7%)	0 (0.0%)	0 (0.0%)
Injury, Poisoning and Procedural Complications				
Post Procedural Haemorrhage	n (%)	4 (3.4%)	0 (0.0%)	2 (3.5%)
Nervous System Disorders				
Cerebral Infarction	n (%)	2 (1.7%)	0 (0.0%)	0 (0.0%)
Respiratory, Thoracic and Mediastinal Disorders				
Pleural Effusion	n (%)	2 (1.7%)	0 (0.0%)	0 (0.0%)
Vascular Disorders				
Haemorrhage	n (%)	3 (2.5%)	0 (0.0%)	1 (1.8%)

The related serious treatment-emergent adverse events for acquired deficient patients are listed in Table 33. For patients who received ATryn, there were seven patients (6%) who reported eight related serious adverse events, from which all of the patients recovered. For patients who received hpAT, there was one patient (7%) who reported two related serious adverse events, from which the patient recovered. No placebo patients reported any related serious adverse events.

Table 33. List of Related Serious Treatment-Emergent Adverse Events - Acquired Deficient Patients (Safety Population)

System Organ Class						
Protocol / Patient Number	Treatment	Preferred Term	Reported Term	Severity	Relation	Outcome
ATryn						
<i>Vascular Disorders</i>						
AT 0502 / (b)(6)	ATryn 75 IU/kg	Hemorrhage	Persistent Bleeding	Severe	Possible	Recovered
AT 0504 / 05-010	ATryn 75 IU/kg	Hemorrhage	Diffuse Bleeding	Severe	Probable	Recovered
AT 0903 / (b)(6)	ATryn 75 IU/kg	Hemorrhage	Increased Bleeding	Moderate	Possible	Recovered
<i>Injury, Poisoning and Procedural Complications</i>						
AT 0504 / (b)(6)	ATryn 75 IU/kg	Post Procedural Hemorrhage	Severe Postoperative Bleeding	Severe	Possible	Recovered
AT 0903 / (b)(6)	ATryn 15 IU/kg	Post Procedural Hemorrhage	Bleeding From Chest Tube	Moderate	Possible	Recovered
<i>Investigations</i>						
AT 0504 / (b)(6)	ATryn 75 IU/kg	Hemoglobin Decreased	Decrease Of The Hemoglobin To A Minimum Of 6.8 g/dL	Severe	Possible	Recovered
hpAT						
<i>Psychiatric Disorders</i>						
AT 0903 / (b)(6)	hpAT 15 IU/kg	Hallucination	Hallucinations	Severe	Possible	Recovered
		Acute Psychosis	Acute Psychosis	Severe	Possible	Recovered

The related treatment-emergent adverse events for acquired deficient patients are summarized in Table 34.

Table 34. Related Treatment-Emergent Adverse Events - Acquired Deficient Patients (Safety Population)

System Organ Class/Preferred Term	Statistic	ATryn (N = 118)	hpAT (N = 14)	Placebo (N = 57)
Blood and Lymphatic System Disorders Hemorrhagic Diathesis	n (%)	1 (0.8)	0 (0.0)	0 (0.0)
Cardiac Disorders Atrial Fibrillation Ventricular Extrasystoles	n (%)	0 (0.0) 0 (0.0)	0 (0.0) 0 (0.0)	1 (1.8) 1 (1.8)
Eye Disorders Visual Disturbance	n (%)	0 (0.0)	1 (7.1)	0 (0.0)
Gastrointestinal Disorders Nausea	n (%)	1 (0.8)	0 (0.0)	1 (1.8)
Infections and Infestations Urethritis	n (%)	1 (0.8)	0 (0.0)	0 (0.0)
Injury, Poisoning and Procedural Complications Operative Hemorrhage Post Procedural Hemorrhage Procedural Pain Wound Secretion	n (%)	1 (0.8) 6 (5.1) 0 (0.0) 5 (4.2)	0 (0.0) 0 (0.0) 0 (0.0) 0 (0.0)	0 (0.0) 0 (0.0) 1 (1.8) 0 (0.0)
Investigations Hemoglobin decreased	n (%)	1 (0.8)	0 (0.0)	0 (0.0)
Psychiatric Disorders Acute Psychosis Hallucination	n (%)	0 (0.0) 0 (0.0)	1 (7.1) 1 (7.1)	0 (0.0) 0 (0.0)
Urinary System Disorders Urethral Hemorrhage Renal Failure	n (%)	1 (0.8) 1 (0.8)	0 (0.0) 0 (0.0)	0 (0.0) 0 (0.0)
Vascular Disorders Hematoma Hemorrhage Hypotension	n (%)	1 (0.8) 5 (4.2) 0 (0.0)	0 (0.0) 0 (0.0) 0 (0.0)	0 (0.0) 2 (3.5) 1 (1.8)

Note: Related includes possible, probable, and definite related.

The most commonly reported ATryn related adverse events were events of different types of hemorrhages and wound secretion.

Acquired deficient patients in the ATryn studies were all treated when going on cardiopulmonary bypass pump for cardiac surgery, a procedure associated with significant morbidity by itself. This is reflected in the number and types of events reported in these patients. The event rates found in ATryn treated patients can be compared with the control arms, but the interpretation of any differences needs to be done cautiously. The number of plasma AT treated patients is very small (n=14), so incidence rates are not reliable in this treatment group. The placebo group is still small (n=57), but more suitable for comparison of event rates. Moreover, although these studies were blinded, the overwhelming response rate in the ATryn treated group may have provided the investigators a hint as to what treatment group the patient was assigned to, potentially biasing the assessment of adverse events.

Comparing frequently reported adverse events (reported in 10% or more of the patients, Table 31), there are a few that seem to appear more frequently in the ATryn treated group as compared to the placebo group. These are post procedural hemorrhage (10% vs. 4%), and hypotension (21% vs. 7%). Severe post procedural hemorrhage, however, was reported at a low, but similar rate for the ATryn and placebo group (3% and 4% for the ATryn and placebo group respectively, Table 32), indicating that the majority of the post procedural hemorrhages were not severe in nature. Severe hypotension was reported only once for each treatment group. Three events of severe hemorrhage (not further specified) were reported for the ATryn group (2.5%) and 1 for the placebo group (1.8%). Given the small numbers, no conclusion can be drawn from this.

