Report on the Deliberation Results

June 3, 2015

Evaluation and Licensing Division, Pharmaceutical and Food Safety Bureau

Ministry of Health, Labour and Welfare

[Brand name]   Acoalan Injection 600

[Non-proprietary name]   Antithrombin Gamma (Genetical Recombination)   (JAN*)

[Applicant]   Kyowa Hakko Kirin Co., Ltd.

[Date of application]   July 31, 2014

[Results of deliberation]

In the meeting held on May 28, 2015, the Second Committee on New Drugs concluded that the product may be approved and that this result should be presented to the Pharmaceutical Affairs Department of the Pharmaceutical Affairs and Food Sanitation Council.

The re-examination period is 8 years. Neither the drug substance nor the drug product is classified as a poisonous drug or a powerful drug. The product is classified as a biological product.

[Conditions for approval]

The applicant is required to develop and appropriately implement a risk management plan.

Japanese Accepted Name (modified INN)

Review Report

May 11, 2015

Pharmaceuticals and Medical Devices Agency

The results of a regulatory review conducted by the Pharmaceuticals and Medical Devices Agency on the following pharmaceutical product submitted for registration are as follows.

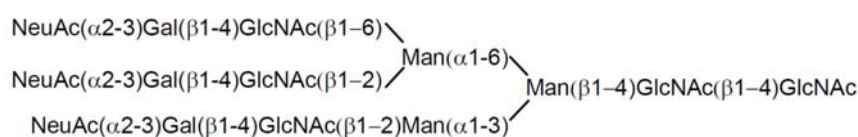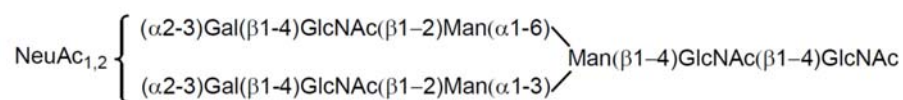|	
[Brand name]	Acoalan Injection 600
[Non-proprietary name]	Antithrombin Gamma (Genetical Recombination)
[Applicant]	Kyowa Hakko Kirin Co., Ltd.
[Date of application]	July 31, 2014
[Dosage form/Strength]	Lyophilized powder for solution for injection: Each vial contains 600 international units of Antithrombin Gamma (Genetical Recombination).
[Application classification]	Prescription drug (1) Drug with a new active ingredient
[Definition]	Antithrombin Gamma is a recombinant human antithrombin produced in glycoprotein 6-α-L-fucosyltransferase-deficient Chinese hamster ovary cells. Antithrombin Gamma is a glycoprotein (molecular weight: ca.57,000) consisting of 432 amino acid residues.
[Chemical structure]	See the attachment
[Items warranting special mention]	None
[Reviewing office]	Office of Vaccines and Blood Products

Amino acid sequence and disulfide bonds:

```
HGSPVDICTA KPRDIPMNPM CIYRSPEKKA TEDEGSEQKI PEATNRRVWE
LSKANSRFAT TFYQHLADSK NDNDNIFLSP LSISTAFAMT KLGACNDTLQ
QLMEVFKFDT ISEKTSDQIH FFFAKLNCRL YRKANKSSKL VSANRLFGDK
SLTFNETYQD ISELVYGAKL QPLDFKENAE QSRAAINKWV SNKTEGRITD
VIPSEAINEL TVLVLVNTIY FKGLWKSKFS PENTRKELFY KADGESCSAS
MMYQEGKFRY RRVAEGTQVL ELPFKGDDIT MVLILPKPEK SLAKVEKELT
PEVLQEWLDE LEEMMLVVHM PRFRIEDGFS LKEQLQDMGL VDLFSPEKSK
LPGIVAEGRD DLYVSDAFHK AFLEVNEEGS EAAASTAVVI AGRSLNPNRV
TFKANRPFLV FIREVPLNTI IFMGRVANPC VK
```

Glycosylation sites: N96, N135, N155, N192

Proposed structures of main sugar chains:

Molecular formula:

$C_{2191}H_{3451}N_{583}O_{656}S_{18}$ (protein moiety)

<div align="center">

Review Results

</div>

<div align="right">

May 11, 2015

</div>

[Brand name]   Acoalan Injection 600

[Non-proprietary name]   Antithrombin Gamma (Genetical Recombination)

[Applicant]   Kyowa Hakko Kirin Co., Ltd.

[Date of application]   July 31, 2014

[Results of review]

Based on the submitted data, the Pharmaceuticals and Medical Devices Agency (PMDA) has concluded that the efficacy of the product in patients with thrombophilia due to congenital antithrombin deficiency or disseminated intravascular coagulation (DIC) accompanied by a decrease in antithrombin has been demonstrated and its safety is acceptable in view of its observed benefits. PMDA considers that the safety of the product in clinical practice needs to be further investigated through post-marketing surveillance.

As a result of its regulatory review, PMDA has concluded that the product may be approved for the indication and dosage and administration as shown below, with the following conditions.

[Indication]   Thrombophilia due to congenital antithrombin deficiency
           Disseminated intravascular coagulation (DIC) accompanied by a decrease in antithrombin

[Dosage and administration] The product should be reconstituted with the supplied water for injection, and the reconstituted solution should be administered either by slow intravenous injection or by intravenous infusion.

1. Thrombophilia due to congenital antithrombin deficiency

  The dosage is 24 to 72 international units (IU)/kg administered once daily.

2. Disseminated intravascular coagulation (DIC) accompanied by a decrease in antithrombin

  The usual adult dosage is 36 IU/kg administered once daily. The dose may be adjusted according to the patient's condition. The maximum daily dose should not exceed 72 IU/kg.

[Conditions for approval] The applicant is required to develop and appropriately implement a risk management plan.

Review Report (1)

March 19, 2015

I. Product Submitted for Registration

[Brand name]	Acoalan Injection 600
[Non-proprietary name]	Antithrombin Gamma (Genetical Recombination)
[Applicant]	Kyowa Hakko Kirin Co., Ltd.
[Date of application]	July 31, 2014
[Dosage form/Strength]	Lyophilized powder for solution for injection: Each vial contains 600 international units of Antithrombin Gamma (Genetical Recombination).

[Proposed indication]   Thrombophilia due to congenital antithrombin III deficiency

Disseminated intravascular coagulation (DIC) accompanied by a decrease in antithrombin III

[Proposed dosage and administration]

The product should be reconstituted with the supplied water for injection, and the reconstituted solution should be administered either by intravenous slow injection or by intravenous infusion.

1. Thrombophilia due to congenital antithrombin III deficiency

   The daily dosage is 1200 to 3600 international units (IU) (or 24 to 72 IU/kg). The dose may be reduced according to the patient's age or condition.

2. Disseminated intravascular coagulation (DIC) accompanied by a decrease in antithrombin III

   The usual daily dosage for adult patients showing a decrease in antithrombin III to ≤70% of the normal level is 1800 IU (or 36 IU/kg) in combination with continuous intravenous infusion of heparin.

   In case of emergency such as obstetric or surgical DIC, the dosage is 48 to 72 IU/kg administered once daily.

   The dose may be adjusted according to the patient's age, body weight, or condition.

II. Summary of the Submitted Data and Outline of the Review by Pharmaceuticals and Medical Devices Agency

The submitted data and the review thereof by the Pharmaceuticals and Medical Devices Agency (PMDA) are summarized below.

1. Origin or history of discovery, use in foreign countries, and other information

Antithrombin (AT) is a major natural coagulation inhibitor that is present in circulating blood. It inactivates thrombin and coagulation factors X, XII, and IX by forming a complex with these coagulation factors.

Diseases and syndromes related to AT deficiency or decreased AT activity include congenital antithrombin deficiency (CAD) and disseminated intravascular coagulation (DIC). CAD is a genetic disorder inherited as an autosomal dominant trait, and CAD patients are at high risk of thromboembolism. Heparin is used to treat the acute phase of thromboembolism and its anticoagulant effect depends on the level of AT activity. For this reason, AT supplementation is required for treating thromboembolism in CAD patients. AT supplementation is also undertaken in high-risk situations for thromboembolism, such as trauma, surgery, pregnancy, and delivery (*Japanese Journal of Thrombosis and Hemostasis.* 2001;12:74-7). On the other hand, DIC is an acquired syndrome characterized by extensive intravascular hypercoagulability with an underlying condition such as infection, hematopoietic malignancy, or solid tumors. DIC is a hypercoagulable state with decreased plasma AT activity, and therefore requires AT supplementation. AT is listed as one of the drugs with the highest overall recommendation score in "Expert consensus based on the evidence for the treatment of disseminated intravascular coagulation due to infection" (*Japanese Journal of Thrombosis and Hemostasis.* 2009;20:77-113) and its supplement (*Japanese Journal of Thrombosis and Hemostasis.* 2014;25:123-5) issued by The Japanese Society on Thrombosis and Hemostasis.

AT preparations approved in Japan consist of the following human plasma-derived AT products: Neuart for intravenous injection (Japan Blood Products Organization), Anthrobin P for injection (The Chemo-Sero-Therapeutic Research Institute), and Kenketsu Nonthron for injection (Nihon Pharmaceutical Co., Ltd.).

Antithrombin Gamma (Genetical Recombination) (hereinafter "rAT-G") is a recombinant human AT developed by Kyowa Hakko Kirin Co., Ltd. rAT-G has been developed as an alternative for human plasma-derived AT preparations in the treatment of CAD and DIC.

The clinical development of rAT-G started in ██ ██ with a phase I single-dose study in healthy adults (Study 3357-█01). The applicant has filed a new drug application based on the results of studies including a bioequivalence study in healthy adults starting in ██ ██ (Study 3357-003) and a phase III study in patients with DIC starting in June 2011 (Study 3357-004). As of March 2015, rAT-G is as yet not approved in any country or region.

2. Data relating to quality

2.A   *Summary of the submitted data*

2.A.(1)   Drug substance

The drug substance is prepared by adding glycine-sodium citrate buffer to Antithrombin Gamma

(Genetical Recombination) (hereinafter "rAT-G"), the active ingredient.

2.A.(1).1) Preparation and control of cell substrates

(a) Preparation of cell banks

██

██ The structural gene coding for rAT-G was fully synthesized by changing the base sequence of the gene for human antithrombin (AT) without altering the amino acid sequence. This structural gene was inserted transgenically into the expression vector to generate the expression construct. ██

██████████████████████████████████

The master cell bank (MCB) and working cell bank (WCB) were successively prepared from this cell line.

(b) Control of MCB and WCB

Isoenzyme patterns, Southern blots, Northern blots, DNA copy numbers, and DNA sequences were analyzed for the characterization of the MCB and cells at the limit of *in vitro* cell age (CAL). Isoenzyme patterns and DNA sequences were analyzed and DNA rearrangement was evaluated for the characterization of the WCB. The results of these analyses demonstrated the genetic stability of the cell bank systems. Non-viral adventitious agents were examined by tests for sterility and mycoplasma. Viral adventitious agents were examined by tests for retroviruses, endogenous viruses (infectivity [S^+L^- focus formation assay], electron microscope observation, and reverse transcriptase assay), and adventitious viruses (*in vitro* test, *in vivo* test [suckling mice, adult mice, and embryonated eggs], hamster antibody production test, mouse antibody production test, and tests for porcine and bovine adventitious viruses). No non-viral or viral adventitious agents were detected other than retrovirus-like particles known to be present in rodents.

Stability studies were conducted for the MCB and WCB, and their storage conditions were appropriately determined based on the results of the studies. A new WCB will be generated as needed, but there is no plan to generate a new MCB.

2.A.(1).2) Manufacturing process

The manufacturing process for the drug substance is shown in Table 2-1. The drug substance is dispensed into polycarbonate containers.

Process validation was carried out using a production scale manufacturing process for the drug substance, and the results has demonstrated that the process is adequately controlled.

Table 2-1. Manufacturing process of the drug substance

Step		In-process control
Culture		
Pre-culture	• Inoculation with WCB • █████████████ •	█████████████
Main culture	• █████████████ • Cell separation	████████████████████████
Purification		
Concentration 1	█████████████	
Virus inactivation	████████████████████	Endotoxins, viable cell count
Purification 1	████████████████	█████████████
Purification 2	████████████████████	████████████████
Concentration 2	█████████████	Endotoxins, viable cell count
Purification 3	████████████████	Endotoxins, viable cell count
Purification 4	████████████████	████████████████████
Concentration/buffer replacement	████████████████	████████████████
Virus removal	████████████████	████████████████
Filling	Filtration	████████████████

The shadowed boxes indicate critical process steps.

2.A.(1).3) Adventitious agent safety evaluation

██
██

██
████████████████████████████████ Neither non-viral nor viral adventitious agents were detected when mycoplasma testing and *in vitro* tests for retroviruses and adventitious viruses were performed in cell cultures (unprocessed bulk). The results of these tests demonstrated that retrovirus-like particles are effectively inactivated and removed during the inactivation, purification, and removal steps.

As shown in Table 2-2, viral clearance studies were performed using model viruses to evaluate the capacity of the manufacturing process to clear viruses. The results of the study demonstrated that the manufacturing process has satisfactory viral clearance capacity.

Table 2-2. Results of viral clearance studies

Manufacturing process	Viral removal factor (log$_{10}$)			
	MuLV	PRV	Reo-3	MVM
███████████████████	██	██	NT	NT
███████████████	██	██	██	██
███████████	██	██	██	██
███████████████	██	██	██	██
Overall virus removal factor	≥ 18.30	≥ 18.16	≥ 12.21	≥ 13.13

MuLV, xenotropic murine leukemia virus; PRV, psuedorabies virus; Reo-3, reovirus type 3; MVM, minute virus of mice
NT, Not Tested
1) Of the values obtained from two independent viral clearance studies, the lower value was adopted for the analysis.
2) Of the values obtained from █ studies using unused resins and the study using used resins, the lowest value was adopted for the analysis.
3) Determined by the 50% tissue-culture-infectious-dose (TCID50) method.
4) Determined by the polymerase chain reaction (PCR) method.

2.A.(1).4) Manufacturing process development (comparability)

Major changes in the manufacturing process for the development of the drug substance are shown below. Manufacturing site changes were also made when the process changes (from Process A to Process B and from Process B to Process C) took place.

- Process A to Process B: ██ ████████████████████████████████████

- Process B to Process C: ████████████████████████████████████
  ██
  ██
  █████████

The comparability of the drug substances before and after these changes in the manufacturing process was confirmed.

2.A.(1).5) Characterization

(a) Structure/Composition

i) Primary structure

- Peptide mapping was performed using lysyl-endopeptidase (Lys-C), peptidyl-Asp metallo-endopeptidase (Asp-N), and trypsin. The results demonstrated that the entire amino acid sequence of rAT-G is identical to the theoretical amino acid sequence deduced from the DNA base sequence.
- The results of Asp-N peptide mapping showed that the N-terminal amino acid of rAT-G is His-Gly-Ser-Pro-Val. ███████████████████████████████████ ██████████████████████████

ii) Secondary structure

- Lys-C peptide mapping suggested that 3 pairs of residues (8-128, 21-95, and 247-430) are linked by disulfide bonds.
- A broad single band was observed when the drug substance was examined by SDS- polyacrylamide

gel electrophoresis under both reducing and non-reducing conditions, suggesting the presence of a sugar chain that is a single-chain glycoprotein.

iii) Tertiary (higher-order) structure

- The alpha helix structure of the drug substance has been elucidated by circular dichroism spectroscopy.
- The results of size exclusion chromatography demonstrated that the drug substance is a monomer and that it contains a small amount of aggregation.

iv) Post-translational modification

a) *N*-linked glycosylation

- Asp-N peptide mapping showed *N*-linked glycosylation of asparagine residues at positions 96, 135, 155, and 192.

- Analysis of the neutral glycan profile revealed that the glycans mainly have bi-, tri-,and tetra-antennary glycan structures. Hybrid-type glycans having several mannose residues attached to the core were also detected.

b) Sialylation

- Sialylation analysis of *N*-linked glycans identified ▮ to ▮ sialic acids attached to *N*-linked glycans, and the number of sialic acids attached to a glycan(SA/N) ranged from ▮ to ▮.

c) *O*-linked glycosylation

O-linked glycosylation was not detected by Lys-C peptide mapping.

d) Others

The results of peptide mapping or mass spectrometry did not show acetylation (N-terminal, Lys, and Arg), phosphorylation (Ser, Thr, and Tyr), carboxylation (Glu), or amidation (C-terminal).

(b) Physicochemical properties

i) Molecular weight

- The molecular weight of the drug substance was determined to be 56.8 kDa by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS).

ii) Isoforms

- ▮ isoforms were found in the isoelectric point (pI) range of ▮ to ▮ by isoelectric focusing.

iii) Spectroscopic properties

- When analyzed by ultraviolet absorbance spectroscopy, the drug substance exhibited an absorption maximum at a wavelength of about 280 nm and the minimum at about 250 nm, which are attributable

to aromatic amino acid residues typically found in proteins.

- ███
███████████████

(c) Biological properties

Measurements by the synthetic substrate method revealed that antithrombin activity was increased depending on the concentration of the drug substance.

(d) Comparison with plasma-derived antithrombin (AT) preparation

A plasma-derived AT (pAT) preparation (Neuart for intravenous injection [Japan Blood Products Organization]; hereinafter, Neuart) was characterized and compared with rAT-G. The results demonstrated that rAT-G and Neuart are similar in terms of the primary structure, secondary structure, higher-order structure, post-translational modifications (structures of sugar chains), and physical properties.

(e) Product-related substances

No molecular entities have been identified as product-related substances.

(f) Impurities

i) Process-related impurities

███
███
███
███
███

All of these process-related impurities have been confirmed to be consistently removed through the manufacturing process. Foreign proteins are adequately controlled with specifications for the drug substance.

ii) Product-related impurities

███
███████████████████████████████

It has been confirmed that all of these product-related impurities are consistently removed in the manufacturing process.

███
███

2.A.(1).6) Control of drug substance

The proposed specifications for the drug substance include description, identification (ELISA and peptide mapping), pH, purity (size exclusion chromatography, hydrophobic chromatography, capillary SDS-gel electrophoresis, and foreign proteins), glycosylation profile, and assay (protein content and specific activity).

2.A.(1).7) Stability of drug substance

The main stability studies of the drug substance are shown in Table 2-3.

