

MEMORANDUM

Department of Health and Human Services
Food and Drug Administration
Center for Biologics Evaluation and Research
Pharmacology / Toxicology Review Memorandum

To: File

From: Evi Struble APPROVED
By Evi Struble, PhD at 9:08 am, Oct 30, 2008

Through: Tim Lee, Susan Abbondanzo, and Basil Golding APPROVED
By Tim Lee at 4:25 pm, Oct 17, 2008

Subject: BLA 125284 GTC Biotherapeutics

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I. Background

ATryn[®] active ingredient is recombinant human antithrombin alfa (rhAT), expressed in and purified from the milk of transgenic goats integrated with the human gene for antithrombin. The product is referred to as tgATIII in this review.

ATryn[®] is formulated with citrate, chloride and glycine. It undergoes nanofiltration and a terminal dry heat treatment step ((b) (4)) for viral removal/inactivation. The FDP is a sterile lyophilized dosage form containing 1750 international units (IU) antithrombin per vial that is intended for intravenous infusion following reconstitution with 10 mL Sterile Water for Injection.

II. Proposed use and doses

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.

Dosage

The dosage is individualized for each patient.

In surgical patients, a usual loading dose (assuming baseline AT activity of 50% and body weight of 75 kg) is 1630 international unit (IU) (or 20-25 IU/kg body weight). A usual maintenance dose (assuming baseline AT activity of 50% and body weight of 75 kg) of ATryn[®] administered by continuous infusion is 368 IU/h (or 4-5 IU/kg/h).

In pregnant women, a usual loading dose (assuming baseline AT activity of 50% and body weight of 75 kg) is 2885 IU (or 35-40 IU/kg body weight) infused over a period of

15 minutes. A usual maintenance dose (assuming baseline AT activity of 50% and body weight of 75 kg) administered by continuous infusion is 695 IU/h (or 9-10 IU/h).

III. Recommendation

This reviewer identified no issues that would prevent this BLA from being approved.

IV. Key Findings and Conclusion

Formulations used in pre-clinical studies differ from the FDP. The pre-clinical formulations contained non-nanofiltered and non-heat treated antithrombin alfa. The exceptions are the reproductive toxicity studies # (b) (4)-007-001 and #6354-13, and bioequivalence study #04-0585P which used the nanofiltered and terminal heat treated product.

PK and BD profiles of non-heat treated and nanofiltered heat treated have been analyzed in two studies (b) (4)-100 (non-heat treated) and 04-0585P (heat-treated and nanofiltered heat treated). There is no difference in PK and BD of non-nanofiltered and nanofiltered tgATIII.

The nanofiltered and terminal dry heated product has an increased aggregation, a decrease in heparin affinity, and an increase in deamidated forms.

Both aggregation and deamidation could cause increased immunogenicity (see Reference 1). Deamidation of (b) (4), which is not a natural amino acid and can potentially be immunogenic. The deamidation can also adversely affect the drug activity (Reference 2). These effects have not been evaluated in animals.

The highest dose used in reproductive toxicity studies is 210 mg/kg/day – or ~35 times the loading dose in pregnant women. At this dose, a slight decrease in pup viability (4%) or an increase in pup mortality was observed. A dose of 21 mg/kg/day or ~3.5 times loading dose in pregnant women is NOAEL.

The highest dose in 14-day repeat toxicity studies in monkeys was 300 mg/kg/day or approximately 2000 IU/kg/day. This dose is approximately 10 times that projected for treatment of HD patients in high-risk situations (maximum daily dose by continuous infusion of 20 mg/kg [for 50% baseline] to 30 mg/kg [for 30% baseline]). At this dose, female monkeys exhibited liver toxicity and internal bleeding both of which not seen in males. NOAEL in monkeys is 36 mg/kg (approximately 250 IU/kg) or approximately equal to the dose for patients in high risk situations (30 mg/kg [for 30% baseline]).

The highest dose of in the 28-day repeat-dose toxicity study in rats was 360 mg/kg/day, over 33 times greater than the dose of 10.7 mg/kg (75 IU/kg) used in two Phase III Heparin Resistance clinical trials, ~ 10 to 20 times that projected for treatment of HD patients in high-risk situations (maximum daily dose by continuous infusion of 20 mg/kg

[for 50% baseline] to 30 mg/kg [for 30% baseline]. The toxicity at this dose was limited to transient limb swelling and local injection site bruising/swelling.