Six serious adverse events, assessed as related to ATryn, were reported for 5 acquired deficient patients (Table 33). These were all some type of bleeding, except for one case of decrease in hemoglobin. No related serious adverse events were reported for the placebo group.

When comparing the reporting rate of all related adverse events (Table 34), only post-procedural hemorrhage and wound secretion appear to be reported more frequently in ATryn treated patients as compared to placebo treated patients (5% vs. 0% and 4% vs. 0%). Related events of hemorrhage are reported at a similar rate for both groups (4% for both). Taken together all types of related bleeding events, the rate for the ATryn treated group would be 12% vs. 4% in the placebo group.

7.3.3 Healthy Volunteers

Table 35 summarizes the number of healthy volunteers experiencing treatment emergent adverse events.

Table 35. Summary of Patients Experiencing Treatment-Emergent Adverse Events - Healthy Volunteers (Safety Population)

Category	ATryn (N=102) ^a	Placebo (N=5)
Any Serious Adverse Event	0 (0.0%)	0 (0.0%)
Any Adverse Event	57 (55.9%)	1 (20.0%)
Death	0 (0.0%)	0 (0.0%)
Any Severe Adverse Event	0 (0.0%)	0 (0.0%)
Any Related Adverse Event ^b	25 (24.5%)	1 (20.0%)
Any Severe Related Adverse Event ^b	0 (0.0%)	0 (0.0%)
Any Adverse Event Leading to Withdrawal	1 (1.0%)	0 (0.0%)

^a Subjects treated on 2 occasions (n=37) are counted twice

^b Related includes possibly, probably, and definitely related.

A little more than half of the volunteers experienced adverse events, while none of the

healthy volunteers experienced serious or severe adverse events. Approximately half of the reported adverse events were assessed as related (25% of healthy volunteers). Only one case led to withdrawal from the study.

The treatment-emergent adverse events reported by at least 5% of healthy volunteers are summarized for the safety population in Table 36.

Table 36. Treatment-Emergent Adverse Events Reported by At Least 5% of Healthy Volunteers (Safety Population)

System Organ Class/Preferred Term	Statistic	ATryn (N=102)	Placebo (N=5)
General Disorders and Application Site Conditions Injection Site Bruising	n (%)	6 (5.9)	0 (0.0)
Nervous System Disorders Dizziness Headache	n (%)	8 (7.8) 20 (19.6)	0 (0.0) 0 (0.0)
Respiratory, Thoracic and Mediastinal Disorders Cough	n (%)	6 (5.9)	0 (0.0)

Highest incidence of adverse events was in the body system of nervous system disorders. Headache was reported in 20% of healthy volunteers. Comparison with the placebo treatment is limited due to the small number of subjects. Application site bruising was the only reported adverse event in the placebo treated subjects. There were no serious or severe treatment-emergent adverse events reported for healthy volunteers.

Related treatment-emergent adverse events reported in 3 or more healthy volunteers are listed in Table 37. Headache was the only related treatment-emergent adverse events reported by more than 5% of healthy volunteers who received ATryn.

Table 37. Related Treatment-Emergent Adverse Events Reported in 3 or More Subjects – Healthy Volunteers (Safety Population)

System Organ Class/Preferred Term	Statistic	ATryn (N = 102)	Placebo (N = 5)
Gastrointestinal Disorders Nausea	n (%)	4 (3.9)	0 (0.0)
General Disorders and Administration Site Conditions Injection Site Bruising	n (%)	5 (4.9)	0 (0.0)
Nervous System Disorders Dizziness Headache	n (%)	3 (2.9) 11 (10.8)	0 (0.0) 0 (0.0)

A comparator for ATryn has only been used in one PK study with healthy volunteers, with a total of 5 subjects receiving placebo. Therefore, comparing incidence rates of adverse events between placebo and ATryn is not feasible.

Adverse events reported in 5% or more of the healthy volunteers were injection site bruising, dizziness, headache and cough. All of these are commonly seen in phase I studies with healthy volunteers. About half of the events were assessed as possibly related to ATryn treatment. None of the events was assessed as serious and/or severe.

Leitner JM *et al.* (59) is the publication of a physician-sponsored trial that was a randomized, double-blind, placebo-controlled study in parallel groups enrolling 30 healthy male volunteers. The active treatment groups received infusions of antithrombin alfa to increase antithrombin levels to 200% and 500% before infusion of 2 ng/kg endotoxin (LPS). Objective was to test the hypothesis that infusion of antithrombin alfa without concomitant heparin would have dose-dependent anticoagulant properties and potentially decrease endotoxin (lipopolysaccharide [LPS])–induced cytokine production. The main study was preceded by a pilot study, where 4 adult male subjects were treated in a cross-over fashion with antithrombin alfa 130 U/kg loading dose & 30 U/kg/h maintenance dose or saline placebo.

None of the volunteers experienced any severe or serious adverse events. No subjects were withdrawn because of an AE. One subject in the pilot trial and one in the main trial developed a mild hematoma on the venipuncture site, which lasted for 5 and 3 days, respectively. Although hematomas are common side effects due to blood drawing, a relation of the intensity and duration of the lesions to ATryn though classified as mild cannot entirely be excluded. One subject developed an exanthema 3 days after the administration of the study medication. The exanthema was mild, self-limiting (lasting for 9 days), affecting the stem, groins, elbows and hair line. The volunteer was seen by the dermatologist, who classified the exanthema as presumably virus related. The symptoms were treated with 2 x 50 mg diphenhydramine per day for 4 days. A relation with ATryn cannot entirely be excluded.

7.3.4 Immunogenicity

As with all recombinant proteins, and sometimes also plasma derived proteins (e.g. plasma derived factor VIII), antibodies directed against the recombinant protein or any of the contaminants may be generated by the patient that is treated with the drug product. For ATryn, antibodies can potentially be generated against antithrombin alfa or contaminating goat milk proteins, including goat AT. Additionally, there is a risk that antibodies against antithrombin alfa cross react with endogenous, i.e. human plasma AT (hpAT) due to the homology of the molecules. Therefore, assays to detect antibodies against antithrombin alfa, hpAT, goat AT and goat milk proteins were developed and used in the clinical development of ATryn. The presence of activity neutralizing antibodies was evaluated using a validated thrombin inhibition assay as a surrogate for a neutralizing antibody assay.