Table 2-3. Results of stability studies of the drug substance

Study	Storage condition	Number of batches	Storage container	Storage period
Long-term	−70 ± 10°C	3	Polycarbonate container/polyethylene bag	21 months
Storage stability	5 ± 3°C	3	Polycarbonate container/polyethylene bag	6 months
	25 ± 2°C	3	Polycarbonate container/polyethylene bag	6 months
Stress	40 ± 2°C	1	Polycarbonate container/polyethylene bag	3 months
Photostability	1,200,000 lux·hr (total illuminance), 200 W·h/m^2 (total near-ultraviolet radiation energy), 5 ± 3°C	1	Polycarbonate container (with or without light-resistance aluminum)	—

Under the long-term storage condition, all the test items met the specifications throughout the study period.

Based on the above, a shelf life of 21 months has been proposed for the drug substance when stored in a tightly-closed polycarbonate container at −70 ± 10°C.

2.A.(2) Drug product
2.A.(2).1) Description and composition of the drug product, and formulation design

The drug product is a lyophilized powder formulation that contains 600 international units (IU) of the

active ingredient in a vial (20 mL colorless glass vial). The drug product contains glycine and sodium citrate hydrate as excipients. In the manufacturing process, █ mL of the drug solution, the amount of pre-lyophilized drug product, is filled in each vial before lyophilization.

As a diluent for reconstitution, Water for Injection (Japanese Pharmacopoeia) in a glass vial (20 mL) is supplied together with the drug product. Each vial contains 12█ mL of the diluent.

2.A.(2).2)   Manufacturing process

The manufacturing process of the drug product comprises drug solution preparation, sterile filtration, filling, lyophilization, and capping. ██████████████████████████████ ████████████████████ Validation of the commercial scale manufacturing process for the drug product has demonstrated that the process is adequately controlled.

2.A.(2).3)   Manufacturing process development (comparability)

In the course of development of the drug product, there was a formulation change (i.e., the amount of the active ingredient per vial was changed from 500 IU to 600 IU). The pre- and post-change drug products, though different in terms of the amount of active ingredient, have the same composition after reconstitution, and the comparability of their quality attributes has been confirmed.

2.A.(2).4)   Control of drug product

The proposed specifications for the drug product include description (including appearance of solution and reconstitution time), identification (ELISA), pH, osmolar ratio, purity (size exclusion chromatography, hydrophobic chromatography), water content, endotoxin, uniformity of dosage units, foreign insoluble matter, insoluble particulate matter, sterility, and assay (titer). Reconstitution time and osmotic pressure ratio have been proposed in the course of the review.

2.A.(2).5)   Stability of drug product

For this application, stability studies were conducted using 3 pilot scale batches. These studies for the drug product are shown in Table 2-4.

Table 2-4. Results of stability studies for the drug product

Study	Storage condition	Storage container	Number of batches	Storage period
Long-term	5 ± 3°C, in the dark	Glass vial/secondary package	3	18 months
Accelerated	25 ± 2°C, 60 ± 5%RH, in the dark	Glass vial/secondary package	3	6 months
Stress	40 ± 2°C, 75 ± 5%RH, in the dark	Glass vial/secondary package	1	3 months
Photostability	1,200,000 lux·hr (total illuminance), 200 W·h/m^2 (total near-ultraviolet radiation energy), 5 ± 3°C	Glass vial; Glass vial/secondary package	1 each	—
Post-reconstitution stability	5°C	Glass vial	1	1 week
	25°C	Glass vial	1	1 day

Under the long-term stability condition, all the tests items met the specifications throughout the study period.

██

██

In the photostability testing, the drug product was tested while being packed in a glass vial with or without the secondary package. ██ ██ ████████████

The post-reconstitution stability study demonstrated that the drug product reconstituted with the supplied diluent is stable for 1 week when stored at 5°C and for 1 day when stored at 25°C.

Based on the above, a shelf life of 18 months has been proposed for the drug product when stored at 2 to 8°C protected from light with the secondary package.

2.A.(3)   Reference materials

The in-house primary reference material is prepared from the drug substance and frozen for storage at ≤−70°C. The specifications for the reference material have been established to qualify the reference material on a periodic basis.

2.B   Outline of the review by PMDA

Based on the submitted data, PMDA concluded that the quality of the drug substance and the drug product is adequately controlled.

3. Non-clinical data
3.(i)   Summary of pharmacology studies
3.(i).A   Summary of the submitted data

The applicant submitted the results of the following studies of Antithrombin Gamma (Genetical Recombination) (hereinafter "rAT-G"): primary pharmacodynamics studies (an *in vitro* study that evaluated the effect on human plasma coagulation time and *in vivo* studies in a rat model of disseminated intravascular coagulation [DIC]), safety pharmacology studies in rats and cynomolgus monkeys, and a pharmacodynamic drug interaction study in rats to evaluate the effect of concomitant administration of unfractionated heparin (UFH).

A drug in the same class as rAT-G was used as a comparator in the primary pharmacodynamics studies and pharmacodynamic drug interaction study. The drug was a human plasma-derived AT product, Neuart for intravenous injection ("Neuart") (████████████████████████████). rAT-G was

intravenously administered in all the *in vivo* studies. In the subsequent sections, values are presented as mean ± standard error, unless otherwise noted.

3.(i).A.(1) Primary pharmacodynamics

The results of the following primary pharmacodynamics studies were submitted.

3.(i).A.(1).1) *In vitro* study (effect on human plasma coagulation time) (4.2.1.1-1, Study d-■-261)

After adding 0 to 3 international units (IU)/mL of rAT-G or Neuart to human plasma spiked with heparin, prothrombin time (PT) and activated partial thromboplastin time (APTT) were measured. Both PT and APTT tended to become longer with an increase in the amount of rAT-G or Neuart added (Table 3-1). The applicant interpreted the results of the study as demonstrating similarity between rAT-G and Neuart in terms of the prolongation of plasma coagulation time.

Tale 3-1. PT and APTT in human plasma

	Amount of addition (IU/mL)	PT (second)	APTT (second)
rAT-G	0	47.90 ± 6.79	69.68 ± 6.21
	1	55.44 ± 7.81	73.94 ± 5.40
	2	61.04 ± 7.18	81.84 ± 6.41
	3	66.16 ± 9.57	86.94 ± 7.19
Neuart	0	45.68 ± 6.56	68.38 ± 4.67
	1	51.74 ± 7.04	78.58 ± 5.72
	2	58.60 ± 8.88	82.78 ± 6.78
	3	64.76 ± 8.10	87.58 ± 4.53

Testing frequency: 5 times each

3.(i).A.(1).2) *In vivo* study (evaluation in a rat model of DIC)

Continuous administration of lipopolysaccharides (LPS) or thromboplastin (Tp) in rats has been reported to induce hypercoagulability, resulting in a DIC-like condition (*Sysmex J.* 2012;35[Suppl.1]:10-20). Therefore, a rat model of LPS-induced and Tp-induced hypercoagulability was generated as a DIC animal model, and the two studies described in the subsections below were conducted using the rat model. The applicant interpreted the results of these studies as demonstrating that the anticoagulant activity of rAT-G was similar to that of Neuart.

(a) LPS-induced hypercoagulability model (4.2.1.1-2, Study d-■-260)

Rats (males, n = 10/group) were treated according to one of the following 6 regimens: 250 IU/kg of rAT-G followed by continuous LPS administration (the rAT-G group), 250 IU/kg of Neuart followed by continuous LPS administration (the Neuart group), the vehicle of rAT-G (Vehicle 1) followed by continuous LPS administration (the control group 1), the vehicle of Neuart (Vehicle 2) followed by continuous LPS administration (the control group 2), Vehicle 1 followed by continuous administration of normal saline as the vehicle for LPS (the normal group 1), and Vehicle 2 followed by continuous administration of normal saline as the vehicle for LPS (the normal group 2). The rats were examined by hematological and other tests 3 hours after the end of LPS or normal saline administration. Compared with the normal groups 1 and 2, the control groups 1 and 2 had decreased platelet count, decreased fibrinogen levels, decreased antithrombin (AT) activity, prolonged PT and APTT, increased lactate dehydrogenase (LD), increased aspartate aminotransferase (AST), increased alanine aminotransferase

(ALT), and increased renal glomerular fibrin deposition [GFD] (the proportion of the number of glomeruli containing fibrin thrombi in 100 glomeruli observed in kidney slices). The differences in these test parameters between the rAT-G or Neuart group and the normal groups were smaller than those between the control groups and the normal groups (Table 3-2).

Table 3-2. Results of hematological and other tests in rats
(LPS-induced hypercoagulability model)

	Platelet (10^4 cell/μL)	PT (sec)	APTT (sec)	Fibrinogen (mg/dL)	AT activity (%)	LD (IU/L)	AST (IU/L)	ALT (IU/L)	GFD (%)
Normal 1 (N = 10)	137.42 ± 5.42*	15.64 ± 0.30*	19.03 ± 0.21*	238.6 ± 4.8*	100.6 ± 2.2*	176.3 ± 10.1*	109.8 ± 4.3*	38.1 ± 1.7*	0.0 ± 0.0*
Control 1 (N = 10)	32.90 ± 3.51	23.30 ± 1.29	32.82 ± 1.56	64.2 ± 4.6	57.0 ± 3.4	11684.0 ± 2199.0	964.3 ± 198.3	317.4 ± 83.8	10.7 ± 3.6
rAT-G (N = 10)	60.63 ± 3.32*	24.90 ± 0.30*	24.90 ± 0.42*	136.5 ± 8.8*	192.4 ± 4.4*	2368.5 ± 469.2*	338.7 ± 31.9*	69.2 ± 6.0*	0.3 ± 0.3*
Normal 2 (N = 10)	130.06 ± 5.87*	15.40 ± 0.16*	18.88 ± 0.21*	232.1 ± 6.8*	101.9 ± 2.1*	187.5 ± 13.2*	112.5 ± 3.7*	38.3 ± 2.0*	0.0 ± 0.0*
Control 2 (N = 10)	31.50 ± 4.48	23.31 ± 0.85	32.36 ± 1.32	62.4 ± 5.4	59.5 ± 3.5	11908.7 ± 3317.6	894.2 ± 192.5	376.3 ± 101.5	13.4 ± 7.2
Neuart (N = 10)	62.73 ± 3.74*	17.01 ± 0.23*	25.21 ± 0.62*	142.5 ± 8.4*	190.1 ± 2.7*	2354.5 ± 269.3*	341.4 ± 22.8*	61.2 5.4*	0.1 ± 0.1*

N: number of rats

*: $P < 0.05$ (tested using one of following: Student's t-test, Aspin-Welch test, and Wilcoxon rank sum test); the normal group 1 and the rAT-G group were compared with the control group 1, and the normal group 2 and the Neuart group were compared with the control group 2.

(b) Tp-induced hypercoagulability model (4.2.1.1-3, Study d-■■-299)

Rats (males, n = 8/group) were treated according to one of the following 6 regimens: 250 IU/kg of rAT-G followed by continuous Tp administration (the rAT-G group), 250 IU/kg of Neuart followed by continuous Tp administration (the Neuart group), Vehicle 1 followed by continuous Tp administration (the control group 1), Vehicle 2 followed by continuous Tp administration (the control group 2), Vehicle 1 followed by continuous administration of normal saline as the vehicle for Tp (the normal groups 1), and Vehicle 2 followed by continuous administration of normal saline as the vehicle for Tp (the normal groups 2). The rats were examined by hematological tests 1 hour after the end of Tp or normal saline administration. Compared with the normal groups 1 and 2, the control groups 1 and 2 had decreased platelet count, decreased fibrinogen levels, decreased AT activity, and prolonged PT and APTT. The differences in these test parameters between the rAT-G or Neuart group and the normal groups were smaller than those between the control groups and the normal groups (Table 3-3).

Table 3-3. Results of hematological tests in rats
(Tp-induced hypercoagulability model)

	Platelet (10^4 cell/μL)	PT (sec)	APTT (sec)	Fibrinogen (mg/dL)	AT activity (%)
Normal 1 (N = 8)	127.2 ± 5.1*	15.7 ± 0.2*	20.8 ± 0.5*	222.3 ± 5.5*	98.0 ± 2.9*
Control 1 (N = 8)	44.9 ± 2.5	24.0 ± 1.2	42.8 ± 2.9	39.6 ± 3.8	81.8 ± 3.1
rAT-G (N = 8)	73.1 ± 3.0*	17.0 ± 0.5*	30.3 ± 1.5*	76.1 ± 8.4*	220.1 ± 8.4*
Normal 2 (N = 8)	120.8 ± 5.6*	15.5 ± 0.2*	20.7 ± 0.2*	220.2 ± 3.7*	101.3 ± 2.0*
Control 2 (N = 8)	44.3 ± 4.1	22.5 ± 1.0	41.2 ± 2.6	42.3 ± 3.3	80.0 ± 2.0
Neuart (N = 8)	74.6 ± 6.0*	16.8 ± 0.5*	28.4 ± 1.4*	86.8 ± 13.3*	213.0 ± 7.9*

N: number of rats

*: $P < 0.05$ (tested using either Student's t-test or Aspin-Welch test); the normal group 1 and the rAT-G group were compared with the control group 1, and the normal group 2 and the Neuart group were compared with the control group 2.

3.(i).A.(2)   Secondary pharmacodynamics

No secondary pharmacodynamic study has been conducted.

3.(i).A.(3) Safety pharmacology

Repeated dose toxicity studies were conducted in rats and cynomolgus monkeys (Studies █08 and █07) to evaluate safety pharmacology of rAT-G in the central nervous, cardiovascular, and respiratory systems [see "3.(iii).A.(2) Repeat-dose toxicity"]. Based on the results of these studies shown below, the applicant explained that rAT-G does not affect the activities of the central nervous, cardiovascular, or respiratory system.

3.(i).A.(3).1 Central nervous system and cardiovascular system

Clinical signs in rats were evaluated in Study █08, and clinical signs, body weight, blood pressure, heart rate, and electrocardiogram (ECG) were evaluated in cynomolgus monkeys in Study █07. After the end of administration in Study █07, 6 cynomolgus monkeys were necropsied in the 1000 IU/kg group (n = 10), the maximum dose group in this study. Hemorrhage was observed in all of the 6 monkeys, one of which had serious anemia. This monkey had shown decreases in blood pressure and body temperature on Day 12 and hypoactivity from Day 13 onward.

The applicant's explanation on the clinical findings in the monkey:

In the 1000 IU/kg group, this monkey had the largest number of bleeding sites (5 sites in total; 2 sites each in both thighs and 1 site in the scrotum) and the highest decreases in red blood cell count, hemoglobin, and hematocrit. However, no hemorrhage was observed in the brain, spinal cord, or heart. The histopathological findings as well as the data on heart rate and ECG suggested that rAT-G had no effect on the central nervous or cardiovascular system. Therefore, the changes observed in this monkey is probably attributed to severe hemorrhage resulting from an excessive pharmacological effect of rAT-G and thus rAT-G is unlikely to affect the central nervous or cardiovascular system.

3.(i).A.(3).2 Respiratory system

The results of clinical observations in Studies █08 and █07 indicated that rAT-G had no effect on the respiratory system even in animals treated at the maximum dose (cynomolgus monkeys, 1000 IU/kg; rats, 2000 IU/kg).

3.(i).A.(4) Pharmacodynamic drug interaction

The results of the following study were submitted as data on the pharmacodynamic drug interactions of rAT-G.

3.(i).A.(4).1) Effect of concomitant administration of unfractionated heparin in LPS-induced hypercoagulability model (4.2.1.4-1, Study d-█-671)

In order to compare the effects of rAT-G or Neuart in combination with unfractionated heparin (UFH) in a rat DIC model with LPS-induced hypercoagulability, rats (males, n = 12/group) were treated according to one of the following 10 regimens: continuous UFH plus rAT-G 250 IU/kg followed by LPS (the rAT-G + UFH group), continuous UFH plus Neuart 250 IU/kg followed by LPS (the Neuart + UFH

group), continuous UFH plus the vehicle of rAT-G (Vehicle 1) followed by LPS (the UFH monotherapy group 1), continuous UFH plus the vehicle of Neuart (Vehicle 2) followed by LPS (the UFH monotherapy group 2), continuous administration of normal saline as the vehicle for UFH plus rAT-G followed by LPS (the rAT-G monotherapy group), continuous administration of normal saline plus Neuart followed by LPS (the Neuart monotherapy group), continuous administration of normal saline plus Vehicle 1 followed by LPS (the control group 1), continuous administration of normal saline plus Vehicle 2 followed by LPS (the control group 2), continuous administration of normal saline plus Vehicle 1 followed by normal saline as the vehicle for LPS (the normal group 1), and continuous administration of normal saline plus Vehicle 2 followed by normal saline as the vehicle for LPS (the normal group 2). The rats were examined by hematological and other tests 3 hours after the end of continuous administration of LPS or its vehicle (normal saline). The rAT-G + UFH group and Neuart + UFH group showed smaller decreases in platelet count and fibrinogen than those in the rAT-G monotherapy group and Neuart monotherapy group. Also, prolongation of APTT was greater in the rAT-G + UFH group and Neuart + UFH group than in the UFH monotherapy groups. Moreover, the increase in AST and decrease in AT activity in the rAT-G + UFH group and Neuart + UFH group were prevented, and the similar results were obtained in the rAT-G monotherapy group and Neuart monotherapy group, showing no effects of concomitant UFH (Table 3-4).

The applicant interpreted the results of this study as demonstrating that the anticoagulant activity of rAT-G was similar to that of Neuart even when used in combination with heparin.