Excipients

The composition of FDP, including its excipients, is shown in Table 1, adapted from the submission. All excipients are present in other FDA approved drugs administered via the same ROA and having the same concentration found in the ATryn[®] formulation (data obtained from Inactive Ingredients Database <http://www.accessdata.fda.gov/scripts/cder/iig/index.cfm>).

Table 1. Qualitative and Quantitative Composition of ATryn[®]

Component	Quality Standard	Function	Amount per Vial	Deliverable Amount per Vial (concentration in g/100 mL)
Antithrombin alfa	(b) (4)	Active Ingredient	262.5 mg (1838 IU ^a)	NLT ^b 250 mg - 1750 IU
Glycine	(b) (4)	(b) (4)	104.8 mg	100 mg (0.1%)
Sodium Chloride	(b) (4)	(b) (4) (b) (4)	82.8 mg	79 mg (0.8%)
Sodium Citrate	(b) (4)	(b) (4)	27.1 mg	26 mg (0.26%)
(b) (4)	(b) (4)	(b) (4)	(b) (4)	(b) (4)

^aEach mg of antithrombin alfa possesses approximately 7 international units (IU) of activity when tested in a thrombin inhibition assay using a house reference standard which has been calibrated against the WHO International Standard for antithrombin. Thus, when reconstituted with 10 mL of diluent as directed each vial contains approximately 1838 IU of antithrombin of which approximately 1750 IU are deliverable from the vial.

^b NLT: Not Less Than

^c USP: United States Pharmacopoeia

^d EP: European Pharmacopoeia

^e NF: National Formulary

^f QS: Quantity Sufficient

^g Not Applicable

Metabolism

ATIII is made in liver cells and metabolized/cleared via three mechanisms:

1. The transgenic form of ATIII is cleared by asialoglycoprotein receptor binding in the liver – the major and most rapid form of clearance;
2. Both plasma and transgenic forms of the ATIII bind to heparin sulfate proteoglycans, get tethered onto vascular endothelium and thus are removed from circulation. Also mannose receptors at the endothelium bind tgATIII containing oligomannose; and
3. Both plasma and transgenic forms of the ATIII, having a MW<70,000 Da, are filtered through kidneys.

V. Comments

Immunogenicity after re-exposure needs to be addressed either in a pre-clinical study or via post-marketing surveillance.

Reproductive Toxicity:

- Animal studies show the safety of tgATIII up to a dose of 210 mg/kg/day in pregnant rats when used around parturition and during lactation.
- There is not enough preclinical data to qualify tgATIII as “Pregnancy Category A”.
 - Study # 6354-131 in rats shows that there is a slight increase in pup mortality when a dose of 210 mg/kg/day (~35X loading dose) is used during most of pregnancy. The same study shows that a dose of 21 mg/kg/day (3.5X loading dose) is safe when used during most of pregnancy in female rats.
 - Correction needs to take place in the PI where under 8.1 is stated *“Reproduction studies have been performed in rats at doses up to 5 times the human dose and have revealed no evidence of impaired fertility or harm to the fetus due to ATryn®.”*

VI. List of non-clinical studies in BLA 125284

Safety toxicology				
Study Type and Duration	Route	Species	Doses (mg/kg)	Study no
Acute Toxicity				
Single-dose toxicity (2 studies)	iv	Rat	21-360	TSI-3-B68
	iv	Rat	36-360	TSI-3-E36
Single-dose toxicity	iv	Dog	21-210	TSI-3-B41

Repeat-dose toxicity:				
28-Day (2 studies)	iv iv	Rat Rat	36-360 36-360	(b) (4)-B42 -102
14-Day	iv	Monkey	36-360	(b) (4)-3-B43
Genotoxicity:				
Ames assay	In vitro	Bacteria	0.05-5 mg/plate	(b) (4)-3-B63
Chromosomal aberration	In vitro	CHO cells	0.5-5 mg/ml	(b) (4)-3-B65
Micronucleus	iv	Mouse	36-360	(b) (4)-3-B64g
Reproductive Toxicity:				
35-37 Days	iv	Mouse	36-360	(b) (4) AI-007-001
5-8 Days	iv	Rat	2.1-210	(b) (4) 6354-13