7.3.4.1 Anti-antithrombin alfa IgG and IgM Assays

In the clinical studies, a total of 235 individuals were treated with ATryn on one (197) or two (38) occasions (Table 38). Although most subjects, including those who received placebo infusions, were evaluated with the immunoassays, only the number of subjects treated with

antithrombin alfa are indicated in Table 38. Dosing was done by single injection, multiple daily injections or continuous infusion. These individuals were healthy volunteers or subjects with hereditary or acquired antithrombin deficiencies (HR patients undergoing CABG).

There were no clinical immunological reactions noted in any of the clinical trials. There have been no clinical symptoms of hypersensitivity, infusion reactions, autoimmunity or loss of efficacy. Only 1 of the 222 evaluable treated subjects developed an apparent non-specific IgG response to antithrombin alfa (Table 38). The specificity of this observed reactivity is questionable due to the very short time period between ATryn first exposure and the development of an IgG antibody response that reacted with denatured antithrombin alfa, hpAT and hSA. Based on the inconsistent results from the various confirmatory assays, it cannot be concluded that there is a confirmed specific IgG immune response to antithrombin alfa in this patient. None of the 93 treated subjects evaluated for an IgM antibody developed a confirmed IgM response to antithrombin alfa.

Table 38. Summary of PIR Assay Results for Antithrombin alfa Treated Subjects from the Clinical Trials

Study No.	Trial Type	Administration (Dose in U/kg)	Sample Time (Days)	Treated Subjects	IgG Positive/ No. Tested	IgM Positive/ No. Tested
GEN/G9601	Define PK (in healthy volunteers)	Single dose (0, 10, 50, 100, 150, 200)	1, 7, 28	15	0/15	ND
GTC AT96-0801	HR Phase I/II	Single dose (10, 25, 50, 75, 100, 125, 150, 175, 200)	1, (between 27 & 62)	30	0/28	ND
GTC AT97-0502	HR Phase III Efficacy in CABG	Single dose (0, 75)	1, 28	27	0/23	ND
GTC AT97-0504	HR Phase III Efficacy in CABG	Single dose (0, 75)	1, 28	28	0/26	ND
GTC AT97-0903	HR Phase III Efficacy in CABG	Single dose (15, 75)	1, 28	33	0/33	ND
AT III-006-00	Non/Heat-treated Crossover PK (in healthy volunteers)	Repeat dose crossover (75, 75)	1, 3, 28	26 (23)*	0/23	0/23
GTC AT PK 011-04	Non/Nanofilter Crossover PK (in healthy volunteers)	Repeat dose crossover (100, 100)	1, 7, 21, 49, 90	24 (14)*	0/24	0/24
AT III-009-00	HD PK	Single dose (individualized/subject)	1, 28, 60	15	0/15	0/15
GTC ATIII 011- 003	HD Compassionate Use	Multiple bolus doses	Various	5 (1)*	0/4	ND
GTC ATIII 01002	HD Phase III Efficacy	Continuous infusion (individualized/subject)	1, 30, 60, 90	14	0/14	0/14
GTC AT HD 012-04	HD Phase III Efficacy	Continuous infusion (individualized/subject)	1, 7, 30, 60, 90	18	1 ^a /17	0/17
			Total	235 (38)*	1^a/222	0/93

* Number of subjects in parenthesis indicate the number of individuals who were treated with antithrombin alfa on two separate occasions.

ND = not done; HD = hereditary deficiency of AT; HR = heparin resistance; CABG = coronary artery bypass grafting; PK = pharmacokinetic study

^a A specific immune response to antithrombin alfa could not be confirmed in this HD patient.

7.3.4.2 Inhibition of Antithrombin Activity

If ATryn treated individuals had mounted an immune response to AT (either anti-recombinant or anti-goat AT antibodies that cross reacted with the endogenous human AT) that is also inhibitory to the activity of the AT molecule, it would present as a decrease in the activity of their endogenous AT.

In the HD studies, AT activity was measured on plasma samples by validated automated thrombin inhibition assays. AT activity levels in all treated individuals returned to baseline and remained at these levels at 30, 60 and/or 90 days following treatment. This finding further substantiates the absence of an antibody response to ATryn or potential contaminating goat AT that neutralizes AT activity or fosters the clearance of endogenous AT from the circulation.

7.3.4.3 Immunodot Blots

None of the samples from the 42 ATryn treated subjects evaluated by immunodot blots (27 HD efficacy, 1 compassionate use and 14 subjects treated twice in GTC AT PK AT011-04) showed any evidence of the induction of antibodies to potential contaminating goat milk proteins, including goat antithrombin. Some normal human serum samples and some ATryn treated subject samples contained cross-reacting antibodies that reacted against the goat milk protein antigens prior to antithrombin alfa infusion. However, these background signals did not change significantly in the treated subjects after exposure to ATryn, indicating the absence of an ATryn induced immune response to any potentially contaminating goat milk proteins in these individuals.

7.3.4.4 Overall Discussion and Conclusions Regarding PIR Results

The absence of a confirmed specific immune response in ATryn treated individuals suggests that antithrombin alfa is immunologically tolerated. Heterozygous congenital deficient patients have a relatively high residual native AT activity (40-60%). There have been reports of a very rare number of homozygous Type I AT deficient individuals that have died soon after birth, since the complete absence of AT appears to be incompatible with life. In Type I AT deficiency, patients have reduced levels (~50%) of immunologically and functionally determined AT, a true physiologic deficiency state where both activity and antigen are low. In most Type II deficient individuals, functionally determined AT is reduced (~50% in heterozygotes) but there may be a low to normal level of immunologically determined AT. These patients have both normal (active) and abnormal (many are inactive or have altered activities) AT molecules. It is therefore not anticipated that supplementation of HD patients with either plasma derived or recombinant AT will lead to antibody production in either of these HD types. This is a major distinction as compared to, for example, factor VIII deficiency.

Even rarer are the homozygous Type II deficient individuals with mutations in the heparin binding region or the substrate binding domain. These individuals may appear normal by antigen screening. Since none of these patients have null or 100% severely truncated forms

of AT, human AT is not a new antigen for them. This is corroborated by the fact that to our knowledge, no publications have described the occurrence of human antibodies directed against endogenous human AT or exogenously administered hpAT concentrates (which might be perceived as foreign in Type II homozygous individuals).