Table 3-4. Results of hematological and other tests in rats
(LPS-induced hypercoagulability model, concomitant administration of UFH)

	Platelet (10^4 cell/µL)	APTT (sec)	Fibrinogen (mg/dL)	AT activity (%)	AST (IU/L)	ALT (IU/L)
Normal 1 (N = 12)	$114.9 \pm 4.5^*$	$16.4 \pm 0.3^*$	$213.1 \pm 4.5^*$	$97.7 \pm 3.9^*$	$101.0 \pm 4.7^*$	$30.5 \pm 1.8^*$
Control 1 (N = 12)	47.3 ± 3.6	30.0 ± 2.1	81.7 ± 7.7	63.3 ± 2.7	287.4 ± 18.3	53.0 ± 2.3
UFH monotherapy 1 (N = 12)	63.5 ± 4.5	$53.3 \pm 2.8^{*\dagger}$	$114.6 \pm 10.6^{\dagger}$	$60.3 \pm 3.5^{\dagger}$	275.3 ± 19.6	$56.5 \pm 4.1^{\dagger}$
rAT-G monotherapy (N = 12)	61.7 ± 4.7	$24.6 \pm 1.4^{\dagger}$	$139.5 \pm 8.1^{*\dagger}$	$188.3 \pm 5.2^*$	$205.6 \pm 5.0^*$	$41.9 \pm 1.9^*$
rAT-G + UFH (N = 12)	$75.3 \pm 4.4^*$	$82.2 \pm 6.8^*$	$184.1 \pm 8.4^*$	$178.8 \pm 6.5^*$	$202.1 \pm 14.0^*$	$41.5 \pm 1.9^*$
Normal 2 (N = 12)	$111.2 \pm 2.7^*$	$17.2 \pm 0.3^*$	$206.2 \pm 6.4^*$	$96.4 \pm 3.5^*$	$98.9 \pm 5.5^*$	$33.8 \pm 2.7^*$
Control 2 (N = 12)	48.4 ± 5.7	28.4 ± 1.9	82.1 ± 14.3	65.0 ± 3.9	299.0 ± 31.7	51.4 ± 4.6
UFH monotherapy 2 (N = 12)	57.5 ± 5.6	$48.7 \pm 2.7^{*\dagger}$	133.2 ± 13.6	$62.0 \pm 3.3^{\dagger}$	$277.7 \pm 19.6^{\dagger}$	$53.5 \pm 3.5^{\dagger}$
Neuart monotherapy (N = 12)	$67.7 \pm 5.0^*$	$24.4 \pm 1.4^{\dagger}$	$142.4 \pm 12.2^*$	$182.8 \pm 5.1^*$	207.5 ± 11.1	40.9 ± 1.2
Neuart + UFH (N = 12)	$75.1 \pm 3.2^*$	$75.2 \pm 4.5^*$	$182.9 \pm 7.1^*$	$176.3 \pm 5.2^*$	$200.3 \pm 13.1^*$	41.7 ± 2.5

N: number of rats (The data on APTT and fibrinogen in the control groups 1 and 2 were obtained from 10 rats due to the missing data for 2 rats.)
*: $P < 0.05$ (tested using one of the following: Student's t-test, Aspin-Welch test, Wilcoxon rank sum test, Tukey test, and Steel-Dwass test); the normal group 1, UFH monotherapy group 1, rAT-G monotherapy group, and rAT-G + UFH group were compared with the control group 1; and the normal group 2, UFH monotherapy group 2, Neuart monotherapy group, and Neuart + UFH group were compared with the control group 2.
†: $P < 0.05$ (tested using either Tukey test or Steel-Dwass test); the UFH monotherapy group 1 and the rAT-G monotherapy group were compared with the rAT-G + UFH group; and the UFH monotherapy group 2 and the Neuart monotherapy group were compared with the Neuart + UFH group.

3.(i).B Outline of the review by PMDA

The data from primary pharmacodynamic studies indicate that rAT-G has antithrombin activity and shows promise as a therapeutic agent having anticoagulant effect in the body. Based on the submitted data from safety pharmacology studies and toxicity studies, PMDA has concluded that there are no particular concerns about the safety of rAT-G.

3.(ii)   Summary of pharmacokinetic studies

3.(ii).A   Summary of the submitted data

Pharmacokinetic data submitted were the results of studies in rats, rabbits, and cynomolgus monkeys. Plasma concentrations of rAT-G and pAT preparations (Neuart; Anthrobin P for Injection ["Anthrobin"], ███████████████████████; and Kenketsu Nonthron for injection ["Nonthron"], Nihon Pharmaceutical Co., Ltd.) were determined by ELISA ████████████████████. Plasma AT activity was measured by the synthetic substrate method. In a study that evaluated rAT-G distribution and excretion into milk, radioactivity levels in plasma and tissues were determined after administration of ^{125}I-rAT-G. In the subsequent sections, values are presented as mean ± standard deviation, unless otherwise noted.

3.(ii).A.(1)   Absorption

3.(ii).A.(1).1   Single-dose studies (4.2.2.2-2, Study d-██-262; 4.2.2.2-4, Study d-██-264; 4.2.2.7-1, Study d-██-081; 4.2.2.7-3, Study d-██-263)

Cynomolgus monkeys (males, n = 5/group) received a single intravenous dose of 200 IU/kg of rAT-G. The plasma concentrations of rAT-G and plasma AT activity were measured before dosing and at 9 time points between 5 minutes and 72 hours post-dose (Study d-██-262), and the obtained values were analyzed to calculate pharmacokinetic parameters (Table 3-5). Also in cynomolgus monkeys (males, n = 5/group) receiving a single intravenous dose of 200 IU/kg of pAT preparations (Neuart, Anthrobin, and Nonthron), the plasma concentrations of the drugs and plasma AT activity were measured before dosing and at 9 time points between 5 minutes and 72 hours post-dose (Study d-██-264), and the obtained values were analyzed to calculate pharmacokinetic parameters (Table 3-6). The results of these 2 studies were analyzed to compare the pharmacokinetic properties of rAT-G and pAT preparations. Following administration of rAT-G and pAT preparations, the plasma concentrations of the drugs and plasma AT activity disappeared in a biphasic manner, with no clear difference in the beta-phase half-life ($t_{1/2\beta}$). However, the clearance (CL) of rAT-G was approximately 1.2- to 1.4-fold higher than those of pAT preparations.

The applicant's explanation:

The residence time of some proteins in plasma has been reported to be reduced with a decrease in the content of terminal sialic acid residues of *N*-linked glycans (*J Biol Chem.* 1968;243:155-9). A single-dose administration study in cynomolgus monkeys with rAT-G preparations differing in sialic acid content suggested that CL increases with a decrease in sialic acid content (Study d-██-263). In the above single-dose administration studies (Studies d-██-262 and d-██-264), sialic acid content was ██ mol/mol protein for rAT-G and ██ to ██ mol/mol protein for pAT preparations, and these differences in sialic acid content are considered to have affected CL.

Table 3-5. Pharmacokinetic parameters of rAT-G[*]

Concentration of rAT-G in plasma					
	$t_{1/2\alpha}$ (h)	$t_{1/2\beta}$ (h)	$AUC_{0-\infty}$ (µg·h/mL)	CL (mL/h/kg)	V_1 (mL/kg)
rAT-G (N = 5)	2.43 ± 0.82	31.0 ± 4.2	15000 ± 1500	1.92 ± 0.19	37.6 ± 4.5
Plasma AT activity					
	$t_{1/2\alpha}$ (h)	$t_{1/2\beta}$ (h)	$AUC_{0-\infty}$ (IU·h/mL)	CL (mL/h/kg)	V_1 (mL/kg)
rAT-G (N = 5)	3.27 ± 0.54	38.9 ± 2.7	107 ± 7	1.87 ± 0.12	41.2 ± 4.8

N, number of animals; $t_{1/2\alpha}$, alpha-phase half-life; $t_{1/2\beta}$, beta-phase half-life; $AUC_{0-\infty}$, AUC from 0 hours to infinity; CL, clearance; V_1, volume of distribution of the central compartment
*: The dose (200 IU/kg) of rAT-G is equivalent to ■ mg/kg.

Table 3-6. Pharmacokinetic parameters of pAT preparations[*]

Drug concentrations in plasma					
	$t_{1/2\alpha}$ (h)	$t_{1/2\beta}$ (h)	$AUC_{0-\infty}$ (µg·h/mL)	CL (mL/h/kg)	V_1 (mL/kg)
Neuart (N = 5)	2.24 ± 1.07	30.2 ± 5.2	18000 ± 2500	1.54 ± 0.21	34.4 ± 2.6
Anthrobin (N = 5)	3.16 ± 0.99	29.9 ± 4.3	21000 ± 3400	1.62 ± 0.28	36.4 ± 4.8
Nonthron (N = 5)	2.91 ± 0.84	32.6 ± 3.5	18900 ± 1500	1.40 ± 0.11	35.6 ± 2.5
Plasma AT activity					
	$t_{1/2\alpha}$ (h)	$t_{1/2\beta}$ (h)	$AUC_{0-\infty}$ (IU·h/mL)	CL (mL/h/kg)	V_1 (mL/kg)
Neuart (N = 5)	2.02 ± 0.32	31.0 ± 3.9	140 ± 19	1.45 ± 0.22	35.9 ± 4.1
Anthrobin (N = 5)	3.90 ± 1.59	42.9 ± 13.2	153 ± 21	1.33 ± 0.17	39.0 ± 4.4
Nonthron (N = 5)	2.88 ± 1.09	37.3 ± 4.9	144 ± 13	1.40 ± 0.12	40.3 ± 3.5

N, number of animals; $t_{1/2\alpha}$, alpha-phase half-life; $t_{1/2\beta}$, beta-phase half-life; $AUC_{0-\infty}$, AUC from 0 hours to infinity; CL, clearance; V_1, volume of distribution of the central compartment
*: The dose (200 IU/kg) of each drug is equivalent to the following: Neuart, ■ mg/kg, Anthrobin, ■ mg/kg; and Nonthron, ■ mg/kg.

In the reproductive and developmental toxicity study in rats (Study ■27), the concentration of rAT-G in plasma at 24 hours post-dose on gestation day 17 was lower than that on gestation day 7. In order to evaluate pregnancy-related changes in the pharmacokinetics of rAT-G, a single dose of 2000 IU/kg of rAT-G was intravenously administered to rats in the non-pregnant, 7-day pregnant, and 17-day pregnant groups (n = 4/group). The concentration of rAT-G in plasma was measured before dosing and at 5 time points between 0.5 hours and 72 hours post-dose (Study d-■-081). Based on these measurements, pharmacokinetic parameters were calculated and analyzed to compare the pharmacokinetics of rAT-G in in the non-pregnant, 7-day pregnant, and 17-day pregnant rat groups (Table 3-7). The plasma concentrations of rAT-G at 24 hours and 48 hours post-dose (C_{24h} and C_{48h}) in the 17-day pregnant rat group were lower than those in the non-pregnant rat group and the 7-day pregnant rat group. The CL calculated from the plasma concentrations of rAT-G in the 17-day pregnant rat group was approximately 1.2-fold those in the non-pregnant rat group and the 7-day pregnant rat group. Taking into account that hypercoagulability generally occurs in late pregnancy (*J Am Assoc Lab Anim Sci.* 2009;48:272-8), the applicant considered that hypercoagulability contributed to an increase in the consumption of AT, an anticoagulant factor, thus resulting in accelerated elimination of rAT-G.

Table 3-7. Pharmacokinetic parameters based on concentrations of rAT-G in plasma

	C_{24h} (µg/mL)	C_{48h} (µg/mL)	$t_{1/2\alpha}$ (h)	$t_{1/2\beta}$ (h)	$AUC_{0-\infty}$ (µg·h/mL)	CL (mL/h/kg)	V_1 (mL/kg)
Non-pregnant (N = 4)	350 ± 85	58.9 ± 18.1	3.15 ± 0.76	10.0 ± 0.9	46400 ± 8200	6.43 ± 1.30	45.9 ± 8.3
7-day pregnant (N = 4)	348 ± 106	58.6 ± 14.6	2.52 ± 0.23	9.5 ± 0.3	43600 ± 4700	6.72 ± 0.71	44.8 ± 2.6
17-day pregnant (N = 4)	199 ± 18	20.9 ± 4.7	3.39 ± 0.97	9.2 ± 2.4	37100 ± 1500	7.82 ± 0.30	50.4 ± 5.6

N, number of animals; C_{24h}, concentration of rAT-G in plasma at 24 hours post-dose; C_{48h}, concentration of rAT-G in plasma at 48 hours post-dose; $t_{1/2\alpha}$, alpha-phase half-life; $t_{1/2\beta}$, beta-phase half-life; $AUC_{0-\infty}$, AUC from 0 hours to infinity; CL, clearance; V_1, volume of distribution of the central compartment

3.(ii).A.(1).2) Repeated dose studies

(a) Repeated dose study in rats (4.2.3.2-2, Study ███████08)

Rats (n = 4/sex/group) received repeated intravenous doses of rAT-G at 500 or 2000 IU/kg/day once daily for 2 weeks. The concentration of rAT-G in plasma was measured before dosing and at 3 time points between 10 minutes and 24 hours post-dose on Days 1 and 14. The plasma concentrations of rAT-G was multiplied by specific activity to determine plasma AT activity (Table 3-8). The AUC from 0 to 24 hours (AUC_{0-24}) on Days 1 and 14 increased in proportion to dose, and the AUC_{0-24} on Day 14 increased to approximately 1.3- to 1.7-fold that on Day 1. The applicant considered that the increase in AUC_{0-24} on Day 14 was similar to the accumulation rate (1.23) calculated using the $t_{1/2\beta}$ determined based on the plasma concentration of rAT-G in the single-dose study in rats (d-██-081). There were no clear sex differences in AUC_{0-24}.

Table 3-8. AUC_{0-24} based on plasma AT activity calculated using concentrations of rAT-G in plasma (IU·h/mL)

Dose (IU/kg)	Day 1		Day 14	
	Males (N = 4)	Females (N = 4)	Males (N = 4)	Males (N = 4)
500	50 ± 5	49 ± 7	83 ± 8	64 ± 3
2000	227 ± 10	202 ± 11	329 ± 32	274 ± 19

N: number of rats

(b) Repeated dose study in cynomolgus monkeys (4.2.3.2-6, Study ███████07)

Cynomolgus monkeys (n = 5/sex/group) received repeated intravenous doses of rAT-G at 100, 300, or 1000 IU/kg/day once daily for 2 weeks. The concentration of rAT-G in plasma was measured before dosing and at 3 time points between 30 minutes and 24 hours post-dose. The plasma concentration of rAT-G was multiplied by specific activity to determine plasma AT activity (Table 3-9). The AUC_{0-24} on Days 1 and 14 increased in proportion to dose, and the AUC_{0-24} on Day 14 increased to approximately 2.2- to 2.6-fold that on Day 1. The applicant considered that the increase in AUC_{0-24} on Day 14 was similar to the accumulation rate (2.41) calculated using the $t_{1/2\beta}$ determined based on the plasma concentration of rAT-G in the single-dose study in cynomolgus monkeys (Study d-██-262). There were no clear sex differences in AUC_{0-24}.

Table 3-9. AUC_{0-24} based on plasma AT activity calculated using concentrations of rAT-G in plasma (IU·h/mL)

Dose (IU/kg)	Day 1		Day 14	
	Males (N = 5)	Females (N = 5)	Males (N = 5)	Males (N = 5)
100	25 ± 2	25 ± 1	61 ± 3	58 ± 9
300	81 ± 18	74 ± 18	189 ± 14	152 ± 8
1000	233 ± 29	226 ± 17	611 ± 113	550 ± 75

N: number of cynomolgus monkeys

3.(ii).A.(2) Distribution (4.2.2.3-1, Study ███ 94)

Rats (n = 3/sex/time point) received a single intravenous dose of 100 IU/kg of ^{125}I-rAT-G, and radioactivity levels in plasma and tissue were measured at 0.5, 8, 24, and 48 hours post-dose. Radioactivity was highest in plasma at any time point and relatively high in the spleen, bone marrow, kidneys, adrenal gland, liver, lungs, stomach, and ovary among tissues excluding blood and plasma. Whole-body activity decreased over time at ≥8 hours post-dose. Based on these results, the applicant considers that rAT-G does not accumulate in specific tissues.

A single dose of 100 IU/kg of ^{125}I-rAT-G was administered intravenously to pregnant rats on gestation day 7 or 17 (n = 3/time point), and radioactivity levels in plasma and tissue were measured at 0.5, 8, 24, and 48 hours post-dose. Radioactivity was highest at 0.5 hours post-dose in the embryo in the 7-day gestation group (25 ± 11 μg eq./g,) and at 8 hours post-dose in the fetus in the 17-day gestation group (4 ± 1 μg eq./g). The radioactivity levels were lower than those in plasma in dams at the same time points (292 ± 5 at 0.5 hours post-dose and 70 ± 2 μg eq./mL at 8 hours post-dose). On the basis of these results, the applicant considers that maternal-fetal transfer of rAT-G is unlikely.

3.(ii).A.(3) Metabolism

No studies on the metabolism of rAT-G have been conducted.

3.(ii).A.(4) Excretion (4.2.2.3-1, Study ███ 94)

Excretion of rAT-G was examined only in milk.

A single dose of 100 IU/kg of ^{125}I-rAT-G was administered intravenously to rats on postpartum day 11 (n = 3), and radioactivity levels in plasma and milk were measured at 0.5, 8, 24, and 48 hours post-dose. At all the time points, radioactivity was detected in milk. On the basis of the results, the applicant considers that rAT-G may be excreted in milk.

3.(ii).B Outline of the review by PMDA

The results of the excretion study suggested the potential excretion of rAT-G in milk. PMDA considers that this information should be appropriately communicated to healthcare professionals through the package insert and other materials. Meanwhile, since rAT-G is a protein product, it is acceptable that the applicant conducted no studies on metabolism or excretion of the drug other than the study on excretion in milk based on "Preclinical Safety Evaluation of Biotechnology-Derived Pharmaceuticals" (Notification No. 0323-1 from the Evaluation and Licensing Division, Pharmaceutical and Food Safety Bureau [PFSD/ELD], MHLW, dated March 23, 2012) ("the ICH-S6 [R1] guideline").

3.(iii) Summary of toxicology studies

3.(iii).A Summary of the submitted data

The results of repeat-dose toxicity, reproductive and developmental toxicity, and local tolerance studies were submitted as the evaluation data on toxicity of rAT-G. The reference data submitted include the

results of a preliminary study for a repeated-dose toxicity study in cynomolgus monkeys.

3.(iii).A.(1)   Single-dose toxicity (4.2.3.2-2, Study ████08; 4.2.3.2-4, Study d-██-224 [reference data])

Single-dose toxicity was evaluated in a 2-week repeated-dose toxicity study in rats (Study ████08) and a preliminary study for a repeated-dose toxicity study in cynomolgus monkeys (Study d-██-224).