PK and BD				
Study Type & Duration	Route	Species	Doses (mg/kg)	Study No.
Single-dose PK	iv	Mouse	6	(b) (4) 95004
Single escalating-dose PK	iv	Rat	12.5-125	(b) (4) &
Single-dose PK with (b) (4)	iv	Rat	75-375	(b) (4) ANAW-100
Single-dose PK Single-dose BD	iv	Rat	50	(b) (4) 04-0585P
Single-dose toxicokinetics	iv	Dog	21-210	(b) (4) 3-B41
Single-dose PK	iv	Monkey	10-360	(b) (4) 3-B87
Single-dose radiolabeled PK	iv	Monkey	3	(b) (4) 2-U93
28-Day repeat-dose toxicokinetics	iv	Rat	36-360	(b) (4) a ANAW-102
Single-dose BD	iv	Rat	75-360	(b) (4) ANAW-100
Single-dose radiolabelled PK and BD	iv	Rabbit	3	Berry <i>et al.</i> , 2007
(b) (4) study	(b) (4)	(b) (4)	(b) (4)	(b) (4)

PK = Pharmacokinetics

BD = Biodistribution;

Pharmacology (Non-GLP Compliant)				
	Study	Type	Species/Cells	Dose
1	NIBSC Study	<i>in vitro</i>	Human MNC & whole blood	1-40 IU/mL
2	Kaneider <i>et al.</i> , 2001	<i>in vitro</i>	Human neutrophils	1 µU/mL to 1 U/mL
3	Kaneider <i>et al.</i> , 2003	<i>in vitro</i>	Human neutrophils & HUVEC	1-50 U/mL
4	Feistritzer, <i>et al.</i> , 2004	<i>in vitro</i>	Human eosinophils	1 µU/mL to 1 U/mL
5	Gritti <i>et al.</i> 2004	<i>in vitro</i>	Human leukocytes	1 U/mL
6	Feistritzer, <i>et al.</i> , 2005	<i>in vitro</i>	Human monocytes	1 U/mL
7	Van Veen <i>et al.</i> , 2006	<i>in vivo</i>	Polymicrobial sepsis in mice	3 IU/mL
8	(b) (4)	<i>in vivo</i>	Rat ^{(b) (4)} sepsis model	50, 100, 250, 500 U/kg
9	(b) (4)	<i>in vivo</i>	Rat ^{(b) (4)} sepsis model	50, 100, 250, 500, or 750 U/kg
10	(b) (4)	<i>in vivo</i>	Rat <i>Klebsiella</i> sepsis model	250 or 500 U/kg
11	Kipnis <i>et al.</i> , 2004	<i>in vivo</i>	<i>Pseudomonas aeruginosa</i> (Pa)-induced acute lung injury in rats	500 U/kg
12	(b) (4) & Minnema <i>et al.</i> , 2000	<i>in vivo</i>	<i>Escherichia coli</i> baboon model	500, 250, and 500 U/kg
13	(b) (4)	<i>in vivo</i>	Rats and baboons	0, 50, 135, 400 U/kg
14	Cowan <i>et al.</i> , 2002	<i>in vivo</i>	Pig to baboon renal transplantation	250 U/kg
15	Cozzi <i>et al.</i> , 2005	<i>in vivo</i>	Pig to monkey renal transplantation	250, 500, and 1000 U/kg
16	Murakami <i>et al.</i> , 2003b	<i>in vivo</i>	Smoke inhalation & pneumonia in sheep	1000 U/kg/24 h
17	(b) (4) submitted	<i>in vivo</i>	Burn and smoke inhalation in sheep	290 U every 4 h for 48 h
18	(b) (4) submitted	<i>in vivo</i>	Burn and smoke inhalation in sheep	0.43 mg/kg/h continuous infusion
19	Davis-Jackson <i>et al.</i> , 2006	<i>in vivo</i>	DIC in neonatal pig	50 µg/kg + 25 µg/kg/h

Abbreviations: DIC = disseminated intravascular coagulation; GLP = Good Laboratory Practice;

HUVEC = human umbilical vein endothelial cells; (b) (4) MNC = mononuclear cells;

NA = not applicable; rhAT = recombinant human antithrombin

VII. References

1. Nigel Jenkins, Lisa Murphy, Ray Tyther (2008), Post-translational Modifications of Recombinant Proteins: Significance for Biopharmaceuticals, *Mol Biotechnol* 39: 113–118
2. Harris, R. J., Kabakoff, B., Macchi, F. D., Shen, F. J., Kwong, M., Andya, J. D., Shire, S. J., Bjork, N., Totpal, K., & Chen, A. B. (2001). Identification of multiple sources of charge heterogeneity in a recombinant antibody. *Journal of Chromatography B: Biomedical Science Applications*, 752, 233–245.