Several factors may confer an immunologically “privileged” status on the AT molecule:

- It is known that the glycosylation pattern of antithrombin alfa differs from that found on plasma derived AT. However, the glycans present on antithrombin alfa are the incompletely processed forms found in the normal mammalian glycosylation pathway. This is the same pathway present in the human body, and some of those intermediates would also be present in the human circulation. It is very unlikely therefore that any immune response would be generated to glycosylation intermediates. Gal α 1,3 gal linkages are responsible for immune reactions in some humans receiving recombinant Cetuximab (60) or porcine organ transplants. No gal α 1,3 gal linkages have been detected in antithrombin alfa. Thus, although there is a difference in the glycosylation pattern of antithrombin alfa, there should not be an immune response due to these carbohydrate differences.
- *In vitro*, antithrombin alfa and hpAT reacted similarly in ELISA and Western blot assays (61). Monoclonal and polyclonal antibodies raised to each protein, cross-react with each other and the molecules have proved so far to be immunologically indistinguishable. This is not surprising since they have identical amino acid sequences.
- Antithrombin is a serpin that performs a number of functions in the body and that undergoes several conformational changes during its lifetime in the circulation, thereby exposing different epitopes of otherwise buried amino acid residues when in these different conformations. The conformational changes that take place during binding to heparin and the subsequent binding to thrombin provide wide significant alterations to the protein. This is highlighted by the circular dichroism profile changes seen upon heparin binding. In addition, the various isoforms of pre-latent, latent and hetero-dimeric and cleaved antithrombin occur in the normal metabolism of the human circulation. The antithrombin molecule may be “privileged” in that the human immune system may be tolerant of the many expected altered forms of the molecule. This would help to insure a minimal response to any antithrombin with the wild-type amino acid sequence.

In conclusion: on the basis of the collective body of laboratory and clinical data, no patients have developed a confirmed immune response to ATryn nor have any patients exhibited clinical signs of any immune response to ATryn.

Nevertheless, considering the infrequency with which patients may require treatment with ATryn on more than one occasion and given the relatively limited number of patients who have already been exposed to ATryn in clinical trials, the only way to gather additional

clinical data assessing the immunogenic potential of ATryn, especially in those patients requiring re-exposure to ATryn, is in a post-marketing setting. Thus, the Applicant intends to implement a patient registry in which physicians may collect serum samples from patients treated with ATryn and have the samples analyzed for the development of IgE, IgM and/or IgG antibodies to recombinant human antithrombin. Testing of the serum samples for antibodies to recombinant human antithrombin will be offered as a free service to physicians and patients.

Based upon the total body of data from clinical trials of ATryn which have been performed in normal healthy volunteers, patients with congenital antithrombin deficiency and patients with acquired antithrombin deficiencies, as well as published data on patients treated with human plasma-derived antithrombin, it is not expected that patients will develop antibodies to either the recombinant or human plasma-derived antithrombin. However, to develop such evidence and provide reassurance to physicians and patients, the Applicant plans to conduct a post-marketing immunosurveillance study in 50 patients or over a period of five years, whichever occurs first.

7.3.5 Safety Conclusions

The safety database for ATryn contains data on 235 subjects/patients that have been exposed to ATryn on at least one occasion. Additionally, data are available on 38 of these subjects/patients who have been exposed on two separate occasions to ATryn.

Except for the healthy volunteer studies and the pharmacokinetic study in HD patients, treatment with ATryn was given to patients during surgical procedures and/or in the peri-operative period, as well as during the peri-partum period to pregnant women. The type of adverse events reported in the clinical studies with ATryn reflects this, i.e. events often associated with surgery and delivery were reported. Of note is that the surgical procedures in acquired AT deficient patients were CABG surgery with use of CPB, and also HD patients had major surgical procedures performed when in the studies. Delivery also included Cesarean sections. Additionally, the pharmacodynamic action of ATryn is anticoagulation, especially when concomitantly given with heparin group anticoagulants. Combined use of AT and heparin (either unfractionated or low molecular weight heparin) during and just after surgery and delivery increases the risks of hemorrhagic complications. Among the reported events assessed related to ATryn are also hemorrhagic events of different kinds. The rate, though, seems in line with what can be expected in the populations studied. Comparison with the control arms in studies where applicable is difficult due to the relatively small numbers studied.

Other minor events reported are infusion site reactions and some other events generally seen in clinical trials, like headache, erythema, dizziness, etc. In general, it can be concluded that ATryn was well tolerated.

Assays have been developed and used for detecting any antibodies to antithrombin alfa or potentially contaminating goat milk proteins, including goat AT. No confirmed specific

immune reaction to any of the components of ATryn has been detected during the clinical development.

As opposed to plasma-derived antithrombin concentrates, there is no risk for transmission of any human blood-borne diseases by ATryn. No transmission of these viruses, nor any of animal origin, have been reported in any of the subjects/patients treated in the studies.

Table 39 provides an overview of all treatment-emergent adverse events reported as possibly related to ATryn, hpAT or placebo in any of the studies, in any of the patients.

Table 39. Related Treatment-Emergent Adverse Events – All Patients (Safety Population)

System Organ Class / Preferred Term	Statistic	Number of Patients		
		ATryn (N=273)	hpAT (N=14)	Placebo (N=62)
Total	n (%)	54 (19.8)	2 (14.3)	7 (11.3)
Blood And Lymphatic System Disorders	n (%)	1 (0.4)		
Hemorrhagic Diathesis		1 (0.4)		
Cardiac Disorders				1 (1.6)
Atrial Fibrillation	n (%)			1 (1.6)
Ventricular Extrasystoles				1 (1.6)
Eye Disorders			1 (7.1)	
Visual Disturbance	n (%)		1 (7.1)	
Gastrointestinal Disorders		7 (2.6)		1 (1.6)
Gastroesophageal Reflux Disease	n (%)	1 (0.4)		
Intra-abdominal Hemorrhage		1 (0.4)		
Nausea		5 (1.8)		1 (1.6)
General Disorders And Administration Site Conditions		14 (5.1)		1 (1.6)
Application Site Bruising				1 (1.6)
Application Site Pruritus		1 (0.4)		
Chest Pain		1 (0.4)		
Feeling Hot		3 (1.1)		
Infusion Related Reaction		1 (0.4)		
Infusion Site Erythema	n (%)	2 (0.7)		
Infusion Site Rash		2 (0.7)		
Infusion Site Swelling		1 (0.4)		
Injection Site Bruising		5 (1.8)		
Injection Site Discomfort		1 (0.4)		
Injection Site Pain		2 (0.7)		
Non-cardiac Chest Pain		1 (0.4)		
Infections And Infestations		1 (0.4)		
Urethritis	n (%)	1 (0.4)		