Following 2-week once-daily repeated intravenous doses of rAT-G at 500 or 2000 IU/kg (28-fold higher than the clinical dose) to rats (n = 10 or 15/sex/group), no deaths or deterioration of the clinical condition caused by the drug was observed in any group. Following repeated doses of rAT-G at 180 IU/kg once daily for 2 weeks or at 1800 IU/kg (25-fold the clinical dose) once daily for 1 week to cynomolgus monkeys (males, n = 2/group), 2 of the 2 monkeys in the 1800 IU/kg group were sacrificed in extremis due to deteriorated clinical condition on the day following the seventh dose. Taking into account that systemic hemorrhage and abnormal hematological changes were observed in these 2 monkeys, the applicant considers the deterioration of their clinical condition to be attributable to hemorrhage caused by the exaggerated pharmacological action of rAT-G. In the 180 IU/kg group, no drug-related deaths or deterioration of clinical condition was observed.

3.(iii).A.(2)   Repeat-dose toxicity

3.(iii).A.(2).1   Two-week intravenous studies in rats (4.2.3.2-2, Study ████08; 4.2.3.2-3, Study ████32)

rAT-G was intravenously administered once daily for 2 weeks at 0 (vehicle), 500, or 2000 IU/kg to rats (n = 15/sex/group for the 0 and 2000 IU/kg groups; n = 10/sex/group for the 500 IU/kg group) in Study ████08. rAT-G was intravenously administered once daily for 2 weeks at 0 (vehicle) or 2000 IU/kg to rats (n = 15/sex/group) in Study ████32. No deaths or no adverse effects related to rAT-G were observed in either study, and the no observed adverse effect level (NOAEL) in these studies was considered to be 2000 IU/kg.

3.(iii).A.(2).2   Two-week intravenous study in cynomolgus monkeys (4.2.3.2-6, Study ████07)

Following 2-week once-daily repeated intravenous doses of rAT-G at 0 (vehicle), 100, 300, or 1000 IU/kg (14-fold the clinical dose) to cynomolgus monkeys (n = 5/sex/group), no deaths were observed in any group. Anemia or hemorrhage was noted in all the groups receiving rAT-G. Decreases in red blood cell count, hemoglobin, and hematocrit, an increase in reticulocyte count, and prolongation of PT were observed in the ≥300 IU/kg groups, and oral mucosal pallor, decreases in body weight and food consumption, systemic hemorrhage, and prolongation of APTT were observed in the 1000 IU/kg group. These clinical findings were considered to be attributable to hemorrhage or resulting severe anemia caused by the anticoagulant action of rAT-G. All the clinical findings observed in the ≤300 IU/kg groups were mild changes with no effects on clinical condition or food consumption, and were considered to be of little toxicological significance. Therefore, the NOAEL of rAT-G was considered to be 300 IU/kg.

3.(iii).A.(3)   Genotoxicity

In accordance with the ICH-S6 (R1) guideline, no genotoxicity studies have been conducted for rAT-G, a protein product containing recombinant AT as the active ingredient.

3.(iii).A.(4)   Carcinogenicity

Based on the "Revision of the Guidelines for Carcinogenicity Studies of Drugs" (PFSB/ELD Notification No.1127001 dated November 27, 2008) ("the ICH-S1C [R2] guideline"), no carcinogenicity studies have been conducted for rAT-G, which is considered unlikely to be carcinogenic for the following reasons:

• The proposed duration of treatment with rAT-G in clinical practice is as short as approximately 5 days.

• The structure of rAT-G is similar to that of endogenous antithrombin [see "2.A.(1).5) Characterization"].

3.(iii).A.(5)   Reproductive and developmental toxicity

3.(ii).A.(5).1)   Fertility and early embryonic development to implantation study in female rats (4.2.3.5.1-1, Study ▮▮▮29)

rAT-G was intravenously administered to rats (females, n = 20/group) at 0 (vehicle), 200, 600, or 2000 IU/kg once daily from 2 weeks prior to mating until gestation day 7. The maternal animals showed no treatment-related general toxicity or effect of rAT-G on maternal reproductive function or early embryonic development.

3.(ii).A.(5).2)   Fertility and early embryonic development to implantation study in male rats (4.2.3.5.1-2, Study ▮▮▮02)

rAT-G was intravenously administered to rats (males, n = 20/group) at 0 (vehicle), 500, or 2000 IU/kg once daily from 15 days prior to mating until the end of the mating period (up to 13 days). The paternal animals showed no treatment-related general toxicity or effect of rAT-G on paternal reproductive function or early embryonic development.

3.(ii).A.(5).3)   Embryo-fetal development study in rats (4.2.3.5.2-1, Study ▮▮▮27)

rAT-G was intravenously administered to rats (females, n = 20/group) at 0 (vehicle), 200, 600, or 2000 IU/kg once daily from gestation day 7 to gestation day 17. There was no treatment-related effect on dams or on embryo-fetal development.

3.(ii).A.(5).4)   Embryo-fetal development study in rabbits (4.2.3.5.2-4, Study ▮▮▮28)

rAT-G was intravenously administered to rabbits (females, n = 18 to 20/group) at 0 (vehicle), 50, 150, or 500 IU/kg once daily (6.9-fold the clinical dose) from gestation day 6 to gestation day 18. No treatment-related deaths were observed in dams in any group. Partial gray discoloration of the placenta (necrosis of the cotyledons) was observed on the chorionic side in 6 of 140 live fetuses in the vehicle

group, 3 of 110 live fetuses in the 50 IU/kg groups, 5 of 130 live fetuses in the 150 IU/kg groups, and 18 of 119 live fetuses in the 500 IU/kg groups. While this clinical sign, which was also observed in the vehicle group, was considered to be a spontaneous change, it occurred more frequently in the 500 IU/kg group compared to other groups. Since fetal development was not affected regardless of the discoloration, the applicant considered that this change has no effects on fetal development. In the 500 IU/kg group, vaginal bleeding and intrauterine blood collection were observed in 1 and 8 dams, respectively, out of 19 dams, and low fetal body weight and delayed ossification of fingers were observed in fetuses. In 1 of 119 fetuses in the 500 IU/kg group, meningocele, open eyelid, and omphalocele were observed. Considering that these abnormalities occurred in 1 fetus only and that their incidences were within the range of historical control data or the values expected from literature-based information, the applicant regards them as contingent events. Based on the above results, the NOAEL in dams was determined to be 500 IU/kg for maternal general toxicity and 150 IU/kg for reproduction and fertility toxicity and embryo-fetal development, taking account of the effects on dams and fetuses observed in the 500 IU/kg group.

3.(ii).A.(5).5)   Rat study on pre- and postnatal development, including maternal function (2.4.3.5.3-3, Study ▉▉24)

rAT-G was intravenously administered to rats (females, n = 20/group) at 0 (vehicle), 200, 600, or 2000 IU/kg once daily from gestation day 7 to postpartum day 21. There was no treatment-related effect on the profile of maternal general and reproductive toxicity and offspring developmental and reproductive toxicity.

3.(ii).A.(6)   Local tolerance (4.2.3.6-1, Study ▉▉32)

Rabbits (males; n = 6/group) received a single intravenous dose of saline or rAT-G 3 mL (equivalent to 57 IU/kg; approximately 0.8-fold the clinical dose) or a single paravenous dose of rAT-G 0.3 mL or saline. In the intravenous dosing group, no rAT-G-related changes were observed at the injection site. In the paravenous dosing group, hemorrhage and inflammatory cell infiltration were observed at the injection site, both of which were considered to be changes related to the dosing technique. These results were interpreted as demonstrating that local tolerance of rAT-G in clinical practice is not of particular concern.

3.(iii).B   Outline of the review by PMDA

PMDA's view:

Concerning the deterioration of clinical condition observed in 2 rats that were sacrificed in extremis in Study d-▉-224 on single-dose toxicity, the applicant attributed it to the exaggerated pharmacological action of rAT-G, which is understandable. Other systemic toxicity and local reactions observed were not of particular toxicological concern. It is acceptable that the applicant has conducted no genotoxicity or carcinogenicity studies based on the ICH-S6 (R1) and ICH-S1C (R2) guidelines. For the reproductive and developmental toxicity studies, PMDA conducted the review described in the sections below.

3.(iii).B.(1)   Findings observed in embryo-fetal development study in rabbits

The applicant's explanation regarding vaginal bleeding and intrauterine blood collection in dams and a delay in fetal development observed in an embryo-fetal development study in rabbits (Study █ 28):
The pharmacological action of rAT-G is anticoagulation. Therefore, these findings are considered attributable to excessive anticoagulation resulting from administration of rAT-G to rabbits with normal coagulability. Similar findings were observed with Neuart, an approved pAT preparation (*Clinical report.* 1986;20:757-64), and therefore the findings in question are considered to be common changes due to overdose of AT preparations. Although rAT-G has never been used in pregnant women, no safety problems have been reported with the use of existing pAT preparations and recombinant AT preparations in pregnant women. Based on the above discussion, the risk of safety issues in pregnant women is considered to be similar between rAT-G and pAT preparations, and there would be no major concern. However, given (1) the dose-dependent increase in the frequency and seriousness of the changes observed in rabbits treated with rAT-G, and (2) the possibility that the estimated human exposure at the proposed therapeutic dose (AUC_{0-24}, 42.8 IU·h/mL) may exceed the exposure in rabbits at the NOAEL in Study █ 28 (AUC_{0-24}, 30.9 IU·h/mL), a risk affecting human embryo-fetal development cannot be ruled out. Therefore, the following advice is to be provided in the package insert: rAT-G can be used in women who are or may be pregnant only when the expected therapeutic benefits outweigh the possible risks.

PMDA accepted the applicant's explanation.

4. Clinical data

4.(i)   Summary of biopharmaceutic studies and associated analytical methods

4.(i).A   Summary of the submitted data

Plasma antithrombin (AT) activity was measured by the synthetic substrate method.

4.(ii)   Summary of clinical pharmacology studies

4.(ii).A   Summary of the submitted data

As the evaluation data for clinical pharmacology of Antithrombin Gamma (Genetical Recombination) ("rAT-G"), the applicant submitted the results of a Japanese phase I single-dose study (5.3.3.1-1, Study 3357-█ 01), Japanese pharmacokinetic comparative study (5.3.1.2-1, Study 3357-002), and Japanese bioequivalence study (5.3.1.2-2, Study 3357-003) in healthy adults; 3 Japanese phase III studies in patients with disseminated intravascular coagulation (DIC) (5.3.5.1-1, Study 3357-004; 5.3.5.2-1, Study 3357-005; and 5.3.5.2-2, Study 3357-006); and a foreign phase I study in patients with congenital antithrombin deficiency (CAD) (5.3.3.2-1, Study 3357-EU-001). In the subsequent sections, values are presented as mean ± standard deviation, unless otherwise noted. Each clinical study (Study 3357-XXXX) is referred to as Study XXXX hereinafter.

4.(ii).A.(1)   Studies using human biomaterials

No studies have been conducted using human biomaterials.

4.(ii).A.(2)   Healthy adults

4.(ii).A.(2).1)   Japanese phase I single-dose study (5.3.3.1-1, Study 3357-█01 [████ ███ to ████ ███])

In this Japanese study, 24 healthy adult male subjects aged 20 to 44 years received a single intravenous dose of rAT-G at 5, 20, 60, or 120 IU/kg (n = 6/group), and plasma AT activity was measured before dosing and at 16 time points between 0 and 168 hours post-dose. In order to eliminate any potential effect of baseline plasma AT activity in subjects, pharmacokinetic parameters were calculated by subtracting the pre-dose value of plasma AT activity (0.904 ± 0.147 IU/mL) from the post-dose value of plasma AT activity in individual subjects. When the difference between pre- and post-dose plasma AT activity was <0.300 IU/mL, the relevant data were regarded as below the lower limit of quantitation. In the 5 IU/kg group, the mean plasma AT activity was below the lower limit quantitation at all the post-dose time points.

The applicant's explanation on the maximum plasma AT activity (C_{max}) and AUC from 0 hours to the last time point where AT activity was detected (AUC_{0-t}) calculated based on plasma AT activity (Table 4-1) as follows:

Analysis of the linearity for C_{max} and AUC_{0-t} revealed that C_{max} and AUC_{0-t} increased in proportion to dose in the range of 20 to 120 IU/kg and 60 to 120 IU/kg, respectively. AUC_{0-t} was not linear in the dose range of 20 to 120 IU/kg probably because the mean plasma AT activity in the 20 IU/kg group was below the lower limit of quantitation at 3 hours post-dose and all the subsequent time points, which may have caused the underestimation of AUC_{0-t} in this dose group.

Table 4-1. Pharmacokinetic parameters based on plasma AT activity

Dose (IU/kg)	C_{max} (IU/mL)	ACU (IU·h/mL)
20 (N = 6)	0.36 ± 0.06	1.17 ± 1.56
60 (N = 6)	1.08 ± 0.06	25.86 ± 11.26
120 (N = 6)	2.07 ± 0.22	77.58 ± 21.27

N, number of subjects; C_{max}, maximum plasma AT activity; AUC_{0-t}, AUC from 0 hours to the last time point where AT activity was detected

4.(ii).A.(2).2)   Japanese pharmacokinetic comparative study (5.3.1.2-1, Study 3357-002 [████ ███ to ████ ███])

In this Japanese study, 20 healthy adult male subjects aged 20 to 44 years received multiple intravenous doses of rAT-G or a plasma-derived AT (pAT) preparation (Neuart for intravenous injection; hereinafter "Neuart") (n = 10/group) at 60 IU/kg once daily for 3 days. Plasma AT activity was measured before dosing and at 3 time points between 1 and 10 hours post-dose on Days 1 and 2 and before dosing and at 10 time points between 1 and 169 hours post-dose on Day 3. In order to eliminate any potential effect of baseline plasma AT activity in subjects, pharmacokinetic parameters were calculated by subtracting the pre-dose value of plasma AT activity (1.08 ± 0.10 IU/mL) from the post-dose value of plasma AT activity in individual subjects. The primary endpoints were C_{max} after the third dose ($C_{max,\ 3rd}$) and AUC from the start of the third dose (i.e., 48 hours after the start of the first dose) to the first time point where plasma AT activity fell below the lower limit of quantitation in any subject (AUC_{48-t}). The results are

shown in Table 4-2. This study data demonstrated that AUC_{48-t} was smaller in the rAT-G group than in the pAT preparation group.

Table 4-2. $C_{max, 3rd}$ and AUC_{48-t} based on plasma AT activity

Parameter	rAT-G (N = 10)	pAT preparation (N = 10)	Ratio (%)* [90% CI]
$C_{max, 3rd}$ (IU/mL)	1.67 ± 0.31	1.77 ± 0.16	92.6 [82.5-104.0]
AUC_{48-t} (IU·h/mL)	58.44 ± 11.72	91.44 ± 9.58	63.2 [56.0-71.3]

N, number of subjects; $C_{max, 3rd}$, maximum plasma AT activity after the third dose; AUC_{48-t}, AUC from the start of the third dose (i.e., 48 hours after the start of the first dosing) to the first time point where plasma AT activity fell below the lower limit of quantitation in any subject
*: Ratio of rAT-G to pAT preparation calculated from the difference between the mean logarithmic values of $C_{max, 3rd}$ and AUC_{48-t}

In a Japanese bioequivalence study (Study 003),72 IU/kg of rAT-G and 60 IU/kg of the pAT preparation were selected. The applicant provided the following justification for these doses:
When simulated using the pharmacokinetic data obtained from Study 002, changes in plasma AT activity after multiple doses of 72 IU/kg of rAT-G given once daily for 3 days were predicted to be similar to those after multiple doses of 60 IU/kg of the pAT preparation given once daily for 3 days.

4.(ii).A.(2).3) Japanese bioequivalence study (5.3.1.2-2, Study 3357-003 [█████ ██ to ███ █])

In this Japanese study, 42 healthy adult male subjects aged 20 to 44 years received multiple intravenous doses of rAT-G at 72 IU/kg or a pAT preparation (Neuart) at 60 IU/kg (n = 21/group) once daily for 3 days. Plasma AT activity was measured before dosing and at 3 time points between 1 and 10 hours post-dose on Days 1 and 2 and before dosing and at 10 time points between 1 and 169 hours post-dose on Day 3. In order to eliminate any potential effect of baseline plasma AT activity in subjects, pharmacokinetic parameters were calculated by subtracting the pre-dose value of plasma AT activity (1.01 ± 0.09 IU/mL) from the post-dose value of plasma AT activity in individual subjects. The primary endpoints, $C_{max, 3rd}$ and AUC_{48-t}, are shown in Table 4-3. The 90% confidence intervals (CI) of the ratios (rAT-G/ the pAT preparation) calculated from the difference in the mean of logarithmically transformed values of $C_{max, 3rd}$ and AUC_{48-t} between the two drugs were within the pre-specified acceptable range for bioequivalence (80%-125%). The applicant considered that these results demonstrated the bioequivalence between 72 IU/kg of rAT-G and 60 IU/kg of the pAT preparation and that, therefore, the rAT-G dose that is 1.2-fold the dose of the pAT preparation on the basis of IU/kg body weight can be expected to be equivalent to pAT preparations in terms of efficacy and duration of action.

Table 4-3. $C_{max, 3rd}$ and AUC_{48-t} based on plasma AT activity

Parameter	rAT-G 72 IU/kg (N = 21)	pAT preparation 60 IU/kg (N = 20)	Ratio (%)* [90% CI]
$C_{max, 3rd}$ (IU/mL)	2.08 ± 0.17	1.98 ± 0.23	105.7 [100.3-111.3]
AUC_{48-t} (IU·h/mL)	98.71 ± 13.94	98.99 ± 19.82	100.5 [91.5-110.4]

N, number of subjects; $C_{max, 3rd}$, maximum plasma AT activity after the third dosing; AUC_{48-t}, AUC from the start of the third dose (i.e., 48 hours after the start of the first dose) to the first time point where plasma AT activity fell below the lower limit of quantitation in any subject
*: Ratios (rAT-G/ the pAT preparation) calculated from the difference in the mean of logarithmically transformed values of $C_{max, 3rd}$ and AUC_{48-t} between the two drugs

4.(ii).A.(3)   Patients

4.(ii).A.(3).1   Foreign phase I study (5.3.3.2-1, Study 3357-EU-001 [July 2009 to December 2010])

In this foreign study, 16 patients with CAD aged ≥18 years received a single intravenous dose of rAT-G at 50 IU/kg, and plasma AT activity was measured before dosing and at 13 time points between 15 minutes and 168 hours post-dose. The C_{max} and AUC_{0-t} for plasma AT activity were 1.074 ± 0.148 IU/mL and 18.282 ± 10.187 IU·h/mL, respectively, and the percent increase of AT activity ([C_{max}/dose] × 100; hereinafter "incremental recovery") was 2.14 ± 0.29%/IU/kg.