Table 39. Related Treatment-Emergent Adverse Events – All Patients (Safety Population)

System Organ Class / Preferred Term	Statistic	Number of Patients		
		ATryn (N=273)	hpAT (N=14)	Placebo (N=62)
Injury, Poisoning And Procedural Complications		12 (4.4)		1 (1.6)
Operative Hemorrhage		1 (0.4)		
Post Procedural Hemorrhage	n (%)	6 (2.2)		
Procedural Pain				1 (1.6)
Wound Secretion		5 (1.8)		
Investigations		2 (0.7)		
Hemoglobin Decreased	n (%)	1 (0.4)		
Hepatic Enzyme Abnormal		1 (0.4)		
Musculoskeletal And Connective Tissue Disorders		1 (0.4)		
Hemarthrosis	n (%)	1 (0.4)		
Nervous System Disorders		14 (5.1)		
Dizziness		3 (1.1)		
Dysgeusia	n (%)	1 (0.4)		
Headache		11 (4.0)		
Hypoaesthesia		1 (0.4)		
Psychiatric Disorders			1 (7.1)	
Acute Psychosis	n (%)		1 (7.1)	
Hallucination			1 (7.1)	
Renal And Urinary Disorders		2 (0.7)		
Hematuria		1 (0.4)		
Renal Failure	n (%)	1 (0.4)		
Urethral Hemorrhage		1 (0.4)		
Skin And Subcutaneous Tissue Disorders		1 (0.4)		
Erythema	n (%)	1 (0.4)		
Vascular Disorders		8 (2.9)		3 (4.8)
Hematoma		2 (0.7)		
Hemorrhage	n (%)	5 (1.8)		2 (3.2)
Hypotension				1 (1.6)
Pallor		1 (0.4)		

Many of the related events are only reported once or twice. Some relevant groupings can however be done. All events that are of some type of bleeding/hemorrhage could be grouped. These are hemorrhagic diathesis, intra-abdominal hemorrhage, operative hemorrhage, post-procedural hemorrhage, hemarthrosis, urethral hemorrhage and hemorrhage. For ATryn treated patients these events are reported in a total of 16 (6%) patients. These were not reported in hpAT treated patients and for 2 (3%) placebo treated patients.

Another relevant group of events are the injection site reactions. Application site pruritis, infusion site erythema, infusion site rash, infusion site swelling, injection site bruising, injection site discomfort, injection site pain were reported in a total of 15 (5%) ATryn treated patients. Application site bruising was reported in 1 placebo treated patient.

For the package insert, it is normally requested to formulate a relevant cut-off of the incidence of related adverse events to be listed for information of the treating physician. However, due to the relatively small size of the database, no relevant cut-off seems to be possible. Therefore, it is proposed to report all adverse events assessed as related to ATryn treatment, while using the grouping of events as discussed above.

The following list of adverse reactions will be presented in the package insert:

Gastrointestinal Disorders

- Nausea (5)
- Gastroesophageal Reflux Disease

General Disorders And Administration Site Conditions

- Infusion site reaction (15)
- Chest Pain
- Feeling Hot
- Infusion Related Reaction
- Non-cardiac Chest Pain

Infections And Infestations

- Urethritis

Injury, Poisoning And Procedural Complications

- Wound Secretion (5)

Investigations

- Hemoglobin Decreased
- Hepatic Enzyme Abnormal

Nervous System Disorders

- Headache (11)
- Dizziness (3)
- Dysgeusia
- Hypoesthesia

Renal And Urinary Disorders

- Hematuria
- Renal Failure

Skin And Subcutaneous Tissue Disorders

- Erythema

Vascular Disorders

Hemorrhage (16)

Hematoma (2)

Pallor

7.4 ATryn – Fulfilling an Unmet Medical Need

ATryn addresses an unmet medical need for a safe and effective recombinant DNA-derived alternative to the current human plasma-derived antithrombin III product, Thrombate III®. The safety and efficacy of ATryn® has been established based upon the outcomes of the clinical trials (i.e., active and historical cohort studies) presented in this document, and which have been discussed and agreed upon with CBER.

The need for a non-plasma derived alternative is based upon patient preference as well as a concern, however small or large, about the potential risk of transmission of infectious agents, such as viruses and prions, from pools of human plasma. ATryn does not carry the risk of transmission of viruses derived from human plasma, such as HIV, hepatitis C, and human parvo B19 or prions known to infect man such as CJD or new variant CJD. The robust ATryn® manufacturing process has been extensively validated to demonstrate the removal/inactivation of potential viruses, as well as prions even though the herd of goats from which antithrombin alfa is derived, has been and remains certified scrapie-free by the USDA, i.e. a strictly controlled source. The latter being the weakest link for plasma derived products.

Finally, while it is presently claimed that Thrombate III® is available in the U.S. marketplace, this has not always been the case (as proven by the compassionate use program that existed for ATryn when Thrombate III® was not available) and there is no guarantee that this product will be readily available in the future. Increased demands of other plasma derived products, and implementation of more restrictions on donor selection, may again lead to a product shortage. GTC Biotherapeutics, Inc. has established through use of recombinant DNA technology a dedicated, steady and reliable source of antithrombin alfa which may be made available unrestricted to physicians, pharmacists and distributors alike. Guaranteeing continuous availability of product in the marketplace also fulfills an unmet medical need.

7.5 Benefit to Risk Assessment

ATryn's active ingredient (antithrombin alfa) is the recombinant form of the naturally occurring antithrombin protein. The proposed indication for ATryn is similar to how the plasma derived form of antithrombin has been used for a long time. Plasma AT has historically been used to restore plasma AT activity levels and by doing so reducing the risk of development of venous thromboembolic complications in patients having a genetic defect that makes them deficient for AT, as well for the treatment of any venous thromboembolic complications that do occur.

Pharmacology studies have shown that antithrombin alfa has very similar pharmacodynamic properties as compared to the naturally occurring form. This is true for both its thrombin

inhibitory as well as its anti-inflammatory properties. The main difference having implications for the clinical use of ATryn is the shorter half life of antithrombin alfa. Given the same pharmacodynamic properties though, the same goal of treatment for ATryn could be maintained, which is to achieve plasma AT activity levels in the normal range of 80-120% of normal.

Consistent with the situation with development of orphan drugs, the clinical efficacy and safety database for ATryn in the proposed indication is limited due to the rarity of the disease. Nevertheless, efficacy has been established by comparing the data from two prospective ATryn studies with an active control arm. This control arm consisted of a historical control group of HD patients treated in the past with a plasma-derived AT concentrate. Non-inferiority of ATryn was established in this comparison. The occurrence of one VTE in an ATryn-treated patient from this trial is not unexpected. Prophylactic treatment with AT is aimed at bringing the risk for the development of a VTE to the proportions that are seen in e.g. other studies with joint replacement surgery. It is likely that this patient who had a hip replacement procedure, which has a high risk for development of thromboses, even without thrombophilia, represents this small proportion of treatment failures of thromboprophylaxis.

Supportive data from a compassionate use study in the same population provides additional re-assurance of the efficacy of ATryn.

ATryn has also been tested in acquired AT deficient patients. Patients with heparin resistance were given ATryn to restore the response to heparin, providing sufficient anticoagulation to initiate CPB. ATryn was very effective in restoring heparin resistance, which provides additional evidence for the pharmacodynamic properties. Similar to acquired AT deficient patients, HD patients may be heparin resistant when they are treated for an acute DVT or other acute VTE. Some patients do not reach an adequate aPTT, or some who do still show progression of the clot. Plasma AT is used in such situations as well. The one patient in the ATryn study with an acute DVT was also treated by continuation of the ATryn therapy, next to a therapeutic dose of LMWH and initiation of Vitamin K antagonists. The DVT never became symptomatic and resolved.

The benefit of preventing HD patients from developing a VTE is evident. VTEs can be life-threatening and can induce significant long-term morbidity. A safe and effective treatment alternative to plasma derived AT is therefore important. In addition, a continuous commercial supply of plasma AT has not been readily available in the US and GTC has needed to supply ATryn on a compassionate use basis to physicians and patients in need.

The risks of treatment with ATryn have been shown to be minimal. Adverse events reported in the clinical studies were usually mild to moderate in severity. Serious adverse events were mostly assessed as not related to ATryn. In fact, the types and severity of events reported in the phase 3 studies in HD and acquired AT deficiency reflect the fact that these patients were treated in the peri-operative period of major surgical procedures as well as in the peri-partum period, including during and after cesarean section.

Adverse events that can be expected due to the anticoagulant properties of ATryn are different types of bleeding. However, since the vast majority of patients were also treated with concomitant anticoagulants, this fact provides an alternative explanation for the occurrence of bleeding. Bleeding never was a reason for discontinuation of treatment with ATryn in HD patients.

A potential safety issue for recombinant proteins is the development of an immunological reaction to the recombinant protein or any of the potential contaminating proteins in the final dosage form. For ATryn, assays were developed and used to detect antibodies directed against antithrombin alfa, goat-AT or goat-milk proteins. No confirmed specific immunological reaction was seen in any of the patients tested, nor was there any clinical adverse event that might indicate such response.

The Applicant therefore concludes that the benefit to risk ratio for ATryn for the indication "prevention of peri-operative and peri-partum thromboembolic events, as well as the treatment of such events, in hereditary antithrombin deficient patients" is positive and supports licensure in the US.

8 REFERENCES

1. Vinazzer H. Hereditary and acquired antithrombin deficiency. *Semin.Thromb.Hemost.* 1999;25(3):257-63.
2. Demers C, Ginsberg JS, Hirsh J, Henderson P, Blajchman MA. Thrombosis in antithrombin-III-deficient persons. Report of a large kindred and literature review. *Ann.Intern.Med.* 1992 May 1;116(9):754-61.
3. Brouwer JL, Veeger NJ, Kluin-Nelemans HC, van der MJ. The pathogenesis of venous thromboembolism: evidence for multiple interrelated causes. *Ann.Intern.Med* 2006 Dec 5;145(11):807-15.
4. Pabinger I, Schneider B. Thrombotic risk in hereditary antithrombin III, protein C, or protein S deficiency. A cooperative, retrospective study. Gesellschaft für Thrombose- und Hamostaseforschung (GTH) Study Group on Natural Inhibitors. *Arterioscler.Thromb Vasc.Biol.* 1996 Jun;16(6):742-8.
5. van Boven HH, Vandenbroucke JP, Briet E, Rosendaal FR. Gene-gene and gene-environment interactions determine risk of thrombosis in families with inherited antithrombin deficiency. *Blood.* 1999 Oct 15;94(8):2590-4.
6. De Stefano V, Leone G. Mortality related to thrombosis in congenital antithrombin III deficiency. *Lancet* 1991 Apr 6;337:847-8.
7. Echelard Y, Meade H, Ziomek C. The first biopharmaceutical from transgenic animals: ATryn. *Modern Biopharmaceuticals* 2005;995-1016.
8. Pollak ES, Abrams CS. Clinical presentation and laboratory diagnosis of heparin-induced thrombocytopenia. *Semin.Thorac.Cardiovasc.Surg.* 2005;17(1):80-4.
9. Spiess BD. Update on heparin-induced thrombocytopenia and cardiovascular interventions. *Semin.Hematol.* 2005;42(Suppl 3):S22-S27.
10. Konkle BA, Bauer KA, Weinstein R, Greist A, Holmes HE, Bonfiglio J. Use of recombinant human antithrombin in patients with congenital antithrombin deficiency undergoing surgical procedures. *Transfusion.* 2003 Mar;43(3):390-4.
11. Aranha H, Larson R. Prions: Mayhem and Management Part Four, Due diligence in the manufacture of biologicals. *BioPharm.* 2002;Supplement:36-40.
12. Edmunds T, Van Patten SM, Pollock J, Hanson E, Bernasconi R, Higgins E, Manavalan P, Ziomek C, Meade H, McPherson JM, et al. Transgenically produced human antithrombin: structural and functional comparison to human plasma-derived antithrombin. *Blood.* 1998 Jun 15;91(12):4561-71.