The applicant's explanation:

The incremental recovery in patients with CAD in Study EU-001 was 2.14 ± 0.29%/IU/kg, which was not markedly different from that in healthy adults in Study ██01 (1.82 ± 0.28%/IU/kg for the 20 IU/kg group, 1.80 ± 0.10%/IU/kg for the 60 IU/kg group, and 1.72 ± 0.18%/IU/kg for the 120 IU/kg group). Therefore, plasma AT activity is likely to be supplemented by rAT-G in patients with CAD as in healthy adults.

4.(ii).A.(3).2   Japanese phase III study (5.3.5.1-1, Study 3357-004 [June 2011 to May 2013])

A Japanese study was conducted in patients aged ≥20 years with plasma AT activity of ≤70%, who had DIC directly caused by infection and diagnosed according to the acute DIC diagnostic criteria established by the DIC special committee of the Japanese Association for Acute Medicine ("the JAAM acute DIC criteria") (*Journal of Japanese Association for Acute Medicine*. 2005;16:188-202). A total of 222 patients with DIC received multiple intravenous doses of rAT-G at 36 IU/kg (n = 110) or a pAT preparation (Neuart) at 30 IU/kg (n = 112) once daily for 5 days. Plasma AT activity was measured in these patients from Day 1 through Day 5 and on Day 6 (Table 4-4).

4.(ii).A.(3).3   Japanese phase III study (5.3.5.2-1, Study 3357-005 [December 2011 to April 2013])

A Japanese study was conducted in patients aged ≥20 years with plasma AT activity of ≤70%, who had a diagnosis of DIC or suspected DIC according to the diagnostic criteria for DIC established by the Study Group on Blood Coagulation Abnormalities under the Specified Disease Program developed by the former Ministry of Health and Welfare (hereinafter, the Ministry of Health, Labour and Welfare [MHLW] DIC criteria") (*Study Report 1987 by Study Group on Blood Coagulation Abnormalities, Specified Disease Program of the Ministry of Health and Welfare*. 1988;37-41). A total of 15 patients received multiple intravenous doses of rAT-G at 36 IU/kg once daily for 5 days. Plasma AT activity was

measured in these patients before dosing from Day 1 through Day 5 and on Day 6 (Table 4-4).

4.(ii).A.(3).4)   Japanese phase III study (5.3.5.2-2, Study 3357-006 [February 2012 to October 2012])

Multiple intravenous doses of rAT-G were administered at 36 IU/kg once daily for 5 days to 5 Japanese patients aged ≥20 years with plasma AT activity of ≤70%, who had a diagnosis of DIC according to the JAAM acute DIC criteria. Plasma AT activity was measured in these patients before dosing from Day 1 through Day 5 and on Day 6 (Table 4-4).

According to the applicant, analyses of the results of Studies 004, 005, and 006 revealed that 5-day multiple-dose administration of rAT-G increased plasma AT activity and that its increase by rAT-G was similar to that by pAT preparations.

Table 4-4. Plasma AT activity in patients with DIC

Study		Before dosing		Day 6	
		N	Plasma AT activity (%)	N	Plasma AT activity (%)
004	rAT-G	107	54.2 ± 11.5	96	107.3 ± 26.1
	pAT preparation	111	53.2 ± 14.1	101	115.0 ± 25.3
005 (rAT-G)		15	54.2 ± 14.1	13	97.5 ± 19.6
006 (rAT-G)		5	53.4 ± 11.1	5	96.8 ± 27.0

N, number of subjects at each time point excluding those from whom data of plasma AT activity were not available

4.(ii).A.(4)   Drug-drug interactions

Drug-drug interactions have not been evaluated.

4.(ii).B   Outline of the review by PMDA

PMDA's view:

The submitted data has demonstrated that rAT-G, when administered at a dose that is 1.2-fold the dose of pAT preparations, is able to maintain plasma AT activity in the same range that pAT preparations do.

4.(iii)   Summary of clinical efficacy and safety

4.(iii).A   Summary of the submitted data

As the evaluation data on clinical efficacy and safety, the applicant submitted the results of 3 Japanese phase I studies in healthy adults, 1 foreign phase I study in patients with CAD, and 3 Japanese phase III studies in patients with DIC. A list of these clinical studies is shown in Table 4-5.

Table 4-5. List of clinical studies on efficacy and safety

Region	Study identifier	Phase	Population	Number of subjects	Summary of dosage and administration method
Evaluation data					
Japan	3357-■01	I	Healthy male adults (aged 20 to 44 years)	24 in total; 6 each in the 5, 20, 60, and 120 IU/kg groups	Single-dose administration of rAT-G 5, 20, 60, or 120 IU/kg
Japan	3357-002	I	Healthy male adults (aged 20 to 44 years)	20 in total; 10 each in the rAT-G and pAT preparation groups	rAT-G 60 IU/kg or pAT preparation 60 IU/kg once daily for 3 days
Japan	3357-003	I	Healthy male adults (aged 20 to 44 years)	42 in total; 21 each in the rAT-G and pAT preparation groups	rAT-G 72 IU/kg or pAT preparation 60 IU/kg once daily for 3 days
Foreign	3357-EU-001	I	Patients with CAD (aged ≥18 years)	16 in total	Single-dose administration of rAT-G 50 IU/kg
Japan	3357-004	III	Patients with DIC that was directly caused by infection and diagnosed according to the JAAM acute DIC criteria (aged ≥20 years)	221 in total; 109 in the rAT-G group and 112 in the pAT preparation group	rAT-G 36 IU/kg or pAT preparation 30 IU/kg once daily for 5 days
Japan	3357-005	III	Patients with DIC diagnosed according to the MHLW DIC criteria (aged ≥20 years)	15 in total	rAT-G 36 IU/kg once daily for 5 days
Japan	3357-006	III	Patients with DIC diagnosed according to the JAAM acute DIC criteria (aged ≥20 years)	5 in total	rAT-G 36 IU/kg once daily for 5 days

The individual clinical studies are summarized below. Each study (3357-XXXX) is referred to as Study XXXX hereinafter.

4.(iii).A.(1)　Japanese phase I single-dose study (5.3.3.1-1, Study 3357-■01 [■■■ to ■■])

An open-label dose-escalation study was conducted at 1 site in Japan to investigate the safety and pharmacokinetics of a single dose of rAT-G in healthy adult male subjects aged 20 to 44 years (target sample size, 24 subjects [6 per group]).

Subjects received a single dose of rAT-G at 5, 20, 60, or 120 IU/kg as an intravenous infusion over 1 hour.

All of the 24 treated subjects were included in the safety analysis population and the pharmacokinetic analysis population. The results of pharmacokinetic analysis in this study are presented in "4.(ii) Summary of clinical pharmacology studies."

Safety was analyzed. During the study period, 15 adverse events were reported in 11 of 24 subjects (45.8%) across the groups. In the 5 IU/kg group, 8 adverse events were reported in 6 of 6 subjects (100%), and they consisted of 6 events of thrombin-antithrombin III complex increased, and 1 event each of retinal haemorrhage and diarrhoea. In the 20 IU/kg group, 5 adverse events were reported in 4 of 6 subjects (67%), and they consisted of 3 events of thrombin-antithrombin III complex increased, and 1 event each of diarrhoea and feeling hot. In the 120 IU/kg group, 2 adverse events were reported in 1 of 6 subjects (16.7%), and they consisted of 1 event each of diarrhoea and thrombin-antithrombin III

complex increased.

Four adverse events for which a causal relationship to the study drug could not be ruled out (i.e., adverse drug reactions) occurred in 3 subjects. These adverse drug reactions consisted of 1 event (diarrhoea) in 1 subject in the 5 IU/kg group, 2 events (diarrhea [1] and feeling hot [1]) in 1 subject in the 20 IU/kg group, and 1 event (diarrhoea) in 1 subject in the 120 IU/kg group; all of these events resolved. "Thrombin-antithrombin III complex increased" was reported in 6 of 6 subjects, 3 of 6 subjects, and 1 of 6 subjects in the 5, 20, and 120 IU/kg groups, respectively. This event was considered attributable to blood sampling technique, and its causal relationship to the study drug was ruled out.

No deaths or serious adverse events were reported.

4.(iii).A.(2) Japanese pharmacokinetic comparative study (5.3.1.2-1, Study 3357-002 [██████ to ██████ ██████])

A randomized, open-label, parallel-group, comparative study was conducted at 1 site in Japan to investigate the pharmacokinetics and safety of multiple doses of rAT-G in healthy adult male subjects aged 20 to 44 years (target sample size, 20 subjects [10 per group]).

Subjects received rAT-G or a pAT preparation (Neuart) at 60 IU/kg as an intravenous infusion over 1 hour once daily for 3 days.

All of the 20 treated subjects were included in the safety analysis population and the pharmacokinetic analysis population. The results of pharmacokinetic analysis in this study are presented in "4.(ii) Summary of clinical pharmacology studies."

Safety was analyzed. During the study period, in the rAT-G group, 4 adverse events (1 event each of alanine aminotransferase increased, aspartate aminotransferase increased, eosinophil percentage increased, and erythema) were reported in 2 of 10 subjects (20%). In the pAT preparation group, 2 adverse events (1 event each of diarrhoea and nasopharyngitis) were reported in 2 of 10 subjects (20%).

There were 2 adverse drug reactions (1 event each of alanine aminotransferase increased and aspartate aminotransferase increased) reported in 1 subject in the rAT-G group. Their outcomes were reported as "recovered/resolved."

No deaths or serious adverse events were reported.

4.(iii).A.(3) Japanese bioequivalence study (5.3.1.2-2, Study 3357-003 [██████ ██████ to ██████ ██████])

A randomized, open-label, parallel-group, comparative study was conducted at 1 site in Japan to investigate the bioequivalence and safety of multiple doses of rAT-G versus pAT preparation in healthy

adult male subjects aged 20 to 44 years (target sample size, 42 subjects [21 per group]).

Subjects received rAT-G at 72 IU/kg or a pAT preparation (Neuart) at 60 IU/kg as an intravenous infusion over 1 hour once daily for 3 days.

All of the 42 treated subjects were included in the safety analysis population and the pharmacokinetic analysis population. The results of pharmacokinetic analysis in this study are presented in "4.(ii) Summary of clinical pharmacology studies."

Safety was analyzed. During the study period, 20 adverse events were reported in 13 of 21 subjects (61.9%) in the rAT-G group, and 6 adverse events were reported in 4 of 21 subjects (19.0%) in the pAT preparation group. The adverse events reported in ≥2 subjects in the rAT-G group were 3 events of C-reactive protein increased in 3 subjects, 3 events of activated partial thromboplastin time prolonged in 2 subjects, and 2 events each of pericoronitis, aspartate aminotransferase increased, and headache in 2 subjects. In the pAT preparation group, no adverse events were reported in ≥2 subjects.

Six adverse drug reactions (3 events of activated partial thromboplastin time prolonged in 2 subjects, and 1 event each of alanine aminotransferase increased, C-reactive protein increased, and rash in 1 subject) were reported in 5 subjects in the rAT-G group. The outcomes of the events were all "recovered/resolved."

No deaths or serious adverse events were reported.

4.(iii).A.(4)   Foreign phase I study (5.3.3.2-1, Study 3357-EU-001 [July 2009 to December 2010])
An open-label, non-controlled study was conducted at 16 sites in 5 countries (Germany, Sweden, UK, France, and Italy) to investigate the pharmacokinetics, safety, and tolerability of a single dose of rAT-G in CAD patients aged ≥18 years (target sample size, 16 subjects).

Subjects received a single dose of rAT-G at 50 IU/kg administered intravenously over 15 minutes.

All of the 16 treated subjects were included in the safety analysis population and the pharmacokinetic analysis population. The results of pharmacokinetic analysis in this study are presented in "4.(ii) Summary of clinical pharmacology studies."

Safety was assessed. During the study period, 21 adverse events were reported in 11 of 16 subjects (68.8%). The adverse events reported in ≥2 subjects were 3 events of nasopharyngitis in 2 subjects, and 2 events each of headache, pruritus, and rash in 2 subjects each.

Eleven adverse drug reactions (2 events each of pruritus and rash in 2 subjects each, and 1 event each of pinguecula, abdominal pain, diarrhoea, nausea, aspartate aminotransferase increased, rash pruritic,

and hot flush in 1 subject each) were reported in 5 subjects. Their outcomes were reported as "recovered/resolved."

No deaths or serious adverse events were reported.

4.(iii).A.(5) Japanese phase III study (5.3.5.1-1, Study 3357-004 [June 2011 to May 2013])
A randomized, open-label, parallel-group, comparative study was conducted at 30 sites in Japan to investigate the efficacy and safety of rAT-G in DIC patients aged ≥20 years (target sample size, 200 subjects [100 per group]). The study enrolled patients with plasma AT activity of ≥50% to ≤70% (which was changed to "≤70%" after the start of the study so that the efficacy and safety of rAT-G could also be evaluated in patients with plasma AT activity of <50%) who met the American College of Chest Physicians and the Society of Critical Care Medicine (ACCP/SCCM) criteria for sepsis and who had DIC directly caused by infection and diagnosed according to the JAAM acute DIC criteria (DIC score of ≥4).

Subjects received rAT-G at 36 IU/kg or a pAT preparation (Neuart) at 30 IU/kg in combination with heparin or heparinoid by intravenous infusion once daily for 5 days. However, single agent use of rAT-G or pAT preparation was prescribed in subjects predisposed to bleeding because of concomitant use of heparin or heparinoid.

All the 222 subjects assigned to treatment groups (110 subjects to the rAT-G group, and 112 subjects to the pAT preparation group) were included in the intent-to-treat (ITT) population, which served as the primary efficacy population. The safety analysis population included 221 subjects (108 in the rAT-G group and 113 in the pAT preparation group), excluding 1 subject in the rAT-G group who had been withdrawn from the study before starting study treatment. One subject assigned to the rAT-G group who had received the pAT preparation by mistake was handled as a subject in the rAT-G group in the ITT population and as a subject in the pAT preparation group in the safety analysis population.

The proportion [95% confidence interval] of subjects who were free of DIC (defined as a DIC score of <4 when calculated according to the JAAM acute DIC criteria) on Day 6 (or at the time of discontinuation for subjects who discontinued the study earlier than Day 6), the primary efficacy endpoint, was 56.4% ([46.6%-65.8%], 62 of 110 subjects) in the rAT-G group and 52.7% ([43.0%-62.2%], 59 of 112 subjects) in the pAT preparation group.

Safety was analyzed. During the study period, 410 adverse events were reported in 89 of 108 subjects (82.4%) in the rAT-G group and 494 adverse events in 99 of 113 subjects (87.6%) in the pAT preparation group. The adverse events occurring at an incidence of ≥5% in either group are shown in Table 4-6.

Table 4-6. Adverse events occurring at an incidence of ≥5% in either group
(Safety analysis population)

	rAT-G (N = 108)		pAT preparation (N = 113)	
	Number of subjects (%)	Number of events	Number of subjects (%)	Number of events
Erythema	18 (16.7)	19	12 (10.6)	14
Diarrhoea	17 (15.7)	18	6 (5.3)	6
Decubitus ulcer	12 (11.1)	13	10 (8.8)	12
Pneumonia	10 (9.3)	10	6 (5.3)	6
Vomiting	9 (8.3)	11	7 (6.2)	8
Constipation	9 (8.3)	9	4 (3.5)	5
Insomnia	9 (8.3)	9	10 (8.8)	10
Anaemia	7 (6.5)	7	8 (7.1)	9
Sepsis	6 (5.6)	6	8 (7.1)	8
Hypokalaemia	5 (4.6)	7	9 (8.0)	9
Hypernatraemia	4 (3.7)	4	6 (5.3)	6
Pleural effusion	4 (3.7)	4	8 (7.1)	8
Nausea	3 (2.8)	4	7 (6.2)	7
Hepatic function abnormal	3 (2.8)	3	6 (5.3)	6
Blood bilirubin increased	3 (2.8)	3	6 (5.3)	6
Delirium	3 (2.8)	3	6 (5.3)	6
Skin exfoliation	3 (2.8)	3	11 (9.7)	17

N: number of subjects

Adverse drug reactions were reported in 24 subjects (44 events) in the rAT-G group and 16 subjects (19 events) in the pAT preparation group.

Adverse events leading to death reported in the rAT-G group consisted of 11 events (4 events of sepsis, and 1 event each of cardiac arrest, thyrotoxic crisis, large intestine perforation, septic shock, Still's disease adult onset, lung neoplasm malignant, and pneumonia aspiration) in 10 subjects. Adverse events leading to death reported in the pAT preparation group consisted of 23 events (7 events of sepsis, 2 events each of condition aggravated, multi-organ failure, septic shock, endocarditis, pneumonia, and respiratory failure, and 1 event each of disease progression, hepatic function abnormal, pneumonia aspiration, and asphyxia) in 22 subjects. A causal relationship to the study drug could not be ruled out for adverse events reported in 2 subjects in the rAT-G group (1 event of septic shock in 1 subject, and 1 event each of sepsis and Still's disease adult onset in 1 subject). These treatment-related events were the progression of underlying or concurrent conditions. Non-fatal serious adverse events reported in the rAT-G group consisted of 16 events (2 events each of gastrointestinal haemorrhage, sepsis, and haemorrhagic transformation stroke, and 1 event each of cardiac arrest, cellulitis, endocarditis, pneumonia, arthritis bacterial, cholecystitis infective, wound dehiscence, small intestine carcinoma, cerebral infarction, and haemothorax) in 14 subjects. Non-fatal serious adverse events reported in the pAT preparation group consisted of 7 events (1 event each of large intestine perforation, cholecystitis acute, peritonitis, cerebral infarction, cerebral haemorrhage, haemorrhagic cerebral infarction, and haemothorax) in 7 subjects. Non-fatal serious adverse events leading to discontinuation consisted of 1 event of haemorrhagic transformation stroke in the rAT-G group and 1 event of peritonitis in the pAT preparation group. A causal relationship to the study drug could not be ruled out for 3 events (1 event each of gastrointestinal haemorrhage, cerebral infarction, and haemorrhagic transformation stroke [a different event from the one leading to discontinuation]) in 3 subjects in the rAT-G group and 1 event (haemothorax) in 1 subject in the pAT preparation group. The outcomes of all these non-fatal serious adverse events were reported as "recovered/resolved" or "recovering/resolving," except for the

following events: 1 event of haemorrhagic transformation stroke (assessed as an adverse drug reaction) and small intestine carcinoma in the rAT-G group were "recovered/resolved with sequelae" and "not recovered/not resolved," respectively; and haemorrhagic cerebral infarction and cerebral infarction in the pAT preparation group were "recovered/resolved with sequelae" and "not recovered/not resolved," respectively.