13. Kaneider NC, Egger P, Dunzendorfer S, Wiedermann CJ. Syndecan-4 as antithrombin receptor of human neutrophils. *Biochem.Biophys.Res.Commun.* 2001 Sep 14;287(1):42-6.
14. Kaneider NC, Forster E, Mosheimer B, Sturn DH, Wiedermann CJ. Syndecan-4-dependent signaling in the inhibition of endotoxin-induced endothelial adherence of neutrophils by antithrombin. *Thromb.Haemost.* 2003 Dec;90(6):1150-7.
15. Feistritz C, Kaneider NC, Sturn DH, Wiedermann CJ. Syndecan-4-dependent migration of human eosinophils. *Clin.Exp.Allergy.* 2004 May;34(5):696-703.
16. Gritti D, Malinverno A, Gasparetto C, Wiedermann CJ, Ricevuti R. Attenuation of leukocyte beta 2-integrin expression by antithrombin-III. *Int.J.Immunopathol.Pharmacol.* 2004 Jan;17(1):27-32.
17. Feistritz C, Mosheimer BA, Tancevski I, Kaneider NC, Sturn DH, Patsch JR, Wiedermann CJ. Src tyrosine kinase-dependent migratory effects of antithrombin in leukocytes. *Exp.Cell Res.* 2005 Apr 15;305(1):214-20.
18. Neviere R, Tournays A, Mordon S, Marechal X, Song FL, Jourdain M, Fourrier F. Antithrombin reduces mesenteric venular leukocyte interactions and small intestine injury in endotoxemic rats. *Shock.* 2001 Mar;15(3):220-5.
19. Kipnis E, Guery BP, Tournays A, Leroy X, Robriquet L, Fialdes P, Neviere R, Fourrier F. Massive alveolar thrombin activation in *Pseudomonas aeruginosa*-induced acute lung injury. *Shock.* 2004 May;21(5):444-51.
20. Davis-Jackson R, Correa H, Horswell R, Sadowska-Krowicka H, McDonough K, Debata C, Gardner R, Penn D. Antithrombin III (AT) and recombinant tissue plasminogen activator (R-TPA) used singly and in combination versus supportive care for treatment of endotoxin-induced disseminated intravascular coagulation (DIC) in the neonatal pig. *Thromb.J.* 2006 May 18;4:7.
21. Minnema MC, Chang AC, Jansen PM, Lubbers YT, Pratt BM, Whittaker BG, Taylor FB, Hack CE, Friedman B. Recombinant human antithrombin III improves survival and attenuates inflammatory responses in baboons lethally challenged with *Escherichia coli*. *Blood.* 2000 Feb 15;95(4):1117-23.
22. Murakami K, Enkhbaatar P, Shimoda K, Chandra A, Thomas K, Cox RA, Hawkins HK, Traber L, Herndon D, Traber DL. Aerosolized recombinant antithrombin attenuates ovine acute lung injury with smoke inhalation and pneumonia. In 2003.
23. Enkhbaatar P, Cox RA, Traber LD, Westphal M, Aimalohi E, Morita N, Prough DS, Herndon DN, Traber DL. Aerosolized anticoagulants ameliorate acute lung injury in sheep after exposure to burn and smoke inhalation. *Crit Care Med.* 2007 Oct 23.

24. Enkhbaatar P, Esechie A, Wang J, Cox RA, Nakano Y, Hamahata A, Lange M, Traber LD, Prough DS, Herndon DN, et al. Combined anticoagulants ameliorate acute lung injury in sheep after burn and smoke inhalation. *Clin.Sci.(Lond)*. 2008 Feb;114(4):321-9.
25. Cowan PJ, Aminian A, Barlow H, Brown AA, Dwyer K, Filshie RJ, Fisicaro N, Francis DM, Gock H, Goodman DJ, et al. Protective effects of recombinant human antithrombin III in pig-to-primate renal xenotransplantation. *Am.J.Transplant*. 2002 Jul;2(6):520-5.
26. Cozzi E, Simioni P, Boldrin M, Seveso M, Calabrese F, Baldan N, Busetto R, Tormene D, Gavasso S, Castagnaro M, et al. Effects of long-term administration of high-dose recombinant human antithrombin in immunosuppressed primate recipients of porcine xenografts. *Transplantation*. 2005 Nov 27;80(10):1501-10.
27. Pratt CW, Church FC. Antithrombin: Structure and function. *Semin.Hematol*. 1991 Jan 1;28(1):3-9.
28. Bucur SZ, Levy JH, Despotis GJ, Spiess BD, Hillyer CD. Uses of antithrombin III concentrate in congenital and acquired deficiency states. *Transfusion (Paris)*. 1998;38:481-98.
29. De Stefano V, Finazzi G, Mannucci PM. Inherited thrombophilia: pathogenesis, clinical syndromes, and management. *Blood* 1996;87(9):3531-44.
30. Mammen EF. Clinical relevance of antithrombin deficiencies. *Semin.Hematol*. 1995;32(4):2-6.
31. Zoller B, Garcia De Frutos P, Hillarp A, Dahlback B. Thrombophilia as a multigenic disease. *Haematologica* 1999;84:59-70.
32. Bauer KA. Management of patients with hereditary defects predisposing to thrombosis including pregnant women. *Thromb Haemost* 1995;74(1):94-100.
33. Lechner K, Kyrle PA. Antithrombin III concentrates - are they clinically useful? *Thromb Haemost* 1995;73(3):340-8.
34. Schwartz RS, Bauer KA, Rosenberg RD, Kavanaugh EJ, Davies DC, Bogdanoff DA. Clinical experience with antithrombin III concentrate in treatment of congenital and acquired deficiency of antithrombin. *Am.J.Med*. 1989 Sep 11;87(Supplement 3B):3B-53S.
35. Schinzel H, Weilemann LS. Antithrombin substitution therapy. *Blood Coagul.Fibrinolysis* 1998;9(Supplement 3):S17-S22.