4.(iii).A.(6) Japanese phase III study (5.3.5.2-1, Study 3357-005 [December 2011 to April 2013])
An open-label, uncontrolled study was conducted at 8 site in Japan to investigate the efficacy and safety of rAT-G in combination with heparin or heparinoid in DIC patients aged ≥20 years (target sample size, ≥10 subjects). The study enrolled patients with plasma AT activity of ≥50% to ≤70% (which was changed to "≤70%" after the start of the study so that the efficacy and safety of rAT-G could also be evaluated in patients with plasma AT activity of <50%) who were diagnosed with DIC or suspected DIC according to the MHLW DIC criteria (DIC score of ≥3 for patients in the leukemia group [leukemia or similar disease, aplastic anemia, or markedly decreased megakaryocytes with severe thrombocytopenia due to treatment with antitumor agents or other causes] and a DIC score of ≥6 for patients in the non-leukemia group).

Subjects received rAT-G at 36 IU/kg once daily, in combination with heparin or heparinoid, by intravenous infusion for 5 days.

All the 15 subjects treated (9 in the leukemia group and 6 in the non-leukemia group) were included in the safety analysis population and the efficacy analysis population. Underlying diseases in the leukemia group were acute myeloid leukemia, myelodysplastic syndrome, and non-Hodgkin's lymphoma in 2 subjects each, and multiple myeloma, aplastic anemia, and leukemic-phase myeloproliferative disorder (polycythemia vera) in 1 subject each. Underlying diseases in the non-leukemia group were non-Hodgkin's lymphoma in 2 subjects and hemophilia B, non-small cell lung cancer, autoimmune hemolytic anemia, and HIV infection in 1 subject each.

The proportion [95% confidence interval] of subjects who were free of DIC (defined as a DIC score of <3 for subjects having leukemia as an underlying disease and a DIC score of <6 for non-leukemic subjects when calculated according to the MHLW DIC criteria) on Day 6 (or at the time of discontinuation for subjects who discontinued the study earlier than Day 6), the primary efficacy endpoint, was 40.0% ([16.3%-67.7%]; 6 of 15 subjects).

Safety was analyzed. During the study period, 63 adverse events were reported in 12 of 15 subjects (80.0%). The adverse events reported in ≥2 subjects consisted of 4 events of febrile neutropenia in 4 subjects, 3 events of generalised oedema in 3 subjects, 3 events of diarrhoea in 2 subjects, and 2 events each of thrombocytopenia, constipation, vomiting, sepsis, delirium, pruritus, and skin ulcer in 2 subjects each. No adverse drug reactions were reported.

Four adverse events leading to death (1 event each of multi-organ failure, sepsis, non-small cell lung cancer, and acute respiratory failure) were reported in 4 subjects. Two non-fatal serious adverse events (1 event each of sepsis and lung infiltration) occurred in 2 subjects. The outcomes of the sepsis and lung infiltration were reported as "recovered/resolved" and "recovering/resolving," respectively.

4.(iii).A.(7) Japanese phase III study (5.3.5.2-2, Study 3357-006 [February 2012 to October 2012])

An open-label, uncontrolled study was conducted at 6 sites in Japan to investigate the efficacy and safety of rAT-G in combination with heparin or heparinoid in DIC patients aged ≥20 years (target sample size, ≥10 subjects). The study enrolled patients with plasma AT activity of ≥50% to ≤70% (which was changed to "≤70%" after the start of the study so that the efficacy and safety of rAT-G could also be evaluated in patients with plasma AT activity of <50%) who were diagnosed with DIC according to the JAAM acute DIC criteria (DIC score of ≥4).

Subjects received rAT-G at 36 IU/kg once daily, in combination with heparin or heparinoid, by intravenous infusion for 5 days.

All the 5 subjects treated were included in the safety analysis population and the efficacy analysis population. The underlying causes were infection and heat illness in 2 subjects each and acute pancreatitis in 1 subject.

The proportion [95% confidence interval] of subjects who were free of DIC (defined as a DIC score of <4 according to the JAAM acute DIC criteria) on Day 6 (or at the time of discontinuation for subjects who discontinued the study earlier than Day 6), the primary efficacy endpoint, was 60.0% ([14.7%-94.7%]; 3 of 5 subjects).

Safety was analyzed. During the study period, 25 adverse events were reported in 3 of 5 subjects (60.0%). The adverse events reported in ≥2 subjects were 4 events of atrial fibrillation in 2 subjects. No adverse drug reactions were reported.

Two adverse events leading to death (1 event each of multi-organ failure and acute respiratory failure) were reported in 2 subjects. One non-fatal serious adverse event (cardiac arrest) occurred in 1 subject, but its outcome was reported as "recovered/resolved."

4.(iii).B   Outline of the review by PMDA
4.(iii).B.(1)   Premise for Review
rAT-G has been developed as a recombinant AT preparation that can be an alternative to pAT preparations used for the treatment of CAD and DIC. The equivalence of rAT-G and pAT preparations in terms of efficacy and duration of action was assessed in a bioequivalence study based on plasma AT activity (Study 003). Then the efficacy of rAT-G for its main indication, i.e., the treatment of patients

with DIC, was evaluated in 3 studies (Studies 004, 005, and 006) at a dose which, based on the results of Study 003, could be expected to maintain plasma AT activity in the same range that pAT preparations do (1.2-fold those of pAT preparations).

PMDA's view:

As with endogenous AT, rAT-G binds to thrombin and serine proteases such as activated coagulation factor X, thereby inhibiting the coagulation system and suppressing thrombus formation. Therefore, the efficacy of rAT-G is considered to be evaluable by a pharmacokinetic comparison based on plasma AT activity between rAT-G and approved pAT preparations with established efficacy. The efficacy of rAT-G was also evaluated in terms of recovery from DIC and other outcomes in rAT-G-treated patients with DIC accompanied by a decrease in plasma AT activity, and the safety was evaluated with a focus on the occurrence of adverse events.

4.(iii).B.(2) Efficacy
4.(iii).B.(2).1) Comparison with existing AT preparations
In Study 003, a comparison was made between 72 IU/kg of rAT-G and 60 IU/kg of the pAT preparation in terms of pharmacokinetic parameters based on plasma AT activity [see "4.(ii) Summary of clinical pharmacology studies"]. PMDA considers that rAT-G, when administered at a dose that is 1.2-fold that of pAT preparations, is likely to maintain plasma AT activity in the same range as pAT preparations do, as shown by the results of Study 003, and therefore shows promise as a therapeutic agent having anticoagulant action.

4.(iii).B.(2).2) Efficacy in patients with DIC
The applicant's explanation on the efficacy of rAT-G in patients with DIC:
When comparing rAT-G and pAT preparations, the efficacy evaluation would be improved by restricting underlying causes of DIC. Accordingly, Study 004 enrolled patients with DIC directly caused by infection diagnosed according to the JAAM acute DIC criteria. Study 004 was conducted in an unblinded manner for the following reasons: (1) rAT-G and pAT preparations are all lyophilized preparations, and thus physical masking would cause inconvenience during reconstitution; (2) study drug would possibly be identifiable by the types of lyophilization cakes; (3) separate blinding of preparation and administration of the injection solution would increase the required staff and time; (4) infection-induced DIC is an urgent severe condition that requires prompt treatment; and (5) given the current state of emergency and intensive medicine in Japan, blinding of Study 004 was considered to be impractical. However, the applicant considered that this unblinded study allowed evaluation of recovery from DIC because DIC was scored according to the JAAM acute DIC criteria, which consists solely of objective indicators. The results of Study 004 showed that the proportions of subjects who were free of DIC on Day 6 and of those who were alive on Day 28 were similar between the rAT-G group and pAT preparation groups. Moreover, the protocol of Study 004 prescribed single-agent use of rAT-G and pAT preparations in subjects predisposed to bleeding because of concomitant use of heparin or heparinoid, but a subgroup analysis based on the presence or absence of concomitant heparin/heparinoid showed similar results

between the rAT-G group and pAT preparation groups regardless of concomitant heparin/heparinoid use (Table 4-7). Study 005 enrolled patients with DIC diagnosed according to the MHLW DIC diagnostic criteria, while Study 006 enrolled patients with DIC diagnosed according to the JAAM acute DIC criteria without restricting the underlying cause to infection. The proportion of subjects who recovered from DIC was smaller in Study 005 than in other studies (Table 4-7). This was considered attributable to the differences not only in the method of DIC score calculation but also in the severity of the underlying condition, because Study 005 enrolled many patients with hematopoietic malignancies. The above study results are interpreted as demonstrating the efficacy of rAT-G in the treatment of DIC accompanied by a decrease in plasma AT activity.

Table 4-7. Results of clinical studies in patients with DIC

		rAT-G		pAT preparation	
		Ratio of recovery from DIC [1]	Survival ratio [2]	Ratio of recovery from DIC [1]	Survival ratio [2]
Study 004	Overall	56.4% (62/110)	87.3% (96/110)	52.7% (59/112)	77.7% (87/112)
	Concomitant use of heparin/heparinoid	56.3% (18/32)	81.3% (26/32)	64.5% (20/31)	80.6% (25/31)
	Without concomitant use of heparin/heparinoid	57.1% (44/77)	90.9% (70/77)	48.1% (39/81)	76.5% (62/81)
Study 005 (Concomitant use of heparin/heparinoid in all subjects)		40.0% (6/15)	73.3% (11/15)		
Study 006 (Concomitant use of heparin/heparinoid in all subjects)		60.0% (3/5)	60.0% (3/5)		

1: Proportion of subjects who were free of DIC on Day 6 (or at the time of discontinuation for subjects withdrawn from the study earlier than Day 6); Recovery from DIC was defined as a DIC score of <4 calculated according to the JAAM acute DIC criteria in Studies 004 and 006 and as a DIC score of <3 for the leukemia group and DIC score of <6 for the non-leukemia group calculated according to the MHLW DIC criteria in Study 005.
2: Proportion of subjects who were alive on Day 28

The efficacy of rAT-G in the treatment of emergency cases such as obstetric or surgical DIC has not been evaluated in the clinical studies. Obstetric DIC and surgical DIC have been reported to account for 1.3% to 4.0% and 2% to 4.6%, respectively, of all cases of DIC in Japan (*Current Review of Clinical Pathology*. 2011;147:123-9; *New Strategies for Treatment of DIC*. 2004;103-8). Given that approximately 73,000 people per year are estimated to have DIC in Japan (*Study Report 1999 by Committee on Blood Coagulation Abnormalities, Study Group on Blood Diseases, Specified Disease Program of the Ministry of Health and Welfare*. 1999;57-64), the number of patients who have obstetric or surgical DIC is calculated to be approximately 900 to 3000 and 1500 to 3400, respectively, per year. For the treatment of obstetric DIC, prompt vaginal delivery, Caesarean section, or blood transfusion may be selected instead of AT preparations depending on maternal or fetal condition (*All about DIC – Basics and Diagnosis*. 2010;1580-3). For the treatment of surgical DIC associated with underlying causes such as trauma and burns, transfusion of platelets or fresh frozen plasma is likely to be selected in the acute-phase with increased fibrinolytic activity, and AT preparations may be used if the resulting hypercoagulability causes organ damage (*Journal of Clinical and Experimental Medicine*. 2011;238:46-55; *Hemostasis and Thrombosis in Clinical Practice*. 2013;196-9; *Biomedicine and Therapeutics*. 2007;41:33-6).

Due to the extremely small number of patients with obstetric or surgical DIC eligible for clinical studies

and due to the variability of the dosing timing and regimen of AT preparations in these patients depending on their condition, the applicant considered it difficult to evaluate the efficacy of rAT-G in the treatment of obstetric or surgical DIC in clinical studies. However, irrespective of underlying conditions, a generalized, persistent, hypercoagulable state is a common feature of DIC. Therefore, similarly to existing pAT preparations, rAT-G is considered to be effective against DIC regardless of the underlying cause including cases that have not been treated with rAT-G.

PMDA's view:

Infection is one of the common underlying causes of DIC, and sepsis-induced DIC is known as typical hypofibrinolytic (hypercoagulation) DIC (*Japanese Journal of Thrombosis and Hemostasis*. 2009;20:77-113). Therefore, enrollment of patients with DIC directly caused by infection in Study 004 is acceptable. However, possible measures to blind Study 004, including the design of the clinical study, should have been thoroughly explored to minimize potential bias in comparing rAT-G and pAT preparations in this study. Nevertheless, the results of Study 004 using a randomized unblinded comparative design allow the efficacy of rAT-G to be evaluated because the primary efficacy endpoint of this study was recovery from DIC, which was based on the DIC score calculated according to the JAAM acute DIC criteria consisting solely of objective indicators. In addition, patient outcome (survival or death) was also included in the evaluation because this secondary efficacy endpoint is considered to be an objective measure, and DIC has a poor survival prognosis as evidenced by a reported mortality of 35.6% according to the JAAM acute DIC criteria (*Clin Appl Thromb Hemost*. 2005;11: 71-6).

In the "Expert consensus based on the evidence for the treatment of disseminated intravascular coagulation due to infection" (*Japanese Journal of Thrombosis and Hemostasis*. 2009;20:77-113), AT preparations are listed with an overall recommendation score of B_1 (recommendation based on moderate evidence of efficacy, or strong evidence of efficacy but with limited clinical usefulness) which is the highest recommendation for an individual drug class. Also the superior efficacy of AT preparations over placebo is supported by the results reported in several randomized placebo-controlled studies, albeit differing in dosage and administration from the clinical studies of rAT-G (*Chest*, 1993;103:882-8; *J Thromb Haemost*. 2006;4:90-7; *Crit Care*. 2013;17:R297). In Study 003, rAT-G has been shown to maintain plasma AT activity in the same range as pAT preparations do, and in Study 004, the proportions of subjects who were free of DIC on Day 6 and of those who were alive on Day 28 were similar between the rAT-G group and pAT preparation groups. Therefore, rAT-G are expected to be a therapeutically effective agent for the treatment of DIC accompanied by a decrease in plasma AT activity.

DIC can be caused by many underlying conditions, including not only sepsis and various severe infections but also acute leukemia, solid cancer, trauma, burns, heat stroke, surgery, abdominal aortic aneurysm, giant hemangioma, collagenosis (associated with vasculitis, in particular), obstetric complications (placental abruption, amniotic fluid embolism), fulminant hepatitis, acute pancreatitis, and rhabdomyolysis (*Japanese Journal of Thrombosis and Hemostasis*. 2008;19:344-7). Therefore, PMDA considers it difficult to include exhaustively all underlying causes in clinical studies. Clinical

studies have not been conducted in patients with obstetric and surgical DIC, but given the scarcity of these patients, there are clearly difficulties associated with the conduct of such studies. Irrespective of underlying conditions, excessive coagulability is a common feature of DIC, and hence it is deduced that the mechanism of action of rAT-G shows promise in terms of efficacy against DIC regardless of the underlying cause. Therefore, PMDA concluded that, like pAT preparations, rAT-G can be indicated for the treatment of DIC without specifying underlying conditions. However, rAT-G has so far been used in only a limited number of patients with DIC, and post-marketing information including the types of underlying conditions should be collected.

4.(iii).B.(2).3)　Efficacy in patients with CAD
The applicant's explanation on the efficacy of rAT-G in patients with CAD:
It has been reported that there were 119 patients with CAD in Japan and that 39% of these patients were treated with AT preparations (Results of Survey for the Required Quantity of Blood Coagulation Factor Products 2013. *Blood Products Research Organization Report*. 2013;138:9-16). Patients with CAD do not need AT supplementation on a daily basis but do so only in the acute phase of thromboembolism or when the patients have any risk factors for thromboembolism, such as trauma, surgery, and pregnancy/delivery (*Japanese Journal of Thrombosis and Hemostasis*. 2001;12:74-7). Therefore, there seems to be only an extremely limited number of CAD patients who need to be treated with AT preparations in Japan. In Europe and the US, similarly to Japan, patients with CAD are not supplemented with AT on a daily basis, which suggests the scarcity of CAD patients requiring treatment with AT preparations. Under these circumstances, the applicant considered it difficult to conduct clinical studies that could evaluate the efficacy of rAT-G in this patient population. CAD is a pathological condition where the patient is constantly deficient in AT. This is apparently similar to DIC. Study 004 in patients with DIC demonstrated the equivalence of rAT-G and pAT preparations in terms of efficacy, and thus rAT-G is also expected to be effective in the treatment of CAD.

PMDA's view:
There are only a limited number of CAD patients, among whom an even smaller number of patients would be in a critical condition with thromboembolic symptoms requiring treatment with AT preparations. Therefore, there are clearly difficulties associated with investigating the efficacy of rAT-G by conducting clinical studies in CAD patients. Study 003 in healthy adults demonstrated that rAT-G maintains plasma AT activity in the same range as pAT preparations do, and Study EU-001 in patients with CAD showed that rAT-G can be effectively used for AT supplementation in patients with CAD as in healthy adults. Meanwhile, according to some reports published in Japan and overseas, patients with CAD were treated effectively with pAT preparations or recombinant AT preparations in Japan and overseas (*Medical Consultation and New Remedies*. 1985;22:2139-45; *Blood*. 1990;75:33-9; *Thromb Haemost*. 2008;99:616-22). In addition to the above findings, the mechanism of action of rAT-G, like pAT preparations, also shows promise in terms of efficacy against thrombophilia due to CAD. However, taking into account that no clinical studies have been conducted to evaluate the efficacy of rAT-G in treating thrombophilia in patients with CAD, as much information as possible on the use results of the

drug in this patient population should be collected in the post-marketing setting.

4.(iii).B.(3)   Safety

The applicant's explanation on the safety of rAT-G:

The following adverse events leading to death were reported in clinical studies in patients with DIC: 11 events in 10 subjects in the rAT-G group and 23 events in 22 subjects in the pAT preparation group in Study 004; 4 events in 4 subjects in Study 005; and 2 events in 2 subjects in Study 006. These events included 3 events (septic shock in 1 subject; and sepsis and Still's disease adult onset in 1 subject) reported in 2 subjects in the rAT-G group in Study 004, for which a causal relationship to the study drug could not be ruled out, but all of them were manifestations of the progression of underlying or concurrent conditions. The non-fatal serious adverse events reported were as follows: 16 events in 14 subjects in the rAT-G group and 7 events in 7 subjects in the pAT preparation group in Study 004, 2 events in 2 subjects in Study 005, and 1 event in 1 subject in Study 006. These events included 3 events (1 event each of gastrointestinal haemorrhage, cerebral infarction, and haemorrhagic transformation stroke) in 3 subjects in the rAT-G group and 1 event (haemothorax) in 1 subject in the pAT preparation group in Study 004, for which a causal relationship to the study drug could not be ruled out. No serious adverse events were reported in Study EU-001 in patients with CAD.

The pharmacological action of AT is anticoagulation. Therefore, bleeding-related adverse events were analyzed. There were no marked differences in the incidence and profile of hemorrhagic adverse events and adverse drug reactions between the rAT-G group and the pAT preparation group in Studies 004, 005, and 006 in patients with DIC (Table 4-8). In Study EU-001 in patients with CAD, 1 hemorrhagic adverse event (vessel puncture site haematoma) was reported in 1 of 16 subjects (6.3%), but its causal relationship to the study drug was ruled out.

Taking into account that shock and anaphylaxis were reported in the pAT preparation group, hypersensitivity-related adverse events were analyzed. In Studies 004, 005, and 006 in patients with DIC, the incidence of hypersensitivity-related adverse events/adverse drug reactions reported in the rAT-G group was higher than that in the pAT preparation group (Table 4-9). However, there were no events suggesting shock or anaphylaxis, and 4 events (2 events of drug eruption, 1 event each of erythema and toxic skin eruption) classified as adverse drug reactions in the rAT-G group were all mild or moderate in severity with the outcome being reported as "recovered/resolved." In Study EU-001 in patients with CAD, 4 adverse drug reactions associated with hypersensitivity (2 events each of pruritus and rash) were reported in 3 of 16 subjects (18.8%). Though a causal relationship to the study drug could not be ruled out for these 4 events, they were all mild and the outcome was reported as "recovered/resolved."

Table 4-8. Bleeding-related adverse events and/or adverse drug reactions reported in at least 2 subjects in either group in Studies 004, 005, and 006 (Safety analysis population)

	Adverse events				Adverse drug reactions			
	rAT-G (N = 128)		pAT preparation (N = 113)		rAT-G (N = 128)		pAT preparation (N = 113)	
	N (%)	Number of events	N (%)	Number of events	N (%)	Number of events	N (%)	Number of events
Overall	31 (24.2)	46	32 (28.3)	47	10 (7.8)	11	9 (8.0)	11
Gastrointestinal haemorrhage	4 (3.1)	4	2 (1.8)	2	2 (1.6)	2	0 (0.0)	0
Haemorrhage subcutaneous	3 (2.3)	5	4 (3.5)	4	2 (1.6)	3	2 (1.8)	2
Post procedural haemorrhage	3 (2.3)	3	2 (1.8)	2	0 (0.0)	0	0 (0.0)	0
Vessel puncture site haemorrhage	3 (2.3)	3	2 (1.8)	2	0 (0.0)	0	0 (0.0)	0
Haematuria	2 (1.6)	2	3 (2.7)	3	1 (0.8)	1	2 (1.8)	2
Mouth haemorrhage	2 (1.6)	2	3 (2.7)	3	0 (0.0)	0	0 (0.0)	0
Wound haemorrhage	2 (1.6)	2	1 (0.9)	1	0 (0.0)	0	1 (0.9)	1
Haemorrhagic transformation stroke	2 (1.6)	2	1 (0.9)	1	1 (0.8)	1	1 (0.9)	1
Faeces discoloured	2 (1.6)	2	0 (0.0)	0	0 (0.0)	0	0 (0.0)	0
Wound secretion	2 (1.6)	2	0 (0.0)	0	0 (0.0)	0	0 (0.0)	0
Haemoptysis	1 (0.8)	1	2 (1.8)	2	0 (0.0)	0	1 (0.9)	1
Haemothorax	1 (0.8)	1	2 (1.8)	2	0 (0.0)	0	1 (0.9)	1
Haemorrhage	1 (0.8)	1	2 (1.8)	2	0 (0.0)	0	0 (0.0)	0
Epistaxis	0 (0.0)	0	4 (3.5)	5	0 (0.0)	0	1 (0.9)	1
Puncture site haemorrhage	0 (0.0)	0	2 (1.8)	2	0 (0.0)	0	1 (0.9)	1

N: number of subjects

Table 4-9. Hypersensitivity-related adverse events and/or adverse drug reactions reported in at least 2 subjects in either group in Studies 004, 005, and 006 (Safety analysis population)

	Adverse events				Adverse drug reactions			
	rAT-G (N = 128)		pAT preparation (N = 113)		rAT-G (N = 128)		pAT preparation (N = 113)	
	N (%)	Number of events	N (%)	Number of events	N (%)	Number of events	N (%)	Number of events
Overall	32 (25.0)	39	16 (14.2)	20	4 (3.1)	4	0 (0.0)	0
Erythema	19 (14.8)	20	12 (10.6)	14	1 (0.8)	1	0 (0.0)	0
Drug eruption	5 (3.9)	5	3 (2.7)	3	2 (1.6)	2	0 (0.0)	0
Pruritus	4 (3.1)	5	0 (0.0)	0	0 (0.0)	0	0 (0.0)	0
Urticaria	2 (1.6)	2	0 (0.0)	0	0 (0.0)	0	0 (0.0)	0
Generalised erythema	1 (0.8)	1	2 (1.8)	2	0 (0.0)	0	0 (0.0)	0

N: number of subjects

PMDA's view:

DIC is a serious condition with a potentially fatal outcome, and serious adverse events including deaths occurred during clinical studies of rAT-G in patients with DIC. A causal relationship could not be ruled out in 2 cases, both of which are considered to have been related to the progression of underlying or concurrent conditions. On the other hand, all hypersensitivity-related adverse drug reactions were non-serious with the outcome being reported as "recovered/resolved," and there were no other adverse events of clinical concern. rAT-G is thus considered to be a tolerable therapy with no specific safety concerns in comparison with existing pAT preparations.

4.(iii).B.(4)   Clinical positioning and indications

PMDA's view on the clinical positioning and indications of rAT-G:

rAT-G has been shown by Study 003 to maintain plasma AT activity in the same range as pAT preparations do, and Study 004 showed that the proportion of patients who recovered from DIC was similar to that achieved with pAT preparations. Therefore, rAT-G is a drug positioned similarly to pAT preparations. Accordingly, it is acceptable to select the indications of rAT-G as "thrombophilia due to congenital antithrombin deficiency" and "disseminated intravascular coagulation (DIC) accompanied by a decrease in antithrombin," similarly to those of existing pAT preparations. A term "antithrombin

43

III" used in the indications of existing pAT preparations is now called "antithrombin," and the indications of rAT-G should be revised accordingly.

Despite viral inactivation of plasma and measures taken to reduce the risk of transmitting viral infections during the manufacturing process, plasma-derived products are still associated with an unquantified risk of viral transmission. Meanwhile, rAT-G is manufactured using no human- or animal-derived materials other than CHO cells, and is therefore considered to carry a lower risk of viral transmission.

4.(iii).B.(5)   Dosage and administration
Study 003 demonstrated that rAT-G, when administered at a dose that is 1.2-fold the dose of pAT preparations, maintains plasma AT activity in the same range as pAT preparations do. Based on the above results, the proposed dosage and administration of rAT-G is as follows (Table 4-10):

Table 4-10. Proposed dosage and administration of rAT-G and approved dosage and administration of pAT preparations

rAT-G	pAT preparations
The product should be reconstituted with the supplied water for injection, and the reconstituted solution should be administered either by slow intravenous injection or by intravenous infusion.	The product should be reconstituted with the supplied water for injection, and the reconstituted solution should be administered either by slow intravenous injection or by intravenous infusion.
1. Thrombophilia due to congenital antithrombin III deficiency The daily dosage is 1200 to 3600 IU (or 24 to 72 IU/kg). The dose may be reduced according to the patient's age or condition.	1. Thrombophilia due to congenital antithrombin III deficiency The daily dosage is 1000 to 3000 IU (or 20 to 60 IU/kg). The dose may be reduced according to the patient's age or condition.
2. Disseminated intravascular coagulation (DIC) accompanied by a decrease in antithrombin III The usual daily dosage for adult patients showing a decrease in antithrombin III to ≤70% of the normal level is 1800 IU (or 36 IU/kg) administered in combination with continuous intravenous infusion of heparin. In case of emergency such as obstetric or surgical DIC, the dosage is 48 to 72 IU/kg administered once daily. The dose may be adjusted according to the patient's age, body weight, or condition.	2. Disseminated intravascular coagulation (DIC) accompanied by a decrease in antithrombin III The usual daily dosage for adult patients showing a decrease in antithrombin III to ≤70% of the normal level is 1500IU (or 30 IU/kg) administered in combination with continuous intravenous infusion of heparin. In case of emergency such as obstetric or surgical DIC, the dosage is 40 to 60 IU/kg administered once daily. The dose may be adjusted according to the patient's age, body weight, or condition.

PMDA considered that the dosage and administration statement should be modified taking into account the dosage regimens selected in the clinical studies of rAT-G, and instructed the applicant to reconsider the statement accordingly.

4.(iii).B.(5).1)   Dosage and administration for treatment of CAD
The applicant's explanation on the proposed dosage and administration for the treatment of CAD:
The dosage of approved pAT preparations for the treatment of CAD includes both a non-weight-based dose of 1000 to 3000 IU/day and a weight-based dose of 20 to 60 IU/kg. Therefore, the dose of rAT-G was set at 1200 to 3600 IU/day (or 24 to 72   IU/kg). Meanwhile, a weight-based dose (50 IU/kg) was used in Study EU-001 in patients with CAD, and there is no clear evidence to recommend fixed-dose administration of AT preparations to patients with CAD. Although the dosage and administration of pAT preparations does not specify any daily dose frequency, it has been reported that patients with CAD should start treatment with a pAT preparation at a dose that allows AT activity to be 100% to 120%, and, thereafter, receive an adequate dose of the pAT preparation every 24 hours in order to maintain plasma

44

AT activity within the normal range (*Medical Consultation and New Remedies*. 1985;22:2139-46). After taking the above into account, and given that there is no clear evidence for recommending divided administration of a daily dose, the applicant has decided to change the dosage of rAT-G for the treatment of CAD to 24 to 72 IU/kg once daily.

The statement about dose reduction according to the patient's age or condition will be deleted, because a dose reduction outside the range of 24 to 72 IU/kg is not recommended. However, given that plasma AT activity has been reported to increase at different rates depending on individual patients or gestation length (*Jn J Obstet Gynecol Neonatal Hematol*. 1995;5:74-81), is the applicant considered it desirable to adjust the dose of rAT-G according to plasma AT activity monitored during treatment. Hence, the following statement will be included: "The dose may be adjusted within the range of 24 to 72 IU/kg according to AT activity monitored during treatment."

PMDA's view:

The applicant's proposal that the dose and administration of rAT-G for the treatment of CAD will be set at 24 to 72 IU/kg once daily is acceptable. Adequate advice on the monitoring of plasma AT activity should be provided in the package insert and other materials.

4.(iii).B.(5).2)   Dosage and administration in treatment of DIC

The applicant's explanation on the dose and administration selected for the treatment of DIC:

The dosage of approved pAT preparations for the treatment of DIC includes both a non-weight-based dose of 1500 IU/day and a weight-based dose of 30 IU/kg. Therefore, the dose of rAT-G was set at 1800 IU/day (or 36 IU/kg). However, the dosage will be changed to 36 IU/kg because a weight-based dose (36 IU/kg) was used in Studies 004, 005, and 006 in patients with DIC, and there is no clear evidence for recommending fixed-dose administration of AT preparations to patients with DIC. Although the dosage and administration of pAT preparations does not specify any daily dose frequency, once-daily administration was selected for the clinical studies of rAT-G. However, divided administration of a daily dose has been reported to be effective in maintaining plasma AT activity (*Shock*. 2007;28:141-7), and the applicant therefore decided not to specify any daily dose frequency for the dosage and administration of rAT-G.

The dosage of approved pAT preparations for the treatment of emergency cases of DIC such as obstetric or surgical DIC has been set at 40 to 60 IU/kg once daily. In the practice guidelines for obstetrics and gynecology, the doses of pAT preparations for the treatment of obstetric DIC associated with placental abruption and massive obstetric hemorrhage are set at 3000 units (60 IU/kg) and 1500 to 3000 units (30 to 60 IU/kg), respectively (Japan Association of Obstetricians and Gynecologists. *Guidelines for Obstetrical Practice in Japan*. 2011; Japan Society for the Study of Hypertension in Pregnancy. *Guideline 2009 for Care and Treatment of Hypertension in Pregnancy [PIH]*. 2009). While there are no established guidelines for the treatment of surgical DIC, large quantities of AT preparations are supposedly required to prevent hypercoagulability due to increased consumption of coagulation factors

in patients with surgical DIC with tissue injury. Therefore, the applicant considers it appropriate to set the dose of rAT-G for the treatment of emergency cases such as obstetric or surgical DIC at 48 to 72 IU/kg, 1.2-fold the dose of pAT preparations. Although dose adjustment was not specified in the protocols of clinical studies of rAT-G (Studies 004, 005, and 006), plasma AT activity in patients with CAD has been reported to increase at different rates depending on individual patients or gestation length (*Jpn J Obstet Gynecol Neonatal Hematol.* 1995;5:74-81), which suggests that the optimal dose may differ depending on individual patients with DIC similarly to those with CAD. Therefore, it is necessary to specify a rule for dose adjustment. The maximum dose of pAT preparations is 3000 units (60 IU/kg) in the above-mentioned guidelines, and there is no clear evidence for recommending a higher dose. In consideration of safety, the applicant selected the maximum daily dose of 72 IU/kg for dose adjustment.

PMDA's view:

The results of 3 studies (Studies 004, 005, and 006) in patients with DIC with plasma AT activity of ≤70% demonstrated the equivalence of rAT-G and pAT preparations in terms of efficacy and revealed no particular safety concerns in comparison with pAT preparations. Therefore, it is appropriate to set the usual dosage of rAT-G at 36 IU/kg once daily based on the dosage regimen employed in the studies in patients with DIC. The statement that rAT-G should be administered to patients showing a decrease in plasma AT activity to ≤70% of the normal level serves as the definition of the intended patient population and, therefore, should be included in the "Precautions for Indications" section instead of the "Dosage and Administration" section. Concomitant use of heparin was not a prerequisite in Study 004, and single-agent use of pAT preparations was also prescribed in patients predisposed to bleeding because of concomitant use of heparin. The results of Study 004 showed similar efficacy between the treatment groups (Table 4-7) and a tolerable safety profile for rAT-G regardless of concomitant use of heparin. These results indicate that the concomitant use of heparin does not need to be specified in the "Dosage and Administration" section.

The dose of pAT preparations for emergency cases is 1.3 to 2-fold (40 to 60 IU/kg) the usual dose (30 IU/kg), and the practice guidelines recommend a high dose of pAT preparations for the treatment of obstetric DIC. Therefore, it is acceptable to specify that the dose of rAT-G may be increased as needed up to 72 IU/kg, double the usual dose (36 IU/kg). The "Dosage and Administration" section for pAT preparations includes the statement, "in case of emergency such as obstetric or surgical DIC," which should be understood as examples of urgent situations. Such information should be provided not in the "Dosage and Administration" section of the package insert but in other informational materials.

4.(iii).B.(5).3) Infusion rate
The applicant's explanation regarding the infusion rate:
The infusion rate was not specified in any clinical studies. The infusion time was set at 1 hour in the 3 studies in healthy adult subjects (Studies ██01, 002, and 003) and 15 minutes in the study in patients with CAD (Study EU-001). Although the infusion time was not specified in the protocol of the studies in patients with DIC (Studies 004, 005, and 006), rAT-G was administered over about 1 hour. Given that

there are no guidelines defining a specific infusion rate or time for AT preparations, the applicant decided to recommend slow infusion of rAT-G in the "Dosage and Administration" section without stating any specific infusion time or rate. rAT-G was intravenously administered using an infusion pump in Study EU-001, and by drip infusion in all the clinical studies conducted in Japan. In clinical practice, AT preparations are likely to be administered by intravenous infusion in most cases. However, a syringe pump that allows accurate dosing of a small amount of the drug solution may be used for intravenous injection to certain patient populations such as children. The use of intravenous injection also seemed necessary, and thus the applicant decided to recommend the administration of rAT-G either by slow intravenous injection or by intravenous infusion, similarly to that of pAT preparations.

PMDA considers that it is acceptable to specify the administration of rAT-G either by slow intravenous injection or by intravenous infusion. Information regarding the infusion time used in the clinical studies of rAT-G should be provided to healthcare professionals using appropriate informational materials.

Based on the review results presented in the above 4.(iii).B.(5).1) to 4.(iii).B.(5).3), PMDA considers that the dosage and administration of rAT-G should be specified as follows:

[Dosage and administration]
The product should be reconstituted with the supplied water for injection, and the reconstituted solution should be administered either by slow intravenous injection or by intravenous infusion.
1. Thrombophilia due to congenital antithrombin deficiency
   The dosage is 24 to 72 international units (IU)/kg, administered once daily.
2. Disseminated intravascular coagulation (DIC) accompanied by a decrease in antithrombin
   The usual adult dosage is 36 IU/kg administered once daily. The dose may be adjusted according to the patient's condition. The maximum daily dose should not exceed 72 IU/kg.