36. Demers C, Ginsberg JS, Hirsh J, Henderson P, Blajchman MA. Thrombosis in antithrombin-III-deficient persons. Report of a large kindred and literature review. *Ann.Intern.Med.* 1992 May 1;116(9):754-61.
37. Pabinger I, Schneider B. Thrombotic risk in hereditary antithrombin III, protein C, or protein S deficiency. A cooperative, retrospective study. Gesellschaft für Thrombose- und Hamostaseforschung (GTH) Study Group on Natural Inhibitors. *Arterioscler.Thromb Vasc.Biol.* 1996 Jun;16(6):742-8.
38. van Boven HH, Vandenbroucke JP, Briet E, Rosendaal FR. Gene-gene and gene-environment interactions determine risk of thrombosis in families with inherited antithrombin deficiency. *Blood.* 1999 Oct 15;94(8):2590-4.
39. De Stefano V, Leone G. Mortality related to thrombosis in congenital antithrombin III deficiency. *Lancet* 1991 Apr 6;337:847-8.
40. Buchanan GS, Rodgers GM, Ware Branch. The inherited thrombophilias: genetics, epidemiology, and laboratory evaluation. *Best.Pract.Res Clin Obstet Gynaecol.* 2003 Jun;17(3):397-411.
41. Walker ID, Greaves M, Preston FE. Investigation and management of heritable thrombophilia. *Br.J.Haematol.* 2001;114:512-28.
42. De Stefano V, Leone G, Mastrangelo S, Tripodi A, Rodeghiero F, Castaman G, Barbul T, Finazzi G, Bizzi B, Mannucci PM. Clinical manifestations and management of inherited thrombophilia: retrospective analysis and follow-up after diagnosis of 238 patients with congenital deficiency of antithrombin III, protein C, protein S. *Semin.Thromb.Hemost.* 1994;72(3):352-8.
43. Pabinger I, Vormittag R. Thrombophilia and pregnancy outcomes. *Journal of Thrombosis and Haemostasis* 2005;3:1603-10.
44. Walker ID. Management of thrombophilia in pregnancy. *Blood Rev.* 1991;5(4):227-33.
45. Ginsberg JS, Greer I, Hirsh J. Use of antithrombotic agents during pregnancy. *Chest* 2001;119(1):122S-31S.
46. Bauer KA. Management of thrombophilia. *J Thromb Haemost* 1, 1429-1434. 2003.
47. Menache D, O'Malley JP, Schorr JB, Wagner B, Williams C, Alving BM, Ballard JO, Goodnight SH, Hathaway WE, Hultin MB, et al. Evaluation of the safety, recovery, half-life, and clinical efficacy of antithrombin III (human) in patients with hereditary antithrombin III deficiency. *Blood* 1990 Jan 1;75(1):33-9.
48. Schulman S, Tengborn L. Treatment of venous thromboembolism in patients with congenital deficiency of antithrombin III. *Thromb.Haemost.* 1992 Dec 7;68(6):634-6.

49. Maclean PS, Tait RC. Hereditary and acquired antithrombin deficiency: epidemiology, pathogenesis and treatment options. *Drugs*. 2007;67(10):1429-40.
50. Rosenberg RD. Actions and interactions of antithrombin and heparin. *New England Journal of Medicine*, 146-151. 1975.
51. Conard J, Horellou M-H, Samama M. Inherited thrombophilia and gestational venous thromboembolism. *Seminars in Thrombosis and Hemostasis* 29[2], 131-141. 2003.
52. Hellgren M, Tengborn L, Abildgaard U. Pregnancy in women with congenital antithrombin III deficiency: experience of treatment with heparin and antithrombin. *Gynecologic and Obstetric Investigation* 14, 127-141. 1982.
53. Konkle BA, Bauer KA, Weinstein R, Greist A, Holmes HE, Bonfiglio J. Use of recombinant human antithrombin in patients with congenital antithrombin deficiency undergoing surgical procedures. *Transfusion (Paris)*. 2003 Mar;43:390-4.
54. Konkle BA, Bauer KA, Weinstein R, Greist A, Holmes HE, Bonfiglio J. Use of recombinant human antithrombin in patients with congenital antithrombin deficiency undergoing surgical procedures. *Transfusion (Paris)*. 2003 Mar;43:390-4.
55. Menache D, O'Malley JP, Schorr JB, Wagner B, Williams C, Alving BM, Ballard JO, Goodnight SH, Hathaway WE, Hultin MB, et al. Evaluation of the safety, recovery, half-life, and clinical efficacy of antithrombin III (human) in patients with hereditary antithrombin III deficiency. *Blood* 1990 Jan 1;75(1):33-9.
56. Rinder C. Cellular inflammatory response and clinical outcome in cardiac surgery. *Curr.Opin.Anaesthesiol.* 2006 Feb;19(1):65-8.
57. Spiess BD. Treating heparin resistance with antithrombin or fresh frozen plasma. *Ann.Thorac.Surg.* 2008 Jun;85(6):2153-60.
58. Ranucci M, Isgro G, Cazzaniga A, Soro G, Menicanti L, Frigiola A. Predictors for heparin resistance in patients undergoing coronary artery bypass grafting. *Perfusion* 1999;14(1):437-42.
59. Leitner JM, Firbas C, Mayr FB, Reiter RA, Steinlechner B, Jilma B. Recombinant human antithrombin inhibits thrombin formation and interleukin 6 release in human endotoxemia. *Clin.Pharmacol.Ther.* 2006;79(1):23-34.
60. Chung CH, Mirakhur B, Chan E, Le QT, Berlin J, Morse M, Murphy BA, Satinover SM, Hosen J, Mauro D, et al. Cetuximab-induced anaphylaxis and IgE specific for galactose-alpha-1,3-galactose. *N.Engl.J.Med.* 2008 Mar 13;358(11):1109-17.
61. Edmunds T, Van Patten SM, Pollock J, Hanson E, Bernasconi R, Higgins E, Manavalan P, Ziomek C, Meade H, McPherson JM, et al. Transgenically produced human

antithrombin: structural and functional comparison to human plasma-derived antithrombin. Blood 1998 Jun 15;91(12):4561-71.