4.(iii).B.(6) Post-marketing investigations
The applicant's explanation on the post-marketing surveillance of rAT-G:
The safety and efficacy of rAT-G in clinical practice will be investigated in a drug use-results survey in patients with DIC excluding obstetric and surgical DIC (use as an emergency measure). The target number of patients will be 1500, which is expected to provide a ≥95% probability of observing at least one case of adverse drug reactions with an incidence of 0.2%, including hemorrhage that is planned to be selected as an investigation item for safety specification in the drug use-results survey. The registration and survey periods for the 1500 patients will be 4 years and 6 years, respectively.

It is difficult to estimate the enrollment of patients with CAD, obstetric DIC, or surgical DIC (use as an emergency measure) due to the scarcity of eligible CAD and obstetric DIC patients and the unpredictability of the number of patients with surgical DIC qualified for use of rAT-G as an emergency measure. Therefore, the applicant plans to conduct a separate drug use-results survey in these patient populations without setting the target number of patients so as to collect as much relevant information

as possible. The registration and survey periods for these patients will be 4 years and 6 years, respectively. The estimated numbers of patients with CAD and those with obstetric DIC who may be enrolled are █ to █ and █ to █, respectively.

PMDA's view:

rAT-G has so far been used in only a limited number of patients with DIC in clinical studies, and there is no clinical experience with rAT-G in Japanese patients with CAD or emergency cases of obstetric or surgical DIC. Therefore, as much safety information as possible on rAT-G in clinical practice should be collected via post-marketing surveillance and the obtained information should be promptly provided to healthcare professionals. For patients with CAD and obstetric or surgical DIC (use as an emergency measure), a post-marketing surveillance should be conducted, preferably as an all-case surveillance study, for a specific period.

III. Results of Compliance Assessment Concerning the Data Submitted in the New Drug Application and Conclusion by PMDA

1. PMDA's conclusion on the results of document-based GLP/GCP inspections and data integrity assessment

Document-based compliance inspection and data integrity assessment were conducted in accordance with the provisions of the Pharmaceutical Affairs Act for the data submitted in the new drug application. As a result, there were no particular problems. Thus, PMDA concluded that there should be no problem with conducting a regulatory review based on the submitted application documents.

2. PMDA's conclusion on the results of GCP on-site inspection

GCP on-site inspection was conducted in accordance with the provisions of the Pharmaceutical Affairs Act for the data submitted in the new drug application (5.3.1.2-2, 5.3.5.1-1, and 5.3.5.2-1). The results showed satisfactory overall GCP compliance in the conduct of clinical studies, and PMDA therefore concluded that there should be no problem with conducting a regulatory review based on the submitted application documents. However, the following issue was found regarding some clinical study sites (medical institutions), albeit with no major impact on the overall study evaluation, and was notified to the heads of the medical institutions in question as an issue requiring corrective action.

Issue requiring corrective action
Clinical study sites (medical institutions)
• Inappropriate study drug management (dispensing of the wrong study drug to some study subjects)

IV. Overall Evaluation

Based on the submitted data, the efficacy of rAT-G in patients with thrombophilia due to congenital antithrombin deficiency or disseminated intravascular coagulation (DIC) accompanied by a decrease in antithrombin is expected and its safety is acceptable in view of its observed benefits. rAT-G is thus considered to be a clinically significant therapeutic option for thrombophilia due to congenital

antithrombin deficiency or disseminated intravascular coagulation (DIC) accompanied by a decrease in antithrombin.

This application may be approved if rAT-G is not considered to have any particular problems based on comments from the Expert Discussion on the efficacy, safety, post-marketing investigations, and other issues.

<center>**Review Report (2)**</center>

<div align="right">May 11, 2015</div>

I. Product Submitted for Registration

[Brand name]   Acoalan Injection 600

[Non-proprietary name]   Antithrombin Gamma (Genetical Recombination)

[Applicant]   Kyowa Hakko Kirin Co., Ltd.

[Date of application]   July 31, 2014

II. Content of the Review

The comments from the Expert Discussion and the subsequent review by the Pharmaceuticals and Medical Devices Agency (PMDA) are outlined in the following sections. The expert advisors for the Expert Discussion were nominated based on their declarations etc. concerning the product submitted for registration, in accordance with the provisions of the "Rules for Convening Expert Discussions etc. by Pharmaceuticals and Medical Devices Agency" (PMDA Administrative Rule No. 8/20 dated December 25, 2008).

(1) Premise for efficacy evaluation

As with endogenous antithrombin (AT), Antithrombin Gamma (Genetical Recombination) ("rAT-G") binds to thrombin and serine proteases such as activated coagulation factor X, thereby inhibiting the coagulation system and suppressing thrombus formation. PMDA concluded that the efficacy of rAT-G was evaluable by a pharmacokinetic comparison based on plasma AT activity between rAT-G and approved plasma-derived AT (pAT) preparations with established efficacy.

The above conclusion of PMDA was supported by the expert advisors.

(2) Efficacy

(2).1) Comparison to existing antithrombin preparations

In Study 003, a comparison was made between 72 IU/kg of rAT-G and 60 IU/kg of a pAT preparation in terms of pharmacokinetic parameters based on plasma AT activity. PMDA concluded that rAT-G, when administered at a dose that is 1.2-fold that of pAT preparations, is likely to maintain plasma AT activity in the same range as pAT preparations do, as shown by the results of Study 003, and therefore shows promise as a therapeutic agent having anticoagulant action.

The above conclusion of PMDA was supported by the expert advisors.

(2).2) Efficacy in patients with DIC

rAT-G has been shown by Study 003 to maintain plasma AT activity in the same range as pAT preparations do. In Study 004, the proportions of subjects who were free of DIC on Day 6 and of those who were alive on Day 28 were similar between the rAT-G group and pAT preparation group. PMDA

concluded that these results suggest the therapeutic efficacy of rAT-G in the treatment of DIC accompanied by a decrease in plasma AT activity.

No clinical studies have been conducted in patients with obstetric or surgical DIC, but given the scarcity of these patients, there are clearly difficulties associated with the conduct of such studies. Irrespective of the underlying condition, excessive coagulability is a common feature of DIC, and hence it can be deduced that the mechanism of action of rAT-G shows promise in terms of its efficacy against DIC regardless of the underlying cause. Therefore, PMDA concluded that, like pAT preparations, rAT-G can be indicated for the treatment of DIC without specifying the underlying condition.

The above conclusions of PMDA were supported by the expert advisors.

(2).3) Efficacy in patients with congenital antithrombin deficiency
PMDA concluded that rAT-G in patients with thrombophilia due to congenital antithrombin deficiency (CAD) shows promise in terms of its efficacy, which is similar to that of pAT preparations, for the following reasons:
- rAT-G has been shown by Study 003 in healthy adults to maintain plasma AT activity in the same range as pAT preparations do;
- Study EU-001 in patients with CAD showed that rAT-G can be effectively used for AT supplementation in patients with CAD as in healthy adults;
- According to literature information, patients with CAD were effectively treated with pAT preparations or recombinant AT preparations in Japan and overseas; and
- The mechanism of action of rAT-G also shows promise in terms of its efficacy against thrombophilia due to CAD.

The above conclusion of PMDA was supported by the expert advisors.

(3) Safety
DIC is a serious condition with a potentially fatal outcome, and serious adverse events including deaths occurred during clinical studies of rAT-G in patients with DIC. A causal relationship could not be ruled out in 2 cases of serious adverse events leading to death, both of which are considered to have been related to the progression of underlying or concurrent conditions. On the other hand,, all hypersensitivity-related adverse drug reactions were mild or moderate with the outcome being reported as "recovered/resolved," and there were no other adverse events of clinical concern. Therefore, PMDA concluded that rAT-G is a tolerable therapy with no specific safety concerns in comparison with existing pAT preparations.

The above conclusion of PMDA was supported by the expert advisors.

(4) Indications

PMDA concluded that the clinical positioning of rAT-G should be similar to that of pAT preparations because of similarity in terms of efficacy and tolerability between rAT-G and pAT preparations and that, therefore, rAT-G can be indicated for the treatment of "thrombophilia due to congenital antithrombin deficiency" and "disseminated intravascular coagulation (DIC) accompanied by a decrease in antithrombin," similarly to existing pAT preparations. A term "antithrombin III" used in the indications of existing pAT preparations is now called "antithrombin," and, therefore, PMDA considers that the indications of rAT-G should be revised accordingly.

PMDA also considers that rAT-G, the first genetically recombinant AT preparation, poses a lower risk of infection transmission compared with pAT preparations.

The above conclusions of PMDA were supported by the expert advisors.

The expert advisors commented that another Japanese term for "disseminated intravascular coagulation" is now more commonly used. PMDA instructed the applicant to revise the indications, and the applicant followed the instruction accordingly.

(5) Dosage and administration
(5).1) Dosage and administration in treatment of CAD

A weight-based dose (50 IU/kg) was selected in Study EU-001 in patients with CAD, and there is no clear evidence for recommending divided administration of a daily dose. PMDA concluded that the dosage in the treatment of CAD should be 24 to 72 IU/kg once daily and that advice on plasma AT activity monitoring should be included in the package insert.

The above conclusions of PMDA were supported by the expert advisors.

(5).2) Dosage and administration in treatment of DIC

PMDA concluded that the usual dosage of rAT-G in patients with DIC should be 36 IU/kg once daily, based on the dosage regimen used in the clinical studies of rAT-G in DIC patients with plasma AT activity of ≤70% (Studies 004, 005, and 006). The dose of approved pAT preparations for emergency cases has been set at 40 to 60 IU/kg, a level 1.3- to 2-fold the usual dose (30 IU/kg), and a high dose of pAT preparations is likely to be administered in the treatment of obstetric or surgical DIC. Therefore, PMDA concluded that the dose of rAT-G should be increased as needed up to 72 IU/kg, double the usual dose (36 IU/kg), similarly to pAT preparations. The proposed statement for the dosage and administration of rAT-G should be modified in line with the following three instructions:

- The statement that rAT-G should be administered to patients showing a decrease in plasma AT activity to ≤70% serves as the definition of the intended patient population and, therefore, should be included in the "Precautions for Indications" section;
- Concomitant use of heparin does not need to be specified as a part of the dosage and administration

for the following reasons: concomitant use of heparin was not a prerequisite in Study 004; single-agent use of pAT preparations was also prescribed in subjects predisposed to bleeding because of concomitant use of heparin; and the results of Study 004 showed similar efficacy between the treatment groups and a tolerable safety profile for rAT-G regardless of concomitant use of heparin.

- The "Dosage and Administration" section for pAT preparations includes the statement, "in case of emergency such as obstetric or surgical DIC," which should be understood as examples of urgent situations. Such information should be provided not in the Dosage and Administration section of the package insert but in other informational materials.

The above conclusions of PMDA were supported by the expert advisors.

(5).3) Infusion rate
The infusion rate was not specified in any clinical studies of rAT-G. The infusion time was set at 1 hour in the 3 studies in healthy adult subjects (Studies █01, 002, and 003) and 15 minutes in the study in patients with CAD (Study EU-001). Although there was no protocol-specified infusion time in the studies in patients with DIC (Studies 004, 005, and 006), rAT-G was administered over about 1 hour.

For approved pAT preparations, no specific infusion time or rate has been defined in the "Dosage and Administration" section other than the instruction that the dose should be administered slowly. PMDA concluded that it is acceptable to recommend slow infusion of rAT-G in the "Dosage and Administration" section without defining any specific infusion time or rate while providing information on the infusion time used in the clinical studies of rAT-G and to specify administration either by slow intravenous injection or by intravenous infusion as the administration method, similarly to that of pAT preparations.

The above conclusions of PMDA on the dosage and administration were supported by the expert advisors.

The following comments were made by the expert advisors:
It will be sufficient to state that rAT-G should be administered either by slow intravenous injection or intravenous infusion, similarly to pAT preparations, to prevent any confusion in clinical practice, and there is no need to provide the infusion times used in the clinical studies of rAT-G.

Taking into account the comments from the Expert Discussion shown in 1) to 3) above, PMDA concluded that the "Dosage and Administration" section should be revised as follows. PMDA instructed the applicant to revise the section in question, and the applicant followed the instruction accordingly.

[Dosage and administration]
The product should be reconstituted with the supplied water for injection, and the reconstituted solution should be administered either by slow intravenous injection or by intravenous infusion.
1. Thrombophilia due to congenital antithrombin deficiency

The dosage is 24 to 72 international units (IU)/kg, administered once daily.

2. Disseminated intravascular coagulation (DIC) accompanied by a decrease in antithrombin

The usual adult dosage is 36 IU /kg administered once daily. The dose may be adjusted according to the patient's condition. The maximum daily dose should not exceed 72 IU/kg.

(6) Risk Management Plan (draft)

As reviewed in "4.(iii).B.(6) Post-marketing investigations" of the Review Report (1), PMDA considered that as much safety information as possible on rAT-G in clinical practice should be collected via post-marketing surveillance and the obtained information should be promptly provided to healthcare professionals because rAT-G has so far been used in only a limited number of patients with DIC in clinical studies. In addition, given that there is no clinical experience with rAT-G in Japanese patients with CAD or as an emergency measure in patients with obstetric or surgical DIC, a post-marketing surveillance in these patient populations should be separately conducted, preferably as an all-case surveillance study, for a specific period.

The above conclusions of PMDA were supported by the expert advisors.

The following comments were made by the expert advisors:

- Although rAT-G should be indicated for the treatment of DIC without specifying underlying conditions, information to be collected through post-marketing surveillance should include information related to underlying causes such as obstetric, surgical, or hematopoietic conditions taking into account that infection was the main underlying cause of DIC that was investigated in the clinical studies.

- The results of a questionnaire survey showed that there were 119 patients with CAD in Japan (Results of Survey for the Required Quantity of Blood Coagulation Factor Products 2013, *Blood Products Research Organization Report*. 2013;138:9-16). However, considering that it was not the main purpose of the survey to identify the number of patients with CAD, the above result may not have accurately reflected the actual number of CAD patients. Given that the incidence of CAD in Japan has been reported to be approximately 0.2% (*Japanese Journal of Thrombosis and Hemostasis*. 2000;11:510), the hypothetical number of CAD patients including those who have not yet been diagnosed is estimated to be approximately 200,000, a much larger number than that shown in the survey. Therefore, as much information as possible on this patient population should be collected.

- For obstetric and surgical DIC, survey methods, including early post-marketing phase vigilance, should be devised so as to cover all relevant cases.

Taking account of the comments from the Expert Discussion, PMDA instructed the applicant to collect as much information as possible through post-marketing surveillance, including underlying or concurrent conditions, plasma AT activity, dose of rAT-G, concomitant drugs, and outcomes. The applicant agreed to follow this instruction.

PMDA reviewed the draft risk management plan (RMP) of rAT-G proposed by the applicant and concluded that the RMP should include the safety and efficacy specifications shown in Table 1 and the additional pharmacovigilance activities and risk minimization activities shown in Tables 2 and 3. Although the applicant has estimated that the number of patients with CAD to be enrolled as ▋ to ▋, a larger number of patients may be enrolled, judging from the number of potential cases. Therefore, the post-marketing surveillance plan including the target number of patients should be reviewed at a certain point in time after the start of the surveillance, taking account of the actual status of enrollment. PMDA provided the applicant with the above instructions, and the applicant agreed to follow them.

Table 1. Safety and efficacy specifications in the draft risk management plan

Safety specifications		
Important identified risks	Important potential risks	Important missing information
Not applicable	• Shock, anaphylaxis • Hemorrhage due to concomitant use of anticoagulants • Hemorrhage	Not applicable
Efficacy specifications		
Efficacy in clinical practice		

Table 2. Outline of additional pharmacovigilance activities and risk minimization activities in the draft risk management plan

Additional pharmacovigilance activities	Additional risk minimization activities
• Early post-marketing phase vigilance • Drug use-results survey	• Early post-marketing phase vigilance

Table 3. Synopsis of use-results survey

	Survey in patients with DIC	Survey in patients with CAD
Objectives	To evaluate the safety and efficacy of rAT-G in patients with DIC accompanied by a decrease in AT in clinical practice	To evaluate the safety and efficacy of rAT-G in patients with thrombophilia due to CAD in clinical practice
Survey method	Central registry system	Central registry system
patient population	Patients with DIC accompanied by a decrease in AT	Patients with CAD
Observation period	For 28 days after the start of treatment with rAT-G	From the start of treatment with rAT-G to 28 days after the completion of the treatment (For up to 12 months)
Target number of patients	• Use in emergency cases such as obstetric or surgical DIC: All patients, if possible • Other use: 1500 patients	All patients, if possible
Main survey items	Patient characteristics, underlying causes of DIC, previous or concurrent diseases, pre-treatment for DIC, use status of rAT-G, concomitant drugs, concomitant therapies, laboratory data, DIC diagnostic criteria, scores (DIC score, SOFA score), efficacy-related information, adverse events	Patient characteristics, pregnancy/lactation (female patients only), previous or concurrent diseases, pre-treatment for thrombophilia or thromboembolism, use status of rAT-G, concomitant drugs, concomitant therapies, laboratory data, efficacy-related information, reason for stopping treatment with rAT-G, adverse events

III. Overall Evaluation

As a result of the above review, PMDA has concluded that rAT-G may be approved with the following conditions, after modifying the indication and dosage and administration as shown below. As rAT-G is a drug with a new active ingredient, the re-examination period is 8 years. Neither the drug substance nor the drug product is classified as a poisonous drug or a powerful drug. The product is classified as a biological product.

[Indication]

Thrombophilia due to congenital antithrombin deficiency

Disseminated intravascular coagulation (DIC) accompanied by a decrease in antithrombin

[Dosage and administration]

The product should be reconstituted with the supplied water for injection, and the reconstituted solution should be administered either by slow intravenous injection or by intravenous infusion.

1. Thrombophilia due to congenital antithrombin deficiency

   The dosage is 24 to 72 international units (IU)/kg, administered once daily.

2. Disseminated intravascular coagulation (DIC) accompanied by a decrease in antithrombin

   The usual adult dosage is 36 IU/kg administered once daily. The dose may be adjusted according to the patient's condition. The maximum daily dose should not exceed 72 IU/kg.

[Conditions for approval]

The applicant is required to develop and appropriately implement a risk management plan.